Applications of Passion Fruit Extracts as Bioactive Pharmaceuticals, Biodiesel Additive for Oxidation Stability and Corrosion Resistance

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DOCTOR OF PHILOSOPHY

by

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Applications of Passion Fruit Extracts as Bioactive Pharmaceuticals, Biodiesel Additive for Oxidation Stability and Corrosion Resistance

Sukumar Purohit

Dedicated to My Family,
Teachers, Friends, and Society

SCHOOL OF ENERGY SCIENCE AND ENGINEERING

INDIAN INSTITUTE OF TECHNOLOGY GUWAHATI

STATEMENT

I do hereby declare that the content embodied in this thesis entitled "**Applications of Passion Fruit Extracts as Bioactive Pharmaceuticals, Biodiesel Additive for Oxidation Stability and Corrosion Resistance"** is the result of investigations carried out by me at the School of Energy Science and Engineering, Indian Institute of Technology Guwahati, Guwahati, India, under the guidance of Prof. Vaibhav V. Goud and Prof. Lingaraj Sahoo. In keeping with the general practice of reporting scientific observations, due acknowledgements have been made wherever the work described is based on the findings of other investigators.

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CERTIFICATE

This is to certify that the thesis entitled **"Applications of Passion Fruit Extracts as Bioactive Pharmaceuticals, Biodiesel Additive for Oxidation Stability and Corrosion Resistance" submitted** by **Mr. Sukumar Purohit (Roll No.: 166151007)**, a research scholar in the School of Energy Science and Engineering, Indian Institute of Technology Guwahati for the award of the degree of Doctor of Philosophy, is a record of the original research work carried out by him under our supervision and guidance. The thesis has fulfilled all requirements as per the regulations of the institute and in our opinion has reached the standard needed for submission. The work documented in this thesis has not been submitted to any other University or Institute for the award of any degree.

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Abstract

Fruits and vegetables are frequently consumed because of their contents - minerals, dietary fibers, vitamins, and antioxidants which are beneficial for the health. In developing countries there are very limited to negligible waste management policies. As a result, most of the waste materials are directly exposed to the open environment causing severe environmental pollution. Agro-waste materials are having enormous potential for valorization as they contain abundant bioactive phytochemicals, including polyphenols, anthocyanins, tannins, glycosides, vitamins, alkaloids, and many more. These compounds have also profound therapeutic values as antidiabetic, antimicrobial, anticancer, and so on. Moreover, the natural antioxidant extracted from this waste can also be utilized as natural additives to second-generation biofuels (biodiesel) for their quality improvement as oxidative stabilizers and corrosion inhibitors. Northeast India is bestowed with two biodiversity hotspots with variety of flora and fauna. Passion fruit is a less popular plant cultivated in these regions. The main edible part of this fruit is its flavourful and nutritious juice. The rest of the parts i.e. rind and seed make upto 60% of the total weight of fruit and are disposed directly without any treatment.Thus, the present work aims to explore the antioxidant potential of these two varieties of passion fruit (yellow and purple passion fruit) from Northeast India for various applications. The entire thesis work has been divided into four major parts.

The first part of the thesis presents the collection, morphological and physico-chemical characterization of the rind and seed of yellow passion fruit and purple

passion fruit collected from Northeast India. The elemental analysis of both samples revealed an abundance of potassium, calcium, magnesium, iron, manganese, and sodium in their composition. Rind samples recorded more carbohydrates and vitamin C, while the seeds of both varieties contained more protein. Further, the physico chemical characterization of passion fruit seed oil revealed that seed oil could be employed for industrial purposes.The fatty acid composition of seed oil estimated using GC showed the abundance of poly unsaturated fatty acid (PUFA). The thermochemical analysis of the oil samples revealed higher thermal stability and high pour point (-17.8°C to -20.0°C).

Further, passion fruit oil samples' biochemical analysis showed good phenolic, flavonoid, and antioxidant properties. The yellow and purple passion fruit seed oil showed significant antibacterial activity against gram-positive and gram-negative bacteria. These characterization studies demonstrated that fruit waste by-products (rind, seed, and seed oil) are nutrient-rich biomass with a high valorization perspective.

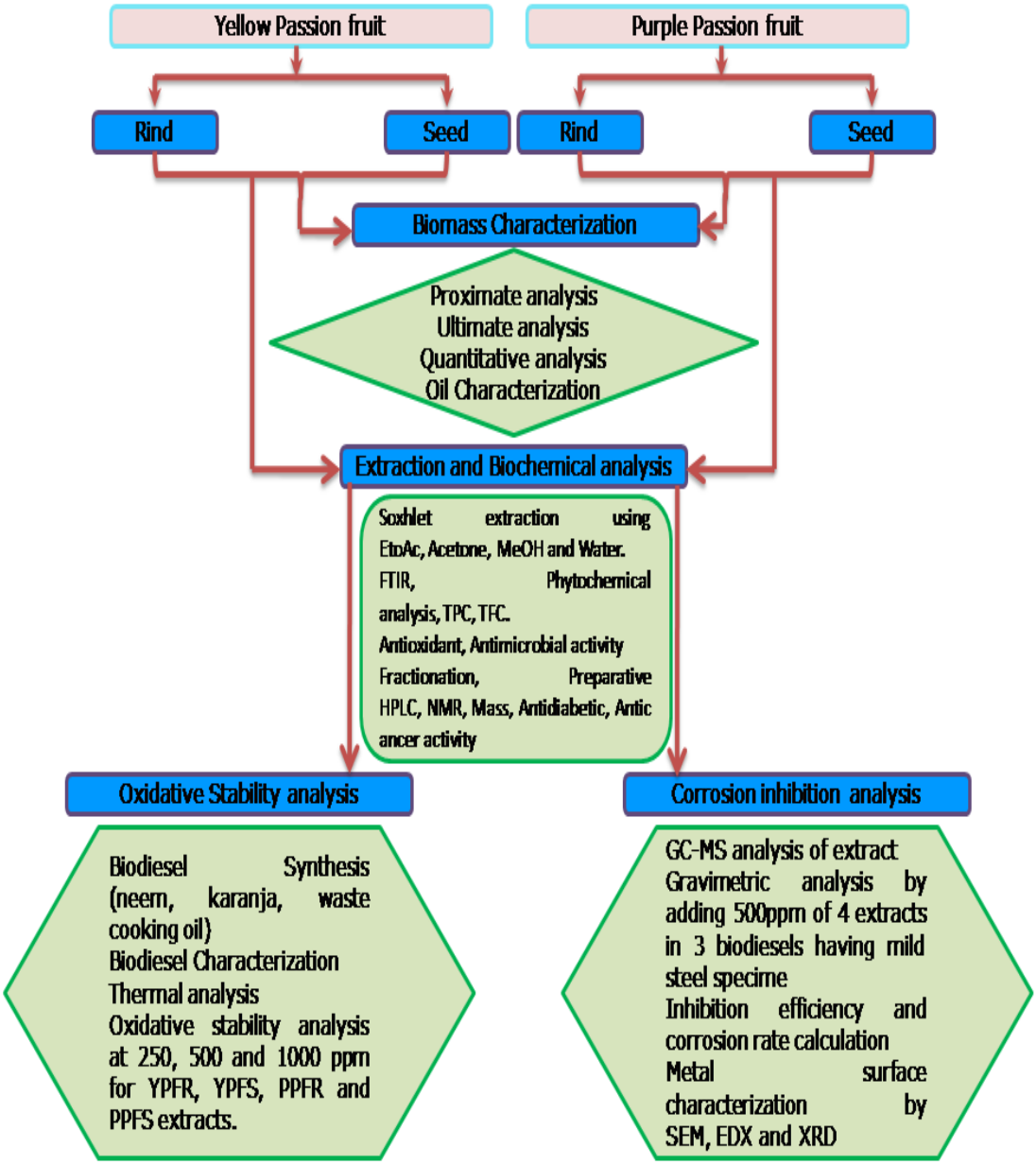
The second part of the thesis discusses the phytochemical profile of the rind and seed extracts from yellow and purple passion fruit and their role as antioxidant and antibacterial agents. In this context, the samples were extracted using four different solvents such as ethyl acetate, acetone, methanol and water. The preliminary phytochemical analysis and FTIR analysis of these extracts confirmed presence of alkaloids, glycosides, tannins, phenolic acids, flavonoids, etc. Methanolic extracts followed by the acetone extracts were found to have superior total polyphenol content, flavonoid content and antioxidant activity. HPLC analysis confirmed

presence of gallic acid, caffeic acid, ferulic acid, p-coumaric acid, quercetin, myricetin and kaempferol in these extracts. These extracts were further found to be prominent antibacterial agents against many gram positive and gram negative bacteria. In the second part, the study was further extended by subjecting the yellow passion fruit rind and seed extracts into fractionation and column chromatography for identifying and isolating other polyphenols. Through different characterization studies (^{1}H NMR, ^{13}C NMR, and mass spectrometry), the identified compounds were found to be Piceatannol, Scirpusin B, Protocatechuic acid and Prunasin. Different biological activities further confirmed, these isolated compounds are promising antioxidant, antidiabetic, antibacterial (checked only for scirpusin B) and anticancer (checked only for scirpusin B) agents.

Further, in the subsequent part of the thesis, role of the antioxidant rich passion fruit extracts for increasing oxidation stability of different biodiesel was elaborated. Biodiesel is prone to oxidation because of high moisture content and unsaturated fatty acid composition. However, natural antioxidants act on free radicals of biodiesel and delay the process of oxidation. In this context, the present work focused on to check the protective effect of different passion fruit extracts on three different synthesized biodiesels such as, Neem biodiesel, Karanja biodiesel and waste cooking oil biodiesel. Initially the biodiesel were prepared by following standard esterification and transesterification processes and the quality of synthesized biodiesel were determined through various physicochemical characterization. Thermal stability of oils and biodiesel were reported using TGA. Fatty acid composition of different biodiesel suggested higher unsaturated fatty acid. Further, Passion fruit extracts showed

remarkable oxidative stability on different biodiesel. All the extracts significantly improved the oxidative stability of all three biodiesels ranging from 1.5 to 2 fold.

Finally, the anti corrosion effects of different passion fruit extracts were checked on mild steel submerged in various biodiesel mediums. As mentioned in the above paragraph, moisture and unsaturated fatty acids present in the biodiesel speed up the oxidation process which further makes biodiesel corrosive. Due to the metal leaching process, metal starts forming sediment on the bottom of biodiesel tank and further deteriorates its quality. Therefore, in this context, the anticorrosion properties of different passion fruit extracts were checked via weight loss method. Before that, GC-MS analysis of different passion fruit extracts confirmed presence of polyphenols, aromatic compounds, and fatty acids.Weight loss analysis revealed, passion fruit extracts showed corrosion resistance effect on mild steel dipped in different biodiesel (Neem biodiesel, Karanja biodiesel and waste cooking oil biodiesel). Further, the anticorrosion efficacy of passion fruit extracts was calculated by determining the corrosion inhibition efficiency and corrosion rate. Different characterization studies such as SEM, EDX and XRD analysis were employed to further check the morphological and compositional changes on the metal surface due to corrosion and inhibition by the extracts.



Graphical Abstract for PhD Thesis Work

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Nomenclature

Abbreviation

ABTS: 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid

AOCS: American oil chemists' society

ANOVA: Analysis of variance

AT: Acetone

ATR: Attenuated total reflectance

CD_3OD: Deuterated methanol

COX: Calculated oxidizablilty value

COX-2: Cyclooxygenase-2

^{13}C NMR: Carbon nuclear magnetic resonance

DPPH: 2,2-diphenyl-1-picryl-hydrazyl-hydrate

DSC: Differtial scanning chromatography

DTT: Dithiothreitol

DMEM: Dulbecco's Modified Eagle Medium

DMSO: Dimethyl sulfoxide

EA: Ethyl acetate

EDX: Energy dispersive X-ray analysis

ESI MS: Electrospray ionisation mass spectrometry

FAME: Fatty acid methyl ester

GAPDH: Glyceraldehyde 3-phosphate dehydrogenase

GAS: Global antioxidant score

GC: Gas chromatography

GC-MS: Gas chromatography-Mass spectrometry

HPLC: High performance liquid chromatography

^{1}H NMR: Proton nuclear magnetic resonance

IP: Inductuin period

KO: Karanja oil

KBD: Karanja biodiesel

MIC: Minimum inhibitory concentration

ML: Methanol

MS: Mild steel

MTT: 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide

MUFA: Mono unsaturated fatty acid

NO: Neem oil

NBD: Neem biodiesel

OC: Oral cancer

OQS: Oil quality score

PI-FACS: Propidium iodide-fluorescence activated single cell sorting

PMSF: Phenylmethylsulfonyl fluoride

PNPG: 4-Nitrophenyl-β-D- glucopyranoside

PUFA: Poly unsaturated fatty acid

PPF: Purple passion fruit

PPFR: Purple passion fruit rind

PPFS: Purple passion fruit seed

RPCC: Reverse phase column chromatography

SEM: Scanning electron microscopy

TFC: Total flavonoid content

TGA: Thermogravimatric analysis

TNFα: Tumor Necrosis Factor alpha

TPC: Total phenolic content

UV-VIS: Ultraviolet-visible

VEGF-A: Vascular Endothelial Growth Factor A

WCO: Waste cooking oil

WCOBD: Waste cooking oil biodiesel

WR: Water

XRD: X-Ray diffraction

YPF: Yellow Passion fruit

YPFR: Yellow passion fruit rind

YPFS: Yellow passion fruit seed

ZOI: Zone of Inhibition

Units

°C: Degree centigrade

cm: Centimetre

mM: Milli molar

µM: Micro moles

v/v: Volume per volume

wt%: Weight percentage

mg/g: Milligram per gram

mg/mL: Milligram per millilitre

µg/mL: Microgram per millilitre

mm: Millimetre

N: Normality

IC_{50}: 50% Inhibitory concentration

IE%: Inhibitory efficiency

W_{corr}: Corrosion rate

mg GAE: Milligram Gallic acid

mg QUE: Milligram Quercetin

sd: Standard deviation

mg-KOH/g: Milligram potassium hydroxide per gram

CHAPTER I

Introduction and literature review

Background

Literature survey

Research gap and objectives

Organization of the thesis

Chapter I

Introduction and literature review

This chapter provides a brief overview of waste biomass valorization for identifying antioxidant rich phytochemicals present in passion fruit rind and seed and their applications in the field of food, pharma and renewable energy sectors. Insights of different passion fruit varieties availablity and distribution in India, and their applications are discussed in this section. Additionaly, phytochemical compositions of by-products such as rind, seed, leaves, etc. and their potential application in the field of energy, health and bioremediation are also discussed. This section also contains a summary of different oil feed stocks considered for synthesis of biodiesel, their limitations and remedy. Further, the review of literature section discusses about various pharmaceutical applications of different extracts and their role in improving oxidative stability of biodiesel and as inhibitors of biodiesel corrosive behavior. Based on the research lacunae, the knowledge gaps have been mentioned followed by the research objectives of the thesis and their organizations.

1.1 Introduction

Food security, health hazards, environmental pollution and fossil fuel depletion are the four major concerns in the current world. Exponential increase in the population has impacted these conditions more severely. However, researchers, analysts and other stakeholders around the globe are working relentlessly to find out suitable alternatives to address these issues. In this section of the thesis, step wise use of food

and agricultural waste in the world and scope of their valorisation in different fields including health and renewable energy is mentioned.

While over 820 million people are underfed, 1/3rd of the total food consumption by human is wasted globally (Al-Tamimi et al., 2023). Food waste index suggests that 931 million ton of food waste were generated in the year 2019 (Sinha and Tripathi, 2021). The United States of America (15 million tonnes), China (32 million tonnes), India (18 million tonnes) and Philippines (6.5 million tonnes) (Wadhwa and Bakshi, 2013) are the major contributors for food waste. Food waste can be classified into household food waste, food processing waste, agricultural residues, fruit and vegetable waste and so on. The house hold waste alone contributes around 42% of waste where the beverage companies and food processing units contribute 26% and 38% respectively (Baiano, 2014). India generates approximately 270 million of agricultural waste residues which is a big concern from the point of environmental safety (Venkatramanan et al., 2021). The agricultural waste primarily includes sugarcane bagasse, rice husk, rice straw and wheat straw, jute fibre, fruits waste including peel, seed, pomace, bark, leaves, and other vegetable waste. Among different classes of food groups, oil seed crop, tubers and root waste contribute up to 26% where the fruit and vegetable waste contribute up to 22% of generated waste (Rao and Rathod, 2019). The quantum of fruit waste generated depends upon the fruit type. For example, mango generates up to 30-50% of waste from its peel and seed, pineapple generates 40-50% of waste from its peel and leaves, citrus fruits generate up to 50% waste from its peel and seeds (Banerjee et al., 2018). Consumption of fruits and vegetables cannot be stopped or reduced because of their numerous health benefit properties and minerals, dietary fibres, vitamins and antioxidants contents. However,

population surge has increased the rate of consumption the fruit and vegetable. As a consequence, significant amount of fruit waste is generated. Very few waste management techniques are employed to manage this issue which is not sufficient for reducing environmental polluton. The fruit-waste enhances microbial contamination, release tremendous amount of green house gases, damages aquatic life and also releases bad odour. Therefore, there is a dire need of developing efficient and sustainable approaches for waste management.

In developing courtiers there are limted policies governing waste management and its conversion to by-products. As a result, these waste materials are directly exposed to the open environment causing severe environmental pollution. However, different methods for waste handling have been highly studied over the last decade. Reuse and recycling of agro-waste for development of value added products come first in the waste management hierarchy. The utilization of agro-waste to produce commercially viable products is called as bio-refinery process. Products developed from bio-refinery are classified as bio-fuels, bio-fertilizers, bio-chemicals, food additives, pigments and so on (Schieber et al., 2001). Extraction of value added products like cellulose; lignin, and hemicelluloses, etc. add on to the waste effective management and further find their applications in various fields. These agro-waste materials have enormous potential for valorisation as it also contain abundant bioactive phytochemicals including polyphenols, anthocynines, tannins, glycosides, vitamin, alkaloids and many more. These compounds have profound therapeutic values (Ben Menni et al., 2022).

Energy crisis is equally concerning as the waste management and environmental pollution issues are affecting. With population growth and increasing energy demand, fossil fuels (crude petroleum oil, natural gases, and coal) alone cannot help to meet the total energy requirement for mankind. Due to increase in transportation and industrialization, energy consumption has been increased exponentially. Economic giant countries like the US, China and India alone contribute to the 2/3rd consumption of energy worldwide (Dudley, 2018). The consequences of excessive use of fossil fuel include air pollution (due to nitrogen oxide emission from vehicles, industries), water pollution (due to crude oil spill), acid rain (due to sulfer dioxide with nitrogen oxide and water), mercury emission (due to combustion of coal), global warming (due to CO_2 emission and ozone layer depletion), excessive land use (in search of coal) which directly impact on wild life and so on. In this way, the ongoing use of fossil fuel causes extreme harm to the local climate and the entire planet. Therefore, alternative of fossil fuel such as biofuels seems an option for alleviating the environmental pollution and fossil fuel crisis.

India comes at number five in energy consumption after China, the United States of America, Russia and Japan (Pal and Mitra, 2017).The main goal for encouraging alternative energy in India is for energy security, economic development and alleviating environmental pollution. Biomass based bio energy is one of the renewable and sustainable energy apart from the wind energy and solar energy. Different bioenergy products from biomass are ethanol, methanol, biogas, bio hydrogen, biodiesel and so on. The concept of biodiesel was first highlighted in the early 1900s which further evolved with time. In 1937, a Belgian scientist developed the transesterification process for biodiesel synthesis from vegetable oil for the first time

(Knothe, 2001). Use of soybean oil based biodiesel blended with ethanol in automobile sector was also reported in early 1940s (Navas et al., 2018). From these pioneer developments, other nations started looking into biodiesel as an alternative fuel for the conventional petroleum oil.

India imports around 80% of crude oil and 50% of edible oil to fulfil the energy demand (Dewangan et al., 2018). Transportation sector in India is largely dependent on petroleum products and therefore, Indian government is taking initiatives to promote the use of biodiesel. For that, the central government is encouraging plantation of non food oil crops in the wastelands (Ravindranath et al., 2011). According to the wastelands database, currently around 467 lakh hectares of wastelands are present within India (Wasteland Atlas of India, Department of Land Resources. MoRD, GOI 2011).To avoid direct competition with edible sources and high import dependency, non edible oils are considered for their use for biodiesel production (Kumar et al., 2012; Yadav et al., 2015). Biodiesel is sustainable and having potential to reduce dependency in the fosil fuel because of its properties like non-toxicity, biodegradablity, and low emission (Rajamohan et al., 2022). Presently in India, different plant based raw materials are used as biodiesel feedstock namely *Jatropha curcas* (jatropha) and *Pongamia pinnata* (karanja). These oils contain several toxins which make them non edible. (Sharma and Singh, 2008, 2009), Other promising oil feedstock for biodiesel production are neem seed oil (*Azadirachta indica*) (Jain et al., 2021; Sayyed et al., 2022; Tasneem et al., 2022) *Madhuca longifolia* (mahua), *Manilkara zapota* (chickoo), *Schleichera oleosa* (kusum), animal fats, and microalgae, etc.(Dewangan et al., 2018).

Utility of waste resources at various stages of the biomass supply chain is increasingly gaining popularity; hence biodiesel synthesis from waste cooking oil has also gained popularity because it is cheap, help in environmental cleaning and renewable, etc. (Jain et al., 2021). Despite many advantages of biodiesels over fossil diesel, there are some limitations which include higher tendency of oxidation and corrosive behaviour that hinder the process of biodiesel to get commercialize in the main stream world (Serqueira et al., 2021). Higher unsaturated fatty acid of biodiesel is susceptible for oxidation (auto oxidation). Oxidation leads to the formation of free radicals (Yaakob et al., 2014), which degrade the biodiesel through production of degradation chemicals like acids, peroxides, alcohols, ketones, aldehydes that causes biodiesel instability. During the storage and transportation, thermal, aerobic and photo contact with biodiesel also causes oxidation leading to change in the biodiesel's physical and chemical properties like viscosity, acid value, iodine number and peroxide value, etc. (Dinkov et al., 2009; Jain and Sharma, 2011). In addition, moisture and unsaturated fatty acids of biodiesels also accelerate corrosion of metal container in which biodiesels are stored. The increase in oxygen level in the biodiesel (due to oxidation) leads to formation of oxygenated compounds which adhere to the metal surface thereby causing metal leaching and degrading its quality (Serqueira et al., 2021). The corrosive behaviour of the biodiesel can corrode the engine pipeline, pistons as well as storage tanks leading to huge economic loss to the industries. It can also pollute the biodiesel by forming sedimentation and leaching of metal into the fuel tank.

However, use of antioxidant to reduce oxidation process and corrosion in biodiesel can be a sustainable approach. Usage of synthetic antioxidants such as BHT (Butylated hydroxytoluene), BHA (Butylated hydroxyanisole) and PG (Propyl

gallate), etc. is not sustainable, non eco-friendly, costly and carcinogenic as compared to the natural antioxidants from plant origin, food by-products and waste vegetable biomass (Jain et al., 2021). Natural antioxidants on the other hand are non toxic, eco friendly and sustainable. They act on free radicals as primary antioxidants or secondary antioxidants. Primary antioxidants break the free radical chain and there by delay the oxidation process of biodiesel where the secondary antioxidants chelate the metal ions and decompose the hydro peroxide formed during the oxidation process (Varatharajan and Pushparani, 2018). Secondary plant metabolites (phenolic acids, flavonoids, terpenes, etc.) bearing OH or NH groups in their structures can donate more hydrogen atoms and break the chain reaction (Varatharajan and Pushparani, 2018).

As mentioned above, fruit and vegetable waste are rich in phytochemicals such as antioxidants, tannins; anthocyanins which could be utilised as an additives for the improvement of the quality of biodiesel. Additionally, value added products isolated from fruit waste products can also be used in various fields including food, cosmetics and pharmaceuticals. *Passiflora edulis* (purple passion fruit) and *Passiflora edulis* Var. *flavicarpa* (yellow passion fruit) are two mostly used passion fruit varieties found in very limited places in India confined to Western Ghats, Himachal Pradesh and Northeastern parts of India like Manipur and Nagaland (Purohit et al., 2021b). The remaining parts of the fruit after juice extraction consisting of peel and seed contain up to 60% of its total weight are thrown as a waste material. Passion fruit industries also generate huge waste (peel and seed) which are not employed for any waste management and eventually increase environmental pollution (Purohit et al., 2021c).

In the following sub section of this chapter, details about the phytochemicals present in passion fruit, extraction of phytochemicals, their biological activities, etc. has been discussed in details. Further, importance of various plant extracts in improving oxidation stability and corrosion in biodiesel is also systematically discussed.

1.2 Review of literature

1.2.1 Discovery of passion fruit and its history in India

Passion fruit (PF) belonging to the family: *Passifloraceae*, is reported to be discovered initaly in the dense forest of Latin America by a Monastic scholar in 16th century (Barbosa Santos et al., 2021). He found the flower presented a glimpse-view of "crowning of the sacred Jesus Christ" and thus he named the flowers as passion flower. Out of its 500-600 species distributed worldwide in various tropical and subtropical regions, most of them are extensively used since centuries. According to the book "Systematics of Fruit Crops", PF have been spread to Europe and Asia from topical America in 19th century (Sharma, 2009). It is believed that, the migration of PF in the southern parts of India, namely Tamil nadu & Kerala took place from Sri Lanka. The purple variety of PF is mostly cultivated in the regions of Manipur and Nagaland where the yellow variety is majorly grown in the regions of western ghat including Coorg, Malabar, Nilgiri hills, etc. PF is commonly known as Krishna phal in Hindi (national language of India), whereas Lota bael in Assam, Sitabon in Manipur, Sohbrap in Meghalaya, Garender in Sikkim, Pesam palam in Tamilnadu. India produces around 85000 metric tons of PF in approximately 14000 ha annually (Purohit et al., 2021a) of which the state of Manipur and Nagaland alone contribute 70%. Traditionally, the dried passion flowers and leaves were used as an addatives in

pain-killing tea. In Nagaland, the leaves of the PF has been traditionally used for the treatment of dysentery and hypertension (Jamir et al., 1999). *P. incarnate* has been locally used for the de-addiction of the morphine in India (Dhawan et al., 2004). The decoction of leaves and roots has also been used for the treatment of hysteria as well as skin diseases. Dhawan and group, have used *P. incarnate* aerial parts (Dhawan et al., 2001a), leaves, stem, flower (Dhawan et al., 2001b) to check their anxiolytic (Dhawan et al., 2003a; Dhawan et al., 2001a), aphrodisiac (Dhawan et al., 2003c), bronchospasm (Dhawan et al., 2003b), antitussive (Dhawan and Sharma, 2002) properties in vivo. There are many varieties of PF found in India which includes *P. edulis* (purple PF), *P. flavicarpa* (yellow PF), *P. quadrangularis* (granadilla), *P. mollissima* (Kunth) (Banana PF), *P. incarnate* (maypop), *P. foitida*, *P. ligularis* (parchita amarilla), *P. subpeltata* (white passion flower), etc. Along with many authentic species of PF, Indian Institute of Horticulture Research, Karnataka, have developed a hybrid variety called "Kaveri" which have a mixed characteristics of purple and yellow PF. This variety is indigenous to India having high production yield (Thokchom and Mandal, 2017). In India, PF juice is mainly consumed in the form of juice, jam, jelly, squash, etc.

1.2.2 Distribution and growing condition of Passion fruit

The most cultivated species of PF include; yellow PF (*P. edulis* var. *flavicarpa*), purple PF (*P. edulis* sims. *edulis*), giant granadilla (*P. ligularis* Juss.), sweet PF (*P. alata* Curtis), banana PF (*P. mollisima* Kunth), Gulupa PF (*P. edulis* sims. fo *edulis*) (Tripathi, 2018). These fruits were discovered from rain forests of south America but over the time they have been distributed to Australia, New Zealand, Italy, France,

Portugal African nations, China, India, Sri Lanka, Japan, Taiwan, Indonesia, etc (Wang et al., 2021a). Many of these fruits grow in 60-80% humid condition with annual rain fall of 1000 to 2500 mm on light sandy loam and sandy clay loam with a pH range of 6.5 to 7.5. The favorable growing temperature and altitude for purple PF is 15-22 ℃ in day time and 12-14 ℃ at night with an altitude of 1,600-2,300 meter above sea level (MASL); for yellow PF is 15-28 ℃ & 0-1,300 MASL; for giant granadillas is 15-23 °C and 1,800-2,600 MASL; for sweet PF is 25-26 ℃ & 100-700 MASL (Fischer and Miranda, 2021); for Gulupa PF is 12-22 ℃ & 1600-2800 MASL (Rodríguez et al., 2019); for banana PF is 13-16 °C and 1.800-3.200 MASL (Fischer and Miranda, 2021). Cloudy atmosphere reduces the plant growth, flowering and hence a minimum of 7-8 h daily sunlight is a must for PF development. Most important part of PF plant (shrubs and/ or climbers) is its fruit which is consumed for the sweet and sour juice with high nutritional value. However, the leaves, seed, waste peels (flavedo and albedo) are also equally beneficial in terms of medicinal properties and for novel formulations. Morphologically leaves are simple, alternate, margined, sometime compound and imperipinnate. The flowers are brilliantly colourful staged on a large receptacle with infinite filamentous structures between the corolla and stamens. The calyx bears 3-5 nos. of free or connected sepals to its base. The flower blooms in the warmest time of day towards the afternoon and pollination is carried out by the pollinators. The fruits are mostly round in shape (banana PF is oval in shape), and granadilla is the largest in size. The average fruit length is 6-10 cm in diameter weighing between the range of 55-120 g with a smooth and glossy texture. The fruit contains numerous seeds covered with juice filled sacs. Different parts and by-products (rind, seed) of PF is mentioned in the Figure 1.1.

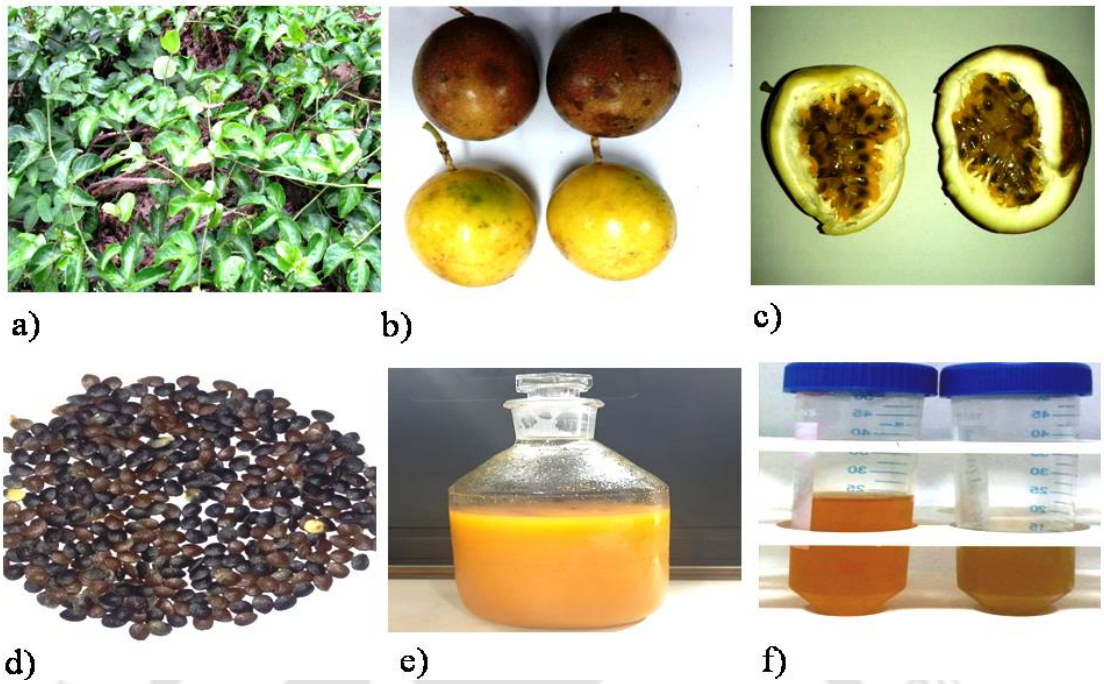


Figure 1.1. Overview of passion fruit and its by-products (a) Passion fruit vine, (b) Purple passion fruit (*Passiflora edulis* sims.) and Yellow passion fruit (*Passiflora edulis* Var. *flavicarpa*), (c) Transverse section of passion fruit showing seeds covered by juice sacs, (d) Dried Seeds, (e) passion fruit juice and (f) Passion fruit seed oil.

1.2.3 Nutritional and phytochemical profile of passion fruit

It has been evident that Agro-food sectors generate maximum amount of by-products which are outstanding source of valuable phytochemical (phenolic acids, flavonoids, carotenoids, tannins, etc.) and nutritional elements such as vitamins, macro nutrients and micronutrients. The application of these natural ingredients in food and pharmaceutical fields can fulfill the nutritional demand of the consumers and helpful in the field of drug discovery. As discussed in the introduction section, PF constitutes approximately 55-60% agro-waste by-products which hold variety of compounds responsible for arrays of biological activities. Traditionally, PF leaves, juice, seeds were used as a folk medicine for the treatment of various diseases (Andrade et al.,

2021) without knowing the composition of those plant parts. Recent research in phytochemical profiling of PF by-products presents understanding on range of bioactive molecules reserved in it. To isolate and identify these chemicals from the plant matrix, many scholars have investigated various extraction techniques with different solvent systems.

1.2.3.1 Nutritional composition of PF by-products

Due to the non-toxic nature and medicinal value, PF grabs more interest in the food industry. It has been used as a substrate for probiotics, for making various food products, etc. (Ramaiya et al., 2019) had stated that consumption of a 275g of passion fruit daily, one would get adequate micronutrients and a cup of PF juice supplies 3-6% of the daily requirement for sodium. PF by-products also contains ample amount of minerals, polysaccharides, vitamins, proteins and other macro and micro nutrients.

The proximate analysis reported for PF revealed that it is rich in dietary fiber, which increases its food value. The edible portion of passion fruit had a yield of 44.81% (w/w) total dietary fiber with 16.47% (w/w) water-soluble polysaccharide (Wongsariya and Kanchanadumkerng, 2021). The total carbohydrate content in PF edible portion was found 15.55 ± 1.18 g/100 g-wet basis. They reported PF extracted polysaccharides a potential probiotic as they support the growth of probiotic bacteria such as *Lactobacillus acidophilus* and *Bifidobacterium longum*. A chemical composition analysis of *P. edulis* seed cake used for biodiesel production showed total carbohydrate content of 750.7 g/kg-dry matter where hemicellulose content was 24.1 g/kg-dry matter; cellulose content was 227 g/kg-dry matter. It also contains non-

fiber carbohydrates 73.6 g/kg-dry matter. With these nutritional value of passion fruit seed cake, can be used as an alternative feedstock for ruminants (Véras et al., 2020). PF contains a considerable amount of heteropolysaccharide pectin. The rheological behavior of pectin attracts the attention of the food industry. Lin and group (Lin et al., 2021) used the flash extraction method for the extraction of pectin from the peel of *P. edulis f. flavicarpa* and found a yield of 7.52% which is a well known ingredient in the food industry. Macromolecules like proteins (composed of large chains of amino acids) fuels energy and carry oxygen throughout the body also regulate the body's metabolic reactions, maintain cell structure, etc. Saravanan and group (Shanmugam et al., 2020) analyzed isolated amino acids by GC-MS from *P. leschenaultia* are alanine, arginine, aspartic acid, cystine, glutamic acid, glycine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, proline, serine, threonine, tryptophan, tyrosine, valine. The amino acid content found was 1.09 ± 0.54 mg Leucine equivalent/g sample. They also reported the concentration of glutamic acid, aspartic acid, and proline higher as compared to others; while the lysin concentration was limited. Beatriz and group (Sanchez et al., 2020) showed pasteurization of *P. setacea* effective conservation method with little loss of its valuable compounds. They identified the presence of three bioactive amines in passion fruit, viz., spermidine, putrescine, agmatine. The inner flesh without seed contains 2.44 ± 0.11 g/100g-wet basis protein content. Moreover, PF is also a source of natural enzymes (e.g., amylase, xylanase). (de Andrade Silva et al., 2020) used *P. edulis* peel as a potential substrate for bioprocessing using *Lichthemia ramose*. Herein they observed the production of amylase, carboxy methylcellulase, xylanase in the substrate at different levels. Though these enzymes have the potential of reuse and can be utilities. There is very

limited literatures are available for enzyme profile of the *Passifloraceae* family which should be addressed in future. Mineral deficiency can cause diarrhea, poor immunity, anemia, colour blindness, bone decay, etc. Recent reports showed presence of sodium, potassium, calcium, magnesium, copper, zinc, manganese, and iron in seed and rind of yellow and purple PF. Potassium (304-397 mg/100g) was the predominant macro element reported in all by-products material followed by manganese (113.9-193.2 mg/100g) and calcium (4.7-139.1 mg/100g). Souza Silva (de Souza Silva et al., 2020) had investigated the physicochemical changes in *P. cincinnata* Mast. at different maturation stages. They also found potassium in higher concentration in ripening stage while calcium level decreases 60% during maturation. Vitamin C content in PF (0.6-132 mg/100g) was documented by (Adeyeye and Aremu, 2017; Asiimwe et al., 2021; Barbosa Santos et al., 2021; Purohit et al., 2021a; Sanchez et al., 2020; SANTOS et al., 2020). Adeyeye Emmanuel investigated the chemical composition of pulp, peel, and seed of *P. edulis* Sims by nitric acid digestion method. The analysis revealed the concentration of vitamin C found higher in juice, while vitamin A mostly found in the outer coat of the fruit. The HPLC analysis of acetone extracts of yellow, purple, and orange passion fruit peel, pulp, and seed showed the presences of provitamin A. The orange passion fruit peel contains the highest provitamin A content (1773 ± 56.4 µg/100g dw) than the others (Dos Reis et al., 2018). Lisa and group (Striegel et al., 2019) reported that *Passiflora* genus is a rich source of folate. Among them, *P. flavicarpa* (271 ± 3.64 µg/100g) contain a higher amount of folate, followed by *P. edulis* (136 ± 21.7 µg/100g), *P. ligularis* (64.0 ± 1.70 µg/100g).

1.2.3.2 Phytochemical composition of PF by-products

PF has been reported as a rich source of polyphenols (phenolic acids, flavonoids, stilbene, etc.), responsible for their antioxidant activity. Total phenolic content (TPC) in different by-products from various species of PF was found to be between 0.36-305.8 mg GAE/g (Barbosa Santos et al., 2021; de Souza Silva et al., 2020; dos Santos et al., 2021c; Reis et al., 2020; SANTOS et al., 2020; Shanmugam et al., 2020; Song et al., 2018; Viganó et al., 2016). MAC de Albuquerque (de Albuquerque et al., 2019) reported three compounds namely, vanillic acid (1 mg/mL food by-product water extract (FWE)), syringic acid (1 mg/mL FWE), and gallic acid (1 mg/mL FWE) identified through HPLC-DAD (High-performance liquid chromatography-Diode array detection) from of *P. edulis* f. *flavicarpa* by-product extract improved adhesion ability of *Lb. rhamnosus* (probiotic strain) to the intestinal cell. G. de S. Silva (de Souza Silva et al., 2020) studied TPC of intermediate and ripening stage pulp of *P. cincinnata* Mast and reported 53.5 ± 4.10 and 41.41 ± 2.52 mg GAE 100/g TPC in both stages respectively. The HPLC-DAD analysis confirmed presence of caffeic acid, caftaric acid, p-coumaric acid, ferulic acid, gallic acid. Most predominant phenolic acids reported from genus Passiflora are chlorogenic acid, syringic acid (Barbosa Santos et al., 2021), coumaryl quinic acid derivatives, rosmarinic acid (Song et al., 2018). Shanmugam and group (Shanmugam et al., 2020) isolated 24 polyphenolic compounds from *P. leschenaultia* using organic solvents (petroleum ether, chloroform, acetone, and methanol) by the soxhlet method. By UHPLC-QqQ-MS/MS analysis they identified artepillin, caffeic acid phenyl ester, ethyl gallate, prototcatechuic acid, vanillin, vanillic acid, cinnamic acid.

Flavonoids are another abundantly found consumable phenolic compound found in PF by-products. Barbosa (Barbosa Santos et al., 2021) extracted phenolics from *P.cincinnata* Mast. (365 mg/kg) and *P. edulis* Sims (475.1 mg/kg) pulp by liquid-liquid extraction. They analyzed the extracted flavonoids using RP-HPLC/DAD and found catechin, epicatechin, epigallocatechin, procyanidin A2, procyanidin B1, procyanidin B2, quercetin-3-glucoside, rutin, myricetin, kaempferol-3-glucoside (flavanols); hesperidin, naringenin (Flavanones).*P. edulis* Sims containsa higher value of catechin (3.08 mg/kg), while *P.cincinnata* Mast. had a higher value of epigallocatechin (3.06 mg/kg). Albuquerque (de Albuquerque et al., 2019) had quantified two flavanols in *P. edulis* f. *flavicarpa* by-product water extract and reported, rutin (7 mg/L of FEW), quercetin (4 mg/L of FEW). Pereira and group (Pereira et al., 2021) had combined the ultrasound extraction method with the pressurized liquid extraction to intensify the phenolic yield from *P. edulis* sp. rind and extracted phenolic compound (2.1 mg GAE/g dw) at a lower temperature compared to pressurized liquid extraction without ultrasound extraction. They identified and quantified the extracts by UHPLC-QE HRMS and found 116 ± 2 µg/g-dry rind isoorientin along with other flavones such as vicenin, isovitexin, vitexin, orientin. These flavonoids compounds are well known antioxidant and antimicrobial agents. Peels of three varieties of *P. edulis* sp.; purple, red, and yellow were studied by (Putra et al.) and they found, the ethanolic extract of purple passion fruit (75.14 ± 0.1 mg/kg) contained highest amount of isoorientin than red (50.16 ± 2.30 mg/kg) and yellow (30.11 ± 1.51 mg/kg) varieties. Rotta (Rotta et al., 2019) used a modified QuEChERS method to extract phenolics from the pulp of *P. edulis*, *P. alata*, and*P. ligularis*. The UHPLC-MS/MS analysis showed presence of 4-hydroxybenzoic acid, chlorogenic

acid, vanillic acid, caffeic acid, p-coumaric acid, ferulic acid, rutin, quercetin, trans-cinnamic acid. *P. edulis* pulp also contained these phenolic acides, but vanillic acid (426 ± 29 µg/kg) and quercetin (416 ± 6 µg/kg) were in abundance, while the content of rutin (289 ± 6 µg/kg) and caffeic acid (64 ± 2 µg/kg) were higher in *P. alata*, and *P. ligularis* respectively. An in-vivo study by Min (Hu et al., 2020) demonstrated that ethanolic extract contained anthocyanin from purple *P. edulis* Sims epicarp contained cyanidin-3-O-glucoside, cyanidin-3-O-rutinoside, peonidin-3-O-rutinoside that have anti-fatigue effects. UPLC-MS/MS analysis showed the presence of three anthocyanins; among them, the cyanidin-3-O-glucoside was present in the highest content i.e., 639.36 mg/g, followed by peonidin-3-O-rutinoside (40.49 mg/g), and cyanidin-3-O-rutinoside (30.55 mg/g). From a conventional extraction method with 80% ethanol acidified with 0.05% citric acid, (Ghada et al., 2020) had obtained 8.3 ± 0.1 mg of cyanidin-3-O-glucoside per gram extract from *P. edulis* Sims. An in-vitro antioxidant activity and phenolic profile investigation of peel and seeds of red-skinned passion fruit using UPLC-QTOF-MS/MS analysis showed the presence of galloyl(di)glucoside, digalloylglucoside, galloylshikimic acid, digalloyldiglucoside, trigalloylshikimic acid, ellagic acid (Tannin) have a good contribution towards antioxidant activity (Nguyen et al., 2019). Alpha tocopherol (1.68%) in PF seed oil using supercritical fluid extraction was also reported previously (Arturo-Perdomo et al., 2021). Supercritical fluid extraction integrated with fractionation (SFE-SFF) extracted more tocols (1019 ± 77.14 mg/ kg extract) from *P. edulis* Sims by-products. The extracts contained δ- tocotrienol (450.62 ± 33.75 mg), β/γ- tocotrienol (418.77 ± 45.99 mg) in higher concentration over β/γ- tocopherol, α- tocotrienol,

α-tocopherol (dos Santos et al., 2021b). They also successfully isolated 38.64 ± 8 mg β-carotene equivalent/kg extract from *P. edulis* Sims by-products.

Dos and group had (Dos Reis et al., 2018) extracted carotenoids from peel, pulp and seed of three variety of PF; (yellow, purple and orange) using acetone and further quantified them through HPLC. They found the orange PF peel (25,516 ± 561.9 μg/100 g of dry weight) contained the highest carotenoids followed by yellow (918.41 ± 36.81) and purple (1244± 52.5) varieties. The major carotenoids identified was β-carotene, followed by lutein, cryptoxanthin, α-carotene, zeaxanthin. Lycopene was only detected in orange PF pulp. A novel extraction technique using vegetable oil as solvent for the ultrasound-assisted (UAE) and microwave-assisted extraction (MAE) extraction for carotenoids from *P. edulis* peel was reported by (Chutia and Mahanta, 2021). They reported 91.4% and 86.7% of total carotenoids using olive oil and sunflower oil respectively. Squalene, a triterpene, from *P. edulis* Sims by-products (913.00 ± 56.65 mg/kg extract) was reported by (dos Santos et al., 2021b) using supercritical fluid extraction (SFE) at 40 °C and 35 MPa. Other phenolic compounds identified from PF by-products in recent times such as flavanols: quercetin hexoside, isorhamnetin glucorhamnoside, isorhamnetin glucoside ((Nguyen et al., 2019); flavones: chrysoeriol derivatives (Song et al., 2018), acacetin rhamnoside (Nguyen et al., 2019), acacetin, apigenin, luteolin; Flavanones: pinocembrin, eriodictyol (Shanmugam et al., 2020), Pinobanksin-3-o-acetate (Song et al., 2018); Isoflavones: daidzeinin (Shanmugam et al., 2020); Stilbenes: trans-resveratrol, cis-resveratrol (Barbosa Santos et al., 2021); terpenes: β-pinene, 3-δ-carene, limonene, eucalyptol, γ-terpinene, p-cymene, camphor, linalool, β-caryophyllene, α-terpineol, geranyl acetone, thymol (Shanmugam et al., 2020), cis-

ocimene, trans-β-ocimene, γ-terpinene, α-terpinolene, 1,3,8-p-menthatriene, allo-ocimene, neo-allo-ocimene, bornylene (Nguyen et al., 2019).

1.2.4 Importance of passion fruit oil

PF seed are often regarded as the agro-industrial waste by the beverage industries after the extraction of juice. The seeds part comprises of 20-25% of the whole fruit composition which is a lage portion getting discarded. The waste PF seeds contain 20-30% of oil which are great source of minerals, nutrients, essential fatty acids, secondary plant metabolites, etc. The oil yield, fatty acids, carotenoids, tocoferols and sterols composition from different PF species (mostly used for industrial or research purpose world) have been presented in Table 1.1.

PF seed oil is a reservoir of unsaturated fatty acid (Linoleic and Oleic acid) with numerous health benefits. However, several groups have also reported many low polar phenol rich compounds in the oil as well as essential oil in PF peel and seeds. Arturo-Perdomo and group (Arturo-Perdomo et al., 2021) attempted to identified different polar compounds using PF seed and found oleamide, octadecanomide isomers. These compounds are said to have anti-inflammatory and antibacterial properties. Gallic acid (2.18 mg/100 g pulp oil) was found to be the most abundant phenolic acid in the PF pulp oil followed by trans-ferulic acid, p-coumaric acid, etc. reported by (Ribeiro et al., 2020). They further identified different flavonoids as quercetin, rutin and naringenin from the pulp oil. These compounds are well known anti cancer, anti-inflammatory, anti-viral, anti-allergic agents. In a different study by (Chóez-Guaranda et al., 2017), 20 essential oils which include monoterpene, sesquiterpene, aldehyde, hydrocarbons, etc. were identified from shell and seeds of

PF. An alkylated phenol called Ionol was found to the higest among other essential oil in shell and seed (13.5and 12.1% respectively). Terpenes like Linalool, α-terpineol, d-nerolidol, etc. were also detected in shells and seed of PF.These compounds have very prominent flavours and aroma, hence used in various health and beauty products. They are beneficial in combating diseases such as inflammation, pain, spasm, etc.

Table 1.1. Oil composition and biological activity of mostly studied *Passiflora* species in recent times.

Properties	*P. edulis*	*P. flavicarpa*	*P. alata*	*P. setacea*	*P. cincinatta*	*P. tenuifila*	*P. ligularis*
Oil Recovery (wt%)	15.7-30[k,m,n,h,p]	26.1- 28[g,h]	19-28.3[a,b,c,d,f]	16-34[c,d,e,f]	13.5-25.2[c,j]	21.8-31.1[c,d,e]	12.3[i]
Tocoferol (mg/100g oil)	17.7-89.1[e,m,p,q,t]	-	116.1[e]	225.6[e]	-	81[e]	-
Carotenoids (µg/100g oil)	2.5-15.2[n,p,r,t]	-	50.8[e]	115.4[e]	-	112.3[e]	-
Sterols (mg/100g oil)	166.3- 348.2[e,m,p,t]	190[a]	192.2-330[a,e]	176[e]	-	223.5[e]	1.8[a]
Fatty Acids (wt%)							
C6:0 (Caproic)	-	1.3[g]	-	-	-	-	-
C8:0 (Caprylic)	0.1[q]	1.4[g]	-	-	-	-	-
C14:0 (Myristic)	0.07-0.1[n,q,v,u]	-	-	-	-	-	-
C16:0 (Palmitic)	7.7-13.2[e,h,l-r,t-v]	10.4- 13.4 [g,h]	10.3- 12.4[b,c,e]	9.5-9.8[c,e]	9.6-9.8[c,j]	7.8-11.2[c,e]	-
C16:1 (Palmitoleic)	0.05-0.29[m,n,q,r,t,u,v]	-	0.6-1.1[b,c,e]	0.13[c]	0.08[c]	0.19[c]	-
C18:0	1.85-5.04[e,h,l-r,t-v]	2.4- 2.9 [g,h]	2.5-3.2 [b,c,e]	3.1-3.3[c,e]	2.6-3.4[c,j]	2.9-4.2[c,e]	-

(Stearic)							
C 18:1 (Oleic)	9.1-21.7[e,h,l-v]	13.8- 17.3 [g,h]	12.9-13.7 [b,c,e]	15.9-19.5[c,e]	11.25-13.11[c,j]	13-15.5[c,e]	17.9[i]
C18:2 (Linoleic)	57.9-78.7[e,h,l-v]	68.9- 69.8 [g,h]	68.6- 72.0[b,c,e]	66.4-69.9[c,e]	73.72-75.48[c,j]	68.9-73.8[c,e]	70.9[i]
C18:3 (Linolenic)	0.15-0.9[e,h,m-r, t-v]	0.36[g]	0.62-0.80[c,e]	0.5-0.9[c,e]	-	0.56-0.58[c,e]	-
C20:0 (Arachidic)	0.10-0.4[n,q,t,u]	-	-	-	-	-	-
C22:0 (Behenic)	0.08-1.1[n,r,s]	0.91[g]	0.020[c]	0.2[c]	-	0.2[c]	-
MUFA (wt %)	12.5-23.3[e,h,l-n,qs,u,v]	13.8- 17.33 [g,h]	14.83[e]	16.4[e]	-	16.4[e]	-
PUFA (wt %)	58.1-77.5[e,h,l-n,qs,u,v]	69.34- 70.3 [g,h]	72.07[e]	70.8[e]	-	70.8[e]	-
SFA (wt %)	10-18.2[e,h,l-n,qs,u,v]	15.8- 15.93 [g,h]	13.10-15.88[c,e]	12.7-13.4[c,e]	-	12.3-12.7[c,e]	-
UFA (wt %)	81.7-90[h,l,n,s]	84.1[h]	84.12[c]	86.5[c]	-	87.6[c]	-
Biological activity	Nanoemultion for skin hydration and elasticity[l] Antioxidant[h,n]Antibacterial[h]	Antioxidant Activity, [g,h] Antimicrobial Activity [g,h]	Antioxidant Activity [b,d] Nanoemultion checked on J774 macrophages cell viability study [c]	Antioxidant Activity[d] Nanoemultion checked on J774 macrophages cell viability study [c]	Nanoemultion checked on J774 macrophages cell viability study [c]	Antioxidant Activity[d] Nanoemultion checked on J774 macrophages cell viability study [c]	-

(a) (Rotta et al., 2018), (b) (Pereira et al., 2017), (c) (de Souza et al., 2021), (d) (Reis et al., 2020), (e) (de Santana et al., 2015), (f) (De Paula et al., 2015), (g) (Pereira et al., 2019), (h) (Purohit et al., 2021c), (i) (Derosya and Syukri, 2020), (j) (de Oliveira Barretto et al., 2019), (k) (Arturo-Perdomo et al., 2021), (l) (Guzmán et al., 2021), (m) (Massa et al., 2021), (n**) (Santos et al., 2020)**, (o) (Alves et al., 2019), (p) (Delvar et al., 2019), (q) (Serra et al., 2019), (r) (dos Santos et al., 2019), (s) (Nga et al., 2019), (t) (da Silva and Jorge, 2017), (u) (Bezerra et al., 2017), (v) (Pardauil et al., 2017).

1.2.5 Biological activities reported from passion fruit waste by-products

1.2.5.1 In vitro analysis

Phytochemicals of PF like, phenolic acids, flavonoids, anthocyanins, sugars, fibres are key components responsible for treating diseases like oxidative stress, immunodeficiency, diabetes, etc. Reacting oxygen species or free radicals cause severe oxidative damage to cell which further increase the risk of degenerative diseases like cancer, diabetes, asthma, cardiovascular disease and inflammation. Antioxidant activity of the plant matrix is dependent on several variables like type of biomass chosen, extraction method, solvents used, pH, particle size of biomass, etc. Similarly there are many methods used for checking antioxidant activity like DPPH, ABTS, metal chelating, FRAP, hydroxyl radical scavenging to name a few. The high unsaturated fatty acid composition in PF seed oil adds to its antioxidant property. A similar study had been carried out by (Pereira et al., 2019) where they have utilized *P. edulis flavicarpa* seed extract through subcritical propane and other method of extraction and achieved a radical scavenging activity of in a range from 53.5-82.8% in DPPH assay. In a different study, IC_{50} value of DPPH assay of P. *edulis* Var *edulis* seed cake extract was reported to be 2734 µg/mL (Oliveira et al., 2016) and pulp of *P. glandulosa* showed scavenging activity of 39.70 ± 3.39 g/mg DPPH. These studies accounting different parts of PF with different species infers that each byproduct of PF has high antioxidant content which can be utilized for value added product development.

Antibiotic resistance in human has become a global concern to deal with pathogenic microbial infection. Several approaches have been considered, among which; use of natural resources as potent antimicrobials is in the top of the list (Jiang et al., 2021). A

study demonstrated the peel and seed extracts of two different PF varieties (yellow and purple) efficiently killed both tested gram positive and gram negative bacteria. Seed extract of *P. edulis flavicarpa* obtained from soxhlet extraction, ultrasound assisted extraction and subcritical propane extraction showed MIC against *E. coli, S. enteritidis, S. aureus* and *B. cereus* from 1.56 to 100% at concentration from 1.95 to 1000 mg/mL (Pereira et al., 2019). Ethanolic extract of peel from P. *edulis* Sims at a concentration of 300 mg/mL had shown antibacterial activity against *S. aureus* and *E. coli* (Nugraha et al., 2018). Inclusion of such antimicrobial agents into food products like beverages or soon may help in treatment of microbial infection.

Type II diabetes have become a global concern because of its exponential growth in its active patients. In case of hyperglycemia, the glucose level in the blood shoots up and the pancreases cannot sufficiently produce insulin to control that. Many approaches have been considered to lower the blood glucose level and one of such example is to inhibit carbohydrate hydrolyzing enzymes like α-amylase and α-glucosidase. In general, the α-amylase breaks the long chain carbohydrate where the α-glucosidase is involved in breakdown of starch and disaccharide to glucose. Inhibition of these enzyme delays release of glucose into blood and eventually helps in maintaining the blood glucose level. Natural or plant based anti diabetic agents have been considered for the treatment of type II diabetes. In recent times, PF has been proved to be an efficient anti diabetic agent. For instance, (Monzón Daza et al., 2021) had checked the leaf extract of *P. ligularis* for its antidiabetic activity and reported the α-amylase inhibitory IC_{50} of 31 ± 1.1 to 409.8 ± 11.4 µM compared to the standard drug Acarbose (234.1 ± 15.9 µM). The possible anti diabetic compounds from the leaves of *P. ligularis* were found to be Quercetin-3-O-β- glucoside,

Kaempferol-3-O-β-glucoside and Ligularoside A. In a different study by (Shanmugam et al., 2020), lyophilized pulp of *P.leschenaultii* was subjected to soxhlet extraction using Pet. Ether, chloroform, acetone, methanol and water and those extracts were tested for anti diabetic activity. The α-amylase and α-glucosidase inhibitory activity for the fresh pulp (32.20 and 19. 81 µM/mL) and acetone extract (38.30 and 37.72 µM/mL) were found to be most effective and their results were comparable with the standard drug (30.95 and 16.45 µM/mL). The peel flour extract of *P. edulis x.*Tai-Nung No.1 variety from China was checked by (Cao et al., 2021) for its α-glucosidase inhibition activity and found IC_{50} of 0.6 ± 0.1 mg/mL. PF parts like peel, pulp and flour can be incorporated to various food formulations which can help in lower the glycemic index.

Cancer has become one of the main diseases in increasing mortality. This crisis compels research communities to delve into more alternatives and opportunities. The aqueous extract of *P. edulis* Sims leaves was employed by (do Carmo et al., 2020) to check gut inflammation on human colorectal adeno-carcinoma cells (Caco-2 cells). *P. edulis* leaf extract showed protective effect on the intestinal epithelial barrier by reducing the pro inflammatory cytokine i.e. IL-8. Extract dose of 5 and 10 mg/mL significantly reduced IL-8 level by 65.3 and 74.5% where the 10 mg/mL dose was comparable with the standard drug budesonide (81.8%). In a similar study by (Carmona-Hernandez et al., 2019), lyophilized and 70% methanol extracted pulp of yellow, purple and sweet granadilla improved Caco-2 barrier dysfunction at 10 mg/mL dose. Polyphenols like Cynidin-3-rutinoside, ferulic acid, catechine showed anti inflammatory activity to the cancer cells. Mota (Mota et al., 2018) checked the in vitro cytotoxicity activity of seed cake extracts on MCF-7 (MCF: Michigan Cancer

Foundation) breast cancer cells. The supercritical extract exhibited dose and time dependent cytotoxicity in MCF-7 cells with an EC_{50} value of 264.6 µg/mL after 72 h of treatment. The possible mechanism of action of cytotoxic nature of the seed cake extract could be because of the fatty acid of oil (palmitic , linoleic and oleic acid) as an effective agent for DNA fragmentation, cell cycle phases modification followed by cancer cell death through apoptosis.

1.2.5.2 In vivo analysis

PF by-products contain various secondary metabolites which are responsible for their biological properties like anti diabetic, gastro protective, hypolipidemic, CNS activity, anti cancer and anti tumor, anti inflammatory, anti fatigue, cardio and hepato protective, analgesic/antipyretic, etc. Aqueous Peel extract containing polysaccharide from *P. eduils* Sims showed hypoglycemic effect on obese diabetic mice. The polysaccharide in extract promoted insulin secretion by up regulating GLP-1 (glucagon like peptide-1) and further increased liver protection at higher doses and thus reduced the blood glucose level from 27-33% in 50-200 mg/kg-doses in a dose dependent manner (Guan et al., 2021).

Worldwide predominance of obesity in human is a matter of concern which increases the threat of lifestyle related diseases like diabetes, hyper cholesteromia, hyper lipidemia, fatigue as well as life threatening diseases like cardiac and hepatic disorders. Noticeable side effects of synthetic anti obesity drugs have driven the population to follow plant based products. Plant based natural products become a thrust area for the treatment and management of hyperlipidemia related diseases. *P. edulis* Var *edulis* juice (4.2 mL/200g body weight/day) was given to pork oil ingested

Wistar rats by (Muntafiah et al., 2021). The PF juice significantly reduced triglyceride level up to 139.67 ± 28.92 mg/dL resulting lowering cholesterol level in hyper cholesterimic mice. PF juice contains polyphenols, fiber, ascorbic acid, beta carotene, etc. which have been proved to have lipid lowering potential. This statement can further be related with the work of (Vuolo et al., 2020) where they have stated ferulic acid, beta carotene, and fibre rich ethanolic extract of *P. edulis* peel exhibited reduction in inflammation by scavenging ROS in lipid phase of high fat rats.

Unhealthy life style, surge in pollution and overuse of pesticides in food, genetic mutations have increased the risk of a prevalent chronic disease like cancer now days. Based on a report by WHO, cancer has become the second most deadly disease worldwide accounting nearly 10 million fatalities per year (Alam, 2021). This crisis urgently demands new ways of therapies to treat such fatal disease. This dire need attracts researchers towards phyto-products and medicine from natural source as they are less toxic and with fewer side effects compared to synthetic drug treatments like chemotherapy. Because of the antioxidant and anti inflammatory properties, many plant based compounds are emerging as alternative drugs for cancer treatment. The 75% ethanolic extract of *P. edulis* Sims bagasse (seed and pulp residue) was tested on prostate cancer TRansgenic Adenocarcinoma Mouse Prostate (TRAMP) mice to check its associative disease conditions such as in vivo antioxidant level, blood enzymatic level to check liver function. Piceatannol, a stilbene compound from the seed of PF was identified from the aqueous extract found to have decreased oxidative stress and inflammation in prostate cancer TRAMP mice (Baseggio et al., 2021). The extract further improved the catalase and GSH level in liver citing improvement of liver function of the treated mice. Aqueous extract of PF bagasse containing

Piceatannol as the main component at a concentration of 10-40 µmol/L was checked in TRAMP mice and found that Piceatannol is efficient in delaying the progression of prostate cancer through cell cycle arrest or delaying it by up regulating the p53 protein expression (Kido et al., 2020).

PF leaves have been traditionally utilized as sedative and anxiolytic by local people. In recent times, many invivo analyses have been carried out to check on CNS activity of different varieties of PF leaves. Recently, whole fruit (peel, seed and pulp) was lyophilized and extracted with 70% ethanol and two different doses (200 and 400 mg/kg) were administered to mice to evaluate the anxiolytic, anti depressant, anticonvulsant and sedative-hypnotic properties. The tested group of mice showed decreased locomotive activity (anxiolytic), negative result in rota rod test (muscle relaxant), and increased sleep time confirmed the sedative-hypnotic nature of the whole fruit extract. Different polyphenols like hydroxyl benzoic acid, ferulic acid, apigeni-C-deoxyhexoside were believed to be the probable compounds found in the whole fruit extract to display such CNS activity (Holanda et al., 2020).

Peptic ulcer mostly caused by *H. pylori* infection, consumption of alcohol and smoking, overuse of non steroidal anti inflammatory drug has been a serious health challenge worldwide (Wang et al., 2021b). Alcohol consumption greatly impacts the peptic health by gastric mucosal injury, bleeding, lesion and perforation. Many synthetic drugs have been employed for the treatment but they come with severe side effects like kidney damage, cardiovascular ailments, etc. Therefore natural source driven alternative anti ulcer drugs have been taking into consideration because of safety and economical aspects. Abboud (Abboud et al., 2019) had reported the soluble

dietary fiber from *P. edulis flavicarpa* peel significantly reduced the gastric lesion of female Wistar rat by 87.17% when compared to the control group. This result was comparable with the standard drug (93.06%). Interestingly, the intraperitonial injection of the soluble dietary fiber also showed gastro protective effect by 72.5% at 1 mg/mL. This experiment refers that, soluble dietary fiber from PF peel not only promote stomach protection via oral route but also by parenteral route. The group had also quantified galacturonic acid (a well know monosaccharide having notable anti ulcer activity) in the peel was concluded as the responsible agent for antiulcer property of PF peel flour.

1.2.6 Application of passion fruit as functional food

PF peel and seed accounts two-third of the fruit get wasted in PF processing. However, these byproducts contain valuable phytochemical and nutritive elements which has immence potential to be used by food industries to add on to the technological properties of the food products (texture, flavous, colour, shelf-life, etc.) as well as up the nutrition quotients (fibres, pectin, flavonoids, minerals and vitamins, etc). Recent development in sustainable utilization of PF byproducts in food sector have been discussed in this section.

Functional healthy beverages are in great demand these days which comprises of mixing of healthy nutritious food with conventional food items that can boost great health benefits. Mendes (Mendes et al., 2021) made kefir (a pribiotic beverage consortium of bacteria and yeast) using two varieties of PF (Caatinga PF and yellow PF) juice and checked their cell viability, GI survival, physicochemical and sensory properties. Cell viability of yeast was increased in yellow PF where bacterial viability

increased by 75% in Caatinga PF kefir (CPK) at 60 days implying increased probiotic activity. Stable acids level (citric acid, malic acid) and low ethanol production (caused by yeast and *Lactobacillus*) in CPK were found stable till 60 days confirmed the product quality. The sensory characteristics were also appreciated. PF juice kefir is a new addition to this beverage category after well known apple and pear kefir. Likely, the physical, sensory and nutritional quality of a liquid yogurt was enhanced by (De Toledo et al., 2018) when they mixed 0-8% of has PF peel and seed flour with drinkable yogurt. The insoluble fibre from peel and seed contributed to the nutritional value where the flour also increases the viscosity of the yogurt. The 2% flour added drinkable yogurt was greatly accepted by the consumers on its sensory aspects. Albuquerque (Albuquerque et al., 2017) used *P. edulis* f. *flavicarpa* peel as a fermentable carbohydrate source with fructo-oligosaccharide (FOS) prebiotic to increase production of folate in soymilk (SM) fermented product using different strains. PF peel with FOS in soymilk showed significant increase in folate in co-culture conditions (ST-M6+LGh = 1466± 37 and TH-4-LGh= 1795 ± 49) as compared to the control (SM+FOS+PF =197±1). The use of PF peel as a carbohydrate source in fermented products with other probiotics increase folate levels. Similar work has also been reported by (Casarotti et al., 2018) where they have used three different fruit by-products like guava, orange, and PF to ferment goat milk, fermented oat beverage, and fermented rice beverage and further checked their fermentation kinetics, post-acidification, probiotic viability and survival under gastrointestinal condition. All types of substrates helped in promoting bacteria during the fermentation step and also in the maintenance of the probiotic population during storage conditions. The PF peel increases the tolerance capacity of bacteria in a harsh

GI environment. Arias and group (Arias-Lamos et al., 2019) attempted a yogurt formulation by mixing PF pericarp to check its quality characteristics. The formulation came out to be fiber and nutrition-rich as well as acceptable sensory characteristics. The epicarp mixing further increased the acidity as well as the viscosity of the yogurt to make it more palatable for consumer. Pectin from the PF peel also help in GI survival od several probioticsand also help in maintaining lactic acid level for longer time (Santos et al., 2017). Therefore, PF peel can be considered as probiotic food carrier for different food preparation.

Not only the direct fruit by-product (pulp, peel, juice), but also the extracts of PF has been employed to protect shelf-life of various food product. Costa (Costa et al., 2020) has mixed the semi-purified *P. cincinnata* Mast. extract with traditional Brazilian cheese (coalho) to check development of microbial load in control and treated groups. The PF aqueous extract was found to be very much effective against *Listeria* species, *S. aureus,* and multidrug-resistant *S. aureus.* and did not hamper more (less MIC) to the indigenous lactic acid bacteria of cheese. This study prevails efficiency of PF aqueous extract as a safe and strong antimicrobial can be added to coalho cheese to increase storage time. In a different study, (Rotta et al., 2020) mixed PF seed extract to a dairy beverages (milk concentrate) formulation containing Omega-3 rich linseed oil. They checked the antioxidant potential and protection towards ω-3 PUFA (Omega-3 family) oxidation by *P. edulis* Sims. seed extract. PF seed extract did not alter the fat droplet size in the formulation (implies physical stability), decreased oxidation (specially Omega-3) during the storage of both pasteurized and sterilized formulations. Their simulation study also evidenced that PF seed extract did not influence the dairy protein or lipid digestibility. Therefore the authors proposed to use

PF seed extract as a natural antioxidant to prevent lipid oxidation of dairy products during storage.

Mechanically separated tilapia (discarded product) has great source of nutrients, high dietary fiber, protein, etc. Dos Santos (dos Santos et al., 2021a) developed a spam like meat product by adding *P. edulis* Sims peel and transglutaminase (an aggregating agent helps in reconstruction of meat) and further evaluated its physicochemical and technological qualities. Less particle size (4.72 ± 0.185 mm), low water content (0.28% ±0.01), and stable pH PF (4.12 ± 0.02) made peel flour an ideal agent to be used in spam-like products. The carbohydrates (0.79 ±1.95 g/g), protein (0.05 g/g), ash (0.06 g/g) content were also found higher in PF peel 2.5 % PF peel with 1% transglutaminase formulation was high in protein (10.4%) and fibre (1.78%) was greatly appreciated for its sensory acceptance and overall acceptance. Ramli and group (Ramli et al., 2020) had marinated PF peel extract with pressured meat to check its antioxidant and antibacterial properties upon storage. The marinated meat was found to be safe and consumable after 9 days of refrigeration where the extract also showed antibacterial activity against foodborne pathogens like *S.aureus*, *serratia* and *E. coli*.

From these above examples it can be noted that, PF agro-waste like peel, seed, albedo, flavedo, pomace have great potential to be utilized in different food preparations to make food items nutritious, healthy and more appealing for consumers. Role of PF treated (after maceration) and untreated flour in stabilizing, emulsifying, thickening, and gelling of on nectar, mayonnaise, ice cream syrup topping, and structured PF respectively was reported by (Coelho et al., 2017). The PF-treated flour was found to

be ultimate stabilizing agent for nectar (30% cloud) compared to commercial agents carrageenan and guar gum (10% and 15% cloud). The emulsifying (gumminess) property of treated and untreated flour was comprable with guar gum. The viscosity of fruit syrup was highest for xanthan gum (commercial) followed by treated and untreated flour. The gelling capacity of PF treated and untreated flour on structured PF was highest when compared to control group. The use of PF flour in various formulations to upgrade their technological properties can give added value to the product and also replace commercial stabilizing or emulsifying agents.

1.2.7 Applications of passion fruit waste by-products in the field of renewable energy

Biofuel produced from sustainable resources help in minimizing the use of fossil fuel and CO_2 emission. Plant biomass based biofuels also reduce the environmental pollution and mitigate global warming. In this section, utilization of PF in energy field (biogas, bioethanol, biodiesel, activated carbon) have been discussed.

Biogas production by using *P. edulis* peel waste as a lignocellulosic substrate using *Aspergillus japonicus* URM5620 was reported by (Silva et al., 2019). PF peel waste (1.0 to 3.0%) was used as substrate and at at 3% substarte (1.243 U/mL cellulose activity), 66% methane was achieved in 30 days. Chen Zhao (Zhao et al., 2016) also reported the biomethane conversion activity by different fruit wastes (peels, seeds, shells). Among other plant residues, *P.coerulea L.* performed considerably well during anaerobic digestion with a methane yield of 194.8 mL/g-VS in 36 days which was much higher than litchi, avocado, cherry residues from the same study. Higher lignin content residues are not recommended to be used for methane production as a

single substrate. *P. coerulea L.* contained very less lignin (9.5%) than other species (30.2 % in litchi, 26.8% in Avocado, and 30.1% in cherry). Therefore *P. coerulea L* was proved to be a genuine alternative substrate for methane production. Biomethane production from PF waste was reported by (dos Santos et al., 2020). The high value of carbon to nitrogen ratio (C/N= 51.6) and high cellulose content (25.4%) makes PF peel an ideal substrate for anaerobic digestion. The biogas generation potential of PF peel with industrial sludge was 264 NmL/g-VS (volatile solids) and methane was 115 NmL CH4/g-VS.

Second-generation bioethanol production by using PF peel following enzymatic hydrolysis and fermentation was achieved by (Megawati et al., 2020). PF peel contained higher glucose concentration (66g/L at 9% v/v enzyme ratio) from where they reported 8.9% bioethanol production. Thus PF peel can be considered as an essential substrate for second-generation bioethanol production.

An indirect approach to contribute to renewable energy sector by producing many oxidative and hydrolytic enzymes using different white-rot fungi utilizing *P. edulis* waste peels was attempted by (Zilly et al., 2012). Among several enzymes, laccases (lignin breaking enzymes) were produced by all the organisms used in the study implied PF peel can be a good carbohydrate source for growing fungi. Laccases enzyme is used for lignin degradation of high lignin content agricultural and vegetable waste from Lignocellulosic biomass.

Activated carbon (AC) can be used as catalysts or raw material for electrode in supercapacitors for the development of batteries. They can also be made from nonrenewable sources like coal, bitumen, etc. but they become very costly and of

large pore size. Andia (Andia et al., 2020) had reported green ACs synthesis from *P. ligularis* seeds and shells (ACs yield from the shell was 35.24% with 40% H_3PO_4 and 38.24% with 80% H_3PO_4). Similarly, (Fu et al., 2021) developed a novel electrode using microwave-assisted MnO_2 nanoparticles well distributed on activated porous carbon material synthesized from PF peel. PF waste based AC and the supercapacitor exhibited the best energy densities (E) and power densities (P) characteristics (42.8 W/kg at 499.7 W/kg and 33.4 Wh/kg at 5012.1 W/kg) compared to literature data.

P. edulis shells have been utilized by (Lin and Zheng, 2021) as an alternative for blending with optoelectronic sludge to produce biochar. According to the authors, this biochar can be co-fired with bituminous coal to achieve high combustion value which can help in reducing the depletion of fossil coal. The calorific value of alone optoelectronic sludge was 13.57 MJ/Kg where PF shell showed 17.11 MJ/Kg indicating higher efficiency.

PF oil has been used as a pharmaceutical agent for ages. But, Pantoja (Pantoja et al., 2013) had synthesized biodiesel from PF oil. They have reported the degree of oil unsaturation (85.8% in PF oil) plays a major role in the quality of biodiesel. Tippayawong (Tippayawong and Chumjai, 2012) also transesterified PF oil to achieve PF oil biodiesel and tested the same in a 4-stroke, single-cylinder air-cooled, Yanmar engine model TFR75LM. The biodiesel showed satisfactory performance in engine tests when compared with conventional diesel in terms of engine torque, engine brake power, CO emission, brake thermal efficiency, and brake specific fuel consumption.

1.2.8 Use of extracts as oxidative stabilizer for biodiesel

Customer reception, standardisation and quality assurance and stability are the main features for introducing biodiesel and its blends into the market. Petroleum diesel is more stable as compared to biodiesel. Due to oxidation in biodiesel, various properties such as acid value, peroxide value, viscosity, density, calorific value and composition (fatty acid) of biodiesel changes. This affects its usability in the practical field. Fuel consumption and engine performance are significantly affected because of degraded fuel. Degradation due to oxidation in biodiesel is a major concern for the usability of biodiesel fuels.However, application of natural plant extracts significantly delay the oxidation process in the biodiesel and there by improves the biodiesel quality. In this context, Leanna Silva et al., (de Sousa et al., 2021) used 3000 ppm of Bilberry, Oregano and basil extracts for enhancing the oxidation stability of soybean biodiesel using Rancimat method. All the extracts significantly improved the oxidative stability of the tested biodiesel up to 6 to 8 h as compared to the control group (3 h). Kumar et al., (Kumar and Singh, 2018) reported the oxidative stability properties of *T. Cordifolia* extract at concemntration ranging from 100-1000 ppm in a Rancimat machine. The said extract significantly improved the oxidative stability of the karanja biodiesel up to 8 hour. Utilization of turmeric improved the oxidation stability of tilapia oil based biodiesel up to 10 hour at a concentration of 500 ppm (Rodrigues et al., 2020). Narayanasamy et al.,(Narayanasamy et al., 2018) used five different plant extracts such as ginger, moringa, oregano, basil and clove to their role in improving the oxidative stability of Mahua oil based biodiesel. All the extracts performed extreamly well for enhancing the oxidative stability of the biodiesel. Oregano extract enhanced the oxidative stability up to 15 hour where the maximu oxidative resistance

was recorded for the clove extract (Narayanasamy et al., 2018). Utilization of sesame seed extracts at 500-1000 ppm concentration was carried out by Hussain et al., (Hussain et al., 2018) using sunflower oil based biodiesel. They checked the alteration of fuel properties such as peroxide values, free fatty acid, acid value, etc. during the oxidation process. The sesame seed extract significantly improved the oxidation stability in the sunflower biodiesel was confirmed after noticing improvment in different fuel properties. Some recent studies reported on role of plant based natural extracts on oxidative stability of biodiesel are presented in the Table 1.2.

Table 1.2. Oxidative stability exhibited by different plant extracts on biodiesel.

Extract	Conc. (ppm)	Biodiesel	IP value (h)	Reference
Potato peel	100-250	*Mesea ferra* L.	5.9-7.1	(Devi et al., 2018)
Rosemary, Oregano and Basil	7000	Soybean	9-10	(Spacino et al., 2015)
β-carotene and Curcumin	500	Soybean	6	(De Sousa et al., 2014)
Quercetin, Basil, Oregano and Rosemary	200 mg/mL	Soybean	8-12	(de Sousa et al., 2021)
Thuja	500	Waste cooking oil	8	(Devi et al., 2019)
Green Tea	1000	Waste cooking oil	7	(Bharti and Singh, 2020)
Corn silk	1000	Neem	10	(Ali and El-Anany, 2017)
Indian gooseberry	1000	Karanja	14.2	(Singh et al., 2019)
Bilberry, Oregano, basil	3000	Soybean	6-8	(Leanne Silva et al., 2021)
Turmeric	500	Tilapia oil	10	(Silva et al., 2020)
Giloy	100-1000	Karanja	2-8	(Kumar et al., 2020)

1.2.9 Application of extracts as corrosion inhibitor for metal

Biodiesel have many advantages over commercial diesel like, sustainability, high flash point, good lubrication efficiency but some of these benefits also cause self oxidation and metal corrosion. Different probable causes for metal corrosion in biodiesel are presented below. As biodiesels are esters that make hydrogen bonds with water molecule and becomes hygroscopic unlike commercial diesel. Presence of water caused hydrolysis resulting formation of fatty acids and glycerol and these compounds promote corrosion when the metal comes in contact with the biodiesel.

Excessive water content also increase the risk of microbial growth leading to microbial corrosion. Improper washing and cleaning of biodiesel after transesterification cannot remove the catalyst (Na, K) also accelerate metallic corrosion. Being viscous in nature, biodiesels dissolve metal parts upon longer contact and thus corrosion happen. Similarly to the above reason, sometimes metals also behave like catalyst promoting increasing acid value of the biodiesel and further result in corrosion of metals. Several approaches have been considered by various groups to inhibit corrosion of metals in biodiesel, acidic or basic medium but application of vegetal extracts as green corrosion is a sustainable approach. List of recent work emphasizing different plant parts used, reaction medium, metal used, inhibition percentage, experiment type and possible compound responsible for the anti corrosion property have been presented in Table 1.3.

Table 1.3. Different plant materials used as green corrosion inhibitors.

Species	conc.	Metal	Medium	Experimental method	corrosion inhibition (%)	Possible phytochemical	Ref.
A.visnaga	150 mg/L	Mild steel	1M HCl	Electrochemical impedance spectroscopy (EIS)	93% in n-butanol and 88% in ethyl acetate extracts	Flavonoids and tannins	(Aourabi et al., 2021)
C.grandiflora	500 ppm	Mild steel	1M H_2SO_4	Weight loss and EIS	87.54%	Myricetin and Rutin	(Prabakaran et al., 2016)
A.occidentale	200 ppm	Aluminium alloy	Soybean biodiesel	Weight loss and EIS	93.2% in 200 ppm	Cardanol	(Deyab et al., 2019)
C.sinensis	100-500 mg/L	Boiler grade steel	1M HCl	Weight loss, EIS, PDP	83.6% in 500 ppm	Caffeine, Catechine, Epigallocatechin	(Pal and Das, 2020)
P.incarnata	500 ppm	Copper sheet	Palm biodiesel	Weight loss	0.322 µm/year corrosion rate	NM	(Chen et al., 2020)
NA	300 mg/Kg	Copper and carbon steel	Residual cooking oil	Weight loss	62% in curcumin and 81% in BHT	Commercial curcumin, propyl gallol and butylated hydroxyl toluene (BHT)	(Serqueira et al., 2021)
T.involuncrata	50-250 mg/L	carbon steel	1M HCl	EIS	74% in 250mg/L	Flavonoids from crude extracts	(Chung et al., 2019)
S.rosmarinus	0.5 g/L	Aluminium	Waste cooking oil biodiesel	Weight loss and PDP	95.7%	NM	(Deyab, 2016)
P.sativum	400 mg/L	Mild steel	1M HCl	Weight loss and EIS	91%	NM	(Srivastava et al., 2018)
NA	100 ppm	Copper	Palm biodiesel and diesel blends	Weight loss	16-18 µm/year corrosion rate	Commercial Tert-butylamine (TBA), Butylated Hydroxy Anisole (BHA)	(Fazal et al., 2018)
R.communis	0.5-2.5 ppm	Copper	Neem Biodiesel	Weight loss	57-95% in different conc.	NM	(Indra Priyatharesini et al., 2021)

NA: not applicable, EIS: Electrochemical impedance spectroscopy, PDP: Potentiodynamic polarization

1.3 Knowledge gap

Physico-chemical characterization is the first step to understand the composition, quality and valorisation potential of any biomass. In addition, extreme geographical condition also impacts the quality and phytochemical content of plant biomass. Considering the increased environmental pollution, it is a necessity to employ biomass waste (fruit and agricultural waste) for their sustainable utilization to make the planet a greener and safer place. Passion fruit waste (peel and seed) have high potential for valorization and commercial application because of its bioactive phytochemicals. Hence, the utilization of extracts obtained from passion fruit rind and seed waste would be a sustainable approach towards addressing environmental as well as health crisis. In India, the cutivation of passion fruit is limited to areas of Northeast India, Western Ghats and to some extent in the regions of Northern Himalayan range. However, research in the field of bioactive phytochemical extraction, characterization and its utilization as nutrition and medicine from Northeastern passion fruit varieties have not been documented. It will be an interesting topic to study the composition, bioactive properties of different passion fruits (yellow passion fruit and purple passion fruit) mainly found in the areas of Northeast India. Moreover, passion fruit oil is a good source of poly unsaturated fatty acids, mainly linoleic acid; there is no literature regarding the detailed physicochemical properties, thermal properties and biochemical activities of oil apart from one or two literature citations by groups focusing on fatty acids compositions of the oil.

It is a well understood fact that the agro-food waste contains huge amount of polyphenols. However, very few phytochemicals are successfully isolated and used

for its commercial applications. In this context, there is a research gap in isolation, identification and analysis of bioactivity of important phytochemicals extracted from different passion fruits varieties of Northeast India.

Considering the global fossil fuel depletion and increasing environmental pollution, it is also necessity to consider alternative fuels such as biodiesel. In this context, utilization of non edible oil for biodiesel synthesis may aid economic uplifting of diverse societies and encourage considering non-conventional energy feedstock. Customer reception, standardisation and quality assurance are the main features for introducing biodiesel and its blends into the market. Moreover, making the biodiesel at par commercial standard is a matter of concern because of its oxidative and corrosive properties. To the best of our knowledge no groups have reported the utilization of passion fruit rind and seed extracts to improve the oxidative stability and reduce the corrosiveness of biodiesel.

Understanding the phytochemical composition, structural and biochemical properties of the yellow passion fruit and purple passion fruit collected from Northeast India will be a new finding. Further, isolation and identification of bioactive phytochemicals from waste biomass from passion fruit can open avenues for waste valorisation. Further, application of polyphenol rich passion fruit extracts as oxidative stabilizer for different biodiesel would be a sustainable approach. Determining the role of antioxidant rich passion fruit extracts as corrosion inhibitor for mild steel immerged in different biodiesel would be a green approach for decreasing corrosion of metal. Overall, this study can furnish utilization of agro waste like rind and seeds from

yellow and purple passion fruit from Northeast India in the field of nutrition, medicine and sustainable additives for biodiesel stability as well as natural anti corrosion agent.

1.4 Objectives of the thesis

On the basis of literature survey and above mentioned research gap, it seems there is a larger scope for utilizing yellow passion fruit (*Passiflora edulis* var. *flavicarpa* Degenerer) and purple passion fruit (*Passiflora edulis* Sims) collected from Northeast India for its sustainable applications. The polyphenol rich extracts from the different passion fruit can be utilised as pharmaceuticals, natural antioxidant additives for enhancing biodiesel oxidation stability and green corrosion inhibitor of metal. Therefore, the PhD thesis with title "**Applications of Passion Fruit Extracts as Bioactive Pharmaceuticals, Biodiesel Additive for Oxidation Stability and Corrosion Resistance**" aims the fulfilment of the following major objectives.

1. Collection, identification, physicochemical characterization of yellow and purple passion fruit collected from Northeast India

    i. Morphological, physicochemical and compositional characterization of the rind and seed obtained from yellow and purple passion fruit.

    ii. Extraction and characterisation of oil extracted from the passion fruit seed.

    iii. Analysis of compositional, structural, biochemical and antibacterial properties of passion fruit seed oil.

2. Extraction of polyphenols from passion fruit rind and seeds and physiochemical and biochemical analysis for their therapeutic use

    i. Extraction, phytochemical analysis and polyphenol identification from rind and seed of yellow and purple passion fruit.

    ii. Analysis of biochemical and antibacterial properties of antioxidant rich extracts.

    iii. Fractionation, isolation, identification and bioactivity analysis of purified compounds from passion fruit rind and seed.

3. Green approach for increasing oxidative stability of different biodiesels using antioxidant rich passion fruit extracts.

    i. Synthesis of biodiesel from Neem oil, Karanja oil and waste cooking oil.

    ii. Estimation of physico-chemical and thermal properties of all the three biodiesels followed by analysing fatty acid composition.

    iii. Determining the effect of passion fruit extract on the oxidative stability of synthesized biodiesel.

4. Improving the sustainability of different biodiesels by controlling their corrosive effects on mild steel by using passion fruit extracts as an additive.

i. Identifying non polar, aromatic phytochemicals and fatty acids from the passion fruit extracts.

ii. Anti corrosion properties of passion fruit extracts on mild steel kept in different biodiesel mediums.

1.5 Organization of the thesis

The content of the thesis has been divided into seven chapters.

Chapter I: **Introduction and literature review**

This chapter provides an overview of the current scenario of developing value added products from agro-waste by-products. Further, importance of passion fruit waste management through extracting phytochemicals and their applications in various fields followed by utilization of second generation biodiesels, benefits, disadvantages, remedies are throughly discussed. This chapter also contain the subsections on research gap, objectives and thesis layout.

Chapter II: Materials and methods

This chapter discusses the detailed materials and methods used for the thesis work. This chapter provides detailed methodology of extraction, phytochemical and biochemical analysis, biodiesel synthesis, oxidation stability, corrosion analysis, etc. Detailed methodology of different instrumentation used for the analysis such as HPLC, GC, GCMS, preparative HPLC, NMR, Mass spectrometry, etc.are explained thoroughly.

Chapter III: **Collection, identification, physicochemical characterization of yellow and purple passion fruit varities collected from Northeast India**

This chapter focuses on the morphological, physicochemical and compositional characterization of the rind and seed obtained from yellow and purple passion fruits collected from the regions of Northeast India. Further, extraction of passion fruit seed oil and their characterization, compositional and structural analysis were carried out. Then various biochemical analysis such as total phenolic content, total flavonoid content and antioxidant activity are also described. Antibacterial activity of theseed oil extracted from differen passion fruitsis estimated and reported in this chapter.

Chapter IV: **Extraction of polyphenols from passion fruit rind and seeds and physiochemical and biochemical analysis for their therapeutic use**

This chapter highlights about the phytochemical analysis and polyphenol identification from the rind and seed extracts of yellow and purple passion fruit. Further, different biochemical and antibacterial analysis of antioxidant rich extracts are mentioned. Then fractionation, isolation and identification of various polyphenols from passion fruit rind and seed are mentioned. Finally, bioactivity analysis like antidiabetic, antibacterial and anticancer activities of the purified compounds is presented in this chapter.

Chapter V: **Green approach for increasing oxidative stability of different biodiesels using antioxidant rich passion fruit extracts**

This chapter focuses on synthesis of biodiesel from three non edible oil sources such as Neem oil, Karanja oil and waste cooking oil. Further, the physico-chemical

characterization, thermal analysis and fatty acid composition of different biodiesel are mentioned. Then, role of different passion fruit extracts in improving the oxidative stability of synthesized biodiesel is discussed.

Chapter VI: **Improving the sustainability of different biodiesels by controlling their corrosive effects on mild steel by using passion fruit extracts as an additive**

This chapter focuses on the identification of non polar, aromatic phytochemicals and fatty acids present in passion fruit extracts. Further their application as a potential corrosion inhibitor for mild steel kept in different biodiesel mediums is discussed. Different corrosion study parameters and characterization such as corrosion inhibition efficiency, corrosion rate, XRD, SEM and EDX are explained in this part of the thesis.

Chapter VII: Overall conclusions and future scope

This chapter summarizes the outcomes of the overall work carried out in the thesis and provide major conclusions drawn and take home massage from the overall work. Scope for the future work based on the outputs of the thesis is further recommended in this chapter.

CHAPTER II

Materials and methods

Materials

Methods

Procedures

Physico-chemical characterisations of fruit biomass, seed oil and products

Chapter II

Materials and methods

In this chapter, details on the experimental procedures followed are discussed. Morphological and physicochemical analysis of carbohydrate, sugar, protein, ash, moisture, CHNS, macro-micronutrients of yellow and purple passion fruits collected from Manipur (Northeastern state), India were performed. Further, the extraction method, phytochemical analysis (qualitative and quantitative), biochemical analysis (phenolic content, flavonoid content, antioxidant activity, HPLC and antibacterial activity) are mentioned. Methods for the fractionation of the extracts, isolation of polyphenols (column chromatography, analytical and preparative HPLC), their identification (^{1}H NMR, ^{13}C NMR, Mass spectrometry, GC-MS) and applications (antioxidant, antibacterial, antidiabetic and anticancer) are discused. This chapter also describes about the experimental procedure of biodiesel synthesis (esterification, transesterification) from non edible oil feed stocks (Neem oil, Karanja oil and Waste cooking oil), their physico chemical properties (acid value, viscosity, density, flash point, etc.), fatty acid composition (GC), and thermal properties (TGA). The procedure for passion fruit extracts doping with synthesized biodiesel using PetroOxy for determining their oxidation stability properties are mentioned. Further, procedure of corrosion study (gravimetric analysis) has been also elaborated in this chapter. The analytical methods of corrosion study through XRD, SEM, and EDX are also discussed in this chapter.

2.1 Materials

2.1.1 Raw materials

Fresh and well-ripened yellow and purple passion fruit were purchased from a local market of Manipur, India. Physical and biological damage in fruits was examined visually and healthy fruits were selected for this study. The fruits were then washed with tap water and dried using a blotting towel. The different parts of the fruit such as rind, seeds and juice were separated manually. The rinds and seeds of both the fruit varieties were kept in an incubator at 40-50ºC for drying. Some of the fresh rind was selected for the water content analysis. After drying, the rind and seeds were packed in airtight low density polyethylene (50-60µm of thickness) zip lock bags and stored in the dark. The dried rind and seeds were later grounded to coarse powder using a mechanical grinder and used for different studies. For the oxidative stability and corrosion study on biodiesel, cold pressed Neem seed oil and Karanja seed oil were procured from local market of Ranchi, Jharkhand while the waste cooking oil (used palmolein oil) was collected from the canteen of the Kapili hostel, Indian Institute of Technology Guwahati, Assam, India for the synthesis of biodiesel. Mild steel specimens were used for the corrosion study which were collected and sized using a shearing machine available in the mechanical workshop of the IITG.

2.1.2 Chemicals

All the chemicals and reagents used for the study were of analytical grades and used directly in pure form. The chemicals and reagents used for specific applications are mentioned below.

Chemicals used for characterization of passion fruit biomass

Chemicals such as, DPPH (2, 2-diphenyl-1-picrylhydrazyl) and ABTS [2, 2'-azino-bis (3-ethylbenzothiazoline-6-sulphonic acid)] were purchased from Sigma Aldrich. Folin-Ciocalteu was bought from Merck India Pvt. Ltd. Aluminium nitrate, Potassium acetate was purchased from Himedia. Standards used for flavonoids and phenolic acids such as gallic acid, caffeic acid, ferulic acid, p-coumaric acid, quercetin, myricetin, kaempferol, isorhamnetin were procured from Merck India Pvt. Ltd and Himedia. Different reagents and salts used for the qualitative phytochemical analysis were purchased from Himedia, SRL ltd, etc. Methanol and Acetonitrile used in the HPLC analysis were of HPLC grade. Bacteriological grade media and Resazurin indicator were procured from Himedia. The fatty acid standard (Supelco 37 component FAME mix) was purchased from Sigma Aldrich.

Chemicals used for passion fruit extract fractionation and bioactivity study

Chemicals like methanol, acetonitrile, hexane, ethyl acetate, butanol were used for the extraction, fractionation, reverse phase column chromatography, and high performance liquid chromatography which were procured from Nacalai Tesque Co. (Kyoto, Japan). Diaion HP20SS resin was purchased from Mitsubishi Chemical Co., Tokyo, Japan. Antioxidant standards were procured from Merck India Pvt. Ltd and Himedia. DMSO and MTT were purchased from Merck Life Science Pvt. Ltd. fetal bovine serum; Penstrep and DMEM were procured from Gibco, USA. Propidium iodide was obtained from Sigma Aldrich. Antibodies used for Immunoblotting were procured from Cell Signalling Technologies, USA. All the chemicals and standards used for the study were of gradient grade quality.

Bacterial strains and cell lines used for various biological activity study

Antibacterial activity of different extracts were tested against eight bacterial strains, i.e., four gram-positive and four gram-negative. All the microorganisms were procured from the Institute of Microbial Technology, Chandigarh, India. Gram-positive bacteria used for the present study were *Bacillus subtilis* (MTCC 1133), *Micrococcus luteus* (MTCC 2848), *Staphylococcus epidermidis* (MTCC 9040) and *Staphylococcus aureus* (MTCC 9886) *and* gram negative bacteria were *Escherichia coli* (MTCC 1687), *Enterobacter aerogenes* (MTCC 8558), *Pseudomonas aeruginosa* (MTCC 8727) *and Klebsiella pneumoniae* (MTCC 4030). After receiving these microbial cultures from the MTCC, the bacterial strains were revived, grown on respective broth and/or agar media for their further use. Glycerol stocks of these bacteria were also prepared for storing purposes and to avoid any contamination in the pure culture.The SAS cell line were obtained from NCCS,Pune, India, and TTn cell line was generously gifted by Dr. Renu Wadhwa, AIST, Japan. DMEM medium supplemented with 10% fetal bovine serum and 1% Penstrep was used to maintain the TTncell lines.

Chemicals used for biodiesel characterization and anticorrosion study

Methanol and ethanol were purchased from Himedia Laboratory. Supelco 37 mix and $CDCl_3$, KoH, Wijs solution was procured from Sigma-Aldrich. The NaOH, KOH, H_2SO_4 and Phenolphthalein indicator were purchased from Himedia Ltd.

2.2 Methods

2.2.1 Morphological characterization of passion fruit biomass

The morphological features of yellow (YPF) and purple (PPF) passion fruit (whole fruits and its parts) were randomly analyzed by selecting thirty fruits of each variety. Whole fruit weight (in gram) was measured by using a laboratory weighing balance. The length and diameter (in cm) of each fruit were determined using a Vernier calliper. The amount of pulp (juice and other residues) per 100g of fresh fruit were measured by weighing the total pulp obtained and the result were presented as pulp/100g of the whole fruit. Rind weight of YPF and PPF were calculated by taking freshly peeled rind and weighing it and the result was presented as the weight of rind/100g-whole fruit. The quantity of juice recovered per 100g of fruit was also determined in percentage. The seeds (separated from the pulp) obtained from 100g of fresh fruits were determined and expressed as seed/100g-whole fruit.

2.2.2 Proximate and ultimate analysis

2.2.2.1 Moisture content

An accurately weighed quantity of about 1 to 2g of powdered sample was taken in a tared glass petridishand uniformly distributed. The Petridish was kept open in vacuum oven and the sample was dried in the temperature range between 100-105℃ for 3 h or until a constant weight was observed. Then it was cooled in desiccators at room temperature,weighed and its weight was recorded. Moisture contents were calculated by using the following formula (2.1).

$$Moisture\ content(wt\%) = \frac{Weight of the sample after the drying}{weight of the sample before the drying} \times 100 \qquad (2.1)$$

2.2.2.2 Ash content

Three types of ash content were calculated for the study such as, Total ash, acid insoluble ash and water soluble ash. Total ashis the total amount of material remaining after ignition. Total ash value was found out after putting about 2 g of the sample in crucible inside a muffle furnace at 450ºC. Total ash value was calculated using the following equation (2.2).

$$Total\ Ash\ (wt\%) = \frac{Weight\ of\ ash\ after\ ignition}{weight\ of\ the\ sample\ before\ ignition} \times 100 \qquad (2.2)$$

Acid insoluble ash is residue obtained after boiling the total ash with dilute hydrochloric acid, and igniting the remaining insoluble matter. The ash was boiled for 5 to 10 min with 25 mL of dilute hydrochloric acid, the insoluble matter was collected in a crucible, ignited and weighed. The percentage yield of acid-insoluble ash was calculated with reference to the air-dried sample from the following equation (2.3).

$$Acid\ insoluble\ ash\ (wt\%) = \frac{Weight\ of\ insoluble\ ash\ in\ acid}{weight\ of\ the\ sample\ before\ ignition} \times 100 \qquad (2.3)$$

For the water soluble ash, the total ash was boiled for five minutes with 25 mL of water; soluble matter was collected in a crucible,evaporated, and weighed. The percentage of water soluble ash was calculated from the following equation (2.4).

$$Acid\ insoluble\ ash\ (wt\%) = \frac{Wt.of\ the\ total\ ash - wt.of\ water\ insoluble\ ash}{weight\ of\ the\ sample\ before\ ignition} \times 100 \qquad (2.4)$$

2.2.2.3 Elemental analysis

Quantitative elemental analysis was performed by using a flame photometer (Systronic 128) for analysis of sodium (Na), potassium (K) and calcium (Ca), whereas AAS (atomic absorption spectrometer) (Varian Spectra 55B) was used for the analysis

of magnesium (Mg), zinc (Zn), copper (Cu), manganese (Mn), and iron (Fe) content. A quantity of 0.5 g of each dry sample was digested with 20mL proportionate mixture of HNO_3 and $HClO_4$ (3:1) at 80 °C for 1h until complete evaporation of the acids. Further the residue was dissolved in 0.1N HNO_3 followed by filtration and then, the samples were subjected for AAS and flame photometry analysis. Standard curve for different elements were prepared from the standard purchased from Merck Ltd.

2.2.3 Quantitative analysis

2.2.3.1 Total Carbohydrate Content

Total Carbohydrate Contents of the rind and seed powder of YPF and PPF were determined following the protocol previously described by (Sadasivam, 1996) with a little modification. In short, 100 mg of powdered material from each sample was taken in a test tubes containing 5 mL of 2.5N HCl. Then the test tubes were kept in boiling water bath for 3 h for hydrolysis and were subsequently cooled to room temperature. Further the samples were neutralized with solid sodium carbonate until the effervescence ceases out. Then volume of the solution was made up to 50 mL with water followed by centrifugation at 10000 rpm for 10 min. After centrifugation, the supernatant was collected and 0.5 and 1 mL aliquots were taken for analysis. Standard solution (1mg glucose in 10 mL of distilled water with a few drops of toluene) was prepared and different concentrations were made by taking 0, 0.2, 0.4, 0.6, 0.8 and 1 mL from the working standard. The final volume was made up to 1 mL with distilled water. Contents of all the tubes were cooled in ice prior to addition of 4 mL of ice cold Anthrone reagent. Test tubes were shaken vigorously and heated for eight minutes in a boiling water bath followed by rapid cooling. Absorbance of the dark

green coloured mixtures was recorded at 630nm. The carbohydrate content was calculated using a standard curve of glucose and finally represented as weight percentages.

2.2.3.2 Reducing sugar content

A quantity of 200 mg of each dry sample (rind and seed powder) was taken in pre heated glass centrifuge tubes at 65ºC kept in a water bath for 10 min containing 5 mL of 80% methanol. Further addition of 5 mL of 80% methanol in between was done for extraction of sugar. After the extraction, supernatants of every sample were collected and evaporated on water bath at 70ºC to get the sugar residue followed by dissolving the residue in 10 mL of distilled water. Suitably diluted aliquot were pipette out for the estimation of reducing sugars following (Sadasivam, 1996) taking glucose standard curve (0-100µg) and presented as weight percentage of reducing sugar content.

2.2.3.3 Total Protein Content

Crude powders (500mg) of all samples of YPF and PPF were defatted with chloroform, methanol and acetone in 2:1:1 ratio followed by drying to evaporate the organic solvents. After drying, all samples were extracted with protein extraction buffer (1M Tris-HCl pH 6.8, glycerol, absolute alcohol and water) for 2-4 h at 4ºC. Next day the homogenates were centrifuged at 15000g for 20 min at 4ºC and the supernatant was collected. The supernatants obtained after centrifugation were suitably diluted and were used for protein estimation by (Lowry et al., 1951) taking BSA standard curve (0-100µg) and converted to weight percentage of protein content.

2.2.3.4 Ascorbic acid content

The extraction and estimation of ascorbic acid (Vitamin C) was carried out by volumetric method using 2, 4-Dinitrophenyl hydrazine reagent following (Sadasivam, 1996) with a little modification. Briefly, 1g of dry sample was incubated for 6 h with 25 mL of 4% oxalic acid with occasional stirring followed by centrifugation at 6000 rpm for 5 min and then supernatant was collected. To the initial amount of 10 mL supernatant, 30 mL bromine water (1-2 drops of bromine in 100 mL of chilled water) was added and shaken well until yellow orange colour was formed. Then the total volume was made up to 50 mL with the addition of 4% Oxalic acid solution. Different aliquots of brominated extracts were made up to total volume of 3 mL by adding millipore water, 1 mL of 2% 2, 4-Dinitrophenyl hydrazine and one to two drops of 10% thio urea to each test tubes and vortex afterwards followed by an incubation at 37 ºC for 3 h. After incubation, orange-red osazone crystals were formed which was further dissolved by adding 7 mL of 80% sulphuric acid. Likewise, 10 mL of ascorbic acid stock solution was converted to its dehydro form by bromination. The absorbance was recorded at 540 nm. Standard curve was plotted using 0-100 µg of ascorbic acid and represented in mg/100 gm plant samples.

2.2.4 Extraction of phytochemicals and oil

Rind and seed powders from YPF and PPF were subjected to different soxhlet apparatus for extraction. Five different solvents were used for extraction such as hexane, ethyl acetate, acetone, methanol and water. All extractions were conducted at the boiling temperature of the solvents mentioned above and continued until a clear siphon tube was visible. After the completion of extraction, the solvents were

evaporated using a rotary evaporator (Buchi R-215 Rotary Evaporator) and extractive values were calculated. Hexane extraction was carried out only for the seed samples to extract the oil. Finally, the extracts were transferred to airtight amber-coloured glass containers and stored at 4ºC until further use.

2.2.5 Physico-chemical characterization of seed oil

Physico-chemical characterization (acid values, iodine values, peroxide values, dynamic viscosity and refractive index) of yellow passion fruit seed (YPFS) and purple passion fruit seed (PPFS) oil were carried out following AOCS (American oil chemists' society) methods (Society and Firestone, 1994). The acid value was calculated from the following equation (2.5).

$$Acid\ value = \frac{volume\ of titrant\ (mL) \times N \times 56.1}{Mass\ of\ the\ sample\ (g)} \quad (2.5)$$

Where, N is the normality of accurately standardised sodium hydroxide solution.

The refractive index of the extracted passion fruit oil was measured using refracto meter (ABBE Refracto meter). It is the degree of deflection of a beam light when passes from one medium to the other is measured.

2.2.6 Fatty acid methyl ester (FAME) synthesis and Gas chromatography

Fatty acid methyl ester of YPFS and PPFS oil was synthesized by following the previously mentioned method. Briefly, 500 mg of oil was mixed with 5 mL of 0.5 N methanolic KOH and was saponified at 60ºC for 5 min. Further, 15 mL of esterification solution (ammonium chloride and sulphuric acid solution in methanol) was added and the entire mixture was further refluxed at 60ºC for 5 min. Then the

mixture was washed twice in a separating funnel containing 25 mL petroleum ether and 50 mL water. Finally, the FAME was obtained after evaporating the petroleum ether fraction. The fatty acid composition in oil FAME was analyzed in a Gas chromatography (PerkinElmer Clarus 590) equipped with a BPX-70 capillary column (50 m x 0.22 mm i.d., film thickness 0.25 µm) and FID detector. The initial oven temperature was 100^{o}C for zero min. Further, the temperature was increased by 3^{o}C min^{-1} up to 240^{o}C and held for 10 min. Helium at a flowrate of 2 mL/min on split mode was used as the carrier gas. The injector and detector temperatures were maintained at 250^{o}C. The fatty acid composition of passion fruit seed oils was identified by comparing with the standard (Supelco 37 component FAME mix) and literature data. Fatty acid quantification of different oil samples was done by extrapolating the standard calibration curve.

2.2.7 Biochemical analysis of oil

The total phenolic content (TPC) of different passion fruit seed oil was determined byusing Folin-Ciocalteu reagent. Firstly, 0.5 mL of hydro-methanolic solution (1:9) of 1 mg/mL oil was mixed to 2.5 mL of 10% Folin-Ciocalteu reagent and 2.0 mL of sodium carbonate. The mixture was incubated for 20 min with continuous shaking in dark before taking absorbance at 765nm. Gallic acid was used for extrapolation of standard curve and the TPC was presented in mg GAE/g-oil. Total flavonoids content (TFC) was performed by adding 500 µL of oil sample (1 mg of oil in 1 mL of 1:9 ratio of oil in hydro-alcoholic solutions) to 100 µL of $Al(NO_3)_3$, 100 µL of 1M CH_3CO_2K, and 4.3 mL of 80% ethanol. Then the test tubes were thoroughly shaken and incubated for 45 min at room temperature in the dark before measuring

absorbance at 415 nm. The TFC was calculated using Quercetin calibration curve and was represented in mg QUE g^{-1} of oil. The in-vitro antioxidant activity of YPFS and PPFS oil was evaluated by DPPH and ABTS methods. For the DPPH analysis, 3 mL of five different concentrations (10-50 µg/mL of oil in methanol) of oil were mixed separately to 1 mL of 0.1 mM L^{-1} DPPH solution and kept for 30 min in the dark followed by recording absorbance at 517 nm. In the ABTS experiment, 7 mM of ABTS was mixed with 2.45 mM of $K_2S_2O_8$ at 1:1 ratio and kept in dark for 12 h. Thereafter, the solution was diluted and the absorbance was brought to 0.7. Then, 1 mL of diluted ABTS solution was mixed with 10-50 µg/mL oil in methanol and incubated for 20 min before taking absorbance at 734 nm. Ascorbic acid (AA) was used as standard antioxidant compound.

2.2.8 Physical and Thermal analysis of oil

2.2.8.1 Thermo-gravimetric analysis (TGA)

The thermal stability and degradation properties of oil samples (6-10 mg) were determined using a thermo-gravimetric analyzer (TG/SDTA851e, METTLER TOLEDO). A non-isothermal condition in a nitrogenous environment (40 mL/min) with increasing heating rates at 10°C/min up to 900°C was considered for the experiment.TG curves and their respective derivative curves (DTG) were plotted to measure the onset temperature and mass loss percentage of the oils. Onset temperature was calculated by extrapolating a horizontal line from the TG curve and the intercept of this line with the tangent corresponding to the onset temperature.

2.2.8.2 Differential scanning calorimetry analysis (DSC)

Pour point determination of oil samples was performed in a DSC instrument (Model DSC1, stare system, METTLER TOLEDO). Approximately 10 mg oil sample was kept in a crucible and kept inside the DSC machine along with a reference crucible. Pour point was determined following previous article with some modifications. At first, (i) the temperature was increased up to 50^{o}C at 2^{o}C/min, (ii) maintained isothermal condition at 50 ^{o}C for 5 min, (iii) decreased from 50 ^{o}C to -30 ^{o}C at 2 ^{o}C/min, (iv) isothermal condition at -30 ^{o}C for 5 min, (v) increased temperature from -30 ^{o}C to 30 ^{o}C at 2 ^{o}C/min, (vi) decreased temperature from 30 ^{o}C to -30 ^{o}C and finally (vii) repeated step '(v)'. Then the pour point was determined by extrapolating DSC plots.

2.2.9 FTIR-ATR analysis of passion fruit seed oil

FTIR-ATR analysis of passion fruit seed oil was carried out using FTIR spectrometer ("IR Affinity1", Shimadzu Corporation, Japan) and measured between 4000 cm^{-1} to 400 cm^{-1} with a resolution of 2 cm^{-1} and 30 scans per spectrum. Approximately 3-5 µL of oil sample was directly added to the small cup inside the instrument for the analysis. Corrections of baseline and frequency variations were done using Shimadzu IR solution 1.5 software.

2.2.10 Antibacterial activity

Antibacterial activity of Passion fruit seed oil was carried out by zone of inhibition study against four gram-positive bacteria such as *Staphylococcus epidermidis* (MTCC 9040), *Staphylococcus aureus* (MTCC 9886), *Bacillus subtilis* (MTCC 1133) and

Micrococcus luteus(MTCC 2848) and four gram-negative strains namely *Escherichia coli* (MTCC 1687), *Klebsiella pneumonia* (MTCC 4030), *Enterobactor aerogenes* (MTCC 8558) and *Pseudomonas aeruginosa* (MTCC 8727). All these bacteria were procured from the Institute of Microbial Technology (Chandigarh, India). The oil samples were sterilized through 0.2 µm filter followed by mixing into a different ratio with Tween 80 (10%, 30% and 50% v/v). To the agar plates, 100 µL of the bacterial broth was uniformly spread and left for drying for some time. After that, three equidistance 0.5 cm holes were bored using a sterilized cork borer. Then different dilutions of the oils were poured into the wells and the plates were again left for some time for diffusion of oil. Finally, the plates were kept overnight in the incubator at ambient temperature (30 ^{o}C and 37 ^{o}C). The next day, the clear zone of inhibition created by different oil samples on different bacterial strains was measured using a scale.

2.2.11 Oil quality score

Oil quality score is a hypothetical concept calculated by averaging the individual standard score obtained from the raw data of different parameters of interests (in our study: Anti-oxidant, total phenolic content, total flavonoid content and unsaturated fatty acid percentage, acid value, iodine value and peroxide value). The standard score was obtained as suggested by dabetic et al. (Dabetic et al., 2020a). The following equation (2.6) was used to calculate the standard score of the oil

$$Standardscore = \left(\frac{Samplescore}{Bestscore}\right) \times 100 \quad (2.6)$$

Where sample score is the raw data of the experiments of the present study and data for best score was considered from published literature on passion fruit.

2.2.12 Qualitative phytochemical analysis

Preliminary phytochemical analysis was carried out for all the different extracts such as ethyl acetate (EA), acetone (AT), methanol (ML) and water (WR) obtained from the rind and seed of YPF and PPF. All the reagents and solutions required for the phytochemical analysis were prepared and kept in different containers at room temperature prior to the experiments. Identification tests for alkaloids, carbohydrates, glycosides, tannins and phenolic compounds, steroids, flavonoids, proteins and amino acids were done for all the extracts. The presence or absence of phytoconstituent in different extracts was confirmed by observing the change in colour as well as the change in the physical properties of the solution under specific conditions. The detailed procedures for the qualitative analysis followed from the practical text book by Khandelwal et al. (Khandelwal, 2008).

2.2.13 FTIR analysis of extracts

FTIR spectra of crude powdered materials and extracts (ethyl acetate, acetone, methanol and aqueous extracts) of YPF and PPF were measured between 4000 cm^{-1} to 400 cm^{-1}at a resolution of 2 cm^{-1} and 30 scans per spectrum were recorded using FTIR spectrometer IR Affinity1, Shimadzu Corporation, Japan. Potassium bromide (KBr) was used as the reference material. The oven-dried powdered materials (biomass) and approximately 50 µg of extracts were ground separately with KBr and

then subjected to the analysis. Baseline corrections and variations of frequency were done using Shimadzu IR solution 1.5 software.

2.2.14Total phenolic and total flavonoid content

Estimation of total phenolic content of different extracts of the rind and seed of YPF and PPF was carried out as per the method described by Aiyegoro and Okoh (Aiyegoro and Okoh, 2010). Gallic acid calibration curve ranging from 10-50 µg/mL ($Y= 0.012x-0.015$, $R^2=0.999$) was used to calculate the total phenolic content present as gallic acid equivalents in milligram per gram (mg GAE/g) of dried extract. Two individual experiments in triplicates were carried out for the assay. Total flavonoid content in each extract was performed according to Asok kumar et al. (Asokkumar et al., 2010). The quantity of flavonoids in each extract was calculated from the standard curve ($Y=0.006x+0.017$, $R^2= 0.998$) of quercetin (10-50 µg/mL) and the values were represented as quercetin equivalents in milligram per gram (mg QE/g) of dried extract.

2.2.15 In vitro antioxidant activity

Antioxidant potential of the rind and seed extracts of YPF and PPF was carried out by DPPH, ABTS and metal chelating assay. The DPPH assay was done according to the method described by Sen et al. (Sen et al., 2013). The ABTS method was followed as per Re et al. (Re et al., 1999) and the metal chelating activity was performed according to Gursoy et al (Gursoy et al., 2009). The sample preparation was done by dissolving the extracts in different concentrations (10-50 µg/mL) in methanol and water (for aqueous extracts only). Ascorbic acid was taken as the reference

antioxidant compound for DPPH and ABTS assay, where EDTA was the standard for metal chelating assay. Antioxidant capacity (percent scavenged) of each extract was calculated from the following equation [$(Abs_{control} - Abs_{sample})/Abs_{control} \times 100$]. Further, each extract's inhibitory concentration (IC_{50} value) was calculated following the concentration versus percentage scavenged graphs obtained for each sample.

2.2.16 Global antioxidant score

Global antioxidant score (GAS) of obtained antioxidant results (from DPPH, ABTS and metal chelating) and total phenolic and total flavonoid contents for YPF and PPF extracts was calculated for comparison between best extracts in terms of antioxidant activity. Different values of antioxidant activity (percent scavenged at 50 µg/mL extract concentration) and TPC and TFC (X) were converted into T_{score} value by the formula; T_{score} = (X-min) / (max-min), where max and min are the smallest and largest values of the variable X of the individual sample(Todorovic et al., 2017).

2.2.17 High performance liquid chromatography analysis

The experiment was performed using an HPLC machine coupled with a UV-Vis detector (Shimadzu Corporation, Kyoto, Japan) and Enable C18 column (A8-ST5C18G120-98). Different experimental parameters such as the flow rate (0.5 mL/min for flavonoids and 1 mL/min for phenolics), injection volume (20 µL) and choice of the mobile phase were kept constant for all the samples. Two different mobile phases were selected for the identification of flavonoids (methanol: acetonitrile: water (40:15:45) with 1% acetic acid) and phenolic acid (Milli Q water with 1% ortho phosphoric acid [solvent A] and acetonitrile with 1% ortho phosphoric

acid [solvent B] in a A:B ratio of 63:37). Detection of flavonoids, especially flavonols (quercetin, myricetin, kaempferol and isorhamnetin), was done at 368nm. In contrast, phenolic acid detection was performed at different wavelengths (gallic acid at 280nm, caffeic acid and ferulic acid at 296nm and p-coumaric acid at 310nm) by injecting 20 µL samples. Different extracts (1 mg/mL) were prepared using HPLC grade methanol, followed by filter sterilization using a 0.45µm filter. Initially, different standard compounds (gallic acid, caffeic acid, ferulic acid, p-coumaric acid, quercetin, myricetin, kaempferol and isorhamnetin) were run at concentrations ranging from 20-100 µg/mL followed by extrapolation of their standard curve. Then, 20 µL of 1mg/mL concentrations of different extracts were injected. The retention time of flavonoids and phenolic acids was matched with the retention time of the respective standard compound. The quantification of the polyphenols was done using the straight-line equation derived from the standard curves. All experiments were done at ambient temperature (25±2^{o}C).

2.2.18 Antibacterial assay

2.2.18.1 Preparation of bacterial culture

A single colony of each bacterial strain was inoculated into a 5 mL broth medium aseptically and incubated. After that, 100 µL of the culture was subcultured in 10 mL broth and kept in ambient conditions until the OD_{600} was 0.5. After the OD_{600} reaches 0.5, serial dilution (10^{-5} dilutions) of each bacterium was done.

2.2.18.2 Minimum inhibitory concentration (MIC)

Determination of MIC of the acetone and methanol extracts of the rind and seed of YPF and PPF was carried out using resazurin indicator. Resazurin solution was prepared by dissolving 10 mg of resazurin powder in 10 mL of autoclaved distilled water followed by vortexing the solution. Different extracts concentrations range from 40 mg/mL to 19.53 µg/mL in 10% DMSO was made with different extracts to check their MIC. In this method, wells of sterile 96 well plates were coated with 30 µL of 3.3X broth media, 50 µL of different concentrations of extracts and 10 µL of the bacterial suspension ($5*10^5$ CFU/mL). The positive control well contained media and 10% DMSO and bacterial suspension, where the negative control well contained media and 10% DMSO only. The plates were incubated for 12-14 h depending upon the bacterial strain's growth rate. The next day, 10 µL of 1 mg/mL resazurin solution was added to all the wells and the plates were again kept in the incubators for some time. MIC was determined by visually observing the colour change from blue to pink. Any colour change from blue to pink or blue to colourless was recorded as the presence of bacteria. The lowest concentration at which the blue colour did not change to pink was considered the MIC value of a particular extract (Sarker et al., 2007). All the experiments were performed in duplicates.

2.2.18.2 Zone of inhibition (ZOI)

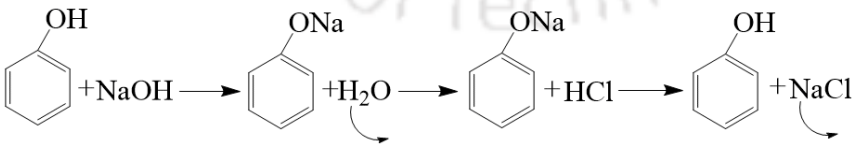
Another method employed for assessing YPF and PPF extracts' antibacterial properties was the agar well diffusion method following previous protocol (Puttipan et al., 2017) with some modifications. A 100 µL of bacterial culture was spread on nutrient agar plates (for *Enterobacter aerogenes, Pseudomonas aeruginosa,*

Micrococcus luteus, Bacillus subtilis and *Staphylococcus epidermidis*), LB agar plates (for *Klebsiella pneumonia* and *Escherichia coli*) and tryptone soya agar (for *Staphylococcus aureus*) plates. After spreading, three equidistant wells of 0.5 mm were bored on each plate with the help of a sterile cork borer. 50 µL of each extract with three different concentrations such as 10 mg/mL, 30 mg/mL and 50 mg/mL were added into each well. The extracts were allowed to diffuse at room temperature in the laminar airflow for another 30 min. Control experiments comprised of inoculums and 10% DMSO only. Finally, the plates were incubated at respective temperature, i.e., 30^{o}C and 37^{o}C for 12-16 h for the growth of different bacterial pathogens. The diameter of the zone of inhibition (in mm) was measured using a geometric ruler. The experiment was performed in duplicate and the mean readings were calculated.Tazar (Piperelline and Tazobactam) from Lupin Ltd. was used as the standard antibacterial drug.

2.2.19 Extraction and fractionation analysis of yellow passion fruit

The seed sample (50g) was initially subjected to soxhlet apparatus using hexane for removing oil. Then the de-fatted seed residue was used for 80% methanolic extraction for 48h. Later, the YPFS hydro-alcoholic extract was recovered after solvent evaporation through rotary evaporator. Finally, the dried extract was stored in 4^{o}C refrigerator for future use. Fractionation of YPFS was carried out in a separating funnel using different solvents such as hexane, dichloromethane (DCM), ethyl acetate (EtOAc) and butanol (BuOH). At first, the extract (6.5g) was completely dried using rotary evaporator to avoid presence of any organic solvents. Afterwards, the extract was mixed with water in a conical flask. To the flask, equal volume of hexane was

added and then it was thoroughly shaken before transferring to the separating funnel. Then the separating funnel was rested for an hour followed by decantation of the water layer into the conical flask and the hexane layer was separately collected and passed through sodium sulphate for extra water entrapment. This step was repeated twice for better recovery of hexane soluble matter from the water layer. Similarly, the water layer was treated with 2 volume of DCM, 2 volume of EtOAc and 2 volume of BuOH one after another to fractionate the extracts. All the organic solvent fractions were evaporated to get the fractionated extracts except for the EtOAc fraction. The EtOAc fraction was subjected to base-acid treatment for enrichment of phenolic compounds in that fraction as mentioned in equation 1. At first, the EtOAc fraction was treated with equal volume of 1M NaOH and kept for 1h in the separating funnel with intermittent shaking. Then the basic layer is separated from the EtOAc layer. The basified EtOAc layer was evaporated and named as EtOAc base fraction. Further, to the polar layer, an equal amount of 1M HCl was added until the solution is completely acidic (litmus paper turned orange). Then the EtOAc acidic fraction was recovered by extracting the compounds three times with EtOAc. Finally, the EtOAc fraction was concentrated using rotary evaporator. All the YPFS fractions were stored in 4^0C refrigerator for subsequent use.



Similarly, 130 g of rind powder was also subjected to the extraction and fractionation study (50 g extract) following the above method.

2.2.20 Column chromatography and HPLC analysis

Firstly, after drying the EtOAc and BuOH fractions, their solvent system optimization (needed for preparative HPLC) was carried out in a Jasco 4000 series HPLC unit (Jasco, Tokyo, Japan) coupled with a PDA detector and $5C_{18}$-MS-II Cosmosil Packed column (length- 150 mm, ID 4.6 mm, Nacalai Tesque Co.,Kyoto, Japan) at a column temperature of 35°C. The gradient condition was 5 to 40% acetonitrile with 0.5% formic acid for 0 to 30 min. A 5 µL of the sample from 10 mg/mL stock was injected for analysis. Detection of different peaks was done at 254 nm and 330 nm. After assuming the tentative polyphenol peaks, both the fractions were subjected to reverse phase column chromatography (RPCC) using HP20SS ion exchange resin for the separation and enrichment of SB from the entire fraction. The resin was regenerated using 2 column volume X 5 times before loading the extract fraction. Further, the column was eluted twice each with water, 20%, 50%, 80% and 100% methanol. All the fractions were collected and further injected in the analytical HPLC to check the highest concentration of the isolated compounds for further isolation through preparative HPLC. Further, the optimised condition (30% methanol with 0.5% formic acid at 254 nm) was considered for the isolation of purified compound using preparative HPLC. A Jasco 875UV preparative HPLC coupled with a UV detector and Prep ODS Inertsil column (10 mm ID, 250 mm length, GL Sciences Inc., Tokyo, Japan) with a column temperature of 35°C was used for the purification analysis. The enriched fractions were then dissolved in DMSO and sonicated thoroughly before injecting into the instrument. The required sample was eluted during the recorder approached the peak and finally all the eluted fractions were mixed and evaporated to achieve the purified compounds. The purity was further confirmed through injecting a

measured volume of purified product into analytical HPLC and then the purified sample was kept in -20°C refrigerator until further use.

2.2.21 Identification of isolated polyphenols

The ^{1}H spectrum of the purified compound was recorded on a JEOL ECA 500 spectrometer (JEOL, Tokyo, Japan) at 500 MHz and ^{13}C-NMR was carried out in a Bruker NMR spectrometer at 800 MHz. The Sample was dissolved in CD_3OD before subjected to the NMR spectroscopy. The mass spectrum was obtained from a JMS-T100TD mass spectrometer (JEOL) on ESI (Electro Spray Ionization) negative mode. Samples were dissolved in methanol before injection into the mass spectrometer.

2.2.21 In vitro analysis

2.2.21.1 Antiradical activity of isolated compounds

In vitro antioxidant activity of the purified compounds was conducted by DPPH and ABTS methods as discussed above in section 2.2.15 except the concentration of the sample which ranged from 2 to 10µg/mL and further the IC_{50} values were calculated. The results obtained for the purified compound was compared with six different standard polyphenols such as gallic acid, caffeic acid, ferulic acid, ascorbic acid, p-coumaric acid and quercetin.

2.2.21.2 Carbohydrate digestive enzyme inhibition activity of isolated compounds

This study was carried out considering inhibition activity of α-amylase and α-glucosidase enzymes following previous protocol (Swaraz et al., 2021) with some modifications. In brief, for α-amylase activity, 50 µL of five concentrations (20-100

µg/mL) of SB were treated with 50 µL of α-amylase (0.5 g/mL in 0.1M PBS, pH 6.8) for 15 min at 37°C. Then, to the solution, 50 µL of starch solution (1% in PBS) was added and the reaction mixture was further incubated for 20 min at 37°C. After the incubation period, 100 µL of DNS solution (1 g DNS + 20 mL NaOH (2M) + 50 mL of H_2O + 30 g of $KNaC_4H_4O_6{\cdot}4H_2O$) was added and the mixture was boiled in the water bath for 15 min. Finally the samples were diluted with 1 mL water and the absorbance was recorded at 540nm. Acarbose (1-5 µg/mL) was used as the standard drug for comparison. The background control (for sample and standard) did not have starch in it, where, the background control (for control group) had PBS instead of sample/standard and starch. Blank group contained only PBS. The inhibition percentage of the enzyme by the purified compound and standard drug was calculated from the following equation .

$$Inhibition\ (\%) = \frac{(Abs_{Cont.} - Abs_{Cont.BG}) - (Abs_{Samp.} - Abs_{Samp.BG})}{(Abs_{Cont.} - Abs_{Cont.BG})} \times 100 \qquad (2.8)$$

Where, Abs cont. means the absorbance of the control group, BG stands for absorbance of background; Abs samp is for absorbance of the sample/standard compound.

In a different experimental set up, enzymatic activity of purified compounds on α-glucosidase was checked following the previous protocol (Swaraz et al., 2021) with minor modifications. Briefly, 50 µL of the sample (1-5 µg/mL) or standard (100-500 ng/mL) were mixed separately with 50 µL α-glucosidase enzyme (0.1U/mL in 0.1M PBS) and the mixture was incubated for 15 min at 37 °C. After that, 50 µL of 1mM PNPG (4-Nitrophenyl-β-D- glucopyranoside) in PBS was added to the solution and further incubated for 20 min at 37°C. Finally 100 µL of Na_2CO_3 (0.1M in PBS) was

added to the mixture to stop the reaction and absorbance was recorded at 405nm. The inhibition percentage of α-glucosidase by different compounds and Acarbose was calculated according to equation 2.8 where the background control (for samples) did not have PNPG and background control (for control) did not have sample/standard as well as PNPG substrate.

2.2.23 Antibacterial activity of Scirpusin B

The antibacterial activity of Scirpusin B (SB) was checked by using eight bacterial strains (four of each gram positive and gram negative) by the minimum inhibitory concentration (MIC) and zone of inhibition (ZOI) study as mentioned in the section 2.2.18. The only changes considered was concentration range where, the concentration range for MIC was ranging from 10 mg/mL to 0.156 mg/mL. Based on the findings of MIC, different concentrations (2.5, 5 and 10 mg/mL) were chosen for ZOI analysis and the ZOI obtained by Scirpusin B was measured using a geometric ruler.

2.2.24 Bioactivity of Scirpusin B against oral squamous cell carcinoma

2.2.24.1 Viability assay

The effect of SB on the viability of oral cancer cell lines was determined using MTT assay. For this assay, 96 well plates were seeded with approx. 2000 cells in each well and then incubated at 37 °C for 24h. Further, the cells were treated with different concentrations of SB (0, 10, 25 and 50 µM) performed at 72h. Then 10 µL of MTT (5 mg/mL) solution was added to each well and incubated for 2h after which, the culture medium was removed and 100 µL of DMSO was added to all the wells and incubated

at room temperature for 1h to dissolve the formazan product. The absorbance of the colored solution was taken at 570 nm at microplate reader, Molecular Devices, Spectramax iD3. The inhibition caused by the given compound on the proliferation of SAS and TTn cell lines was determined by the percentage viability (Bordoloi et al., 2019).

2.2.24.2 Colony formation assay

The clonogenic potential of oral cancer cells treated with SB was determined by a colony formation assay. The cells were seeded with density of 1500 cells/well in 6-well plates and treated with different concentration of SB (0, 25 and 50 µM). The cells were then cultured for 10 days with media replacement in every alternate day. After this period, cell fixation was made with ethanol followed by well cleaning using PBS and finally stained with crystal violet (SRL Pvt. Ltd., India). Colonies were scanned for each concentration of SB and survival fraction was determined (Aswathy et al., 2021).

2.2.24.3 Propidium iodide flow cytometric assay

Propidium iodide (PI) flow cytometry analysis was carried out by seeding SAS and TTn cell lines in 6-well plates with a density of 5×10^4 cells per well and incubated for 24h. The cells were treated with the indicated concentrations of SB (0, 25, 50 and 75 µM) for 72h. Then the cells were collected, washed with PBS, and centrifuged. The cell pellets were suspended in 495 µL PBS and incubated with 5 µL of PI (1mg/mL) for 10 min. The effect of SB on the cells was analyzed using FACS Celesta™, BD Biosciences (Khwairakpam et al., 2020).

2.2.24.4 Western blot analysis

To determine the effect of SB on different oncogenic proteins, whole cell lysates were prepared from the SAS and TTn cell lines treated with SB by exposing it to lysis buffer (20mM HEPES, 2mM EDTA, 250mM NaCl, 0.1% NP40) in the presence of protease inhibitors (2µg/mL Leupeptin hemisulfate, 2µg/mL aprotinin, 1mM PMSF, 1mM DTT). The lysates were centrifuged at 13,000 g for 10 min to remove insoluble material and the supernatants were collected and stored at -20 ℃. The lysates were resolved by SDS-PAGE and the proteins were electro transferred onto nitrocellulose membranes, blotted with relevant antibodies. The bands for different proteins were visualized with Clarity Western ECL Substrate (Bio-Rad, Hercules, CA, the USA) in a ChemiDoc™ XRS System (Bio-Rad). Glyceraldehyde 3-phosphate dehydrogenase (GAPDH) was used as the loading control housekeeping gene (El-Naggar et al., 2021).

2.2.25 GC-MS analysis of passion fruit methanolic extracts

Methanolic extracts from rind and seed of YPF and PPF were subjected to GC-MS (Agilent Technologies 7980 A with 5977 A mass selective detector, HP5-MS column, and dimension 30 m × 320 µm with film thickness of 0.25 µm) for identification of various polyphenols and volatile compounds. GC-MS operation condition was as follows; initial hold at 40 ℃ for 2 min, ramping up to 125 ℃ at 3 ℃ per min (hold for 3 min), ramping up to 250 ℃ at 3 ℃ per min (hold for 3 min), and finally ramping up to 300 ℃ at 10 ℃ per min with a final hold of 3 min. Helium at a flow rate of 1 mL/min at a split ratio of 10:1 was used as carrier gas. Quadrupole mass spectrometry detractor was used for the study with a temperature of 250 ℃,

ionization potential of 70eV and scanning range of 50 to 550 atomic mass units. Compounds present in the tested extracts were identified by Version 2.0 g of NIST2017 mass spectral library (Agilent Technologies, Palo Alto, CA, USA) using MSD Chemstation F.01.01.2317 software. Extracts were dissolved in methanol (5 mg/mL) followed by filtration through 0.2 µm syringe filter prior to the injection into GC-MS instrument.

2.2.26 Transesterification process

2.2.26.1 Experimental set up

Experiments were conducted in a 1000 mL glass reactor insulated with a glass jacket for temperature maintenance and equipped with a mechanical stirrer and timer. The three neck top was sealed properly by the condenser on one neck, temperature probe in second neck and a glass stopper on third neck (sample pouring neck).

2.2.26.2 Esterification process using acid catalyst

Because of higher acid value of Neem oil (NO) and Karanja oil (KO), acid esterification step was carried out. The acid value of WCO was within acceptable limit (< 2mg-KOH/g) hence one step transesterification was considered for WCO. The initial acid value of the NO and KO was 38.42 and 24.82 mg KOH/g (approx. 19 and 13% FFA respectively) which is far beyond the capacity (< 4% FFA) of transesterification reaction using alkali catalyst. The high acid value causes soap formation, which further affects the separation of biodiesel and results in low yield. In this step, approximately 600 mL of oil, pre-heated to 60 °C was added to a mixture of methanol (6 times the molar concentration of oil) and H_2SO_4 (0.5wt% w.r.t. oil)

maintained at 60 °C under continuous stirring. The reaction was allowed to continue for 2 h and then the reaction mixture was transferred to a separating funnel for gravity driven phase-separation overnight. The water settled at the bottom was drained and the remaining mass (mixture of esterified oil, excess methanol, and acid) was subjected to multiple cycles of hot water wash until the waste stream was visually clear. The residual moisture was removed by means of vacuum drying overnight. The acid value of the esterified product was then estimated titrimetrically.

2.2.26.3 Transesterification process using base catalyst

The experimental setup for the transesterification reaction was the same as it was used for the esterification of the feedstock. The reactor was fed with methanol (6 times the molar volume of oil) and KOH (1 wt% of esterified oil) and heated at 60°C with constant stirring for 15 min prior to the addition of preheated oil. The reaction was allowed to continue for 2 h. After completion of the reaction, the mixture was allowed to attain ambient temperature and was then transferred to a separating funnel for the separation of phases overnight. The bottom layer containg glycerol was discarded and the top layer containg methyl ester was washed with warm water to remove excess alkali, methanol, and traces of glycerol. The remaining mass was then subjected to drying using a vacuum oven.

2.2.27 Physico-chemical characterization of the synthesized biodiesel

Physico-chemical characterization such as moisture content, acid value, iodine value, viscosity of the synthesized biodiesels and oils werecarried out following AOCS methods (American oil chemists society). Density measurement was performed by

gravimetric analysis of the biodiesel using de-ionized water as a reference in a 25 mL density bottle (Sigma-Aldrich) thermostatically at room temperature. Pour point (ASTM D 97), cloud point (ASTM D 97), flash point (ASTM D 93) and calorific value (Toshniwal bomb calorimeter (IS 1350 e1, India)) of the specimens was also carried out.

2.2.28 Conversion analysis of synthesized biodiesel (^{1}H NMR)

Proton nuclear magnetic resonance spectroscopy (^{1}H NMR) was used to estimate the percentage conversion of the feedstock oil to biodiesel. A small quantity of biodiesel was solubilised in $CDCl_3$ bearing internal standard (Tetra methyl silane). The conversion of biodiesel was determined by the following equation:

$$C\ (\%) = (2A_{ME}/3A_{\alpha\text{-}CH2}) \times 100$$

where, C is the conversion percentage of triglycerides to methyl ester, $A_{\alpha\text{-}CH2}$ is the integration value of methylene protons at chemical shift 2.3 ppm, and A_{ME} is the integration value of methoxy protons of methyl ester at a chemical shift of 3.6 ppm (Knothe, 2006).

2.2.29 FAME composition

The fatty acid esters of Neem, Karanja and Waste cooking oil, were characterized by gas chromatography (Perkin Elmer Clarus 590). A 100 mg of different biodiesel were filtered separately through 0.2 µM syringe filter and mixed with 10 mL of analytical grade hexane. One micro litre of the prepared sample was then injected into the GC for analysis. The instrument was coupled with a flame ionization detector, split-injecter and a cyanopropyl polysilphenylene-siloxane (BPX70) column. The injection

and detector temperature were kept at 250^{o}C, and helium as carrier gas was passed at 1 mL/min. The column oven was programmed to start the analysis at 60^{o}C for 2 min, followed by a ramping at 10^{o}C min^{-1}up to 200^{o}C, and finally at 5^{o}C min^{-1} up to 240^{o}C where it was held constant for 7 min. The chromatographic peaks were identified and quantified by injecting Supelco 37 component FAME mix as calibration standard.

2.2.30 Thermo gravimetric analysis of biodiesel

Thermal gravimetric analysis (TGA) and differential thermal analysis of oils and their respective mono-alkyl esters were determined using a Perkin-Elmer 4000 thermogravimetric analyzer instrument. Approximately 10 mg of sample was taken for the analysis under a non-isothermal condition wherein the samples were heated up to 700^{o}C (at 10^{o}C/min heating rate) in a nitrogenous environment (40 mL/min). The thermo gravimetric curves (TG) and their respective derivative curves (DTG) were plotted to compare the degradation steps, onset temperature, and mass loss percentage.

2.2.31 Oxidative stability estimation of biodiesel by PetrOxy

Methanolic extracts of yellow passion fruit rind (YPFR), seed (YPFS), purple passion fruit rind (PPFR) and seed (PPFS) were mixed separately with three different biodiesel; Neem biodiesel (NBD), Karanja biodiesel (KBD) and waste cooking oil biodiesel (WCOBD) with concentration ranging from 250 to 1000 ppm. Initially, the extracts of said concentrations were dissolved in methanol and mixed with different biodiesels separately using a vortex and ultrasonic bath for 15-30 min. The fuel stability (Induction period: IP) of different biodiesels was estimated using oxidation

stability equipment (PetrOxy by Anton Paar) following the ASTM D7545 test method (temperature: 140^{o}C and pressure 700 kPa). Approximately 5 mL of the test specimen with and without extracts was loaded in the test cell, maintained at a temperature of 140^{o}C under an oxygen pressure of 700 kPa. The time elapsed until a 10% drop in oxygen was noted as the induction period of the sample. All the analyses were performed in triplicate, and the induction periods are reported as mean ± s.d.

2.2.32 Anti-corrosion behavior of extracts in biodiesel

2.2.32.1 Preparation of mild steel specimens

Mild steel (MS) specimens were collected from the mechanical workshop of the Indian Institute of Technology Guwahati. The MS sheet was cut into small pieces (5.0×1.1×0.5 for KBD and NBD and 2.4×1.2×0.1 for WCOBD) with a mechanical shearing machine. Due to the unavailability of sufficient MS (from the same lot of MS) at the time of collection, two different dimensions were utilized. The metal was thoroughly washed with acetone, followed by surface polishing with varying grades of emery papers (320, 220, 180, 100, and 80). After that, they were again washed with acetone, dried, and stored in a vacuum oven to avoid oxidation until further use.

2.2.32.2 Weight loss corrosion analysis

In this study, 500 ppm methanolic extracts of YPFR, YPFS, PPFR, and PPFS were added separately into a 50 mL glass vial containing 20 mL of different biodiesel (NBD, KBD, and WCOBD). The control were also kept for three different biodiesel without adding any corrosion inhibitor. Further, the polished MS specimens of known area and weight were immersed in the biodiesel sample. The specimens were

immersed in biodiesel without exposure to light and air for 15 days (360 h). After the mentioned period, all the steel specimens were carefully removed from the biodiesel samples, thoroughly washed with acetone, and properly dried before taking their weight measurements. The corrosion rate and inhibition efficiency (IE%) were calculated by following equations (2.9 and 2.10):

$$Corrosion\ rate\ (W_{corr}) = \frac{534 \times (W1 - W2)}{\rho t A} \quad (2.9)$$

$$Corrosion\ inhibitory\ efficiency\ (IE\%) = \frac{W_{corr}^{control} - W_{corr}^{sample}}{W_{corr}^{control}} \times 100 \quad (2.10)$$

Where, W1 and W2 are the weight of specimens without and with inhibitors (mg), Density (ρ) in g/cm^3, time of exposure in *t*,surface area (*A*) in inch^2, $W_{controlcorr}$ and $W_{samplecorr}$ are the corrosion rate values of control (without inhibitor) and sample groups (with inhibitor). Further, XRD, FESEM, and EDX analysis of the without and with inhibitor MS were carried out following standard protocols to understand their compositional and morphological characteristics. The corrosion rate was calculated as mm/year.

2.2.33 Statistical analysis

All the experiments under biochemical analysis and antibacterial activity of passion fruit oil were performed in duplicate of two individual experiments and reported as mean ± standard deviation. ANOVA analysis of the data sets in the biochemical analysis was performed to check the significance level using MATLAB 2019a software. The Pearson correlation test between the values of DPPH, ABTS, TPC, and TFC analysis of different oil samples was performed using SPSS 20 software to

determine the correlation coefficient between means. Pearson correlation test was conducted to understand the interrelation among other antioxidant methods and their probable mechanism in contributing antioxidant activity to the passion fruit seed oil. The TPC, TFC, and antioxidant activity of different extracts from passion fruit were performed in triplicate in two individual experiments. The antibacterial activity of the sample was performed in duplicate of two individual experiments. All the data are reported as mean ± standard deviation. The significance level in the TPC, TFC, and antioxidant activity of the passion fruit extracts was checked by one-way ANOVA using MATLAB 2019a software.Results of the antioxidant, antibacterial (ZOI), and antidiabetic activity of the isolated compounds from the fractionation study are presented in mean ± SD of two independent experiments in duplicates. In the anticancer activity section, the results of the MTT assay, PI-FACS, and colony-forming assays are presented in mean ± SE of three individual experiments in triplicate, and the p values for the PI-FACS and colony-forming assay were calculated using the student's T-test.

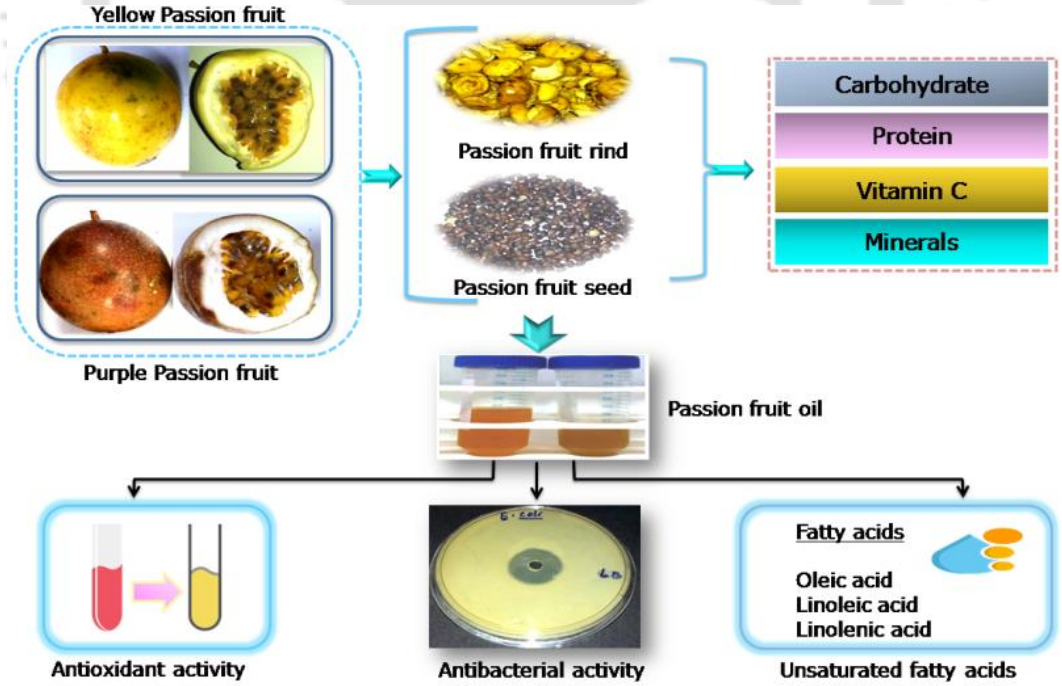
CHAPTER III

Collection, identification, and physicochemical characterization of yellow and purple passion fruit varieties collected from Northeast India

Morphological characteristics of yellow and purple passion fruit

Proximate, ultimate, and elemental composition

Physico-chemical and biochemical characterizations of passion fruit seed oil



Chapter III

Collection, identification, and physicochemical characterization of yellow and purple passion fruit varieties collected from Northeast India

This chapter mentions systematic physicochemical characterization of rind, seed and seed oil from yellow passion fruit (YPF) and purple passion fruit (PPF) collected from Northeast India. Various proximate, ultimate and elemental compositions of rind and seed are performed. Quantitative analysis of the samples' carbohydrate, protein, reducing sugar and vitamin C are also determined. The seeds contain up to 25% of oil with a significant unsaturated fatty acids content. Thus, possible applications of seed oil in the field of pharmaceutical and cosmetic sectors were understood through different physicochemical characterization. The fatty acid composition of seed oil was confirmed using GC analysis, where the thermo physical nature of the oil was derived from TGA and DSC analyses.

Further, biochemical analyses of oils, including phenolic content, flavonoid content, antioxidant activity, and antibacterial activity, were determined. The quality of the passion fruit oils was determined following the oil quality score method. The global antioxidant score for the best oil in terms of superior antioxidant activity was also deduced. The major polyphenol responsible for the antioxidant activity of the oil was further established through Pearson correlation analysis. These characterization

studies demonstrated rind, seed, and seed oil from YPF and PPF are nutrient-rich biomass with high valorization potential.

3.1 Morphological characteristics of YPF and PPF

The Plant systems are complex, with natural variations in their appearance and structure. The morphological character of passion fruit was recorded to distinguish taxonomical issues. The morphological results of YPF and PPF revealed that the fruit weight, length, diameter, and rind weight of YPF were higher than the PPF, as presented in Table 3.1. The juice and pulp content of PPF was higher than YPF. A similar kind of trend has been reported by Joy (Joy, 2010) on the passion fruits from Kerala, which indicates that there is no significant difference in the morphology of passion fruits obtained from Manipur and Kerala.

Table 3.1. Morphological characterization of YPF and PPF.

Characteristics	YPF	PPF
Fruit Weight (g)	100.87 ± 7.71	59.99 ± 3.36
Fruit Length (cm)	9.09 ± 0.62	6.53 ±1.32
Fruit Diameter (cm)	6.89 ± 2.22	6.13 ± 1.46
Pulp Weight (g/100g)	30.62 ± 3.82	41.66 ± 5.22
Rind Weight (g/100g)	57.60 ± 7.57	51.83 ± 6.44
Seed Weight (g/100g)	11.66 ± 2.20	9.85 ± 2.04
Juice Recovery (%)	27.56 ± 0.78	34.27 ± 1.31

Note: Values are presented as mean ± SD of two individual experiments in duplicates.

3.2 Proximate analysis of YPF and PPF

Moisture content is an essential parameter for crude drugs' storage stability and preservation. The moisture content (wet and dry basis) of both the rind and seed of YPF and PPF are shown in Table 3.2. The moisture content of the rind in both varieties was observed to be higher than the seeds. An increase in the ash value indicates contamination, substitution, or adulteration. The total ash consists of inorganic substances like silicates, phosphates, carbonates, and silica of calcium, potassium, sodium, and magnesium. In contrast, acid-insoluble ash value mainly deals with the contamination of siliceous materials like soil and sand. Water-soluble ash is nothing but the total ash which is soluble in water. The values of total ash, insoluble acid ash, and water-soluble ash in YPF and PPF rind and seed are presented in Table 3.2, which indicates that all the values are very low and under acceptable limits. The results suggest that there is not much physiological (plant tissue) and non-physiological (sand or soil) contamination in the studied samples.

3.2 Ultimate and elemental analysis of YPF and PPF

The quantification analysis of different compounds in YPF and PPF rind and seed powder is presented in Table 3.2. The total carbohydrate content of all four samples was found to be in the range of 2.19 ± 0.5 to 3.23 ± 0.2%. YPFR and PPFR showed higher carbohydrate content than YPFS and PPFS. Reducing sugar was also found to be higher in YPFR and PPFR than in YPFS and PPFS. The ascorbic acid was analyzed, and maximum content was observed in YPFR and PPFR compared to their respective seed powders. However, the ascorbic acid content of YPFS was higher than PPFR, leaving PPFS the lowest among the four. Ascorbic acid is an essential vitamin

for the body in many aspects, such as the transformation of cholesterol into bile acid in the liver, as a potent antioxidant and anti-carcinogenic agent. Thus it is necessary for the proper development of the body (Okwu, 2005). The total protein content was highest in seeds than in the rinds, and the maximum was recorded in the YPFS (22.77 ± 1.92%) followed by PPFS (21.82± 1.69%). This could be due to the fact that proteins are one of the significant components of seeds, as also reported in pumpkin and watermelon seeds (Lazos, 1986). Minerals are vital elements for the proper functioning of the human body. Major minerals like Na, K, Ca, and Mg and trace minerals like Fe, Zn, Mn, and Cu are essential for the management of hemoglobin formation (Fe), immunity, and antioxidant nature (Fe, Zn), an electrolyte (Na, Mn), maintenance of bones (Ca), etc. The analysis of different elements in rinds and seeds of YPF and PPF was carried out using flame photometry and atomic absorption spectroscopy, as described earlier and presented in Table 3.2. In the present study, Potassium was found to be majorly present (304 ± 1.41 to 397.5 mg/100g-biomass) in all the plant material, followed by Magnesium (113.99 ± 0.04 to 193.19 ± 0.02 mg/100g biomass) and Calcium (4.7 ± 0.07 to 139.1 ± 0.07 mg/100g-biomass). The potassium content of the samples in this study was higher than the reported values by Gondim et al., (Gondim et al., 2005). Trace minerals such as Manganese, Zinc and Copper in the passion fruit rind and seed were found to be in a comparable range with the reported literature (Dos Reis et al., 2018).The iron content of YPFS (3.85 ± 0.01 mg/100g-biomass) and PPFR (3.17 ± 0.02 mg/100g-biomass) was found to be higher than the YPFR and PPFS samples. The mineral composition mainly, Mg, Ca, and K of yellow and purple passion fruit rind and seed from Northeast India was found to be less compared with the Brazilian variety but higher in Zn, Mn, and Na (except PPF)

(Dos Reis et al., 2018). The variation in the mineral composition in the same passion fruit varieties but from different geographical origins could be because of varying soil type, fertilizer used, ripeness, harvesting time, sampling method, or laboratory analysis method (Marles, 2017). Overall, the rind and seed of both YPF and PPF were good sources of different minerals which can be further explored for various product developments.

Table 3.2. Proximate and ultimate analysis of YPF and PPF.

Sr. No.	Parameters	YPFR	YPFS	PPFR	PPFS
1	Water Content, Fresh Basis (% w/w)	82.25 ± 1.88	13.16 ± 0.75	81.73 ± 2.21	13.67 ± 0.68
2	Moisture Content, Dry Basis (% w/w)	13.18 ± 0.64	12.34 ± 0.5	12.76 ± 0.78	12.23 ± 0.5
3	Total Ash (% w/w)	6.96 ± 0.41	8.21 ±0.35	6.47 ± 0.11	8.85 ± 0.27
4	Acid Insoluble Ash (% w/w)	2.32 ± 0.32	3.38 ± 0.62	2.37 ± 0.16	3.45 ± 0.44
5	Water Soluble Ash (% w/w)	1.72 ± 0.08	2.41 ± 0.22	1.55 ± 0.17	2.39 ± 0.33
6	Total Carbohydrate (wt %)	3.23 ± 0.2	2.19 ± 0.5	3.06 ± 0.3	2.35 ± 0.3
7	Reducing Sugar (wt%)	2.73 ± 0.2	0.78 ± 0.2	2.37 ± 0.2	0.87 ± 0.2
8	Total Protein (wt%)	3.59 ± 1.08	22.77 ±1.92	3.28 ± 1.17	21.82 ±1.69
9	Vitamin C content (mg/100g)	132±5.2	5.6 ± 0.6	102±6.74	6.3 ± 0.3
10	*Elemental analysis*	YPFR	YPFS	PPFR	PPFS
	Sodium (mg/100g)	3.44 ± 0.02	3.52 ± 0.01	3.79 ± 0.02	3.96 ± 0.01
	Potassium (mg/100g)	304 ± 1.41	397.5 ± 2.12	316 ± 1.41	348.5 ± 3.53
	Calcium (mg/100g)	119.7 ± 0.14	7.01 ± 0.04	139.1 ± 0.07	4.7 ± 0.07
	Magnesium (mg/100g)	122.6 ± 0.21	176.1 ± 0.01	113.9 ± 0.04	193.2 ± 0.02
	Manganese (mg/100g)	0.72 ± 0.01	3.08 ± 0.01	0.87 ± 0.01	3.32
	Zinc (mg/100g)	1.63 ± 0.02	4.90	2.08 ± 0.21	5.23 ± 0.01
	Iron (mg/100g)	2.41 ± 0.01	3.85 ± 0.01	3.17 ± 0.02	2.88 ± 0.02
	Copper (mg/100g)	0.11	0.27	0.20	0.32

Note: Values are presented as mean ± SD of two individual experiments in duplicates. Yellow passion fruit (YPF), purple passion fruit (PPF), yellow passion fruit rind (YPFR), yellow passion fruit seed (YPFS), purple passion fruit rind (PPFR) and purple passion fruit seed (PPFS). w/w: weight/weight, wt%: weight percentage

3.3 Extraction of YPF and PPF seed oil

The oil from YPFS and PPFS seed was extracted using n-hexane, and the percentage yield is presented inTable 3.3. YPFS was found to have the maximum yield, followed by PPFS. Passion fruit oil contributes about 22-30% of the total weight of the seed. Similar results were also reported previously i.e. 22.40% (Giuffrè, 2007), 24.8% (Debideen and Sammy, 1978), and 29.4% (López-Vargas et al., 2013), etc. The varied geo-climatic condition of Northeast India does not impact oil yield compared to other regions of the world.

Table 3.3. Oil yield of YPFS and PPFS.

Sample names	Extractive Values (wt%)
YPFS oil	24.6 ± 0.2
PPFS oil	24.4 ± 0.2

Note: Yellow Passion Fruit Seed (YPFS), Purple Passion Fruit Seed (PPFS). Values are presented as mean ± standard deviation of two individual extraction yield experiments.

3.4 Physico-chemical characteristics of extracted oil

After drying the seeds, their moisture content was calculated. The moisture content of seed samples was found to be 12.34 ± 0.5 and 12.23 ± 0.5 for YPFS and PPFS, respectively. Further, both seed oils were examined for different physico-chemical properties such as acid value, iodine value, peroxide value, dynamic viscosity, refractive index, and melting point, and the results are presented in Table 3.4. The

Colour of the oil sample was observed to be yellow, and the odor was pleasantly fruity. The acid value of oils ranged between 2.0 to 4.8 mg-KOH/g-sample, which was within the permissible limit according to AOCS guidelines. Similarly, the peroxide value of the oils was also found within the allowable range (4.5 and 4.3 mg eq 1000/g for YPFS and PPFS, respectively).The quality of any oil in terms of rancidity and stability can be determined by the acid value, peroxide value, and free fatty acid value (Malacrida and Jorge, 2012a). A low percentage of acid value and the peroxide value of passion fruit seed oils indicated the good quality and edibility of the oil. However, long-chain fatty acids are more prone to oxidation in their free form. So, the lower the free fatty acid value better is the oil quality. The iodine value of oil determines the percentage of unsaturated matter in the oil (Mazumdar et al., 2012). The iodine value of passion fruit seed oil was found in the range of 126.9 to 131.4 g-I_2/100g-oil. The above result indicates that passion fruit seed oil can be categorized as semi-drying (siccative) oil. So, it can be used for food and chemical processing. Viscosity is an important parameter to check the oil for its use in food and pharmaceutical preparation. The viscosity of oil decreased with an increase in temperature due to the increased average kinetic energy of the molecules in a liquid. Hence, the increased kinetic energy of molecules quickly decreases their attractive force resulting in lower fluid viscosity. The dynamic viscosity of all passion fruit seed oil was measured at 25-40^{o}C, and presented in Table 3.4. The viscosity of yellow passion fruit seed oil was moderately higher than the purple passion fruit seed oil at both 25^{o}C and 40^{o}C. The Refractive index of all passion fruit seed oil was found to be 1.472 to 1.475, which is within the range of refractive index for most vegetable oils (Uquiche et al., 2008). This value indicated long-chain unsaturated fatty acid in

passion fruit seed oil (Mazumdar et al., 2012).The refractive index of oil suggested their intermolecular interaction. It can also be strongly correlated with the density of the oil.

Table 3.4. Physicochemical properties of YPFS and PPFS Oils.

Properties	YPFS Oil	PPFS Oil	Reference
Acid value (mg KOH/g-sample)	2.2	4.4	AOCS (Te 1a-64)
Iodine value (g-I_2/100 g-sample)	126.9	131.4	AOCS (Tg 1e64)
Peroxide value (mg eq 1000 g-sample)	4.5	4.3	
Viscosity at 25 °C (Pa-S)	0.038	0.024	-
Viscosity at 40 °C (Pa-S)	0.022	0.014	-
Refractive index at 25 °C	1.472	1.475	-

3.5 Fatty acid composition of seed oil

The GC chromatogram for the fatty acid composition of YPFS and PPFS seed oil is represented in Figure A1 (Appendix), and the relative percentage of fatty acid compositions is depicted in Table 3.5**.** The FAME composition of oils indicated the presence of unsaturated fatty acid, which is strongly correlated with a high iodine value. Moreover, in this study, the refractive index indicates the presence of long-chain unsaturated fatty acids in all the passion fruit seed oil,which was confirmed by the presence of linoleic acid (69.8% and 70.1%) followed by oleic acid (13.8% and 16.1%), respectively constituting 84.1-86.6% major unsaturated fatty acid. Quantification of these fatty acids was done by comparing the peak area with the peak area of the standard Supelco 37 component FAME mix, and the results are presented

in Table 3.5. Based on the higher percentage of unsaturated fatty acid, passion fruit seed oil could come under the polyunsaturated oil class, and the oils classified under this class are sunflower oil, sesame oil, and corn oil (Beare-Rogers et al., 2001). The oxidative stability of oil samples was performed to check the tendency of passion fruit seed oil against auto-oxidation, and the values are presented in Table 3.5. The COX value was calculated by considering the percentages of unsaturated fatty acid (Oleic acid, Linoleic acid, and Linolenic acid in our study) present in the oil. Higher unsaturated fats lead to an increase in COX values of oil. The results indicated no significant difference in the COX value in anyvariety. Moreover, passion fruit seed oil of all varieties is stable and can also be employed for the prevention of oxidative deterioration in other vegetable oil. Dabetic et al (Dabetic et al., 2020b) reported similar COX values in grape seed oil. However, higher COX values in the10-11 range were also reported in flaxseed hull oil (Herchi et al., 2016).

Further,these fatty acids in passion fruit oil are immensely helpful in preventing cardiovascular diseases, high blood pressure, boosting the immune system, etc. (Pham-Huy et al., 2008). Linoleic and Linolenic acids repair the cell membranes after the damage caused by oxidants and different free radicals. Similar results in terms of fatty acid composition in passion fruit seed oil collected from Brazil are also reported in a separate study by Malacrida and Jorge(Malacrida and Jorge, 2012a). The beverage industries generally throw these nutritious seeds away;thus, based on the FAME composition, it appears that the passion fruit seed oil can further be utilized by different pharmaceutical and cosmeceutical industries for various formulations.

Table 3.5. Fatty acid composition of seed oil.

Fatty acids	YPFS MP Relative %	(a)	PPFS MP Relative %	(a)
Palmitic acid (C16:0)	13.4	49.4	11.4	73.1
Stearic acid (C18:0)	2.4	9.1	1.9	12.7
Oleic acid (C18:1)	13.8	51.1	16.1	104.3
Linoleic acid (C18:2)	69.8	265.9	70.1	465.3
Linoleic acid (C18:3)	0.54	2.2	0.36	2.5
Saturated fatty acid (SFA)	15.8	NA	13.3	NA
Unsaturated fatty acid (UFA)	84.1	NA	86.6	NA
Mono unsaturated fatty acid (MUFA)	13.8	NA	16.1	NA
Poly unsaturated fatty acid (PUFA)	70.3	NA	70.5	NA
COX value	7.4		7.4	

Note: (a) is the quantitative values of different fatty acids in mg/mL of FAME calculated from standard (Supelco 37 mix). Different standard curve equations used for quantitative analysis are Y=66729X (C16:0), Y=64604X (C18:0), Y=66245X (C18:1), Y=64478X (C18:2), Y=59830X (C18:3). Standard curve was obtained from the peak area of three different concentration of Supelco 37 mix. NA (Not applicable)

3.6 Biochemical analysis of oil

3.6.1 Total phenolic and flavonoid content

The total phenolic and flavonoid content of oil extracted from YPFS and PPFS seeds was estimated according to the method described in the material and methods section. The results are presented in mg Gallic acid equivalent per gram of extract (mg-GAE/g-sample) for phenolic content and mg Quercetin equivalent per gram of extract (mg QUE/g) for flavonoid in Figure 3.1. TPC of YPFS was found to be the highest

(39.11 ± 0.84 mg GAE/g), followed by PPFS (19.8 ± 0.2 mg GAE/g). The TFC of YPFS was also found to be the highest (65.33 ± 2.24 mg QUE/g) compared to PPFS (51.66 ± 0.7 mg-QUE/ g-sample). The results obtained for TPC and TFC of different oil samples are significantly different ($p \leq 0.01$). Recently,(da Silva and Jorge, 2017) have documented the tocopherols, sterols, and phenolic acid content of passion fruit from Brazil. They observed higher contents of tocopherols, sterols, and carotenoidsthan other polyphenols in these varieties' seed oil. A similar trend of higher total flavonoid content than total phenolics was observed for the sample used in the present study.

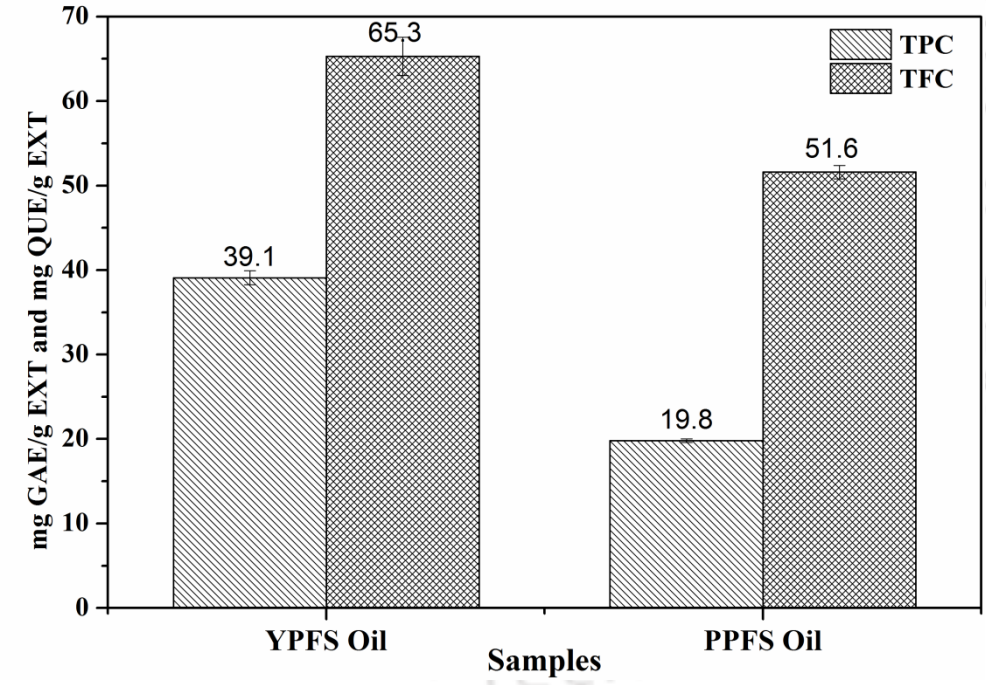


Figure 3.1. Total phenolic and total flavonoid content of passion fruit seed oil.

3.6.2 In vitro antioxidant activity

The in vitro antioxidant activity of different passion fruit seed oil was carried out by DPPH and ABTS methods, as described in the material and method section. The

antioxidant activity of all samples is represented as IC_{50} value in Figure 3.2. The IC_{50} value in DPPH assay for YPFS (70.7 ± 1.7 µg/mL^{-}) was found to be minimum followed by PPFS oil (128.7 ± 9.5 µg/mL). Lower the IC_{50} value; higher is the antioxidant activity. DPPH is a purple colour free radical, which is scavenged by the antioxidant capacity of a drug by accepting a hydrogen atom (Saito and Kawabata, 2005). According to the hypothesis given by (Li et al., 2011), in the case of protocatechuic acid, it could be believed that the DPPH may have been scavenged by any antioxidant molecule present in the oil to form a stable DPPH-H molecule. According to recent reports, passion fruit oil contains different polyphenols such as caffeic acid, p-coumaric acid, and salicylic acids (da Silva and Jorge, 2017), which shows that passion fruit seed oil is a potent antioxidant. The results from the ABTS assay also followed a similar trend as that of the DPPH assay for all seed oil samples (IC_{50} value from ABTS assay of YPFS oil (85.3 ± 5.5 µg/mL) and PPFS oil (145.6 ± 10.1 µg/mL)). $ABTS^{+\cdot}$a is a free radical cation produced after the reaction between the $ABTS^{+\cdot}$ and potassium persulphate. Plant extract (oil in our case) scavenged the $ABTS^{+\cdot}$ radical cations to ABTS in a concentration depended on manner by donating electrons (Re et al., 1999). The different polyphenols present in the passion fruit oil could have donated electrons and showed the synergistic antioxidant activity on ABTS free radicals. All the IC_{50} values were found to have a significant difference ($p \leq 0.005$) among each other. Ascorbic acid was used as a standard, and its IC_{50} value was significantly lower than the test oil samples. The antioxidant activity of any compound is highly dependent on its polyphenols content; hence, these results could be relevant to the TPC and TFC results (Figure 3.1). Antioxidant-rich compounds are beneficial for human health in terms of immunity booster, cardiac friendly, anti-

cancer agent, lowering blood cholesterol level, etc.(Kaur and Kapoor, 2002; Rumbaoa et al., 2009).

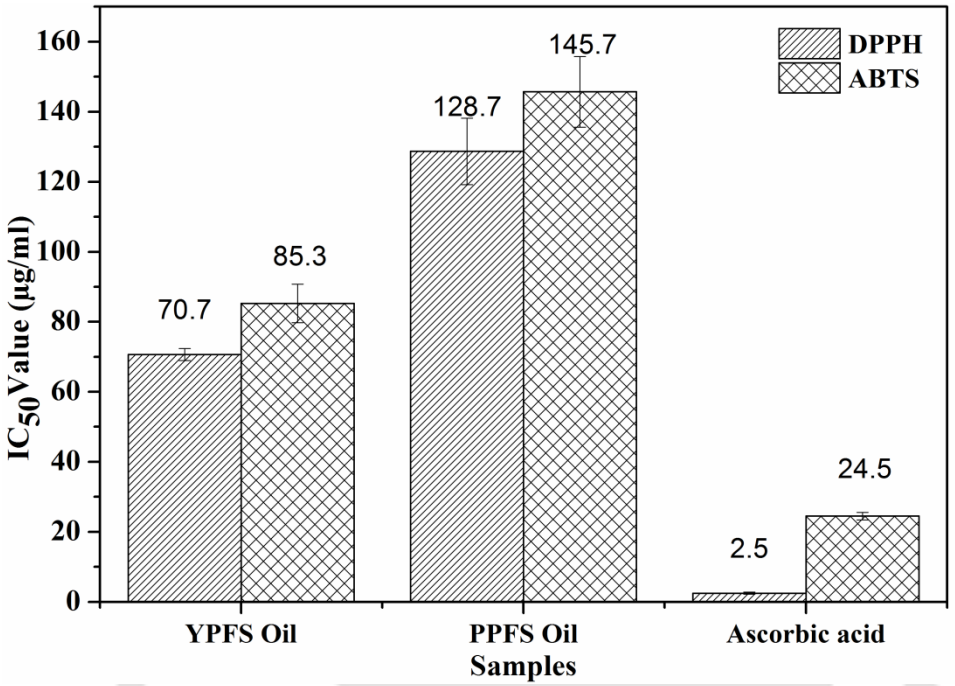


Figure 3.2. Antioxidant activity of passion fruit seed oil.

3.6.3 Global antioxidant score (GAS)

The comprehensive knowledge about the total antioxidant capacity of the YPFS and PPFS oil can be obtained by GAS. The global antioxidant score is a test method to combine an extensive array of antioxidant experiments with improving the discrimination of a sample by establishing its global antioxidant capacity. Initially, individual T_{score} values were calculated, and the mean values of those T_{score} values were presented as GAS for each sample. Four different antioxidant assays, such as DPPH, ABTS, TPC, and TFC, were performed, and obtained results were represented in the mathematical form (materials and methods section). The GAS value of YPFS

and PPFS was observed to be two. These obtained values were used to compare the antioxidant activities of different passion fruit varieties, and the result showed an insignificant difference between the varieties. The passion fruit oil of all the varieties was observed to have a higher GAS value than grape seed oil (Dabetic et al., 2020b).

3.7 Thermo physical analysis of oil

3.7.1Thermo gravimetric analysis

TGA was performed to evaluate the thermal stability of oil samples, and the results of the TGA are represented in Figure 3.3. The thermal stability of oil was estimated between onset temperature (temperature where decomposition starts) and offset temperature (the temperature atwhich no further decomposition occurs) under an inert (N_2) atmosphere (40 mL/min). This study used a heating rate of 10 °C/min to get oil's firm thermal decomposition behavior(Borugadda and Goud, 2014). The TG plot represents a thermal degradation compound, whereas the DTG curve provides information on the number of steps of the decomposition process. The onset temperature of YPFS MP and YPFS were noted at 335.7 °C and 334.4 °C, respectively. While, the offset temperature of oil samples was in the range of 465.5-467.4 °C. A permissible amount of weight loss was observed within 100 °C, which might be attributed to moisture in the sample. The main thermal decomposition of oil samples happened between 333.4-467.4 °C. In the first stage (100-330.0 °C), highly volatile unstable compounds like polyunsaturated fatty acids, other oxygenated compounds, and hydro-peroxides present in the oil undergo decomposition (Dunn and Moser, 2005). A sharp decline in the weight loss of the sample between 335.7- 467.4 °C, suggests the decomposition of monounsaturated fatty acids, saturated fatty acids,

and phytoconstituent. The thermal decomposition for all three oil took place in a single continuous step, which suggested the breakdown of polyunsaturated fatty acids (Jayadas and Nair, 2006). The thermal stability of the oil sample depends on the degree of unsaturation, chain length (mono as well as polyunsaturated), and branching of the chain (Sajeeb and Rajendrakumar, 2019). The high onset temperature of the oil sample indicated that the antioxidant molecules present in the oil refrain from thermal oxidation. The published report indicated that passion fruit oil contains different hydroxybenzoic acid and hydroxycinnamic acids (da Silva and Jorge, 2017), which are well-known antioxidants and can reduce thermal oxidation following their protective mechanism. Even recently, published literature indicates that the phenolic acids present in extra virgin olive oil could sustain antioxidant activityafter frying potatoes at 180°C for 10 min (Cheikhousman et al., 2005). Thus, studying oxidative stability is essentialin defining the useful lifetime and storage conditions in real-life applications.

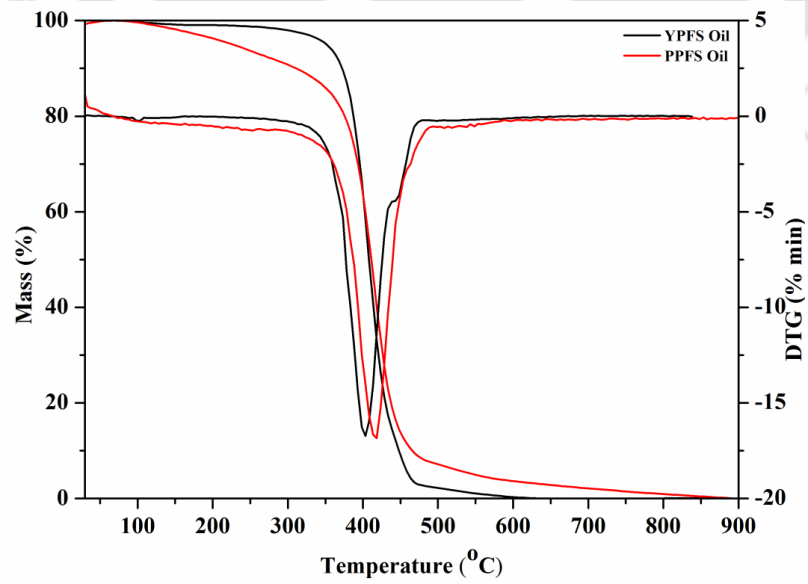


Figure 3.3. TG and DTC analysis of passion fruit seed oil.

3.7.2 Differential scanning calorimetry

The DSC thermogram of YPFS and PPFS seed oil are presented in Figure 3.4. Both yellow and purple passion fruit seed oil collected from Manipur was found to have similar pour points (-18.20^{o}C and -18.05^{o}C, respectively). Vegetable oils are composed of different types of triglycerides esters and various fatty acids such as saturated, monounsaturated, and polyunsaturated. Triglyceride esters and fatty acid composition determine the pour point of the oil. Pour point is considered as an essential property of vegetable oil for its usability. Comparison of pour point values of oil samples obtainedin the present study with that for the many edible and non-edible oils tabulated in the work of (Borugadda and Goud, 2014) reveals better cold flow properties for YPFS and PPFS seed oil.

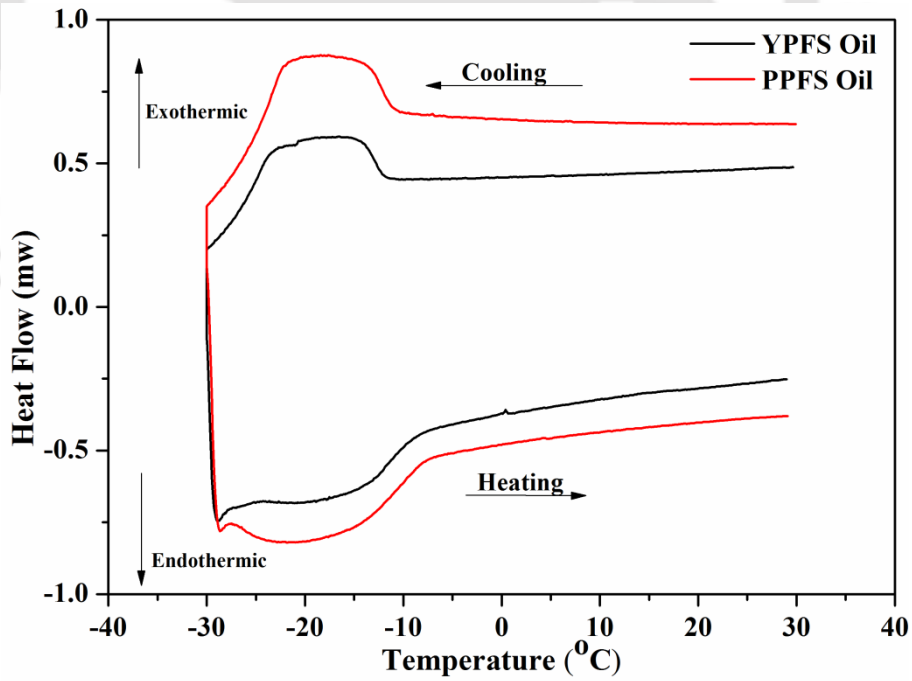


Figure 3.4.DSC thermogram of passion fruit seed oil.

3.8 FTIR-ATR analysis

The qualitative FTIR-ATR spectrum analysis of oil samples' and vibrational bands that appeared in the IR region are presented in Figure 3.5. FTIR spectra of all oil samples showed identical spectrum and functional groups. The present study confirmed the presence of triglyceride esters, aromatic compounds, carbonyl groups, etc. No spectral band of the hydroperoxide group near 3400-3300 cm^{-1} conferred good-quality oil (Ogbu and Ajiwe, 2016). In all, seven significant peaks were identified in the oil samples. The peak at 3011.8 cm^{-1} corresponds to stretching of =C-H bonds (presence of aromatic rings), the other peak at 2924.0 cm^{-1} is attributed to the presence of CH_2 symmetrical stretching vibration, and the peak at 2854.8 cm^{-1}corresponds to aliphatic CH_2 asymmetric stretching vibration. The characteristic peakat 1742.1 cm^{-1} is attributed to thecarboxylic acid in methyl esters; it also corresponds to the presence of fatty acids such as oleic and linoleic acid in the compositional analysis. Further, the peak at 1459.0 cm^{-1} corresponds to the =C-H group, whilepeak at 1161.5 cm^{-1} and 1095.9 cm^{-1} showed strong evidence of the C-O esters.

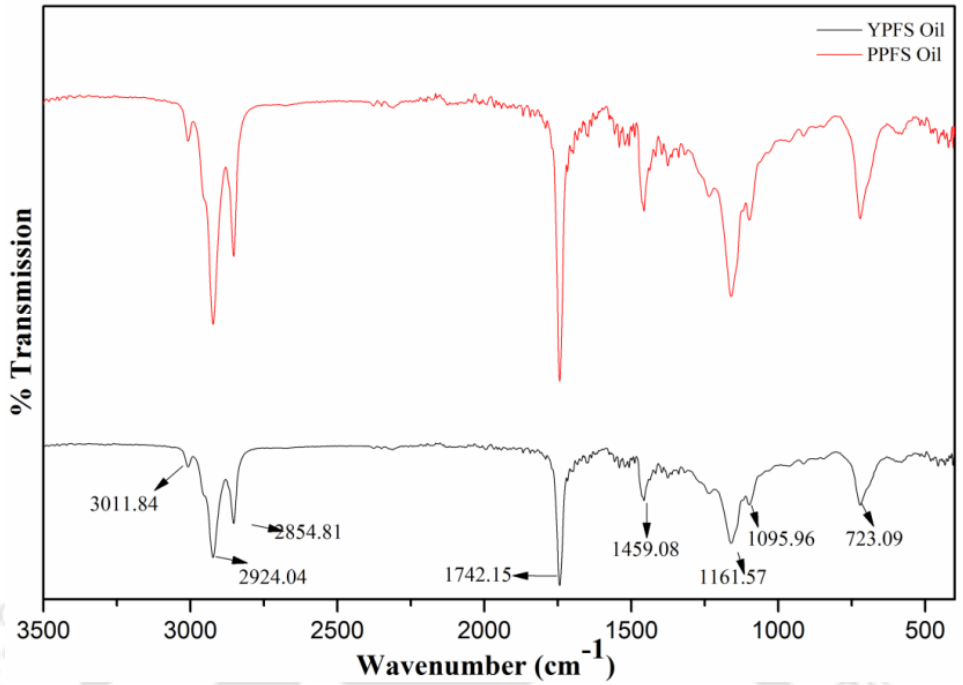


Figure 3.5. FTIR –ATR analysis ofpassion fruit seed oil.

3.9 Antibacterial activity of extracted oil

The antibacterial activity of passion fruit seed oilwastested by the zone of inhibition, and the results are presented in Table 3.6 and Figure 3.6. Different gram-positive bacteria, such as *S.aureus, B. Subtilis, S.epidermidis, M. luteus* and gram-negative bacteria such as *E. coli* , *P. aeruginosa*, and *E. aerogenes* were used for the antibacterial activity because of their role as foodborne pathogen (Mendez et al., 2012; Siddique et al., 2020; Zayko et al., 2020; Zou et al., 2020). The bacteria like *S.epidermidis* is grown on the food processing surface of the food industries (Zou et al., 2020). *Klebsiella pneumonia* is a known human pathogen that causes severe infections in the liver, lungs, bladder, brain, etc. All oil samples were found to be effective against both gram-negative as well as gram-positive bacteria except some specific bacteria. All the oil samples showed noticeable antibacterial activity against

*Klebsiella pneumonia, Bacillus subtilis, Pseudomonas aeruginosa,*and *Micrococcus luteus*. The seed oil of PPFS showed profound bactericidal activity against *Staphylococcus aureus,*while YPFS failed. No zone of inhibition was observed for any oil sample in *Escherichia coli* and *Enterobacter aerogenes*. This could be because of the very tough peptidoglycan cell wall of these gram-negative bacteria to which the oil could not penetrate (Fathi-Achachlouei et al., 2020). The profound antibacterial activity of the oil samples could be because of the fatty acid composition, active antioxidant, and flavonoid content in the oil. Similar results have also been reported by (Cordeiro et al., 2019) for *Virola surinamensis* seed oil and citronella oil (Timung et al., 2016). The possible mechanism of antibacterial activity could be cell wall lysis, inhibition of DNA gyrase, or inhibition of cytoplasmic membrane function by the flavonoids and phenolics present in passion fruit seed oil (Cushnie and Lamb, 2005). Ferreira and group (Ferreira et al., 2011) studied passion fruit seed oil (refined, cold-pressed, Soxhlet apparatus extracted oil) against *S.epidermidis, S. aureus, P. aeruginosa,*and *E.coli*. Still, they could not find any antibacterial activity, while passion fruit seed oil from Northeast India showed satisfactory antibacterial activity against *S.aureus* and *P. aeruginosa*.

Table 3.6. Zone of inhibition study of YPFS and PPFS oils.

Bacteria	Control	YPFS Oil	PPFS Oil
EC			
10*	ND	ND	ND
30*	ND	ND	ND
50*	ND	ND	ND
KP			
10*	ND	9±0.7	8
30*	ND	9±0.7	12±0.7
50*	ND	10	10±1.41

EA			
10*	ND	ND	ND
30*	ND	ND	ND
50*	ND	ND	ND
PA			
10*	ND	10.5	6±0.7
30*	ND	11.5±0.7	9±0.7
50*	ND	11.5	9
SA			
10*	ND	ND	9
30*	ND	ND	10
50*	ND	ND	12
BS			
10*	ND	9.5±0.7	5±0.7
30*	ND	11	7±1.41
50*	ND	11±0.7	9±0.7
SE			
10*	ND	ND	ND
30*	ND	ND	ND
50*	ND	ND	ND
ML			
10*	ND	ND	10±0.7
30*	ND	5	10
50*	ND	6	10

Note*: Escherichia coli* (EC), *Klebsiella pneumonia* (KP), *Enterobactor aerogenes* (EA), *Pseudomonas aeruginosa* (PA), *Staphylococcus aureus* (SA), *Bacillus subtilis* (BS), *Staphylococcus epidermidis* (SE) and *Micrococcus luteus* (ML). 10*, 30*and 50* are 10%, 30% and 50% v/v oil/Tween 80. ND (Not detected). Values are presented in mean ± standard deviation of two different experiments. All values are presented in millimetre (mm).

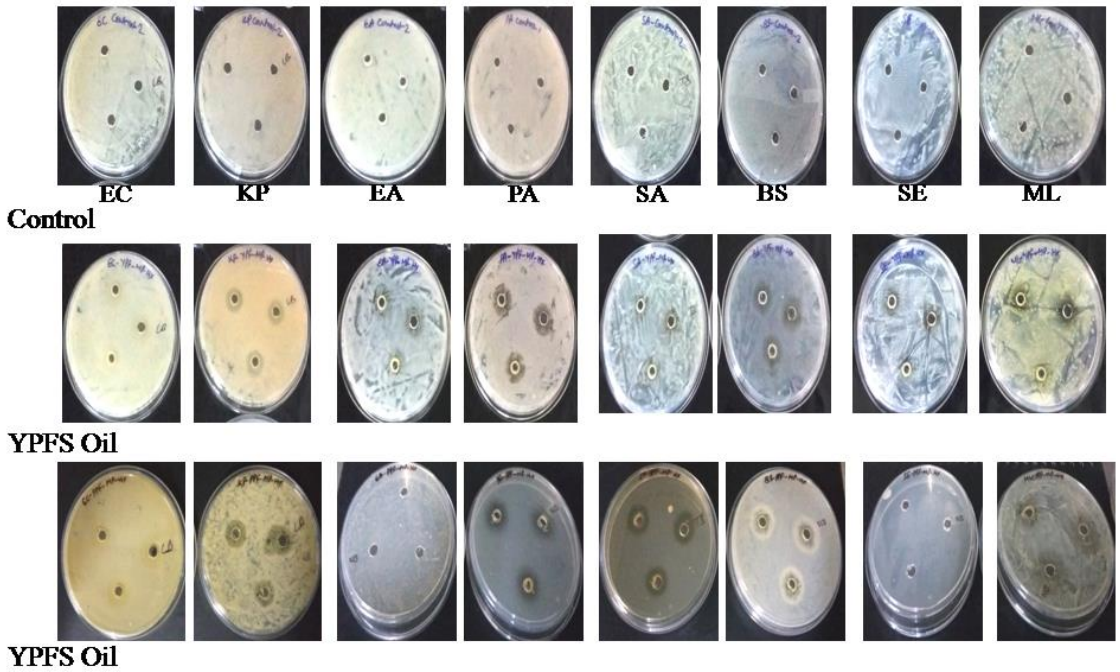


Figure 3.6. Zone of inhibition (ZOI) analysis of YPFS Oil and PPFS Oil. Control lane consist of ZOI of *Escherichia coli*(EC), *Klebsiella pneumonia*(KP), *Enterobactoraerogenes* (EA), *Pseudomonas aeruginosa* (PA), *Staphylococcus aureus*(SA), *Bacillus subtilis*(BS), *Staphylococcus epidermidis*(SE) and *Micrococcus luteus*(ML). Lane 2 and lane 3 consist of ZOI analysis of YPFS and PPFS seed oil, respectively, with the above-mentioned bacteria in the same order.

3.10 Pearson correlation

The Pearson correlation between the mean value of TPC, TFC, DPPH, and ABTS of different passion fruit oil is presented in Table 3.7. The Pearson correlation analysis was performed to understand the possible interrelation between the four biochemical assays with each other for the antioxidant behavior of the oil samples. The TPC values of oil samples were positively correlated withTFC values (r = 0.973) but negatively correlated with DPPH (r = -0.958) and ABTS (r= -0.978). Similarly, there was a significant negative correlation between TFC with DPPH (-0.998*) and ABTS

(-1.000*). This analysis suggested that flavonoid compounds present in the oil significantly increase the antioxidant activity more than phenolic compounds. The negative correlation of TPC and TFC with DPPH and ABTS assay indicated that the sample with higher polyphenols compounds showed the highest antioxidant activity and lower IC_{50} value. A similar pattern of negative correlation between TPC and TFC with DPPH and ABTS was also documented by (Barros et al., 2008; Gan et al., 2013).

Table 3.7. Pearson correlation coefficient between TPC, TFC, DPPH, and ABTS.

	TPC	**TFC**	**DPPH**	**ABTS**
TPC	1	0.973	-0.958	-0.978
TFC	0.973	1	-0.998*	-1.000*
DPPH	-0.958	-0.998*	1	0.996
ABTS	-0.978	-1.000*	0.996	1

Note: Total Phenolic content (TPC), Total Flavonoid content (TFC), 2,2-diphenyl-1-picrylhydrazyl (DPPH), 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid (ABTS)
* Correlation is significant at the 0.05 level (2-tailed).

3.11 Oil quality score

Recently, different scores such as Aggregate Nutrient Density Index, Global Food Security Index etc.have been employed for food products to compare their quality (Dabetic et al., 2020b). The lowest oil quality score better is the quality of the oil. In the present study, two different sets of OQS% were calculated from the antioxidant and physicochemical properties, and further, a cumulative result of both these OQS% are presented in Figure 3.7 (a), (b), and (c), respectively. Oil varieties can be compared to determine the best variety by considering their different antioxidant and

physicochemical activities under the oil quality score (%). The OQS proposes an insight not only into the biological quality of the oil, but also determines the oil quality based on its physicochemical properties.From Figure 3.7 (a) and (b), it was evident that YPF seed oil quality was the best among the tested samples. The minimum OQS% of YPFS was because of the lowest DPPH and ABTS IC_{50} values and lowest acid and peroxide values. The pattern obtained in Figure 3.7 can be correlated with other findings of the present study, such as antioxidant and physicochemical properties. Figure 3.7 (c) is the cumulative OQS% of both antioxidant and physicochemical properties where the average of all standard scores of all varieties had been depicted. The values of different OQS are presented in Table A1 (Appendix). Calculated values revealed the best passion fruit seed oil sequence as YPFS MP > PPFS MP.

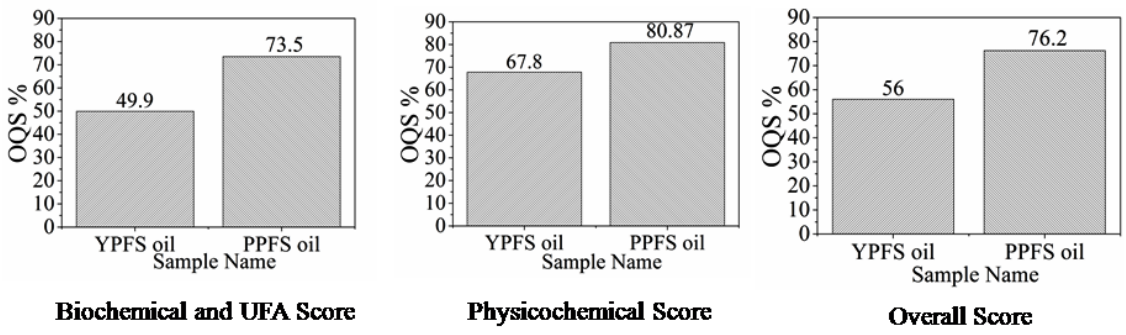


Figure 3.7. Oil quality score of passion fruit seed oil.

3.12 Summary

The rind and seed (mainly seed coat) of passion fruit are usually considered futile and remain unutilized for regular food. The present study provides a clear vision of the nutritional and medicinal value of the rind and seed of yellow and purple passion fruit in Northeast India. The proximate analysis carried out in the study will help to

standardize these fruit parts for different pharmaceutical approaches. A high amount of macro and microelements, ascorbic acid, and crude protein in the YPF and PPF powder makes them a rich source of nutrition that can be further utilized in the development of many value-added products and food supplements. Passion fruit seed is considered a waste by-product by the beverage industries after its juice extraction, resulting in the generation of substantial biodegradable agricultural waste. This study demonstrated the usability of seed oil extracted from yellow and purple passion fruit seeds for their antioxidant, antibacterial, and thermal properties. There is no detailed reporting on such wide parameters mentioned above on yellow and purple passion fruit seed oil from Northeast India. The acid and peroxide value of seed oil indicates a good quality oil. Passion fruit seed oil could also be considered for cooking purposes because of its high thermal stability. The higher percentage of polyunsaturated fatty acid present in this oil could be utilized for different food supplements as well as cosmetic formulations. The presence of a considerable amount of flavonoid content also corroborates the high antioxidant nature of the oil. The Passion fruit seed oil was found to be very effective against different food-borne bacteria and human pathogenic bacteria. The results showed profound antibacterial activity against gram-positive bacteria (*S.aureus* and *P. aeruginosa*), whereas the same bacteria were non-susceptible against some Brazilian passion fruit oil varieties. Oil quality was comprehensively graded through oil quality score ranking by integrating all biochemical parameters and physicochemical properties. The results obtained from the present work demonstrate the candidature of passion fruit seed oil as an economical alternate substrate for different value-added products. Awareness among local people and passion fruit beverage industries on its health benefit and usability

could also help decrease the passion fruit waste generated from household use and food industries.

CHAPTER IV

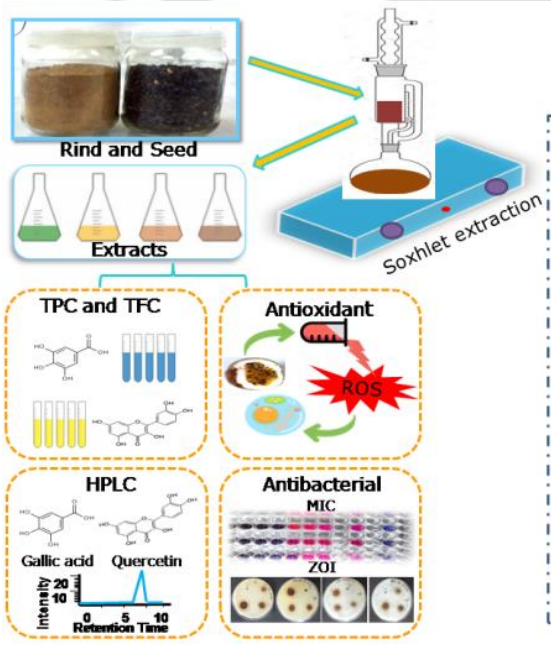
Extraction of polyphenols from passion fruit rind and seeds and physiochemical and biochemical analysis for their therapeutic use

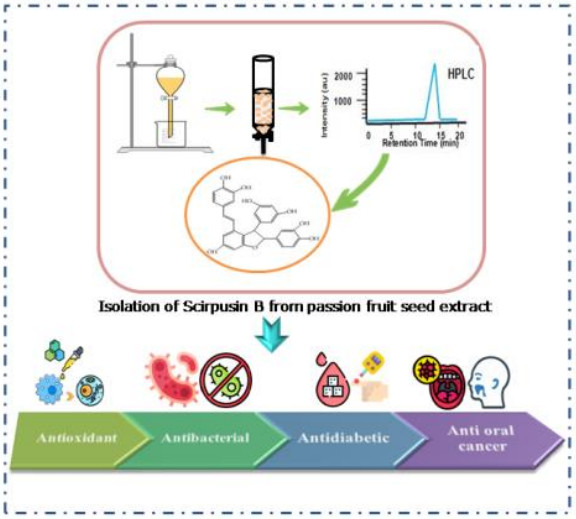
Extraction and identification of polyphenols from passion fruit rind and seed

Biochemical applications of the polyphenol rich extracts

Fractionation and isolation of polyphenols from yellow passion fruit rind and seed

Biological activities of the purified compounds





Chapter IV

Extraction of polyphenols from passion fruit rind and seeds and physiochemical and biochemical analysis for their therapeutic use

The passion fruit industry generates huge amounts of biodegradable waste in the form of rind and seed after juice extraction. These wastes have a high potential for valorisation. For instance, the isolation and identification of bioactive phytochemicals and their application in the health and nutrition sectors can substantially add value to the produced waste. Therefore, this chapter focuses on extracting phytochemicals from the rind and seed of YPF and PPF. The samples were extracted using four different solvents: ethyl acetate, acetone, methanol, and water. Further, the preliminary phytochemical and FTIR analysis of these extracts confirmed the presence of alkaloids, glycosides, tannins, phenolic acids, flavonoids, etc. To validate these results, the extracts' total phenolic and total flavonoid content were determined, followed by their antioxidant activity by three different methods: DPPH, ABTS, and metal chelating assays. Using HPLC analysis, the best extracts from each sample were then subjected to identify some polyphenols.

Further from the minimum inhibitory concentration and zone of inhibition study, the antibacterial activity of the bioactive compounds was determined. This study was further extended by subjecting the YPF rind and seed extracts to fractionation and column chromatography to identify more polyphenols. The isolated compounds were

then characterized and identified by ^{1}H NMR, ^{13}C NMR, and mass spectrometry. Further, different biological activities of isolated compounds (antioxidant, antidiabetic, antibacterial (only scirpusin B), and anticancer (only scirpusin B)) was performed.

4.1 Extraction of rind and seed from YPF and PPF

The extraction of phytochemicals from the rind and seeds of YPF and PPF was carried out following Soxhlet extraction, as discussed in the previous chapter (chapter 3). The extractive values of YPF and PPF extract for different solvents are presented in Table 4.1. The percentage yield of extracts for different solvents indicates the solubility pattern of the kind of phytochemicals present in them. The yield of methanol and aqueous extracts were the highest compared to ethyl acetate and acetone extracts, signifying the presence of more polar phytoconstituents in the rind and seed samples.

Table 4.1. Extractive values in different solvents.

Sample name	Ethyl acetate	Acetone	Methanol	Water
YPFR	3.72 ±0.62	6.16 ± 0.64	32.39 ± 0.10	47.85 ± 0.52
YPFS	22.64±1.04	25.66 ± 2.60	35.77 ± 0.45	18.21 ±1.48
PPFR	4.66±1.62	6.30 ± 0.45	39.02 ± 1.44	49.17 ± 1.09
PPFS	21.94±1.51	25.06 ± 2.75	32.35 ± 1.26	14.94 ± 1.87

Note: Yellow Passion Fruit Rind (YPFR), Yellow Passion Fruit Seed (YPFS), Purple Passion Fruit Rind (PPFR), and Purple Passion Fruit Seed (PPFS). Values are presented as mean ± SD of two individual extractions for each sample by using each solvent. Values are presented as a weight percentage.

4.2 Preliminary phytochemical analysis

The phytochemical constituents of ethyl acetate (EA), acetone (AT), methanol (ML), and water (WR) extracts of YPFR, YPFS, PPFR, and PPFS wereestimated following the protocols given in the materials and method section, and the results are presented in Table A2 (Appendix) and Table A3(Appendix), respectively. The analysis revealed te presence of carbohydrates, glycosides, alkaloids, tannins, and phenolic compounds, amino acids, and proteins in the extracts. This qualitative assay revealed alkaloid content in PPF and YPF from Dragendorff's and Wargner's test. Positive results in the Keller-killiani test for glycoside indicated the presence of deoxy sugar in all the extracts. Cardiac glycosides mainly contain deoxy sugars, so these extracts could also be explored for their cardio tonic properties. The presence of steroid in YPF and PPF rind and seed extracts were qualitatively confirmed from Salkowski reaction. The appearance of red color in the reaction mixture (due to the formation of bi-sulphonic acid of bicholestadiene) revealed the presence of steroids in rind and seed extracts. The presence of flavonoids in different extracts was confirmed by the $Pb(C_2H_3O_2)_2$and NaOH+ HCl tests. Tannins and phenolic compounds were confirmed qualitatively from $Pb(C_2H_3O_2)_2$, 5% $FeCl_3$, diluted HNO_3, and diluted $KMnO_4$ reactions. The rind and seed extracts of YPF and PPF showed positive results in the above-mentioned reactions. Based on the qualitative results, further quantitative measurements of phenolics and flavonoids in the passion fruit rind and seed extracts were also performed and described in the later section. Confirmation of different secondary plant metabolites in YPF and PPF rind and seed extracts further increased the pharmaceutical importance of passion fruits.

4.3 FTIR analysis

The FTIR analysis of YPF and PPF and their extracts exhibited a similar transmittance pattern except for some variations (Figure 4.1). FTIR analysis results confirmed the presence of alcohols, phenols, alkanes, alkenes, aliphatic amines, carboxylic acids, esters, ethers, and aromatic functional groups. Powder samples and solvent extracts of YPFR and YPFS showed transmittance at 872.07 $cm^{-1,}$ 734.02 cm^{-1} confirming C-H stretching and 1462.2 cm^{-1} (C-C stretch in ring) signifies the presence of aromatic compounds. The presence of aromatic compounds or benzene rings in YPF extracts could indicate the presence of flavonoids in extracts (Yazaki et al., 2009). The IR spectrum at 1066.6 cm^{-1}, 1070.9 cm^{-1} in the YPFR (YPFR EA, YPFR ML) and PPFR (PPFR AT) correspond to the presence of alcohol and carboxylic acid groups (Jacques et al., 2007). The presence of COOH group in the extracts contributes to its antimicrobial properties (Ahmad and Ali, 2013). Some other vibrational bands in the YPFS (all extracts and powder), PPFS (all extracts and powder) and PPFR (PPFR EA, PPFR AT, and PPFR WR) at 1171.05 cm^{-1} and 1162.5 cm^{-1} and 1222.6 cm^{-1}, respectively, attributed to the attachment of alcohol, carboxylic acids, esters or ethers compounds. The sharp peak of carbonyl stretching of alkyl-esters at 1748.5 cm^{-1} to 1745.5 cm^{-1} of YPFS (all extracts and powder) and PPFS (all extracts and powder) confirm the presence of oil (Rodrigues et al., 2015). Similarly, the C=O stretching at 1728.1 and 1736.2 cm^{-1} correspond to the methyl esters of pectin found in the powder and other extracts of the rind of yellow and purple passion fruit (Liu et al., 2006). Aliphatic C-H stretching group between 2905.62 $cm^{-1}$to 2930.81 cm^{-1} was observed in all the extracts and powder samples of YPF and PPF. A similar transmittance pattern for passion fruit was also reported by Jacques et al.,(Jacques et al., 2007).

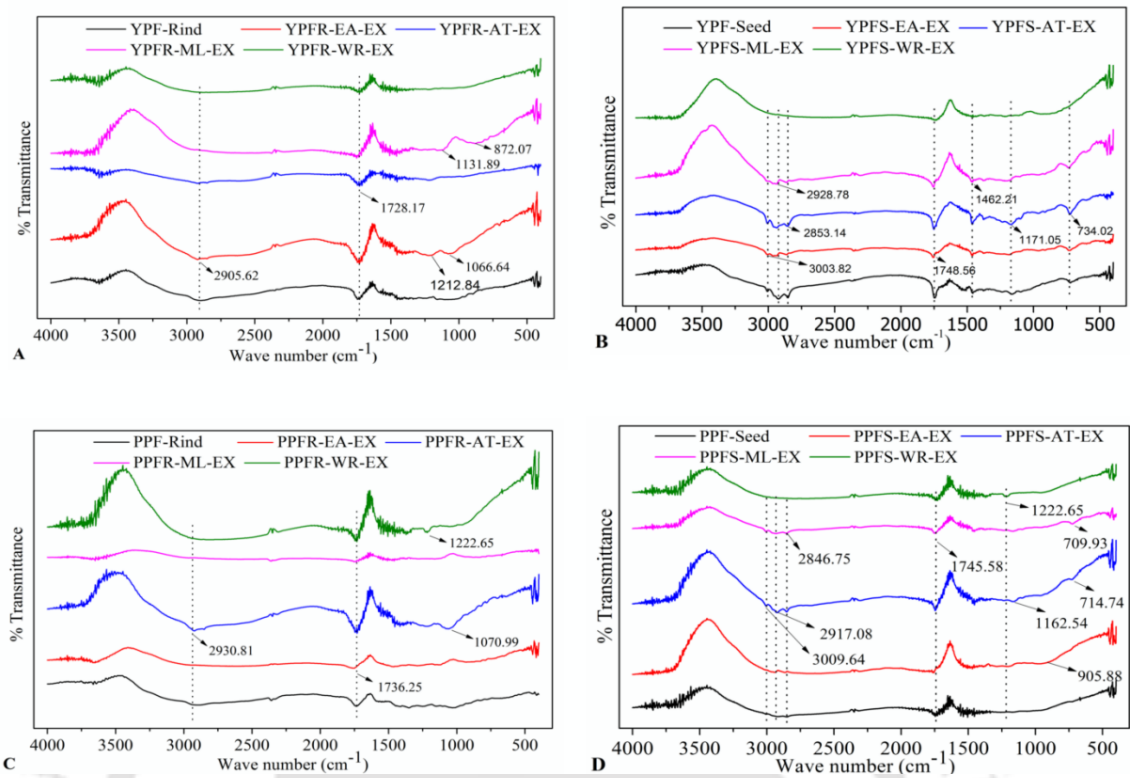


Figure 4.1.FTIR spectra of (A) yellow passion fruit rind (YPFR), (B) yellow passion fruit seed (YPFS), (C) purple passion fruit rind (PPFR), and (D) purple passion fruit seed (PPFS) biomass and extracts respectively. Absorption spectra were recorded between 4000 cm^{-1} to 400 cm^{-1} using an FTIR spectrophotometer. FTIR spectrums in black represent the spectrum of powder biomass, red represents ethyl acetate extract (EA-EX), blue represents acetone extract (AT-EX) extracts, pink represents methanol extract (ML-EX), and the green represents water extract (WR-EX).

4.4 Biochemical analysis

4.4.1 Total phenolic and total flavonoid content

After confirming the presence of polyphenols in the extracts from preliminary phytochemical screening, a further attempt was made to quantify the total phenolic and flavonoid content in YPF and PPF.

All extracts' total phenolic content (TPC) is presented in Figure 4.2, and the values are presented in mg GAE/g (mg Gallic acid equivalent per gram of the extract). The standard curve of Gallic acid is illustrated in Figure A2 (Appendix). Methanol extracts of YPFR, YPFS, PPFR, and PPFS showed the highest total phenolic content, followed by their respective acetone extract. All ethyl acetate and aqueous extracts also possessed a minimal TPC value. The methanolic extracts of YPFR and YPFS showed TPC in the range of 66.52 ± 4.36 mg GAE/g of extract and 62.30 ± 9.07 mg GAE/gof extract, respectively, which was higher than the methanolic extract of PPFR (61.37 ± 1.90) and PPFS (54.63 ± 0.23). The results of this assay revealed that the phenolic content in all the extracts of YPFR and YPFS was higher than in PPFR and PPFS extracts. No significant difference ($p > 0.05$) was observed in TPC content between YPFR, YPFS, PPFR, and PPFS extracts. However, the ANOVA analysis confirmed that extract in different solvents had a significant effect ($p < 0.05$) on TPC content. The qualitative phytochemical analysis also corroborated the presence of phenolic compounds, which attributes to the presence of tannins. FTIR analysis of the same extracts also revealed the presence of aromatic compounds. Tannins and coumarins are vastly attributed to the treatment of different diseases like immune deficiency, carcinoma, heart diseases, etc. (Kähkönen et al., 1999). The rich phenolic substances also impede lipid oxidation in oils and fatty foods (Kaur and Kapoor, 2002; Rumbaoa et al., 2009), which improves food quality by extending the shelf life of food. It is also a well-documented fact that the phenolic compounds of different plant extracts show antimicrobial and antifungal properties (Manach et al., 2004; Moure et al., 2001). Hence, it could be concluded that YPFR and YPFS could be potent sources of phenolic compounds, followed by PPFR and PPFS extracts. The

total flavonoid content (TFC) of all extracts is presented in Figure 4.2, and the values are expressed in mg QUE/g (mg Quercetin equivalent per gram of extract). Quercetin standard curve is illustrated in Figure A3(Appendix). The methanolic extract of YPFR (73.38 ± 2.04) and acetone extract of PPFR (71.72 ± 2.04) possessed the highest total flavonoid content compared to all the other extracts. Flavonoids are thermo labile substances, which could be the reason for the significantly less flavonoids in aqueous extract yield. Moreover, both varieties' rind and seed extracts also had a significant effect ($p < 0.05$) on the TPC content. The rind extract of both yellow and purple passion fruit had high flavonoid contents than their respective seeds. Flavonoids have many health-promoting properties because of their high antioxidant potential and also help in inducing many crucial human protective enzyme systems. The protective effects of flavonoids have been discussed in many studies as an anti-bacterial and antiviral agent (PANDEY, 2007). Apart from that, flavonoids also potentially affect degenerative diseases like cancers and cardiovascular disorders (Cook and Samman, 1996; Rice-evans et al., 1995).

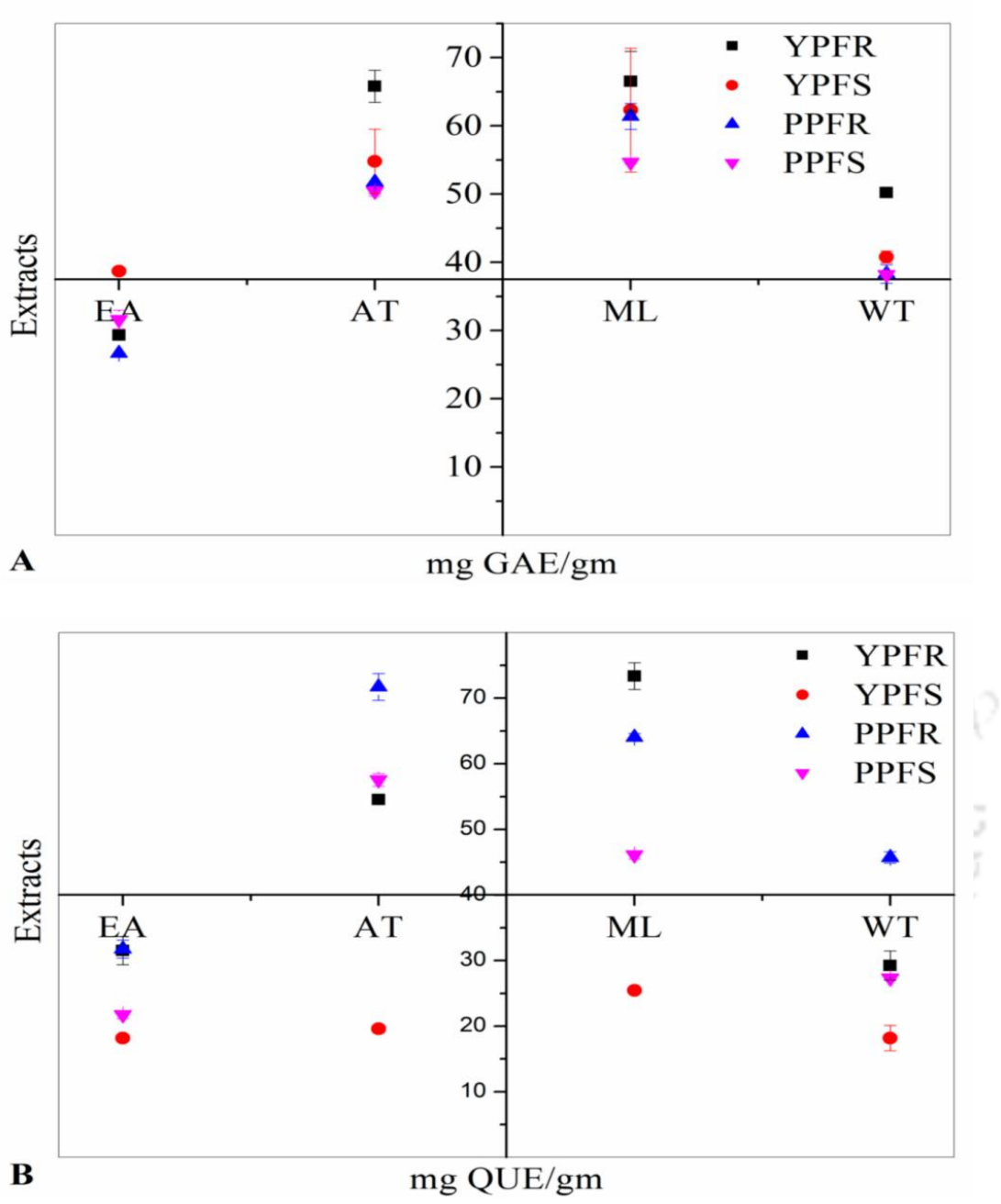


Figure 4.2. Determination of (A) total phenolic and (B) total flavonoid content of different extracts (EA- Ethyl acetate, AT- Acetone, ML- Methanol and WT- Water) obtained from yellow passion fruit rind (YPFR), yellow passion fruit seed (YPFS), purple passion fruit rind (PPFR) and purple passion fruit seed (PPFS). TPC of all extracts are presented as mg GAE/gm of extract where TFC is presented as mg QUE/gm of extract. Error bars on the graph depict the standard deviation obtained from the mean of two individual experiments in triplicate.

4.4.2 In vitro antioxidant activity

All extracts from YPFR, YPFS, PPFR, and PPFS showed a concentration-response relationship for the DPPH assay. DPPH response in terms of IC_{50} values for all the extracts of YPF and PPF and standards are represented in Figure 4.3. The IC_{50} value is the concentration of the drug required to scavenge the free radical by 50%. IC_{50} values of all the extracts were calculated from the percentage scavenged graphs and compared with ascorbic acid values. A significant effect ($p < 0.05$) of different solvent extraction was observed for IC_{50} values. Among all the extracts, methanolic extract of YPFR (44.05 ± 1.41 µg/mL), YPFS (58.42 ± 4.12 µg/mL), PPFR (126.56 ± 0.53 µg/mL), and PPFS (108.55 ± 2.30 µg/mL) showed the highest DPPH radical scavenging activity followed by their acetone extracts. The qualitative and quantitative analysis of methanolic and acetone extracts detected significant content of phenolic and flavonoid compounds, which was the reason for the better antioxidant potential of the extracts, as mentioned above. The significant antiradical effect of samples was also observed with the strong scavenging capacity of YPF and PPF extracts on DPPH free radicals. This was possibly due to the polyphenolic compounds' hydrogen-donating ability in the extracts. ABTS assay was also performed for all the extracts to check their *in vitro* antioxidant level by determining their IC_{50} value. ABTS response in terms of IC_{50} value of all the extracts of YPF and PPF and standards are represented in Figure 4.3.The ABTSresponse followed a similar trend, methanolic extracts of YPFR (62.11± 3.89 µg/mL), YPFS (82.27 ± 1.65 µg/mL), PPFR (73.92 ± 3.54 µg/mL), and PPFS (68.24 ± 3.14 µg/mL) exhibited lowest IC_{50} values hence, showing highest antioxidant activity followed by respective acetone extracts. Ethyl acetate and aqueous extracts were found to have lesser

antioxidant activity than their methanolic and acetone counterparts. However, no significant effect ($p < 0.05$) was observed between the rind and seed extract of YPF and PPF. The metal chelating assay was carried out to check the secondary antioxidant potential of the crude extracts of YPF and PPF. Ferrous ion causes lipid oxidation by making a complex with ferrozine by forming Fe^{2+} complexes (Gülçin, 2006), but in the extracts, this complex formation was inhibited. The inhibition of the formation of ferrous ions by the extract helped to prevent oxidation. Metal chelating response in terms of IC_{50} values for all extracts of YPF and PPF and standards are presented in Figure 4.3. In the present study, both YPFR (111.14 ± 1.61 µg/mL) and YPFS (106.51 ± 4.76 µg/mL) methanolic extracts have shown the best metal chelating activity, followed by their acetone extracts. In the case of PPF, acetone extracts of PPFR (72.98 ± 4.45 µg/mL) and PPFS (57.84 ± 1.12 µg/mL) showed the best metal chelating ability, followed by their respective methanol extracts. The acetone extracts of PPF exhibit an adequate iron-binding capacity, revealing that its antioxidant potential could be related to its iron-binding capacity, which prevented free radical generation through the Fenton reaction (Gülçin et al., 2010).

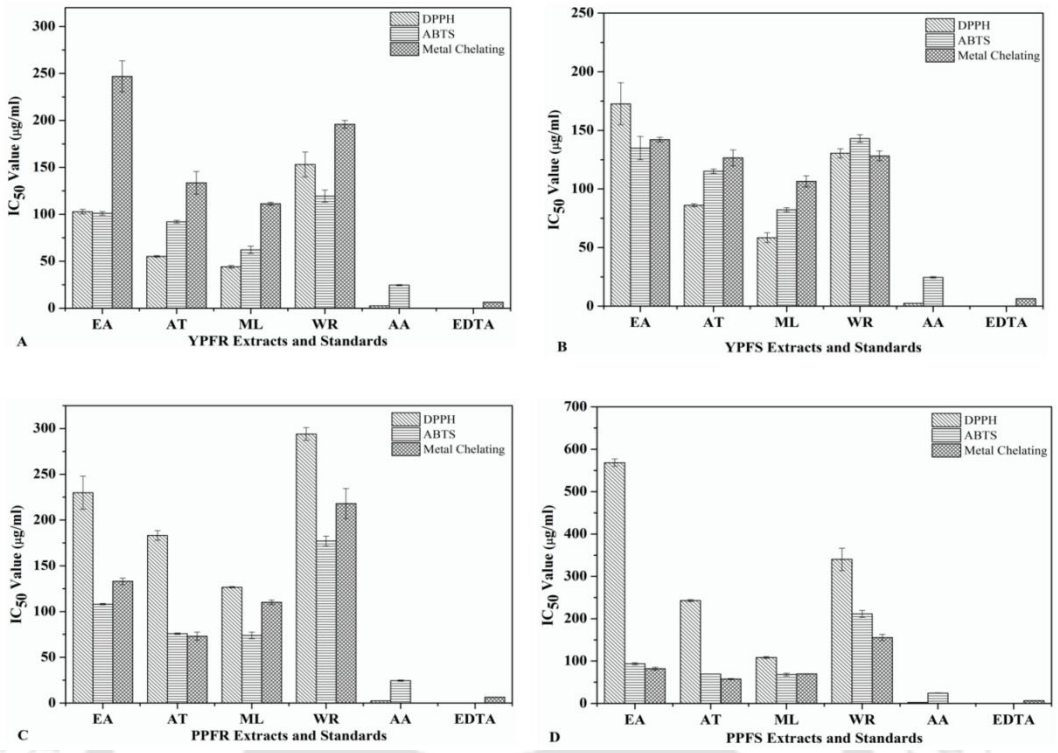


Figure 4.3. In vitro antioxidant activity in terms of IC_{50} value (µg/mL) of different extracts (EA- Ethyl acetate, AT- Acetone, ML- Methanol, and WT- Water) from yellow passion fruit rind (YPFR), yellow passion fruit seed (YPFS), purple passion fruit rind (PPFR) and purple passion fruit seed (PPFS) have been presented. IC_{50} value of AA (ascorbic acid) is used as standard in DPPH and ABTS assay where EDTA (Ethylenediaminetetraacetic acid) is used for metal chelating assay. Error bars indicate the standard deviation from the mean of IC_{50} values calculated from two independent experiments in triplicate of all samples and standard compounds.

4.5 Global antioxidant score

Analysing the results of different antioxidant activity along with their polyphenol content sometimes become difficult and gives a gloomy idea choosing the best extract on the basis of their antioxidant capacity. The global antioxidant score (GAS) was calculated to easily compare the best solvent extract between YPFR, YPFS, PPFR,

and PPFS samples. In this study, the GAS value was determined from the summation of five different T_{scores} obtained from each variable, and the results are presented in Table 4.2. The GAS values for all the solvent extracts were found in the range of 0.14 to 5. The GAS value shows that the overall antioxidant capacity of YPFR ML and YPFS ML was maximum (GAS value=5), followed by PPFR AT and PPFR ML. The PPFR AT extract showed maximum flavonoid content than its methanolic extract, but the in vitro antioxidant activity of the methanolic extract was higher than the acetone extract. The GAS value of 4.56 of PPFR AT was found to be the maximum, followed by 4.26 of PPFR ML confirming the maximum antioxidant capacity for PPFR AT extract.

Similarly, in the case of PPFS ML, the phenolic content was higher than acetone, water, and ethyl acetate extracts, but the flavonoid content was lesser than the acetone extract. However, the GAS value was found to be higher in PPFS ML. The GAS has been published previously to compare polyphenols from vegetables and fruits by some groups (Dabetic et al., 2020b; Van Leeuw et al., 2014). Therefore, analysing the antioxidant capacity using several mathematical equations, such as GAS, could be an essential method for determining antioxidant activity in food and beverages.

Table 4.2. Global Antioxidant Score.

Sample	Extract	DPPH[a]	ABTS[a]	Metal Chelating[a]	TPC[b]	TFC[c]	GAS
YPFR	**EA**	36.28	23.59	8.67	29.32	31.53	0.44
	AT	45.59	28.22	18.93	65.81	54.56	3.21
	ML	48.26	43.57	27.65	66.53	73.39	5.00
	WR	28.55	26.21	25.22	50.22	29.25	1.56
YPFS	**EA**	24.60	19.59	14.27	38.71	18.19	0.15
	AT	26.92	24.38	15.42	54.81	19.61	1.50
	ML	45.45	30.79	28.79	62.31	25.47	5.00
	WR	27.31	17.68	23.80	40.79	18.19	0.87
PPFR	**EA**	15.58	36.01	20.48	26.67	31.75	1.41
	AT	17.74	39.73	35.03	51.74	71.72	4.56
	ML	18.30	41.35	26.12	61.38	64.06	4.26
	WR	12.76	15.81	18.68	38.30	45.72	0.68
PPFS	**EA**	4.35	27.20	36.62	31.61	21.75	1.03
	AT	12.31	38.53	45.82	50.42	57.56	4.14
	ML	20.90	42.81	36.60	54.64	46.11	4.28
	WR	7.65	15.36	22.85	38.19	27.33	0.64

Note: [a] is value of Percentage scavenged at 50 µg/mL extract concentration, [b] is mg GAE eqv/g extract, [c] is mg QUE eqv/g extract. GAS value was calculated following Tscore = [(X-min) / (max-min)] formula where X is the variable of the individual samples and min and max are the smallest and largest value of the variable X. Yellow passion fruit rind (YPFR), yellow passion fruit seed (YPFS), purple passion fruit rind (PPFR) and purple passion fruit seed (PPFS) and EA, AT, ML and WR are ethyl acetate, acetone, methanol and water extracts respectively.

4.6 HPLC analysis

The main objective of the HPLC analysis was to identify some vital flavonoids and phenolic compounds present in the rind and seed extracts of YPF and PPF. Based on the *in vitro* antioxidant activity and the highest polyphenol content, acetone and methanol extracts of YPF and PPF were selected for the HPLC analysis. Mainly the HPLC test was performed for the determination of phenolic acids (gallic acid, caffeic acid, ferulic acid, and p-coumaric acid) and flavonoids, specifically flavonols (quercetin, myricetin, kaempferol, and isorhamnetin) in the sample. Previous reports on HPLC analysis of *P. subpeltata* leaves (Saravanan et al., 2014) and *P. ligularis Juss* fruit pulp (Saravanan and Parimelazhagan, 2014) demonstrated the significant presence of some of the above polyphenols, which encouraged us to check these polyphenols and their quantity in YPF and PPF extracts. The retention time of the extracts is mentioned in Table A4(Appendix). The results of the quantitative analysis of all the above-mentioned phenolic acids and flavonoids are given in Table 4.3. The extracts' HPLC chromatograms of phenolic acids and flavonoids are presented in Figures A4 and A5 (Appendix). It was observed that gallic acid was present in all the extracts in various concentrations ranging from 17.52 to 83 mg/g, predominantly in YPFS acetone extract. The caffeic acid was not detected in any of the extracts, whereas ferulic acid was found only in the methanolic extracts of YPFR and PPFR. On the other hand, P-coumaric acid was found only in the methanolic extracts of YPFS, PPFR, and PPFS. The HPLC analysis of YPF and PPF extracts showed the presence of quercetin, myricetin, and kaempferol; however, none of the extracts showed the presence of isorhamnetin. Acetone extracts of both YPFR and PPFR possessed the highest quercetin level, followed by their methanolic extracts.

Maximum myricetin content was observed in the acetone extracts of PPFS, followed by YPFR AT, PPFR AT, YPFR ML, PPFR ML, and PPFS ML, respectively. Kaempferol was only found in YPFR ML and PPFS ML. As mentioned above, a slight shift in the retention time of standards and samples could be due to the presence of derivative compounds in the extracts. A similar kind of shift in the standard and extract retention time has also been reported previously by (Saravanan and Parimelazhagan, 2014). As flavonoids and phenolic acids are mostly attributed as antioxidants, cardio-protective, anti-hypertensive, anti-apoptotic, anti-carcinogenic, and antimicrobial, thus the present study gives a better idea regarding different essential compounds present in the yellow and purple passion fruit found in the Northeast India.

Table 4.3. Phenolic acids and Flavonol quantity in extracts.

Phytochemicals	Standards	YPFR AT	YPFR ML	YPFS AT	YPFS ML	PPFR AT	PPFR ML	PPFS AT	PPFS ML
Phenolic Acid									
	Gallic Acid	52.381	32.020	83.001	31.341	41.321	25.475	22.915	17.522
	P-Coumaric Acid	ND	ND	ND	6.733	ND	6.422	ND	9.062
	Caffic Acid	ND	ND	ND	ND	ND	ND	ND	ND
	Ferulic Acid	ND	3.436	ND	ND	ND	2.220	ND	ND
Flavonoids									
	Quercetin	5.824	3.814	ND	ND	4.295	2.287	ND	ND
	Myercetin	8.535	5.832	ND	ND	6.564	4.560	10.848	1.647
	Kaemferol	ND	5.587	ND	ND	ND	ND	ND	5.681
	Isorhmnetin	ND	ND	ND	ND	ND	ND	ND	ND

Note: Values are presented in mg/g of dry extract. ND (not detected), yellow passion fruit rind-acetone extract (YPFR AT), yellow passion fruit rind-methanol extract (YPFR ML), yellow passion fruit seed-acetone extract (YPFS AT), yellow passion fruit seed-methanol extract (YPFS ML), purple passion fruit rind-acetone extract (PPFR AT), purple passion fruit rind-methanol extract (PPFR ML), purple passion fruit seed-acetone extract (PPFS AT) and purple passion fruit seed-methanol extract (PPFS ML).

4.7 Antibacterial activity by different passion fruit extracts

Although there are many reports in the literature on the antibacterial properties of leaves, fruits, and other parts of the Passiflora plant (Dhawan et al., 2004; Lingaraju et al., 2015; Mohanasundari et al., 2007; Ramaiya et al., 2014; Sasikala et al., 2011), however literature on the antibacterial assay of extracts of yellow and purple passion fruit from Northeast India is limited. Hence, the antibacterial activity of acetone and methanolic extracts of rind and seed from YPF and PPF was performed following MIC and ZOI assays.

4.7.1 Minimum inhibitory concentration (MIC) assay

The minimum inhibitory concentration of YPF and PPF extracts was determined by using a resazurin indicator. MIC of acetone and methanol extracts of YPFR, YPFS, PPFR, and PPFS against the four gram-negative and four gram-positive bacteria is presented in Table 4.4. Resazurin is an oxidation-reduction indicator for various cytotoxicity assays (Schmitt et al., 2013). It is a blue-colored non-fluorescent, and non-toxic dye that becomes pink on bacterial growth and is reduced to resofurin by oxidoreductases in the bacterial cells. MIC of all the extracts was checked at a concentration range of 40 mg/mL to 19.53 µg/mL in 10% DMSO. Methanolic extract of YPFS and PPFS showed the best MIC in the range of 20 mg/mL to 0.625 mg/mLfor all the bacteria.

Similarly, acetone extracts of YPFS and PPFS were effective against all the bacteria. YPFR AT and PPFR AT were ineffective against any gram-negative bacteria at 40 mg/mL concentration. Methanolic extract of YPFR and PPFR showed similar results

for both types of bacteria. In contrast, YPFR ML was ineffective against Enterobacter aerogenes and Pseudomonas aeruginosa, and PPFR ML were ineffective against *Escherichia coli* and *Klebsiella pneumonia* up to 40 mg/mL extract concentration. Roughly all passion fruit extracts subjected to MIC showed better antibacterial activity against gram-positive bacteria than gram-negative one. This could be due to a rigid cell wall in gram-negative bacteria than a simpler cellmembrane of gram-positive bacteria. Oliveira et al. have also reported similar results (Oliveira et al., 2016). The mechanism of antibacterial activity of plant extracts is not well known so far, but some literature have reported that phenolic acids and flavonoid compounds are mainly responsible for the antibacterial properties of the plant extracts (López-Vargas et al., 2013). More antibacterial activity of the methanolic and acetone extract can be related to the high phenolic content of these extracts (Karimkhani et al., 2019).

Table 4.4. Minimum inhibitory concentration of YPF and PPF extracts.

	Gram negative bacteria				Gram positive bacteria			
Extracts	EC	KP	EA	PA	SA	BS	SE	ML
YPFR AT	ND	ND	ND	ND	5	10	2.5	2.5
YPFR ML	40	40	ND	ND	10	20	2.5	5
YPFS AT	20	40	20	20	10	10	5	5
YPFS ML	10	20	5	5	5	1.25	2.5	2.5
PPFR AT	ND	ND	ND	ND	40	ND	ND	10
PPFR ML	ND	ND	20	20	10	20	2.5	5
PPFS AT	40	40	ND	ND	10	40	2.5	5
PPFS ML	2.5	10	0.625	1.25	2.5	0.625	0.625	0.625

Note: Values are presented in mg/mL of extract concentration. ND (not detected), yellow passion fruit rind-acetone extract (YPFR AT), yellow passion fruit rind-methanol extract (YPFR ML), yellow passion fruit seed-acetone extract (YPFS AT), yellow passion fruit seed-methanol extract (YPFS ML), purple passion fruit rind-acetone extract (PPFR AT), purple passion fruit rind-methanol extract (PPFR ML), purple passion fruit seed-acetone extract (PPFS AT) and purple passion fruit seed-methanol extract (PPFS ML). *Escherichia coli* (EC), *Klebsiella pneumoniae* (KP), *Enterobacter aerogenes* (EA) and *Pseudomonas aeruginosa* (PA), *Staphylococcus aureus* (SA), *Bacillus subtilis* (BS), *Staphylococcus epidermidis* (SE) and *Micrococcus luteus* (ML).

4.7.2 Zone of inhibition assay

After performing MIC, acetone and methanolic extracts of YPFR, YPFS, PPFR, and PPFS were subjected to agar well diffusion assay to check the inhibition zone (ZOI) by using the same eight bacterial strains that were used for MIC. The ZOI images and the inhibition zone (in mm) of each extract for each bacterium are presented in Figure 4.4 and Table A5 (Appendix), respectively. The zone of inhibition depends on the type of extract, plant part, and species of the bacteria. The control group with 10% DMSO showed an absence ofan inhibitory zone. Three different concentrations of extracts, such as 10 mg/mL, 30 mg/mL, and 50 mg/mL in 10% DMSO, were used for the assay to check the concentration-dependence activity of the extracts. Among all the extracts, YPFR AT, YPFS AT, YPFS ML, and PPFS ML showed the best antibacterial properties. YPFS ML extract was effective against all the bacteria. It was the only extract that showed activity (18.5 mm ± 0.7 at 50 mg/mL) against *Klebsiella pneumonia*. The YPFR ML (9 mm at 50 mg/mL) and PPFR AT (10 mm at 50 mg/mL) showed moderate antibacterial activity against *Micrococcus luteus. In contrast,* PPFR ML showed minimal inhibitory action (4 mm ± 1.41 at 50 mg/mL) to *Pseudomonas aeruginosa*. PPFS AT was the only extract that could not show any inhibition zone against any of the bacterium. One of the important findings of the current study is the methanol extracts of seeds of both varieties which exhibited profound antibacterial activity against both gram-positive and gram-negative bacteria. The antibacterial nature of the seed extracts could be due to the presence of different bactericidal proteins as well as phytoconstituents. A stilbene compound called piceatannol (found in passion fruit seeds) is also responsible for antibacterial activity (Osamudiamen et al., 2020). The presence of gallic acid and other polyphenols in the

rind and seed of passion fruit is responsible for the antibacterial activity. The results obtained in the present work are comparable with the other studies on the antibacterial properties of passion fruit (Kannan et al., 2010; Lingaraju et al., 2015; Ramaiya et al., 2014; Ripa et al., 2009). The antibacterial study can also be compared with the preliminary phytochemical study, in vitro antioxidant activity, and HPLC analysis of the present work.

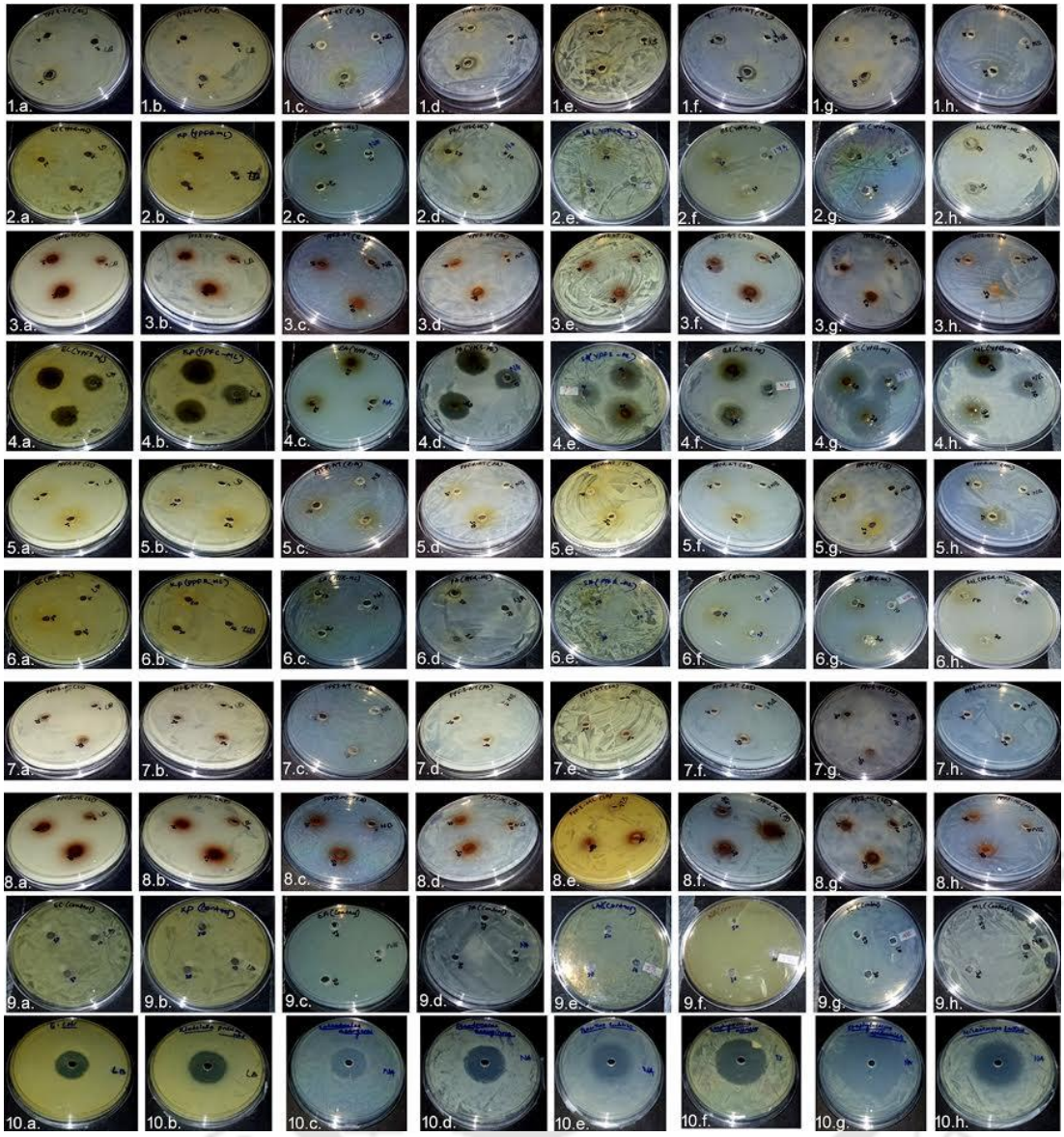


Figure 4.4. Zone of inhibition study by agar well diffusion method. 1.a. to 1.h. represent yellow passion fruit rind-acetone extract (YPFR AT) with different gram negative *Escherichia coli* (EC), *Klebsiella pneumoniae* (KP), *Enterobacter aerogenes* (EA) and *Pseudomonas aeruginosa* (PA) and gram positive *Staphylococcus aureus* (SA), *Bacillus subtilis* (BS), *Staphylococcus epidermidis* (SE) and *Micrococcus luteus* (ML) bacteria. Likewise 2.a. to 2.h., 3.a. to 3.h., 4.a. to 4.h., 5.a. to 5.h., 6.a. to 6.h., 7.a. to 7.h., 8.a. to 8.h represent yellow passion fruit rind-methanol extract (YPFR ML), yellow passion fruit seed-acetone extract (YPFS AT), yellow passion fruit seed-methanol extract (YPFS ML), purple passion fruit rind-acetone extract (PPFR AT), purple passion fruit rind-methanol extract (PPFR ML), purple passion fruit seed-acetone extract (PPFS AT) and purple passion fruit seed-methanol extract (PPFS ML) with the same order of bacteria. 9.a. to 9.h. is different negative control experimental plates for all bacteria. 10.a. to 10.h. represents zone of inhibition of all above mentioned bacteria by Piperelline and Tazobactam antibiotics.

4.8 Isolation and identification of polyphenols from YPF rind and seed

The process of extraction and isolation of polyphenols from YPF is shown in Figure A6 (Appendix). The sample amount used for extraction, fractionation, and yield of the isolated compound is presented in Table A6 (Appendix). From the hydroalcoholic extraction of YPFS, 7.2g (19.2%) extract was recovered. Further, as mentioned in the materials and method section, 6.5g extract was subjected to fractionation using different solvents. After the fractionation process, the EtoAc and BuoH fractions yielded maximum extracts i.e., 1.54g and 2.68g, respectively. From the gradient HPLC chromatograms of BuoH and EtoAc fraction, two stilbene molecules (piceatannol (PT) and scirpusin B (SB) were presumed (Figure 4.5). The EtoAc fraction was found to have a lower concentration of stilbene thanthe BuOH fraction, which could be because of the lower solubility tendency of these compounds towards EtOAc. Acid-base treatment was applied to the fraction to pull the maximum of the said compounds from the EtOAc fraction (mentioned in chapter 3).

Further, the EtOAc and BuOH fractions were passed through Diaion HP20SS resin, and major eluted fractions were collected using a gradient range of methanol with water. The HP20SS resin helps separate and elute smaller molecular weight compounds like polyphenols, sugars, and many chemicals for preparative or industrial applications. Both the major peaks were further resolved through an analytical HPLC, and the optimized solvent system was followed for the isolation of the compounds using a preparative HPLC presented in Figure 4.6. The major compound in the YPF seed was identified as Scirpusin B(SB) eluted solvent from the EtOAc and BuOH

fractions as a pale brown-colored amorphous powder weighing approximately 98.52 mg. The second major compound was found to be Piceatannol (PT), weighing approximately 57.34 mg as a yellow-colored amorphous powder. SB was first reported from the rhizome of *Scirpus flaviatilis* by Nakajima et al., (Nakajima et al., 1978). This trans-stilbene molecule is a dimer of piceatannol, a hydroxylated resveratrol analog (Banik et al., 2020). Resveratrol is the abundant stilbene found naturally in plants with a wide array of biological activity (Wang and Yao, 2016). Very few properties of SB have been reported so far, which include vaso relaxation (Sano et al., 2011), antioxidant (Xiang et al., 2005), anti-HIV (Yang et al., 2005), etc. PT is a stilbene molecule found in prunus species and passion fruit seeds (Banik et al., 2020). PT has also proven to be an oxidative stress quencher for many cancer diseases, which displays more scopes for evaluating these compounds for their preclinical and clinical studies.Apart from SB and PT, another compound identified in the YPFS was Protocatechuic acid (PCA). However, the recovered PCA was significantly less (<20-25mg). Therefore, PCA was confirmed from the HPLC by matching the retention time with the standard PCA (Figure 4.6).

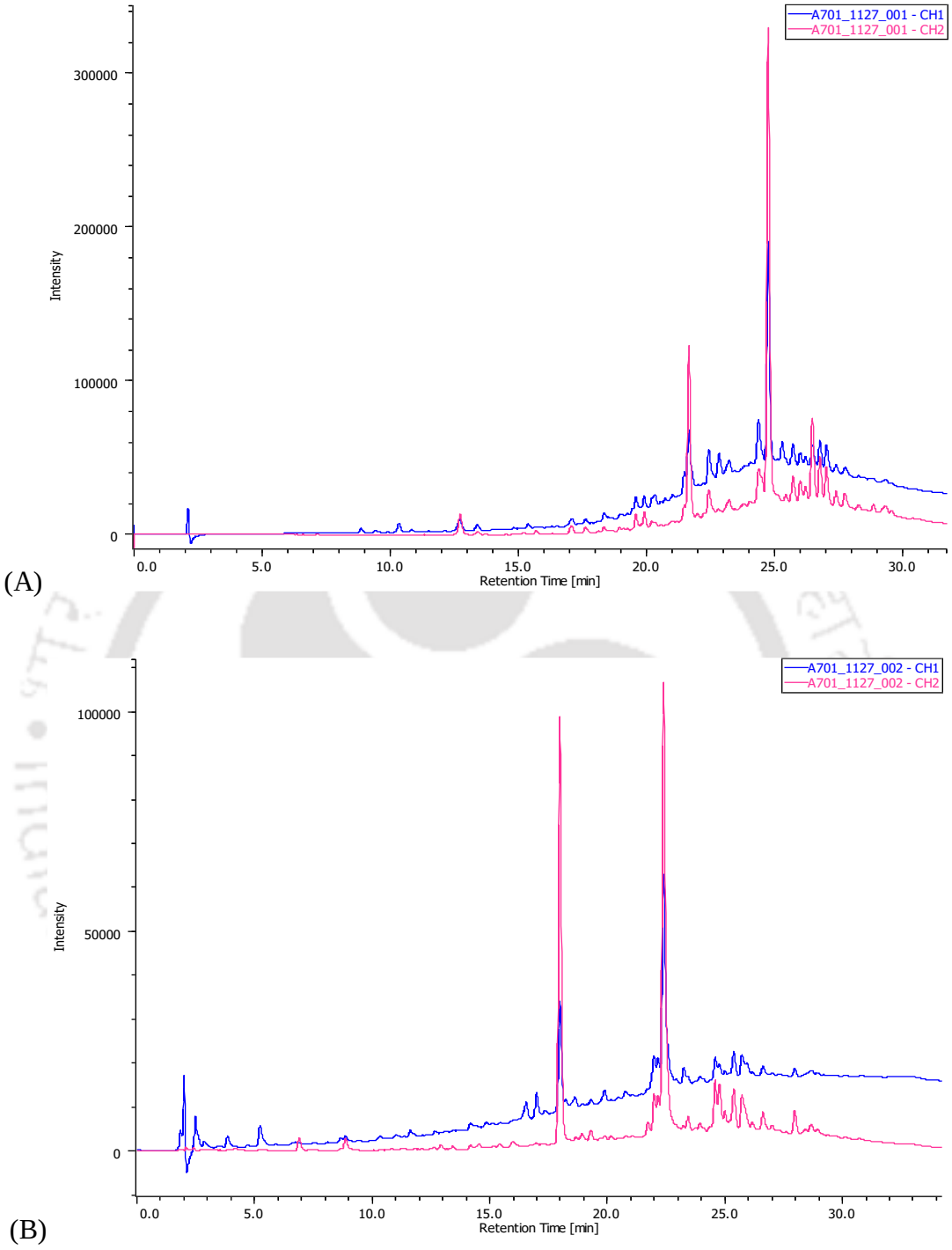


Figure 4.5. Gradient grade HPLC of different fractions obtained from the YPFS-EToAc fraction (A) and YPFS-Butanol fraction (B). The X-axis represents the retention time where the Y-axis represents the intensity of the peaks. The analysis was carried out at two different wavelength, i.e. 280nm (blue peaks) and 330nm (pink peaks).

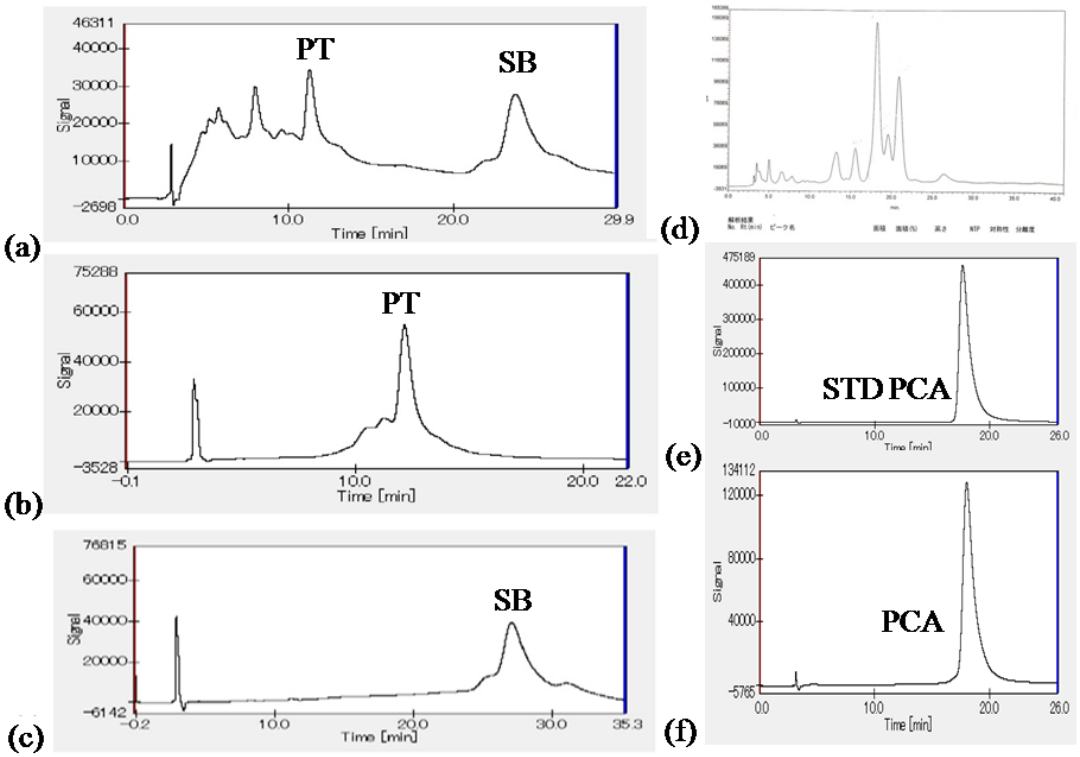


Figure 4.6. HPLC chromatogram of isolated PT and SB (a), purified PT (b), purified SB (c), chromatogram of isolated PCA (d), standard PCA (e) and purified PCA from YPF seed (e).

The different fractions from the YPFR extracts were also subjected to gradient-grade HPLC to check the high-intensity peaks. The ethyl acetate (EtOAc) and butanol fractions were further selected to isolate different compounds. Despite having 2.73g and 1.37g of butanol and EToAc fractions, the concentration was extremely low for many of the peaks for further characterization. Only one compound from the EToAC fractions (Figure 4.7a) was isolated and purified (Figure 4.7 b and c) and further found to be Prunasin (PS). As per the world health organisation's report, more countries are now recognising utilization of plant-based pharmaceuticals. In this context, therapeutic PT and SB from waste PF seed could be an ideal candidate to treat many diseases. This study also reports maximum recovery (approximately 0.2%

of total seed) of SB from PF seed of Northeast India for the first time, which further implies the commercial importance of the waste biomass.

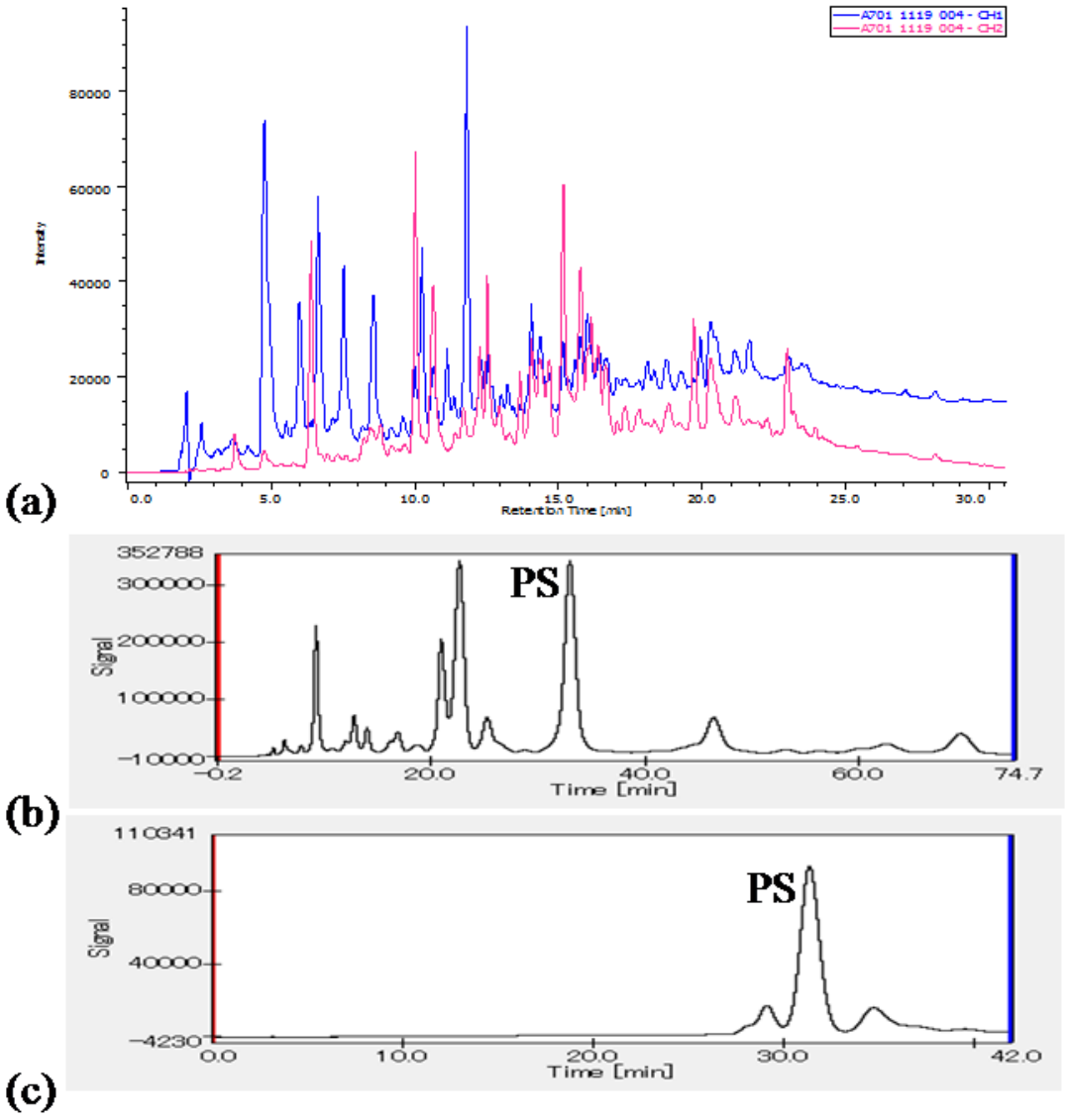


Figure 4.7. Gradient HPLC of the YPFR ethyl acetate fraction using 5-40% acetonitrile (a), major peaks containing PS from the ethyl acetate fraction after reverse phase column chromatography (b) and purified Prunasin (PS) from preparative HPLC (c).

4.9 Identification of the purified compounds

4.9.1 Identification of Piceatannol

The NMR and mass spectra of PT is presented in Figure A7-i,ii (Appendix) and A8-I (Appendix), respectively. The negative mode ESI-MS revealed the pseudo molecular ion peak at *m/z* 243.08 at $[M-H]^-$. ^{1}H NMR (500 MHz, CD_3OD) δ: 6.15 (1H, brt, *J* = 2.2 Hz), 6.43 (2H, brd, *J* = 2.3Hz), 6.72 (1H, brd, *J* = 2.5 Hz), 6.74 (1H, s), 6.75 (1H, s), 6.82 (1H, Dd, *J* = 1.9 Hz), 6.83 (1H, Dd, *J* = 2.2 Hz), 6.87 (1H, s), 6.90 (1H, s), 6.97 (2H, d, *J* = 2.2 Hz). ^{13}C NMR (CD_3OD) δ: 40.3, 49.5, 102.5, 105.7, 113.7, 116.3, 120.1, 126.9, 129.6, 130.9, 141.2, 146.4 2, 159.5. The result suggested that the purified compound is a stilbene compound named piceatannol ($C_{14}H_{12}O_4$).

4.9.2 Identification of Scirpusin B

1H NMR carried out identification of the isolated SB, ^{13}C NMR and mass spectrometry in ESI negative mode, and the spectra are presented in Figures A7-iii,iv(Appendix), and A8-ii(Appendix), respectively. ^{1}H NMR (500 MHz, CD_3OD) *δ*: 4.38(d, J=5.8Hz, 1H), 5.33(d, J=5.9 Hz, 1H), 6.20(d, J=2.0 Hz, 2H) 6.21-6.23(m, 1H), 6.30(d, J=1.8Hz, 1H), 6.58(d, J=16.1Hz, 1H), 6.62(dd, J=8.2Hz, 1.8Hz, 1H), 6.66(d, J=1.9Hz, 1H), 6.68(d, J=8.1Hz, 1H), 6.74(d, J=1.8Hz, 1H), 6.78(d, J=8.1Hz, 1H), 6.80(d, J=1.9 Hz, 1H), 6.82(d, J=6.3 Hz, 1H). ^{13}C NMR (800 MHz, CD_3OD) *δ*: 39.9. 49.4, 57.5, 94.3, 96.3, 101.7, 103.9, 106.8, 113.1, 113.5, 115.7, 115.8, 117.9, 119.3, 119.5, 123.1, 130.3, 130.4, 134.4, 136.5, 145.7, 145.8, 145.9, 146.0, 147.1, 159.1, 159.4, 162.3. Mass spectroscopy *m/z* ESI^- was 485.16, actual mass: 486.16.

4.9.3 Identification of Protocatechuic acid

The ^{1}H NMR and negative mode ESI-MS spectrum of PCA are presented in Figure A7-v (Appendix) and A8-iii (Appendix), respectively. This compound showed the pseudo ion peak at *m/z* 153.02 at $[M-H]^{-}$. ^{1}H-NMR (600 MHz, CD_3OD) δ: 6.93 (1H, d, *J* = 8.0 Hz), 7.39 (1H, dd, *J* = 8 Hz, *J* = 1.5 Hz), 7.42 (1H, d, *J* = 1.5 Hz). The amount of this compound was significantly less; hence the compound was identified using HPLC analysis by comparing the retention time with the standard compound. From the proton NMR and mass spectroscopy, the compound was assumed to be a dihydroxy benzoic acid, and further, it was confirmed as the Protocatechuic acid ($C_7H_6O_4$) from the HPLC analysis. The ^{1}H NMR data align with the previous report (López-Martínez et al., 2015).

4.9.4 Identification of Prunasin

The NMR and mass spectra of Prunasin is presented in Figure A7-vi, vii (Appendix) and A8-iv (Appendix), respectively. The positive mode ESI-MS of this compound showed the pseudo ion peak at *m/z* 613.22 at $[2M+NA]^{+}$. ^{1}H-NMR (800 MHz, D_2O) δ: 3.28- 3.92 (3H, m), 4.25 (1H, d), 4.69 (1H, d), 5.90 (1H, s), 7.45- 7.61 (2H, m). ^{13}C NMR (D_2O) δ: 62, 67, 69, 73, 75, 77, 78, 102, 119, 128, 129, 130, 135, 136. From the above observations, the isolated compound was found to be a cynogenic glycoside called prunasin ($C_{14}H_{17}O_6N$).

4.10 Invitro analysis of the identified compounds from YPF

The invitro analysis includes in vitro antioxidant activity, in vitro antidiabetic activity, and antibacterial activity of different identified polyphenols from YPF. The yield of

Prunasin was extremely low; therefore no activity studies had been carried out. Moreover, for Piceatannol (PT) and Protocatechuic acid (PCA), only the in vitro antioxidant activity and in vitro antidiabetic activity has been reported in the following section. However, the antioxidant, antidiabetic and antibacterial activity of Scirpusin B (SB) have been reported in the following sections.

4.10.1 Invitro antioxidant activity

The invitro antioxidant activity of PT, SB, and PCA was estimated through DPPH and ABTS methods and compared with known polyphenols like hydroxybenzoic acids, hydroxycinnamic acids, ascorbic acids, and quercetin. The result of this analysis is presented as IC_{50} values (concentration needed to scavenge 50% of free radicals) in Table 4.5. All the isolated compounds showed superior antiradical activity against all the tested standard compounds except gallic acid. The PT (3.16 ± 0.02 µg/mL) and SB (5.19 ± 0.02 µg/mL) isolated from YPFS, exhibited approximaterly three folds and two folds higher antioxidant activity than ferulic acid (9.80 ± 0.01 µg/mL) and p-coumaric acid (10.19 ± 0.01 µg/mL). Both the isolated compounds further showed remarkable better DPPH antioxidant activity than caffeic acid (5.77 ± 0.04 µg/mL) and quercetin (7.85 ± 0.01 µg/mL). In the ABTS assay also, the same trend was noticed. However, PCA was found to be the best antioxidant compound among the three isolated compounds, with an IC_{50} value of 1.72 ± 0.03 µg/mL.These compounds reduced the free radicals of DPPH and ABTS concentration-dependent manner. Both DPPH and ABTS assays work on the principle of electron transfer or hydrogen ion transfer mechanism (Purohit et al., 2021c). Free radicals receive electrons or hydrogen ions from the antioxidant molecule and get reduced, resulting in discoloration of the

solution indicating antiradical properties. Various reports suggest hydroxyl and methoxy groups have a major tendency to donate electrons (Parcheta et al., 2021), and because of abundant hydroxyl groups in PT and SB, superior antioxidant activity can be correlated. Natural stilbenoids found in large plant species are mainly secondary plant metabolites that protect the plants from the stress conditions like UV radiations, heat, microorganisms, etc. (Pecyna et al., 2020). The present study provides more comprehensive information on the usability of PT, SB, and PCA as antiradical agents, which showed more antioxidant activity than many known standard polyphenols (caffeic acid, ferulic acid, p-coumaric acid, ascorbic acid, and quercetin). The results of the present study reveal that the SB from PF seed could be established as a natural antioxidant in the mainstream chemical world.

Table 4.5. Invitro antioxidant and antidiabetic activities by Scirpusin B.

Activity	PT	SB	PCA	Acarbose	GA	FA	CA	p-CA	AA	Q
Antioxidant										
DPPH	3.16± 0.01	5.19± 0.02	1.72± 0.03	NA	0.89± 0.01	9.80± 0.01	5.77± 0.04	10.19± 0.01	3.95	7.85± 0.01
ABTS	3.02± 0.01	3.89± 0.03	1.81±0.01	NA	0.79± 0.01	6.02± 0.01	3.98± 0.03	6.36± 0.04	6.22± 0.04	6.40± 0.01
Antidiabetic										
α-amylase	42± 0.07	76.38± 0.25	2.12± 0.2	0.65± 0.01	NA	NA	NA	NA	NA	NA
α-glycosidase	2.17± 0.04	2.32 ± 0.04	1.89± 0.02	0.79± 0.03	NA	NA	NA	NA	NA	NA

Note: Data are presented as IC_{50} value after taking the mean of two individual experiments in duplicate. All the values (µg/mL) of antioxidant and antidiabetic experiments are presented as mean ± SD. NA represents for not applicable. PT; Piceatannol, SB; Scirpusin B, PCA; Protocatechuic acid, GA; Gallic acid, FA; Farulic acid, CA; Caffeic acid, p-CA; p-Coumaric acid, AA; Ascorbic acid, and Q; Quercetin.

4.10.2 Invitro antidiabetic activity

The glucose digestive enzyme inhibition potential of PT,SB, and PCA was evaluated by α-amylase and α-glucosidase inhibition assays, and the results are presented as IC_{50} values in Table 4.5. PCA exhibited significant α-amylase activity (2.12± 0.2µg/mL) than PT (42± 0.07µg/mL) and SB (76.38± 0.25µg/mL). However, all three isolated compounds showed similar α-glucosidase activity ranging from 1.89-2.32 µg/mL. A previous report (Tran et al., 2014) suggested that SB isolated from *Cyperus rotundus* rhizome could only inhibit α-glucosidase but failed α-amylase inhibition in their study.

In the present study, different sets of repeated experiments were conducted to optimize the concentration range of SB for α-amylase activity and revealed its inhibition activity against α-amylase enzyme at a concentration as high as 76.38 ± 0.25 µg/mL (IC_{50} of acarbose against α-amylase was 0.65 ± 0.01 µg/mL). Inhibition of glucose absorption and delaying the postprandial glycemic level has been one of the approaches to treat diabetes (Jayawardana et al., 2022). Therefore, inhibition of polysaccharide digestive enzymes like α-amylase and α-glucosidase are considered nowadays. Stilbinoids (resveratrol and its analogs) are proven to have antidiabetic activity by several reports. Most of the stilbene molecules, trimers, or oligomers, showed better activity (Dirir et al., 2021) because of the presence of maximum hydroxyl groups, which can help increasethe bioavailability of the compound (Tran et al., 2014). The results of this study conferred that; PT, SB, and PCA from YPFS can be explored as a promising hypoglycaemic agents for various preclinical and clinical studies.

4.10.3 Antibacterial activity of Scirpusin B

In vitro antibacterial activity of SB was carried out following MIC and ZOI methods. Their results are presented in Table 4.6 and Figure A9 (Appendix)for MIC, Figure 4.8,and Table A7(Appendix)for ZOI. Natural stilbenes are known to be potential antimicrobial agents (Gutierrez-Escobar et al., 2021), but very few have been studied so far (Shih et al., 2021). Interestingly, there is no reporting on the antibacterial activity of SB on foodborne and human pathogenic bacteria to date. Therefore, in this work, the antibacterial activity of SB was checked using eight different bacteria (four gram-positive and four gram-negative). From section 4.7 of this chapter, the methanolic extract of YPFS showed tremendous antibacterial activity against these same pathogens. Back then, it was believed that the antibacterial properties of the methanolic extract of YPFS could be because of the detection of gallic acid and p-coumaric acid (from HPLC analysis) (Purohit et al., 2021c). This is the first report on SB, one of the major antibacterial agents in the YPFS extract. SB manifested minimum inhibitory concentration ranging from 0.156 mg/mL to 1.25 mg/mL for all tested organisms. Gram-positive bacteria like Staphylococcus epidermidisand Micrococcus luteus showed the best MIC (0.156mg/mL), which can be attributed to their non-rigid cell wall structure into which SB can easily penetrate to cause cellular leakage and death.

Further, from the ZOI analysis, the antibacterial activity of SB was checked using different concentrations (2.5, 5, and 10 mg/mL). SB showed profound antibacterial activity even at the lowest tested concentration i.e. 2.5 mg/mL for all the strains. At such low concentration also, SB exhibited better ZOI in *Escherichia coli* (12 ± 1.41

mg/mL)**,** *Enterobacter aerogenes* (11 ± 1.41 mg/mL), and *Bacillus subtilis* (13 mg/mL) compared to our earlier published report (10 mg/mL of methanolic YPFS extract) (Purohit et al., 2021b). Marginally lower activity in some bacterial strains by SB compared to YPFS methanolic extract, could be because of the synergistic effect of other polyphenols and several other unidentified compounds present in the methanolic extract. Overall, this study can support SB isolated from waste PF seed as a potent antibacterial agent.

Table 4.6. The minimum inhibitory concentration of Scirpusin B.

Identified compound	Gram-negative bacteria				Gram-positive bacteria			
	EC	KP	EA	PA	SE	ML	SA	BS
Scirpusin B	1.25	1.25	0.625	0.625	0.156	0.156	1.25	1.25

Note: Values are presented in mg/mL. EC (*Escherichia coli*), KP (*Klebsiella pneumoniae*), EA (*Enterobacter aerogenes*), PA (*Pseudomonas aeruginosa*), SE (*Staphylococcus epidermidis*), ML (*Micrococcus luteus*), , SA (*Staphylococcus aureus*) and BS (*Bacillus subtilis*).

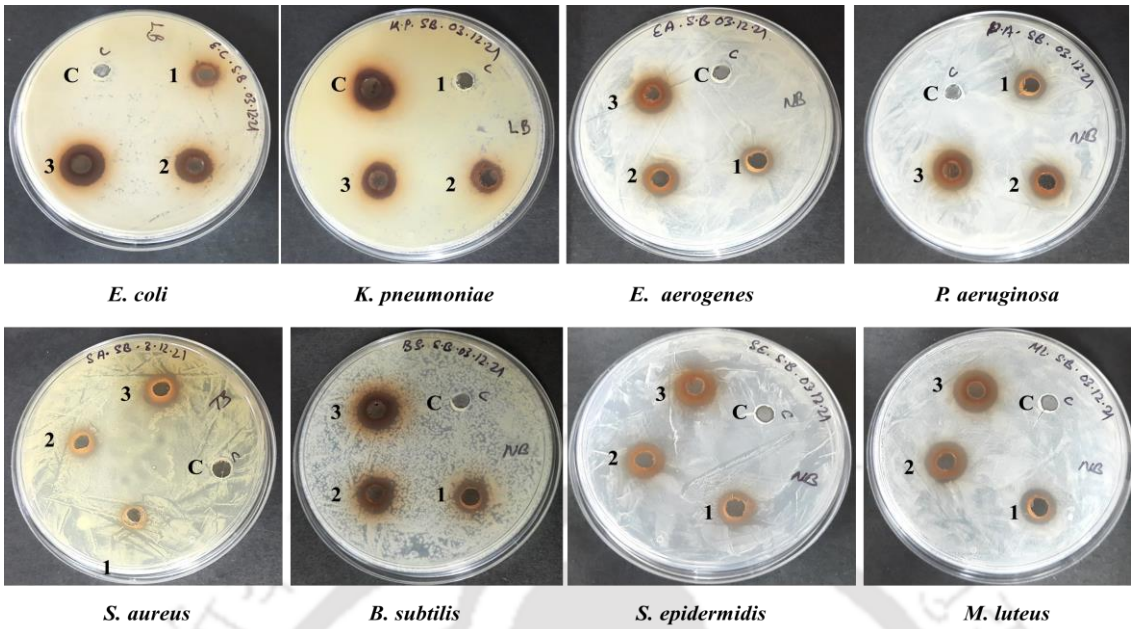


Figure 4.8. Zone of Inhibition study of Scirpusin B on different gram-negative and gram-positive bacteria. Control (C) and different concentrations of scirpusin B are represented as 1 (2.5 mg/mL), 2 (5 mg/mL), and 3 (10 mg/mL) inside the images.

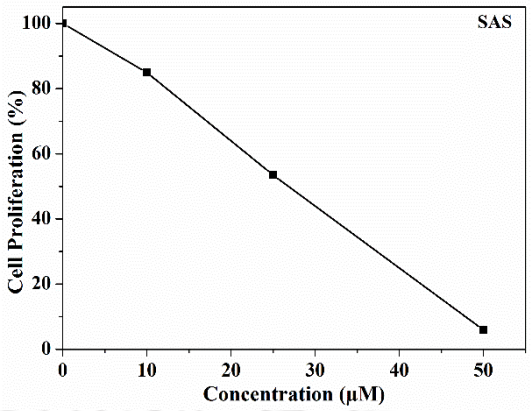
4.11 Bioactivity of SB against oral squamous cell carcinoma

The anticancer activity of purified SB was determined using two different oral cancer cell lines following many experiments, and the results are presented in Figure 4.9. Firstly, we have determined the effect of SB on the viability of oral cancer cell lines (SAS and TTn) through the MTT assay,and the obtained results are presented in Figures 4.9 a1 & 4.9 a2. In this experiment, it was observed that SB reduced the viability of SAS and TTn cell lines in a dose-dependent manner. The IC_{50} for SAS and TTn cell lines were observed to be ≈27 µM and ≈32 µM, respectively. This study can be compared with the study reported by Yamamoto et al., where they observed the inhibition of cell proliferation in lung cancer cells (NCI-H522) (Yamamoto et al., 2019). Further, SB's anticancer effect on killing oral cancer cells was confirmed by colony formation assay (Figure 4.9 b1 & 4.9 b2). Colonogenic assay is an in vitro

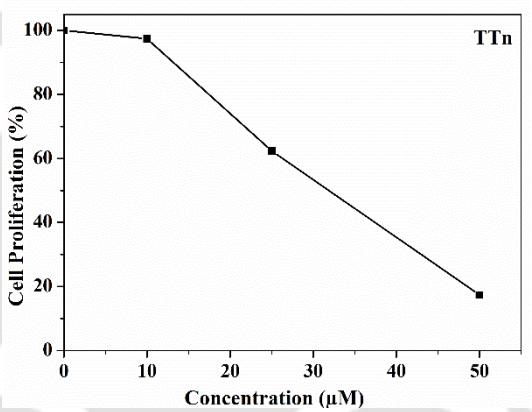
survival assay used to determine a single cell's ability to undergo unlimited cell division to form a colony (Hartmann et al., 2020). In this assay, the treatment of SAS and TTn cell lines with SB at 25 µM and 50 µM inhibited the colony-forming ability of SAS and TTn cell lines in a dose-dependent manner compared to the control. In line with our study, the previous investigation had also demonstrated the anti-proliferative effect of SB in colon cancer (HCT-116) cell lines. The ability of SB to kill SAS and TTn cell lines through PI-FACS analysis was further demonstrated in Figure 4.9 c1 and 4.9 c2. The cell lines were treated with 25, 50, and 75 µM concentrations of SB for 72 hr, and it was observed that SB induced cell death in both the oral cancer cell lines in a dose-dependent manner. The mechanism of action of SB against different oral cancer cell lines was further understood by protein expression analysis (Figure 4.9 a-e for SaS and Figure 4.9 f-j for TTN cell lines). It is well-established that the pathogenesis of oral cancer is a multi-stage process (Rishabh et al., 2021). The risks factors of oral cancer, such as tobacco, areca nut, alcohol etc., are known to cause oxidative stress and inflammation in the oral mucosa through the expression of pro-inflammatory cytokines such as tumor necrosis factor (TNF-α), interleukin- 6 (IL-6), etc. and the induction of reactive oxygen species (ROS) which leads to oral submucosal fibrosis (Girisa et al., 2021). It is also well-known that oral sub-mucosal fibrosis has a high potential for malignant transformation to oral squamous cell carcinoma (Girisa et al., 2021; Ray et al., 2016). Therefore, the agents that can suppress pro-inflammatory cytokines and free radicals have high potential in preventing and treating oral cancer. Our results showed that SB significantly suppressed TNF-α expression and scavenged ROS (Figure 4.9 a and f), suggesting its chemopreventive potential against oral cancer. Further, increasing lines of studies

indicated that the formation of new blood vessels, known as angiogenesis, is essential for the growth of oral cancer, as it helps to provide oxygen and nutrition for the development of cancer cells, and it is induced by a protein known as the vascular endothelial growth factor (VEGF) (Mărgăritescu et al., 2009; Rapone and Ferrara, 2020). This factor is secreted by oral cancer cells,which binds to its receptor VEGFR and induces tube formation in endothelial cells (Rapone and Ferrara, 2020). However, our results showed that SB remarkably suppressed the expression of VEGF-A (Figure 4 b and g) in a concentration-dependent manner. Previous literature suggests that survivin, cyclooxygenase-2 (COX-2), and cyclin D1 are involved in the survival, proliferation, and chemo-resistance of oral cancer cells (Bordoloi et al., 2019; Jaiswal et al., 2015; Lakshminarayana et al., 2018).

Interestingly, it had been observed that SB inhibited the expression of these proteins in a dose dependent manner (Figure 4 c-e and h-j), which helps to stop oxygen and nutrients supply to the oral cancer cells (Zirlik and Duyster, 2018) leading to their death. Taken together, our results showed that SB is a potential anticancer agent for the prevention and treatment of oral squamous cell carcinoma. However, further preclinical studies can support these findings more. This study also highlighted the importance of a natural stilbene (SB) isolated from waste PF seed for various therapeutic activities.



(a1)



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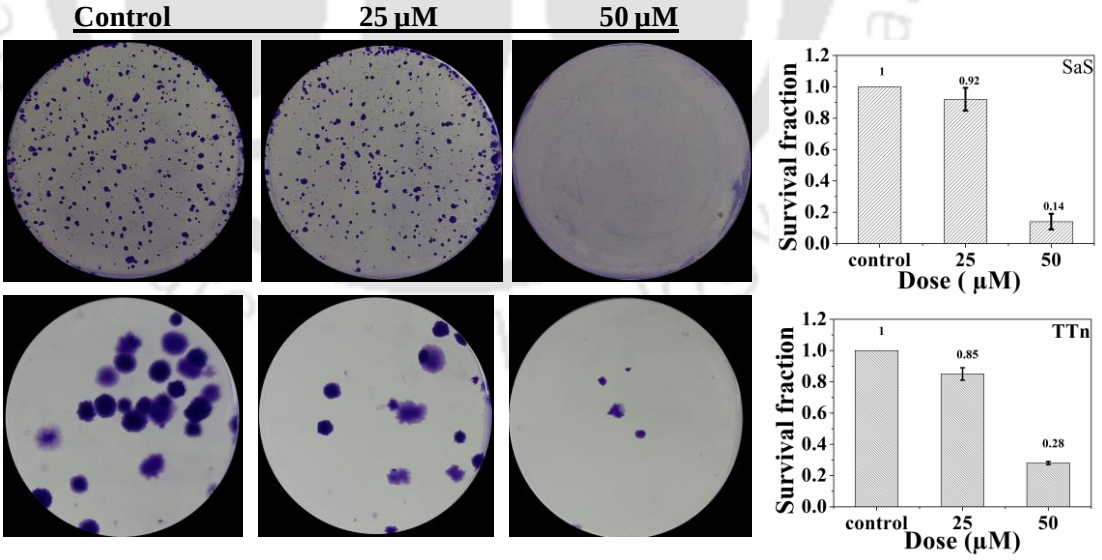
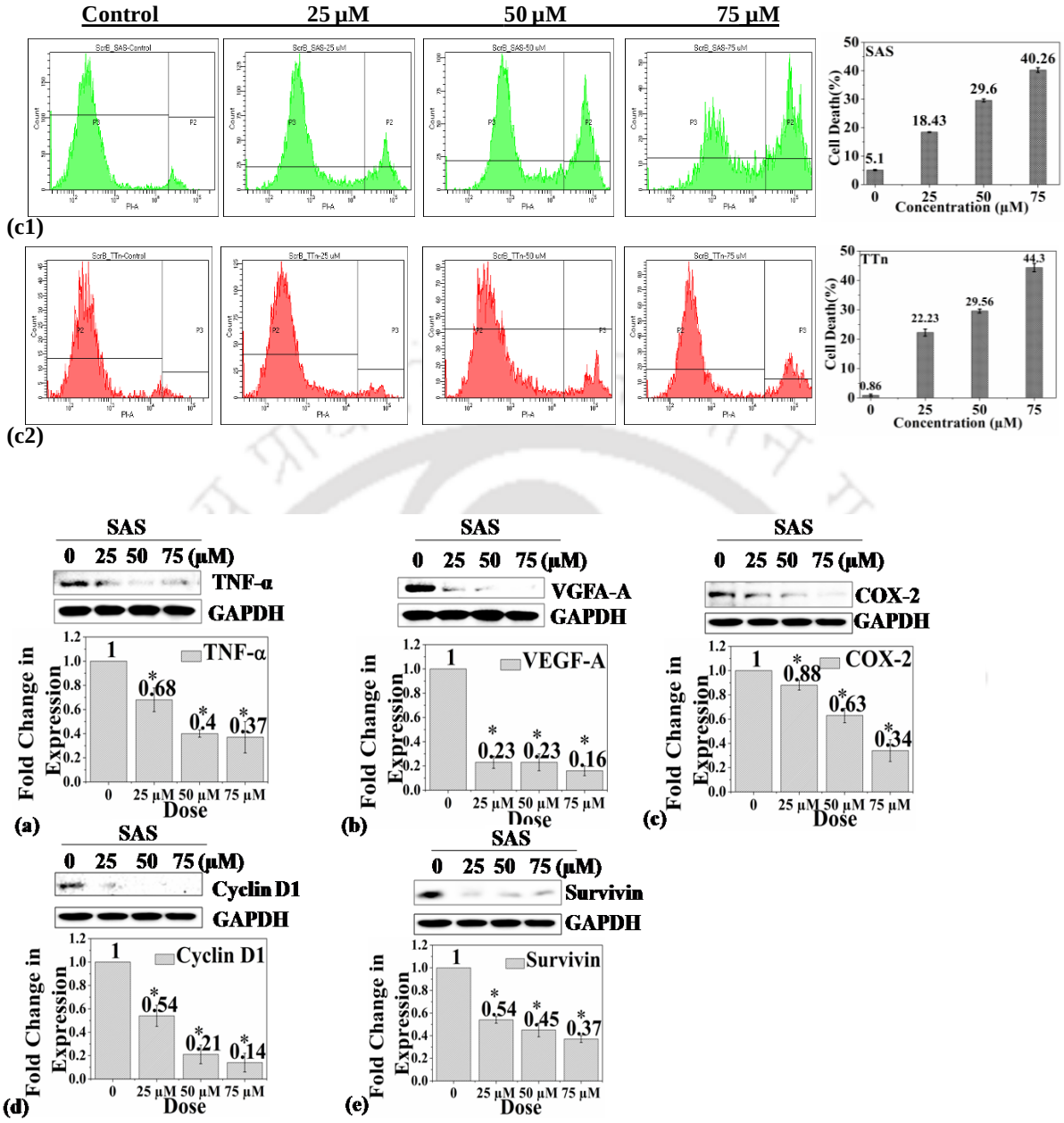




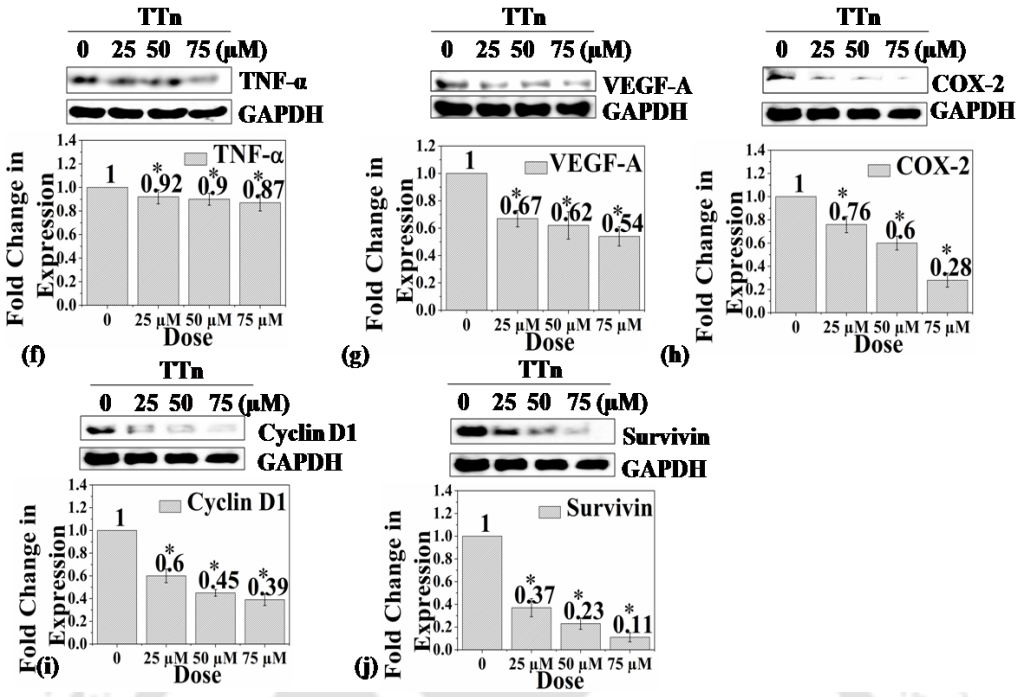


Figure 4.9. Effect of scirpusin B on different oral cancer cells. Inhibition of cell proliferation by scirpusin B on SAS (a1) and TTn (a2) cells. Inhibition of clonogenic potential of scirpusin B on SAS and TTn cell lines (b1 and b2) represented in survival fraction of both cell lines. Scirpusin B induced cell death assay by PI-FACS on SAS and TTn cells (c1 and c2). Inhibition of oral cancer hallmark proteins by Scirpusin B on SAS and TTn cells by western blot analysis. Inhibition of different hallmark proteins in SAS cells by SB is presented from (a) to (e) (a; SAS-TNFα, b; SAS-VGFA-A, c; SAS-COX2, d; SAS-Cyclin D1 and e; SAS- Survivin). Similarly, Inhibition of different hallmark proteins in TTn cells by SB is presented from (f) to (j) (f; TTn-TNFα, g; TTn -VGFA-A, h; TTn -COX2, i; TTn -Cyclin D1 and j; TTn - Survivin).All the experiments carried out are presented as mean ± SD (except western blotting) of three individual experiments. $p < 0.05$ vs control are reported for PI-FACS and colony assay.

4.12 Summary

The present study provides insight intothe utilization of futile passion fruit by-products forextracting various phytochemicals and further isolating them for various

pharmaceutical applications. Most importantly, the rich antioxidant potential and high phenolic and flavonoid contents of extracts will help develop different formulations for the treatment of life-threatening diseases like cancer, immunodeficiency disorders, and cardiac disorders. Northeast India's Passion fruit contains many polyphenols such as gallic acid, quercetin, and myricetin. The antibacterial study of YPF and PPF extracts will be beneficial for treating various diseases caused by bacterial pathogens. The outcome of the present research discloses the unexplored nutritional and medicinal power of the rind and seed of YPF and PPF cultivated in Northeast India. However, polyphenols like Piceatannol, Scirpusin B, and Protocatechuic acid from the seeds and Prunasin from the rind of yellow passion fruit were isolated effectively. Scirpusin B was found to be the maximum,with a recovery of ~0.2% of the total seeds (98.52 mg/ 50g). The isolated PT, SB, and PCA were found to have superior antiradical activity against many contemporary standard antioxidants suggesting higher commercial and therapeutic values. The inhibition of digestive enzymes like α-amylase and α-glucosidase by these compounds can help in understanding their antidiabetic potential. Noteworthy antibacterial activity of SB against various pathogenic and food-borne bacteria can address the concern dealing with multi-drug resistance of serious pathogenic bacteria. In addition, significant anticancer activity by SB against different oral cancer cell lines was also confirmed by suppression of cancer cell proliferation, inflammation, etc. However, more evidence in preclinical and clinical studies is required to evaluate SB's pharmacokinetic and pharmacodynamic behavior for different cancer treatments..

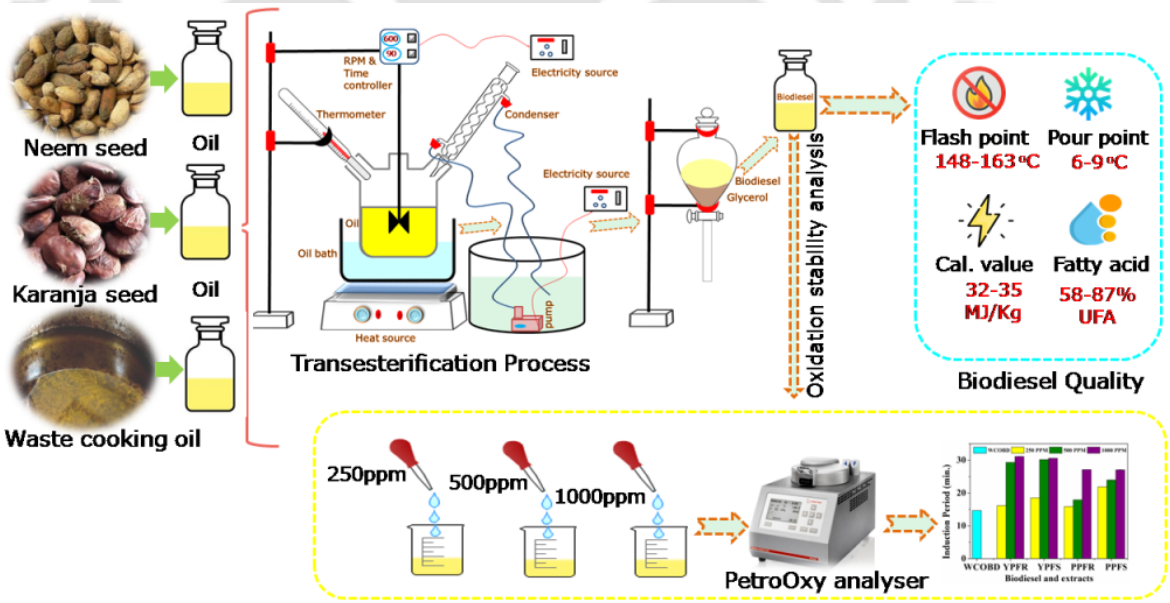
CHAPTER V

Green approach to increase the oxidative stability of different biodiesels using passion fruit extracts rich in antioxidants

Biodiesel synthesis

Physico-chemical characterization of synthesized biodiesel

Role of passion fruit extracts as an oxidative stabilizer on different biodiesel



Chapter V

Green approach to increase the oxidative stability of different biodiesel using passion fruit extracts rich in an antioxidants

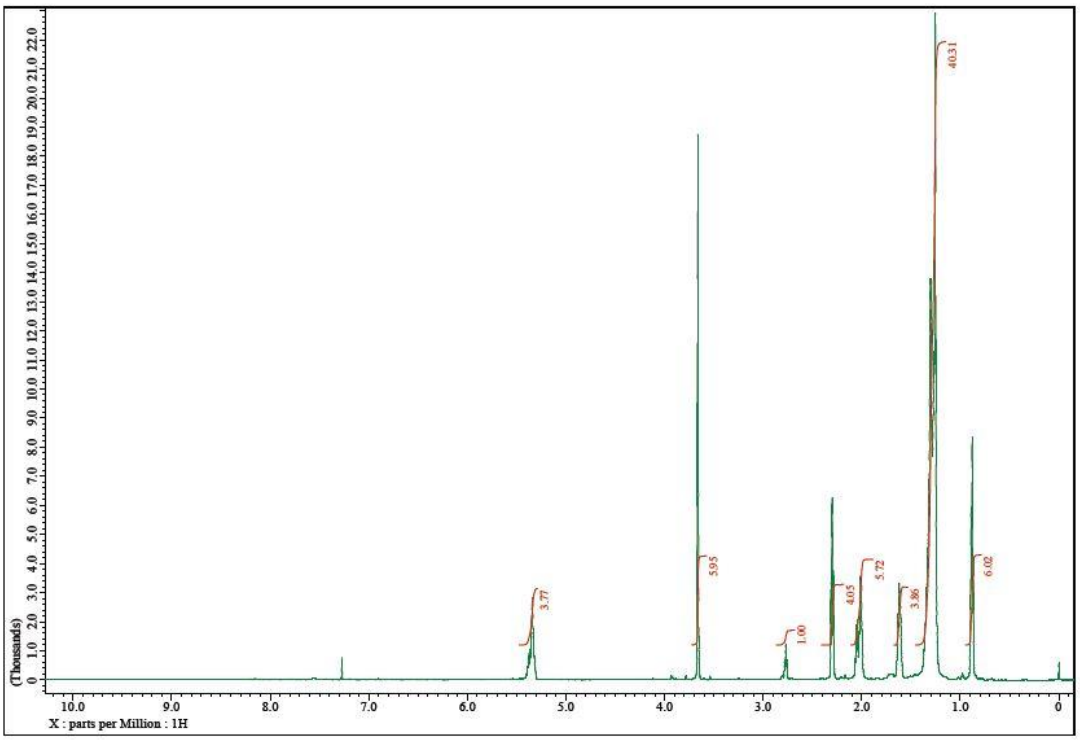
This chapter focused on the protective effect of different passion fruit extracts on three synthesized biodiesels: Neem biodiesel, Karanja biodiesel,and waste cooking oil biodiesel (Palmolein oil). For that, these biodiesels were prepared by following standard esterification and transesterification processes, and the conversion of oil to methyl esters was confirmed from ^{1}H NMR. Further, different characterization studies of oils and biodiesel were carried out to ensure their quality. The thermal stability of the oils and biodiesel wasestimated using TGA. The fatty acid composition of different biodiesel suggested higher unsaturated fatty acid confirming as a better quality biodiesel. Further, the oxidative stability of different biodiesel was improved by doping different concentrations (250 ppm, 500 ppm, and 1000 ppm) of four best extracts from yellow and purple passion fruit. All passion fruit extracts significantly improved the oxidative stability of all three tested biodiesels ranging from 1.5 to 2 fold.

5.1 Biodiesel synthesis and their physicochemical characterization

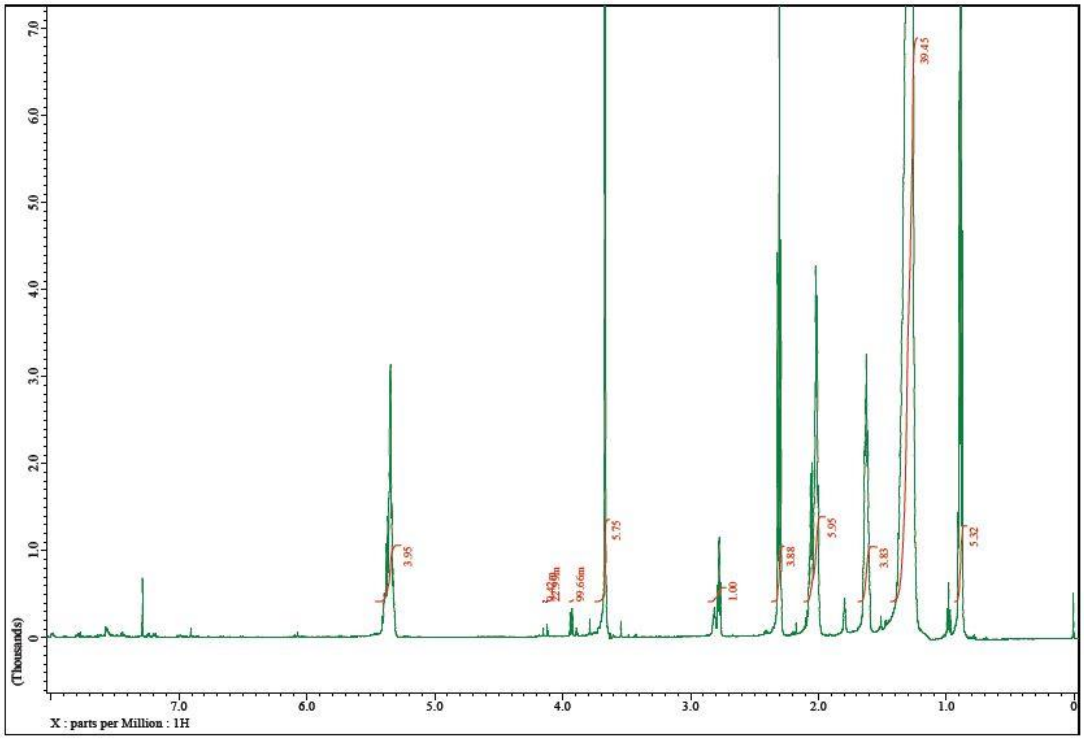
Three different biodiesel with varying fatty acid composition and free fatty acid content i.e. Neem oil, Karanja oil, and Waste Cooking oil, were considered for

biodiesel synthesis. Both Neem oil and Karanja oil showed off-limit acid values (38.42 mg-KOH/g-sample and 24.82 mg-KOH/g-sample, respectively), which urged a two-step transesterification (esterification followed by transesterification), while the lower acid value of waste cooking oil (1.12 mg-KOH/g-sample) indicates that single step transesterification process. After completion of the transesterification process, ^{1}H NMR of the samples was carried out to ascertain the oil-to-methyl ester conversion. The ^{1}H NMR spectrum of all three biodiesel is presented in Figure 5.1. A sharp signal at 3.7 ppm indicates the presence of methylic esters group confirming biodiesel synthesis. Oil to biodiesel conversion of NBD, KBD, and WCOBD was found to be 98.18%, 98.79%, and 99.28%, respectively, and was confirmed from ^{1}H NMR spectrums as mentioned in the section 2.2.28. These values meet the EN 14214 specifications (minimum 96.5%) for oil-to-biodiesel conversion, implying the synthesized biodiesels are of acceptable quality.

(a)



(b)



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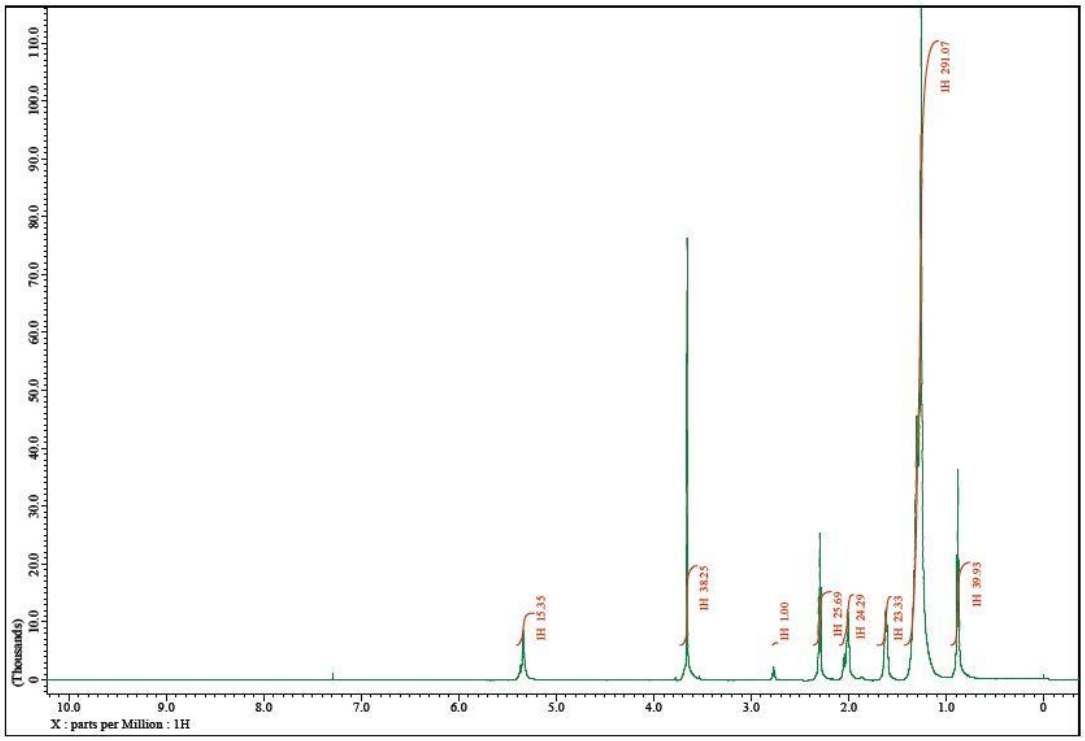


Figure 5.1. Oil to methyl ester conversion by ^{1}H NMR for(a) NBD, (b) KBD, and (c)WCOBD.

Further essential physicochemical properties of different oil feedstock and their respective biodiesels are presented in Table 5.1. Less than 0.45% moisture content of all samples indicated their optimum quality and usability. Higher moisture content in biodiesel may lead to microbial growth and promote oxidation, thereby degrading the quality of the biodiesel. The acid value of different biodiesel was under the acceptable limit set by ASTM 6751–02 standards (Table 5.1).The acid value measures the degree of rancidity or spoilage in oil due to the degradation of fatty acids and the formation of acids. Oxidation is one of the significant reasons forthe quality degradation of biodiesel by enhancing the acid value and thereby making the biodiesel more corrosive in nature (Hassan and Kalam, 2013). The kinematic viscosity of tested

biodiesel was as per ASTM 6751–02 recommended limits (1.9 to 6.0 cST). More viscous biodiesel can cause profound adverse effects on the engine performance, which include the demand for high pressure for fuel injection leading to more energy consumption, formation of larger droplets resulting in poorer spray, etc. This issue can result in substandard combustion and decreased engine efficiency (Verduzco, 2013). The density of the obtained biodiesels for this study was under the acceptable range as per EN 14214 (i.e. 860-900 kg/m^3). The flash point of different biodiesel was determined by ASTM D 93 method. The obtained flash point values for NBD, KBD, and WCOBD were found to be 163°C, 156°C, and 148°C, respectively, higher than commercial diesel, indicating their safety profile during transportation and storage. Further, the cloud and pour points of different biodiesel were found in accordance with the ASTM 6751-02 standard. Crystallization of fatty acid methyl ester component (cloud point) clogs fuel lines and filters, and further, upon complete solidification (pour point) it creates more challenges to the flow properties of biodiesel (Bhale et al., 2009). As a whole, the quality of different biodiesel was found to be optimum as per different international standards for biodiesel, confirmed by the physicochemical characterization.

Table 5.1. Physicochemical characterization of oil feedstock and biodiesel.

Properties	N. oil	NBD	K. oil	KBD	WC. oil	WCOBD	ASTM (6751–02)
Moisture (wt %)	0.54	0.32	0.41	0.37	0.75	0.45	—
Acid value (mg KOH/g)	38.42	0.41	24.82	0.44	1.12	0.48	0.5
Iodine value (g I_2/100 g oil)	67.4	68.5	65.3	67.8	105	106.4	—
Density (Kg/m^2)	—	879.28	—	857.93	—	866.87	—
Kinematic viscosity (cST)	—	5.26	—	4.91	—	4.44	1.9-6.0
Dynamic viscosity (Pa.S)	—	0.004	—	0.004	—	0.003	—
Flash point (^oC)	>180	163	>180	156	>180	148	≥130
Cloud point (^oC)	—	15	—	12	—	11	—
Pour point (^oC)	—	9	—	8	—	6	−15 to 10
Calorific value (MJ/Kg)	31.61	35.88	31.22	32	31.63	33	—

Note: N.oil: Neem oil, K.oil: Karanja oil, WC.oil: Waste cooking oil

5.2 Fatty acid composition of synthesized biodiesel

The fatty acid composition of the synthesized biodiesel is presented in Table 5.2, and their respective GC chromatograms are presented in Figure A10(Appendix). In all the synthesized biodiesel samples, unsaturated fatty acid was found maximum in the range of 57-87% (relative weight percentages), followed by saturated fatty acid (13-42.47%). Eicosenoic acid, Docosanoic acid, Erucic acid, and Eicosapentaenoic acids were the other fatty acids identified in NBD and KBD. WCOBD showed varied fatty acid composition compared to the other two biodiesel. Linoleic acid was predominantly higher,ranging from 42.29 to 54.74% in all the biodiesel. Vegetable oils with higher monounsaturated fatty acids are promising alternatives to fossil fuels. Therefore, the present study suggests that synthesized biodiesel can be an alternative energy source. However, microbial deterioration and oxidation are the primary process by which fatty acid or its ester gets degrades. Auto oxidation is the most common process and is defined as the spontaneous free radical reaction of fatty acids with atmospheric oxygen. Other unavoidable factors, including heat, light, air, and metal ion catalyst, can further increase the oxidation rate and thereby deteriorate the biodiesel quality. However, the utilization of natural antioxidants can substantially reduce auto-oxidation in biodiesel and thereby enhance its shelf life (Bharti and Singh, 2020; Santos et al., 2012).

Table 5.2. Fatty acid composition of synthesized biodiesel.

Fatty acid	NBD	KBD	WCOBD
C14:0 (Myristic)	—	—	0.90
C16:0 (Palmitic)	15.97	11.98	41.28
C18:1 (Oleic)	5.45	6.80	4.61
C18:2 (Linoleic)	48.71	54.74	42.29
C18:2N (Linoleic)	0.54	—	—
C18:3 (Linolenic)	23.86	20.21	10.64
C20:0 (Arachidic)	0.05	—	0.29
C20:1 (Eicosenoic)	1.54	3.65	—
C21:0 (Docosanoic)	1.82	1.02	—
C20:3N (Dihomo-γ-linolenic)	—	0.23	—
C22:1 (Erucic)	1.55	0.9	—
C20:5 (Eicosapentaenoic)	0.5	0.46	—
SFA (%)	17.84	13	42.47
UFA (%)	82.16	87	57.53
MUFA (%)	8.54	11.34	4.61
PUFA (%)	73.6	75.64	52.93

Note: All the data presented here are in relative weight percentage. SFA; Saturated fatty acid, UFA; Unsaturated fatty acid, MUFA; Mono unsaturated fatty acid, PUFA; Poly unsaturated fatty acid.

5.3 Thermal analysis

Thermo gravimetric (TG) analysis of different oils and their biodiesel was performed to study their thermal stability. Thermal stability in terms of onset temperature (temperature from where the thermal degradation of a compound initiates) and different stages of thermal degradation of oil and biodiesel samples are presented in

Figure 5.2. The onset temperature of neem oil, karanja oil, and waste cooking oil was found to be 234.95ºC, 232.20ºC, and 350.64ºC, respectively. On the contrary, the onset temperature of the respective methyl esters was much lower i.e., 172.9º C, 171.5º C, and 172.9º C. There is no standard for thermal stability; however, a sample above 150ºC is considered thermally stable (Kivevele, 2020). The better thermal stability of oil could be because of the greater molecular tension produced by its bulky triglyceride composition and higher viscous nature compared to biodiesel (Durrett et al., 2008; Lujaji et al., 2010). The higher viscosity of oil also promotes a delay in thermal oxidation (Durrett et al., 2008). Multiple degradation patterns in the DTG curve (Figure 5.2) indicated about thermal degradation of different compounds present in the oil. For instance, the first degradation step indicated mono and poly-unsaturated fatty acids decomposition followed by the second degradation of saturated fatty acids (Dunn and Moser, 2005; Vickers, 2017). The TGA analysis also confirmed that the biodiesel were free from methanol and water (no degradation pattern noticed in the DTG curve up to 100ºC).

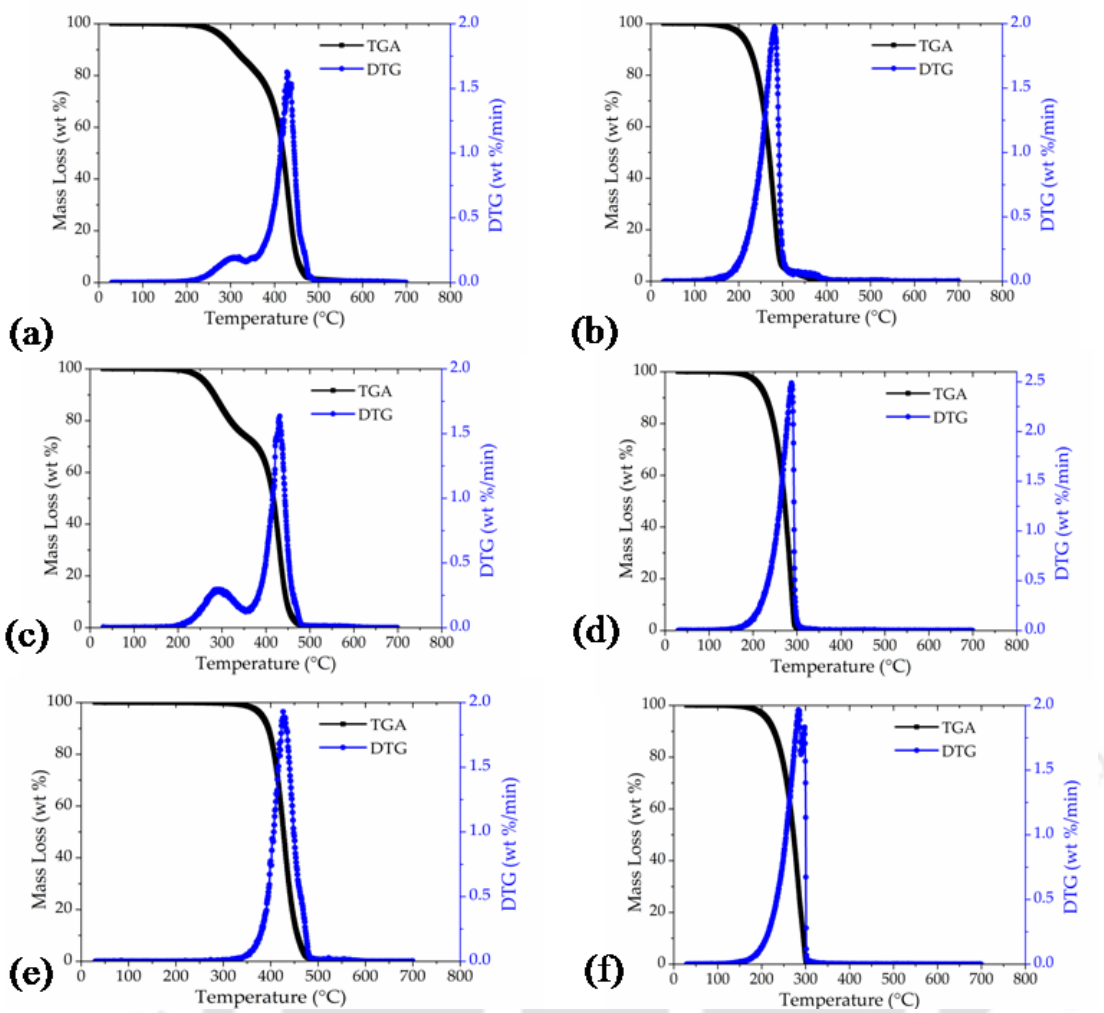


Figure 5.2. TG and DTG plots of (a) Neem oil, (b) Neem biodiesel, (c) Karanja oil, (d) Karanja biodiesel, (e) Waste cooking oil, and (f) Waste cooking oil biodiesel.

5.4 Effect of passion fruit extracts on oxidative stability of biodiesel

The effect of rind and seed extracts (250, 500, and 1000 ppm) from two different varieties of passion fruit (yellow passion fruit and purple passion fruit) on the oxidative stability of biodiesel (NBD, KBD, and WCOBD) is presented in Figure 5.3. The values in the graphs are presented as induction period as a measurement of the oxidative stability in biodiesel. The induction period of neat biodiesel (IP= 16.67 ± 0.02 min for PetrOxy; and 5.21 h for Rancimat for NBD, 20.3 ± 0.01 min for

PetrOxy; and 7.21 h for Rancimat for KBD and 14.65± 0.01 min for PetrOxy; and 4.21 h for Rancimat for WCOBD) was in accordance with the ASTM D6751 specifications (*IP* ≥ 12.37 min for PetrOxy; 3 h for Rancimat) but failed to meet IS15607 and EN14214 specification (21.78 min for PetroOxy and 8 h for Rancimat). The inferior induction period of the neat biodiesel could be attributed to the unsaturated fatty acid composition. Oxidation of unsaturated fatty acid methyl esters results in increased acid value and thereby promotes the leaching of metal into the biodiesel, which again indirectly causes oxidation in the biodiesel. However, in recent times, natural plant extracts have been considered green oxidative stabilizers for delaying oxidation rate in many biodiesels (de Sousa et al., 2021; Kerkel et al., 2021; Neuana et al., 2021). In this context, we had previously reported the kinetics study of yellow passion fruit seed extract as an antioxidant additive for waste soybean cooking oil biodiesel (Jain et al., 2021). The study indicated that YPFS extract significantly improved waste cooking oil biodiesel stability which further encouraged us to check the oxidation stability activity of other PF extracts (YPFR, YPFS, PPFR, and PPFS) in different biodiesel feedstock like NBD, KBD, and WCOBD. The present study showed that PPFS extracts enhanced the IP values in both NBD and KBD, followed by YPFS, YPFR, and PPFR extracts. On the contrary, the YPFR extract increased IP value of WCOBD followed by YPFS, PPFS, and PPFR extracts. The different observations in terms of varied antioxidant activity on IP of various biodiesel in the present study could be because of the uneven fatty acid composition of the feedstock (Tang et al., 2008). However, adding different PF extracts significantly increased the induction period (2 to 3 fold) compared to neat biodiesel. Moreover, increasing the concentration of extracts from 500 ppm to 1000 ppm could not result in any

significant improvement in the induction period in NBD and KBD. The extracts used to increase the oxidative stability in the present study also exhibited superior activity compared to recent literature data.

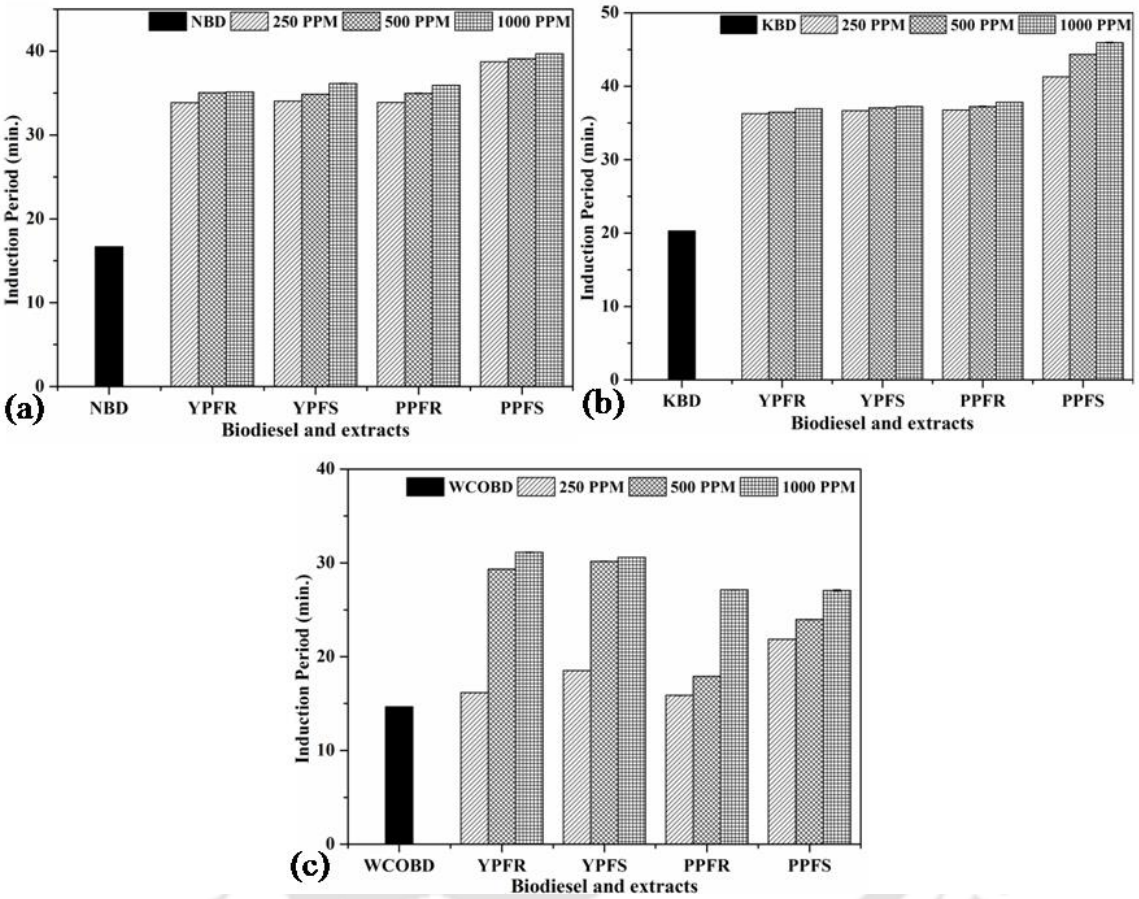


Figure 5.3. Induction periods (IP) of neat biodiesel and biodiesel with different concentrations of passion fruit extract (250 ppm, 500 ppm, and 1000 ppm). (a) Neem biodiesel, (b) Karanja biodieseland (c) Waste cooking oil biodiesel.

A comparison table of different types of biodiesel used, extractconcentration, and IP value reported by other groups with the present study is mentioned in Table A8 (Appendix). Until recently, Rancimat was the only means for calculating the IP of biodiesel. However, the Rancimat method can only detect the highly volatile

oxidative products, hence conferring incomplete results (Jain et al., 2021). On the contrary, PetrOxy provides complete information on biodiesel's oxidative stability as it functions under high pressure in a closed chamber and thereby avoids the volatility of biodiesel. Although PetrOxy is an accepted method by ASTM and EN, no limit is set, unlike the Rancimat method. Therefore, the IP value of PetrOxy (in min) was converted into the IP value of Rancimat (in h), followed by the formula given by (Botella et al., 2014), as mentioned in the material and methods section. The converted values from PetroOxy to Rancimat for the present study are presented in Table 5.3.

Table 5.3. Conversion of PetroOxy Induction Period to Rancimat method.

	NBD			KBD			WCOBD		
	250*	500*	1000*	250*	500*	1000*	250*	500*	1000*
YPFR	14.41	15.05	15.10	15.69	15.81	16.05	5.02	12.01	12.97
YPFS	14.51	14.95	15.63	15.92	16.12	16.22	6.27	12.47	12.67
PPFR	14.44	15.00	15.51	15.96	16.22	16.52	4.86	5.94	10.84
PPFS	17.00	17.19	17.51	18.38	19.99	20.84	8.04	9.16	10.80

Note: Asterisk symbol represents the concentration of plant extract in ppm (parts per million). The other values in the table indicate the converted oxidation stability values from PetroOxy (min) to Rancimat (h)

In the present study, all the extracts at only 250 ppm concentration (irrespective of biodiesel) exhibited increased IP in the range of 14.41-18.38 h (Table 5.3) compared

to the neat biodiesels (4.21-7.21 h), confirming their profound anti-oxidative properties. This small extract concentration can be easily dissolved in a small amount of methanol and further meet the international guidelines for a permissible limit of methanol in biodiesel (0.2 weight %). Therefore, less extract concentration with more oxidation resistance approaches should be adapted for the commercial application of bio-extracts in biodiesel. Overall, this study furnished successful enhancement of IP by different passion fruit extracts on other biodiesel.

5.5 Summary

The synthesized biodiesel was found to be of optimum quality, confirmed by different physicochemical properties. High flash point value ensured the safety of the biodiesel during transportation and storage. The TGA analysis showed that the synthesized biodiesel was also found to be thermally stable above 170^{o}C. Overall this study indicated the utilization of non-edible oil feedstock to produce second-generation biofuels. Importantly, extracts ofyellow and purple passion fruit waste by-products (rind and seed) showed a 2-3 fold improvement in the oxidative stability of all tested biodiesel.

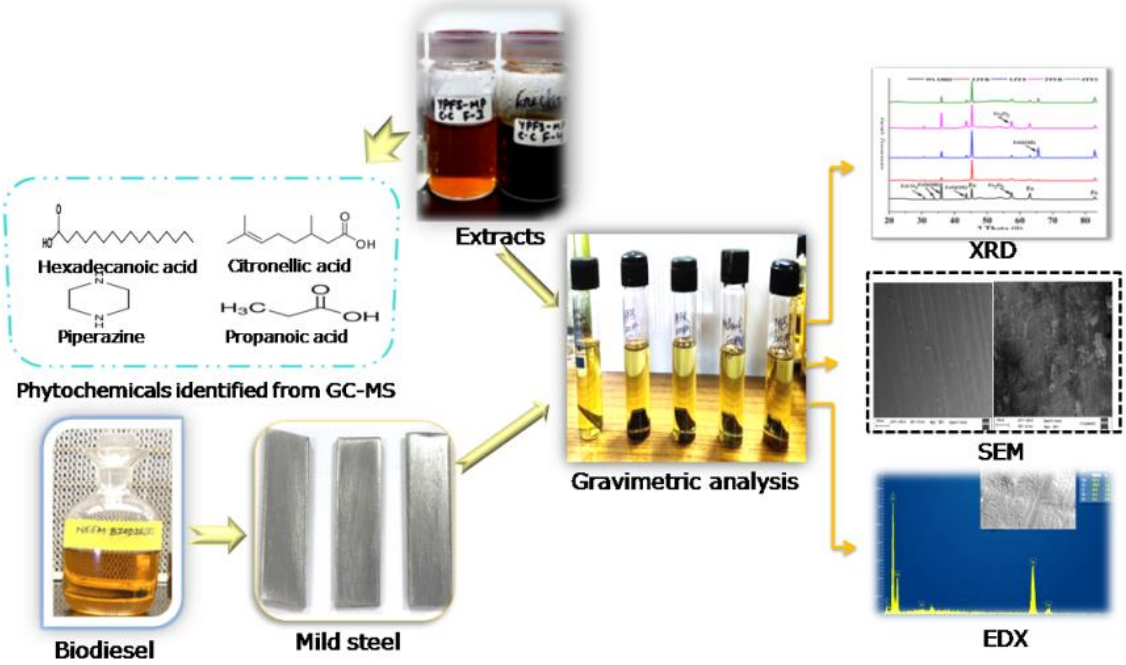
CHAPTER VI

Improving the sustainability of different biodiesels by controlling their corrosive effects on mild steel by using passion fruit extracts as an additive

Phytochemical analysis of extracts by GC-MS analysis

Corrosion inhibition study on mild steel submerged in different biodiesel mediums

Surface and compositional characterization of metal before and after corrosion study



Chapter VI

Improving the sustainability of different biodiesels by controlling their corrosive effects on mild steel by using passion fruit extracts as an additive

In the process of oxidation, several oxygenated compounds are formed in biodiesel. In addition, moisture and unsaturated fatty acids in biodiesel also accelerate the oxidation process causing the corrosiveness of the metal part. The leaching of the metal further deteriorates the quality of biodiesel quality. Overuse of synthetic antioxidants such as BHT, BHA, and PG as corrosion inhibitorsis not sustainable because of their cost, carcinogenicity, and less eco-friendliness. Therefore, the application of bio-based extracts from plant origin, food by-products, and waste vegetable biomass are considered green corrosion inhibitors for their sustainable, non-toxic, cheap, and eco-friendly nature.In this chapter, the anticorrosion properties of different passion fruit extracts were checked by doping them with biodiesel containing mild steel specimens. Various polyphenols, aromatic compounds, and fatty acids were identified from methanolic extracts of the rind and seed samples by GC-MS analysis.

Further, 500 ppm of each extract was doped with each biodiesel (Neem biodiesel, Karanja biodiesel, and waste cooking oil biodiesel) with mild steel specimens for gravimetric analysis. The corrosion inhibition efficiency and corrosion rate calculations determined the anticorrosion behavior of the extracts. The

morphological and compositional changes on the metal surface due to corrosion and inhibition by the extracts were further determined by SEM, EDX, and XRD analysis.

6.1 Extract preparation and GC-MS analysis

In the previous chapter (chapter 4), extract yield, total phenolic content, and antioxidant potential of YPF and PPFextractswere mentioned,and methanolic extracts were found to have superior antioxidant activities than other extracts. Various polyphenols, including gallic acid, caffeic acid, ferulic acid, quercetin, myricetin, etc. were identified using HPLC analysis. However, many other phytochemicals were not identified from HPLC analysis. Therefore, further identification of many polyphenols and volatile aromatic compounds wascarried out using GC-MS analysis. Various reports suggest volatile and aromatic compounds can also be considered an oxidative stabilizers for biodiesel and green corrosion inhibitor for different metals (Bagga et al., 2016). In this context, the methanolic extracts (5 mg/mL) from rinds and seeds of passion fruit were subjected to GC-MS analysis, and the phytochemicals identified are presented in Table 6.1. 2-hydroxy- Propanoic acid methyl ester was predominantly present in all four extracts ranging from 0.95 to 21.65 wt%. It is a fragment of dihydroxy phenolic acid, a well-known antioxidant molecule (eg. Protocatechuic acid) (Siquet et al., 2006). Antioxidant behavior of the synthetic propanoic acid was also reported by Christophe Siquet and group (Siquet et al., 2006). Different major phytochemicals identified were 2,4-Dithiapentane found in YPFR, Piperazine in YPFR, YPFS, PPFR (Homopiperazine) and PPFS, Maltol in YPFS, 5-hydroxy methyl furfural in YPFS and PPFR, Citronellic acid in PPFR. There are evidences of these chemicals as antioxidants and antioxidant precursors (Al-Huqail et al., 2018;

Bassuony, 2015; Özil et al., 2018; Yi and Kim, 1982), but their presence in the rind and seed of yellow and purple passion fruit is reported for the first time in this study. However, the other major chemical, like 1,4-Butanediol-diacetate (aromatic compound), could be because of the flavourful characteristic of the rind and seed of passion fruit. Further, the GC-MS analysis also confirmed the presence of hexadecanoic acid (Palmitic acid), tetradecanoic acid (Myristic acid), and octadecanoic acid (Stearic acid) in all the extracts. Bharath et al., 2021 reported the role of Palmitic acid as a potential antioxidant agent in their study (Bharath et al., 2021), where Myristic acid and Stearic acid were also proven as green corrosion inhibitors (Mohammed et al., 2021). Therefore, it can be concluded that these antioxidant-rich polyphenols, non polar and volatile compounds identified from different passion fruit extracts through GC-MS analysis can improve biodiesels' oxidative stabilityand further lessen their corrosive behavior. These phytochemicals (promising corrosion inhibitors) can also directly adhere to the metal surface, forminga protective layer on the metal surface through nitrogen, oxygen, sulfur, and phosphorus hetero atoms and aromatic π electrons, thereby making a barrier that prevents the access of biodiesel to the metal surface.

Table 6.1. Phytochemical investigation by GC-MS.

Extract Code	RT (min)	Identified compounds	Area (%)
YPFR			
	6.585	1,4-Butanediol, diacetate	17.84
	6.793	Propanoic acid, 2-hydroxy-, methyl ester, (+/-)	13.29
	7.276	2,4-Dithiapentane	6.60
	12.186	(+-)-3-hydroxybutyric acid, acetate	0.27
	18.968	Piperazine, 1,4-dimethyl-	0.34
	28.247	5-Hydroxymethylfurfural	2.99
	52.182	Tetradecanoic acid	1.29
	59.143-59.655	n-Hexadecanoic acid	8.19
	65.353-65.873	Octadecanoic acid	2.82
YPFS			
	6.629	1,4-Butanediol, diacetate	20.07
	6.837	Propylene Glycol	15.30
	8.189	Propanoic acid, 2-methyl-	0.98
	14.444	2,5-Furandione, dihydro-3-methylene-	1.27
	16.116	2,4-Dihydroxy-2,5-dimethyl-3(2H)-furan-3-one	1.08
	18.909	Piperazine, 1,4-dimethyl-	0.55
	20.863	Maltol	1.58
	24.161	4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl-	2.81
	38.365	1,5-Pentanediol	1.42
	59.663	n-Hexadecanoic acid	2.25
	64.127	10-Octadecenoic acid, methyl ester	1.63
PPFR			
	6.614	3-Methoxy-2,2-dimethyloxirane	21.65
	6.815	Propanoic acid, 2-hydroxy-, methyl ester, (+/-)	10.25
	7.12	1-Butanamine, 3-methyl-	15.90
	7.283	2,2-Dimethoxybutane	5.32
	14.429	2,5-Furandione, dihydro-3-methylene-	0.92

	23.589	Homopiperazine	0.21
	24.183	4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl-	4.85
	28.254	5-Hydroxymethylfurfural	2.96
	32.243	(R)-(+)-Citronellic acid	0.61
	59.67	n-Hexadecanoic acid	3.15
	65.881	Octadecanoic acid	0.75
PPFS			
	6.644	1,4-Butanediol, diacetate	26.48
	6.852	Propanoic acid, 2-hydroxy-, methyl ester, (.+/-.)-	17.51
	7.327	N-(2-Sulfanylethyl)-2-oxopropanamide	15.98
	18.909	Piperazine, 1,4-dimethyl-	0.59
	24.154	4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl-	2.35
	59.648	n-Hexadecanoic acid	1.19

6.2 Weight loss study by gravimetric analysis

Corrosion inhibition properties of various passion fruit extracts on MS specimens submerged in three different biodiesels (NBD, KBD, and WCOBD) are presented in Figure 6.1.The corrosion inhibition analysis wascarried out through the gravimetric method, and the results are presented in Table 6.1. The pre-weighted MS specimens weredipped in three biodiesels containing 500 ppm of PF extracts (YPFR/PPFR/YPFS/PPFS) for 15 days (360 h), and further, their inhibition efficiency and corrosion rates were calculated as mentioned in the materials and methods section.

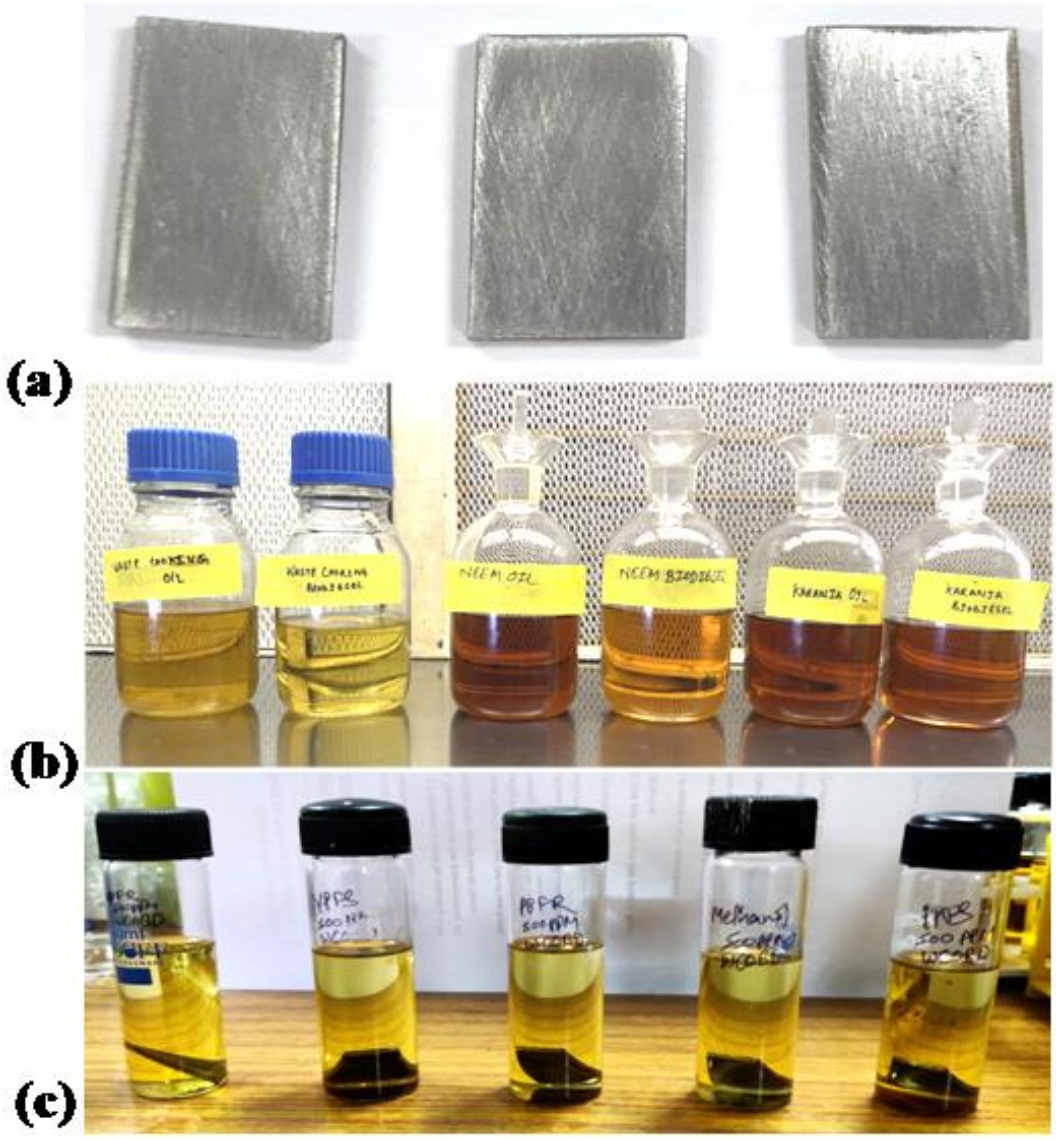


Figure 6.1. Mild steel specimen used for the corrosion study (a); different oils and their biodiesel, i.e. waste cooking oil and biodiesel, neem oil and biodiesel, and karanja oil and biodiesel (b); mild steel immersed in biodiesel solution containing different passion fruit extracts (c).

It can be observed that the neat biodiesels (without inhibitor) were corrosive in nature for MS specimens, resulting highest corrosion rate of 0.21, 0.23, and 0.17 mm/year in NBD, KBD, and WCOBD, respectively (Table 6.2). However, upon adding 500 ppm of different plant extracts, the corrosion rates were improved compared to the neat

biodiesel, as mentioned in Table 6.2. The YPFR extract exhibited the best corrosion resistance activity against other extracts in all biodiesel ranging from an inhibition efficiency of 71.19% to 74.74% with a minimum corrosion rate of 0.04 to 0.06 mm/year followed by YPFS, PPFS, and PPFR extracts. Different degrees of corrosion nature of these biodiesels can be correlated with the degree of unsaturated fatty acids present (Fazal et al., 2010). For example, during the oxidation process in biodiesel, the fatty acid methyl esters form free radicals adjacent to their double bonds, further attach oxygen molecules from the atmospheric air (Sarin et al., 2009). Due to this event, many corrosive by-products like aldehydes, ketones, formic acid, and caproic acid are formed, which further degrade the biodiesel quality and corrode the metal (Niczke et al., 2007; Tsuchiya et al., 2006). This theory can be correlated with our study that KBD is more corrosive than NBD and WCOBD because of its higher unsaturated fatty acid composition. However, not only does the fatty acid composition affect the corrosiveness of biodiesel, but also the moisture content and acid value increase the corrosion rate in biodiesel. Waste cooking oils are already heat treated and used several times for frying purposes which cause an increase in moisture content and generation of many free radicals, thereby polluting the biodiesel's property. This could be one of the reasons for the corrosive behavior of neat WCOBD. The present study aimed to check the duel effects of different PF extracts as an oxidative stabilizer and corrosion inhibitor. As stated earlier, the higher concentration of plant extract dissolved in a limited prescribed solvent may result in sediment formation upon long storage with less activity. Therefore, in the present study, 500 ppm extract concentration was chosen for the corrosion inhibition study (because no significant improvement was observed in 1000 ppm extracts for the

oxidation stability study). As mentioned in the GC-MS analysis, different PF extracts containing phenolic acids (Siquet et al., 2006) and fatty acids (Mohammed et al., 2021) can contribute to forming a protective layer on the MS surface and thereby reducingthe corrosion rate. Mohammed et al., reported that phytochemicals containing COOH and C=C groups bind with the active sites and further behave like the centre of adsorption, thus forming a protecting layer on the metal surface (Mohammed et al., 2021). Adsorption of natural extracts can be understood by electrostatic attraction between charged molecules and the charged metal, interaction of uncharged electron pairs in the molecule with the metal, etc. These findings were further validated with morphological and compositional analysis of treated and untreated samples.

Table 6.2. The corrosion inhibition efficiency and corrosion rate by different extracts on mild steel in different biodiesels.

Test solution	Weight loss (mg)	IE (%)	W_{corr} (mm/year)
MS-NBD	0.7	0	0.21
MS-NBD-YPFR	0.2	71.19	0.06
MS-NBD-YPFS	0.4	43.84	0.11
MS-NBD-PPFR	0.5	41.88	0.12
MS-NBD-PPFS	0.3	55.65	0.09
MS-KBD	0.8	0	0.23
MS-KBD-YPFR	0.2	71.31	0.06
MS-KBD-YPFS	0.4	49.66	0.11
MS-KBD-PPFR	0.6	24.26	0.18
MS-KBD-PPFS	0.3	61.14	0.09
MS-WCOBD	0.8	0	0.17
MS-WCOBD-YPFR	0.2	74.74	0.04
MS-WCOBD-YPFS	0.3	62.87	0.06
MS-WCOBD-PPFR	0.5	37.56	0.10
MS-WCOBD-PPFS	0.3	62.19	0.06

Note: IE%, Corrosion inhibition efficiency; Wcorr, Corrosion rate; MS, Mild Steel; NBD, Neem Biodiesel; KBD, Karanja Biodiesel; WCOBD, Waste cooking oil Biodiesel; YPFR, Yellow passion fruit rind extract; YPFS, Yellow passion fruit seed extract; PPFR, Purple passion fruit rind extract; PPFS, Purple passion fruit seed extract.

6.3 Metal specimen characterization before and after corrosion study

6.3.1X-ray diffraction analysis

The XRD analysis is one of the reliable methods to check the formation of corrosive products on the metal surface due to the harsh effect of biodiesel. In this study, XRD analysis of normal MS and MS in biodiesels without inhibitors (YPFR, YPFS, PPFR, and PPFS) and with inhibitors were carried out, and the results are presented in Figure 6.2. Characteristics XRD peak for MS can be observed in all the samples at 45°, 65°, and 82° (Figure 6.2a). However, in the case of neat biodiesels, many degradation product peaks appeared at 30.84°, 33.74°, 36.06°, 43.62°, 57.32°, and 67°. Interestingly, very minimal degradation products were observed in the extract-treated samples. The formation of degradation products can be directly related to the corrosion inhibitory efficiency. For instance, XRD analysis of MS in NBD doped with PPFR extract (minimum corrosion inhibition efficiency) exhibited intense peaks at 36.6°, 44.3°, and 66.1° compared to other extracts. The XRD pattern noticed on the mild steel surfaces could be because of the formation of different degradation products such as $FeCO_3$, FeO (OH) and Fe_2O_3. Similar results for commercial antioxidants on copper and carbon steel have also been discussed in earlier published reports (Hoang et al., 2020; Serqueira et al., 2021).

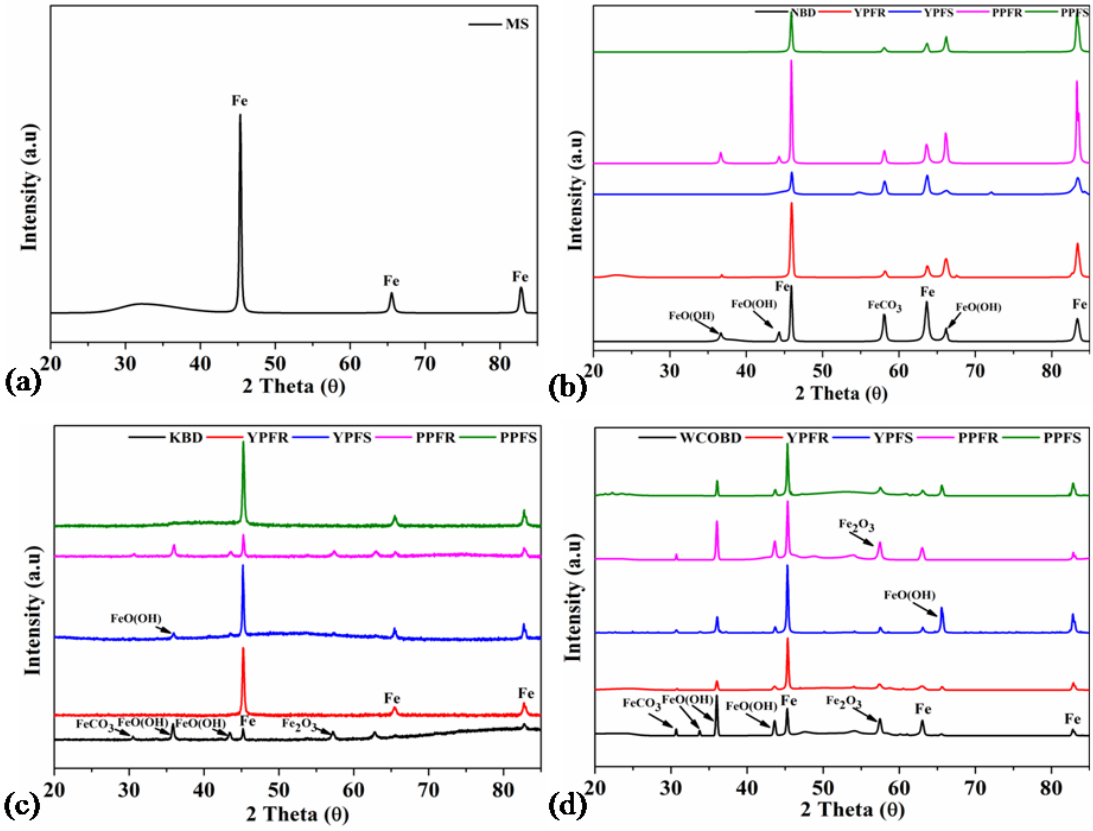
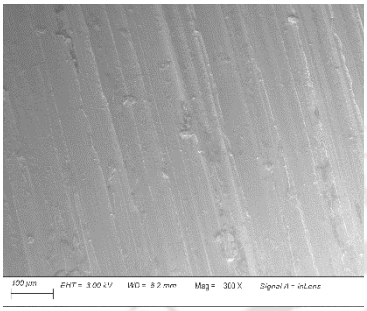


Figure 6.2. XRD pattern of MS (a); NBD with different inhibitor (b); KBD with different inhibitor (c); WCOBD with different inhibitor (d).

6.3.2 Scanning electron microscopy

Scanning electron microscopy images of MS, MS with inhibitor, and without inhibitor in biodiesel are presented in Figure 6.3. The longitudinal scratch mark in the SEM image of MS (Figure 6.3a) is because of the polishing of the metal with different grades of emery papers. In different neat biodiesel samples, pits and cracks were detected due to biodiesel's harsh corrosive environment. However, relatively fewer cracks were observed in the specimen with the inhibitor. From the corrosion inhibitory efficiency calculation (Table 6.2), it can be inferred that none of the extracts resisted corrosion beyond 74%, and this can be the possible reason for some

pits and cracks in the extract-treated specimens. These findings can further be correlated with the XRD results of samples with fewer degradation products because of the presence of protective layers.



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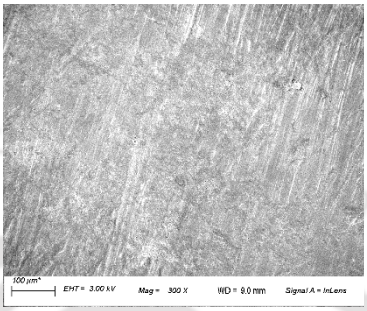

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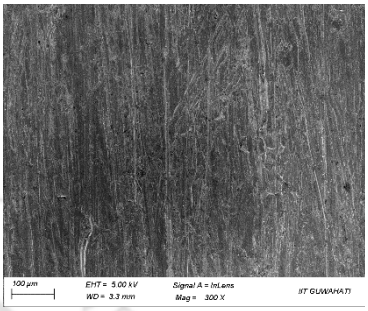

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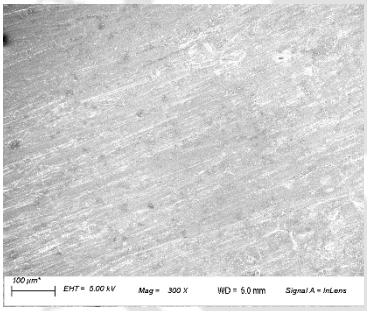

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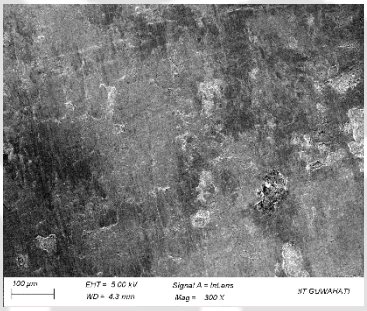

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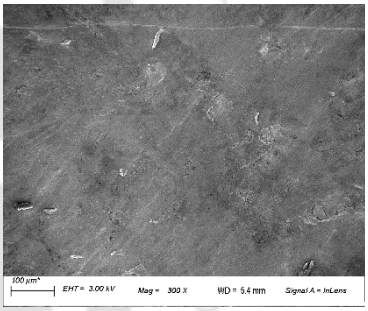

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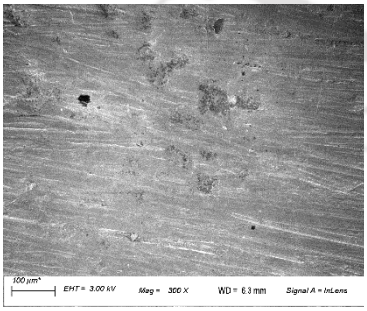

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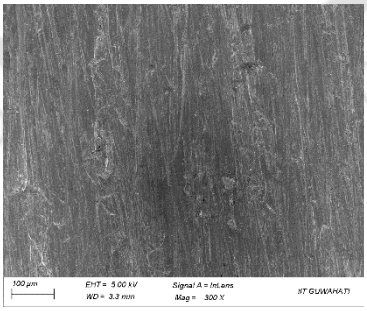

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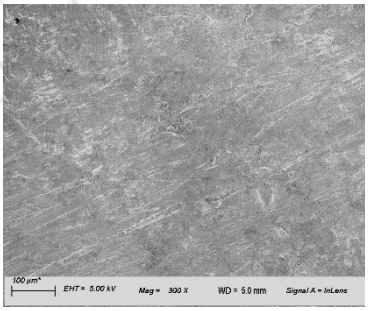

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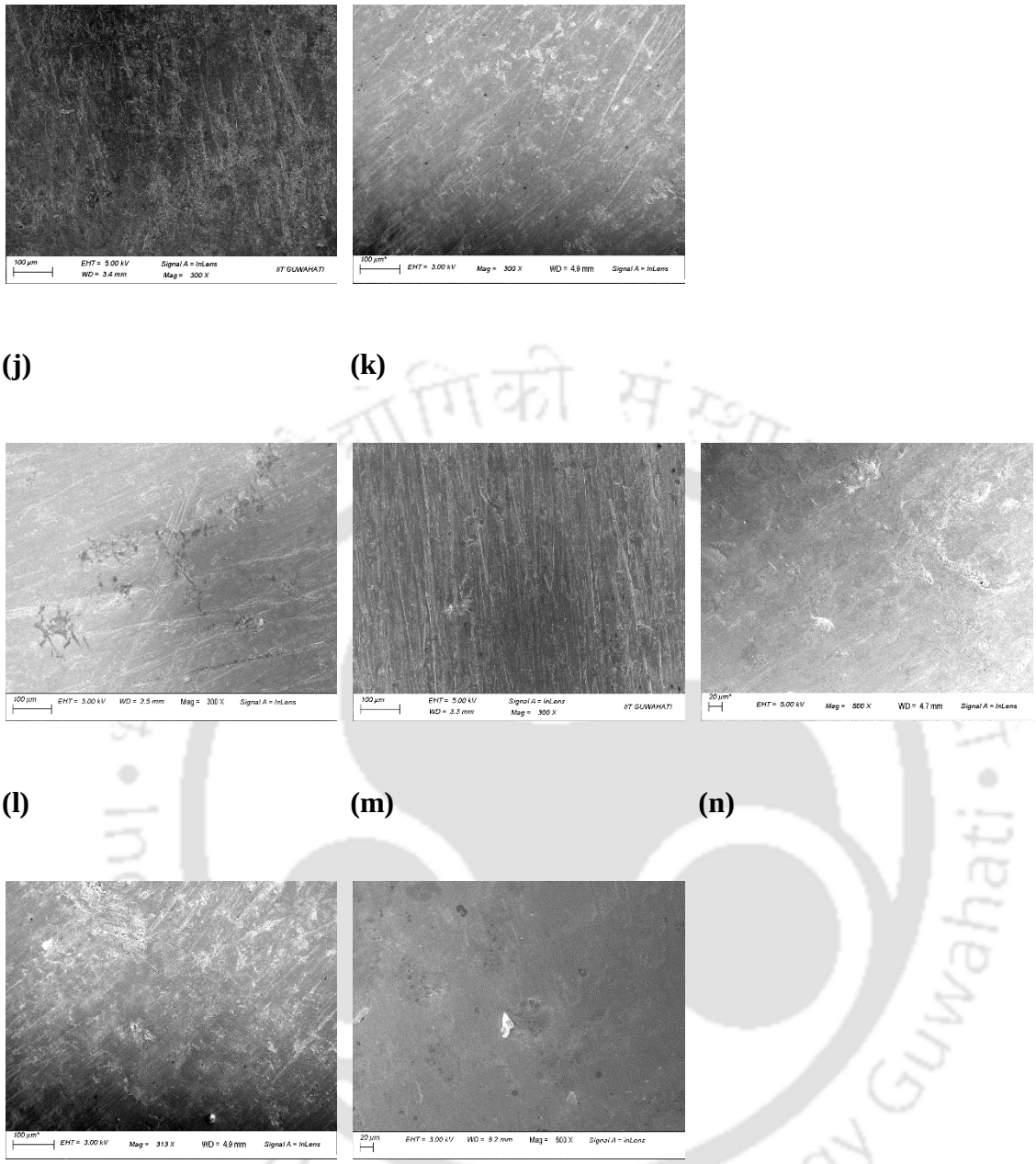


Figure 6.3. Scanning electron microscopy of mild steel specimens after immersion in different biodiesels without and with inhibitors. MS (a), MS -NBD (b); MS-NBD-YPFR (c); MS- NBD-YPFS (d); MS-NBD-PPFR (e); MS-NBD-PPFS (f); MS-KBD (g); MS-KBD-YPFR (h); MS-KBD-YPFS (i); MS-KBD-PPFR (j); MS-KBD-PPFS (k); MS-WCOBD (l); MS-WCOBD-YPFR (m); MS-WCOBD-YPFS, (n); MS-WCOBD-PPFR, (o); MS-WCOBD- PPFS (p).

6.3.3 Energy dispersive X-ray analysis

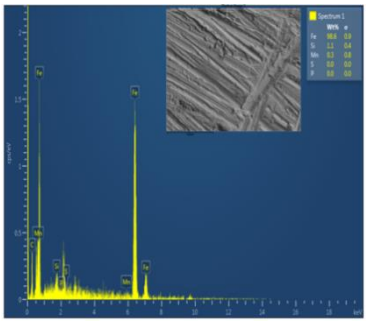
A decrease in iron percentage due to corrosion and an increase in oxygenated and carbonaceous compounds on the metal surface was determined by EDX spectroscopy (Table 6.3), and the spectroscopic data are presented in Figure 6.4.

Table 6.3. Elemental analysis of mild steel surface exposed to biodiesels.

Sample code	Elements (% mass)		
	Fe	O	C
MS	98.6	-	-
NBD	63.3	28	8.4
NBD-YPFR	78.7	8.4	11
NBD-YPFS	66.3	11.6	20
NBD-PPFR	62.5	22.8	14.5
NBD-PPFS	68.1	16.5	14.7
KBD	52.5	26.8	20.3
KBD-YPFR	75.8	4.1	16.5
KBD-YPFS	65.4	12.8	21.7
KBD-PPFR	62	23.3	14
KBD-PPFS	66	17	16
WCOBD	56.5	29.2	13.8
WCOBD-YPFR	78.6	3.5	16.4
WCOBD-YPFS	67.6	16.8	11.8
WCOBD-PPFR	61.6	29.2	8.7
WCOBD-PPFS	73.5	4.2	20.6

Note: MS, Mild Steel; NBD, Neem Biodiesel; KBD, Karanja Biodiesel; WCOBD, Waste cooking oil Biodiesel; YPFR, Yellow passion fruit rind extract; YPFS, Yellow passion fruit seed extract; PPFR, Purple passion fruit rind extract; PPFS, Purple passion fruit seed extract

The composition of untreated MS was found to be 98.6% of Fe, 0.3% Mn and the rest weight balance with C and Si. On the contrary, Fe weight percentage significantly decreased to 52.5%, 63.3%, and 56.5% for neat KBD, NBD, and WCOBD, respectively. The specimens dipped in this biodiesel (without inhibitor) also showed elevated oxygen content due to the formation of oxides as a by-product of metal corrosion. The same results can also be validated with the XRD analysis of the present work. However, protection provided against corrosion on a metal surface by different passion fruit extracts through the deposition of carbon and oxygen was also confirmed from the analysis. For instance, YPFR extracts added to KBD, NBD, and WCOBD exhibited oxygen content of 4.1%, 8.4%, and 3.5%, carbon content of 16.5%, 11%, and 16.4%, and iron content of 75.8%, 78.7%, and 78.6%, respectively. This observation demonstrated that YPFR extract could significantly reduce corrosion on tested metal sheets through a protective layer on the metal surface. Besides the YPFR sample, YPFS and PPFS extracts also inhibited corrosion in MS kept in different biodiesel mediums through the presence of different antioxidants listed in Table 6.1. A similar pattern of antioxidants and carbon deposition in addition of different commercial antioxidants (Curcumin, Butyl hydroxy anisole, tert-butyl hydroquinone and Propyl gallate) was reported by (Serqueira et al., 2021).



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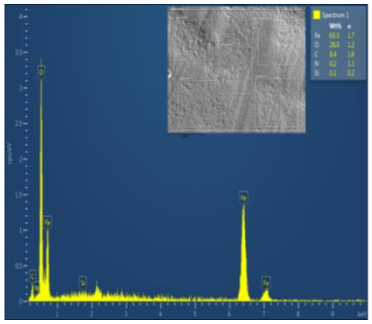

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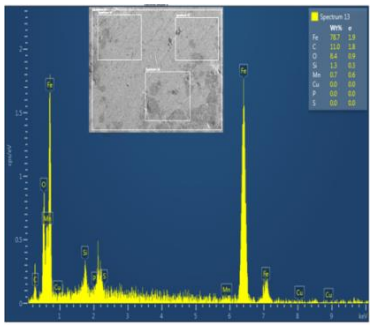

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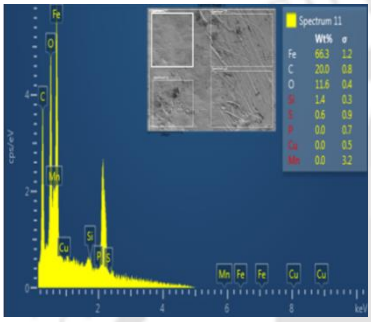

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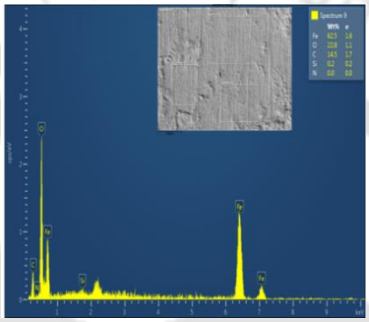

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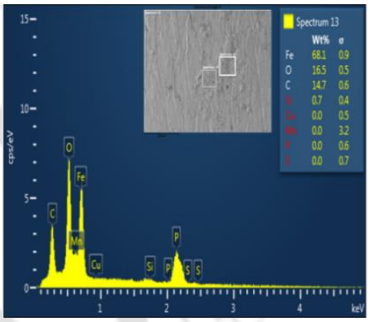

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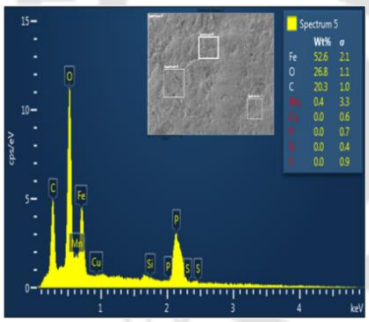

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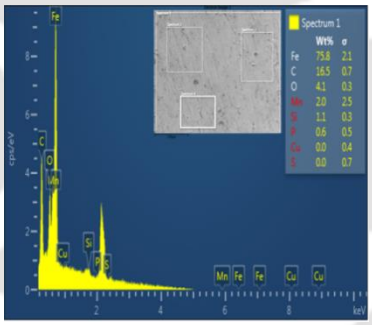

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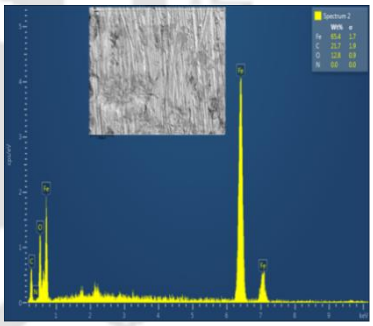

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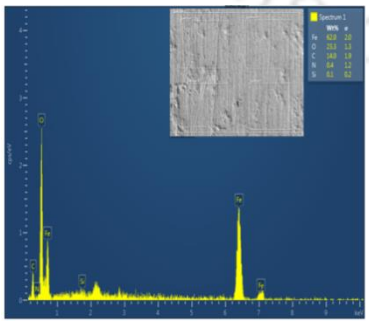

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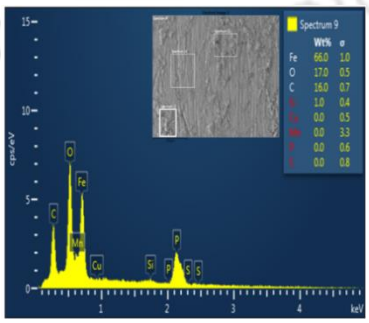

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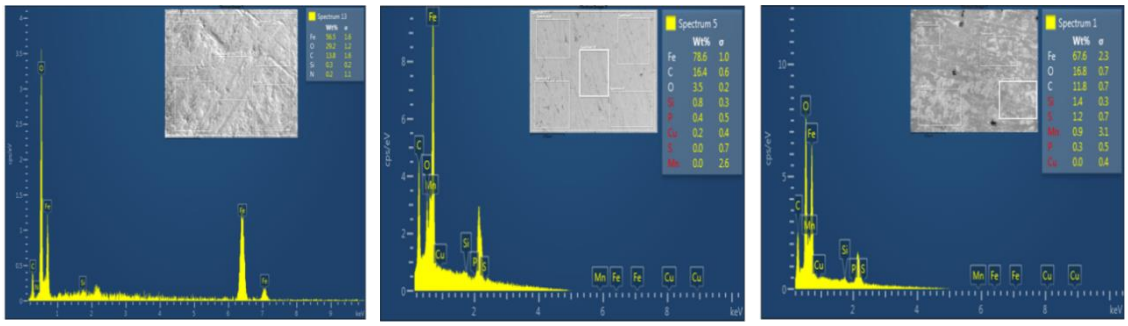
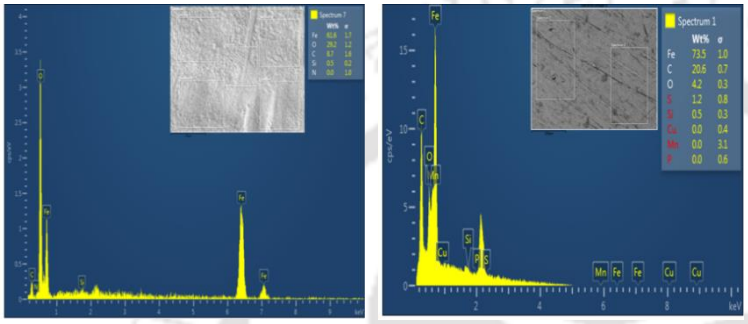


(l) **(m)** **(n)**



(o) **(p)**

Figure 6.4. Elemental analysis of mild steel surface before and after immersion in different biodiesels. MS , (a); MS-NBD, (b); MS-NBD-YPFR, (c); MS-NBD-YPFS, (d); MS-NBD-PPFR, (e); MS-NBD-PPFS, (f); MS-KBD, (g); MS-KBD-YPFR, (h); MS-KBD-YPFS, (i); MS-KBD-PPFR, (j); MS-KBD-PPFS, (k); MS-WCOBD, (l); MS-WCOBD-YPFR, (m); MS-WCOBD-YPFS, (n); MS-WCOBD-PPFR, (o); MS-WCOBD-PPFS, (p)

6.4 Summary

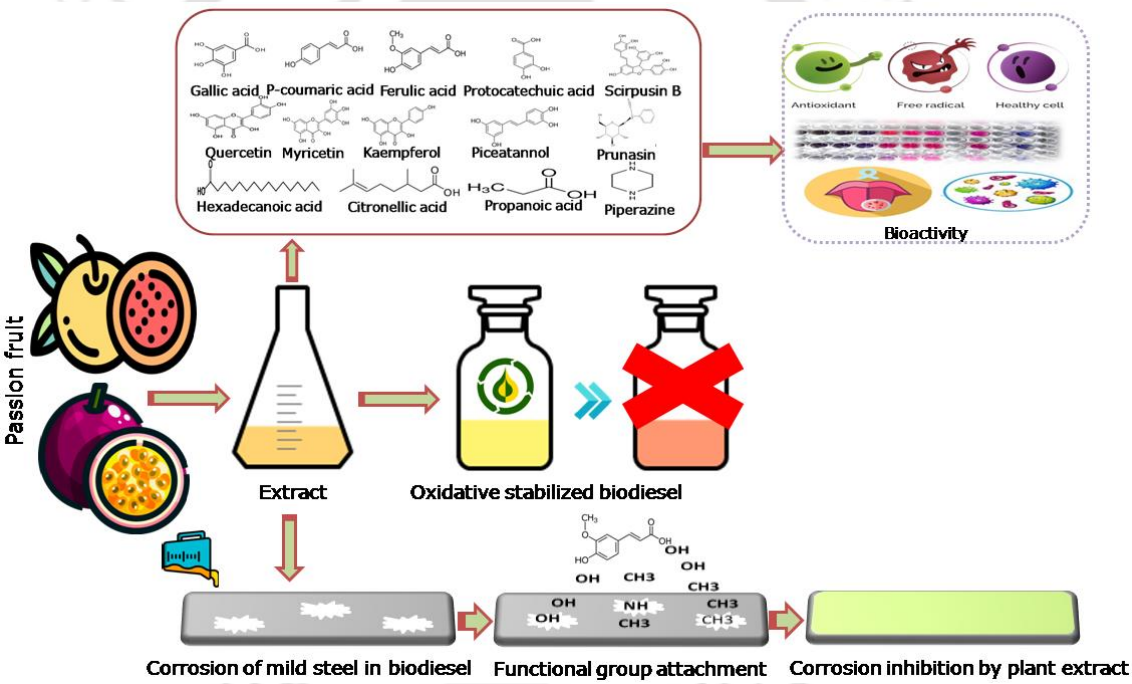
This study discussed the role of various phytochemicals present in the waste rind and seed extracts from YPF and PPF as green corrosion inhibitors. Phytochemicals like 2-hydroxy- Propanoic acid, 2,4-Dithiapentane, Piperazine, Maltol and fatty acids like Palmitic acid, Myristic acid, and Stearic acid were confirmed from GC-MS analysisin all discussed extracts. These extracts significantly reduced the oxidation in the unsaturated fatty acid chains, possibly as primary (by breaking the free radical chains) and secondary antioxidants (by chelating the metal ions). Moreover, these extracts were also found to be potential green corrosion inhibitors as they form a protective layer on the metal surface through nitrogen, oxygen, and carbon present in them, thereby reducing the direct contact of metal to the corrosive biodiesel. The YPFR extract protected mild steel from corrosion in all biodiesel mediums ranging from 71-74%. The use of waste passion fruit by-product extracts can be a one-step solution for enhancing the oxidation stability of biodiesel and reducing corrosion in metal in close contact with biodiesel, thereby increasing the quality of biodiesel considered for commercial application. This study also displaysthe valorization of fruit waste for reducing environmental pollution and add on to the economic development of society.

CHAPTER VII

Overall conclusions and scope for future work

The overall conclusion of the thesis

Scope for future work



Chapter VII

Overall conclusions and scope for future work

This chapter outlines the brief outcome of the thesis work's relevant conclusions, starting fromthe stepwise collection of biomass to its valorisation in different fields, including food, nutrition, health, and renewable energy sectors. The subsequent section of this chapter also outlines some important points for future research in the relevant field.

7.1 Overall conclusions

Valorizing waste biomass is a sustainable approach that helps economic development and a green environment. Research to find novel value-added products from cheap and futile waste by-products has gained popularity among researchers, including pharmacists, environmentalists, and renewable energy scientists. This present study also represents the utilization of passion fruit waste by-products like rind and seed for various applications.

- Two varieties of passion fruit, namely yellow and purple passion fruits, were subjected to various morphological, physico-chemical, and quantitative analyses. The physicochemical analysis revealed that rind could be an excellent source of carbohydrates and vitamin C, whereas the seeds are high in protein. Both the by-products are rich in potassium, calcium, and magnesium, indicating the nutritional value of the waste passion fruit rind and seed. These

nutritious waste by-products can be further utilized for various formulations. In addition, the high oil content in passion fruit seed also manifested its application in food and cosmetics. Various findings of the present work can support this statement. For instance, both passion fruit seed oil varieties contain the highest quantity of unsaturated fatty acids (84-86%), classifying them in the sunflower oil, sesame seed oil, and canola oil category. The oils were thermally stable above 300°C, confirmed by the TGA analysis indicating their usability in the cooking process. Further, both oils are rich in polyphenols like flavonoids and possess appreciable antioxidant activity indicating their applications in many formulations. The study confirmed that passion fruit seed-derived oils are good broad-spectrum antibacterial agents.

- Extraction is an essential technique for understanding the phytochemical profile of any plant matrix. This study demonstrated the extraction of the passion fruit rind and seed using different solvents based on the polarity of compounds to check their antioxidant and antibacterial properties further. Methanolic extracts of rind and seed from YPF and PPF found to have superior polyphenol content compared with other solvents such as acetone, ethyl acetate, and water. HPLC analysis of methanolic and acetone extracts confirmed the presence of different polyphenols like gallic acid, caffeic acid, ferulic acid, quercetin, myricetin, etc. These antioxidant-rich polyphenols further exhibited profound antioxidant and antibacterial activity, indicating their therapeutic values and application in various fields. These findings further encouraged us to isolate and quantify some of the polyphenols from the

rind and seed of passion fruit. A total of four polyphenols were isolated and identified from the rind and seed of yellow passion fruit, namely Prunasin, Piceatannol, Scirpusin B, and Protocatechuic acid. Piceatannol, Scirpusin B, and Protocatechuic acidwere further proved to have superior antioxidant activity against many commercial antioxidant standards suggesting their commercial importance in the field of natural product chemistry. These compounds also inhibited carbohydrate digestive enzymes such as α-amylase and α-glycosidase, confirming their antidiabetic potential.

- Moreover, for the first time, Scirpusin B was found significantly effective against various food-borne and pathogenic gram-positive and gram-negative bacteria. Further, Scirpusin B was also found effective against different oral cancercell lines (Sas and TTn) by inhibiting cell proliferation by 83-95%. Scirpusin B inhibited oral cells by suppressing the cancer hallmark proteins like TNF-α, Survivin, VEGF-A, COX-2, etc. This study indicated thatextracts and purified polyphenols from waste passion fruit rind and seed have a high potential for bioactive pharmaceuticals.

- The reports in literature suggested the high antioxidant potential of passion fruit extracts. Therefore, their useas a natural antioxidant additive to improve the oxidation stability of biodiesel was studiedfurther. Three different oils (Neem oil, Karanja oil, and waste cooking oil) were successfully transesterified, and their conversion from oil to biodiesel (97-98%) was confirmed from ^{1}H NMR. Synthesized biodiesel qualities were (acid value, viscosity, flash point, moisture, iodine number, etc.) in accordance with the

ASTM standards. The GC analysis of biodiesel indicated the presence of the highest unsaturated fatty acid in Neem biodiesel and Karanja biodiesel than in waste cooking oil biodiesel. These biodiesels were found to be thermally stable up to 171^{o}C. Methanolic extracts of passion fruits significantly improved the oxidative stability of biodiesels by 2-3 folds compared to the neat biodiesel. The seed extract of purple passion fruitgavethe best oxidation stability to the Neem and Karanja biodiesel,whilethe rind extract of the yellow passion fruit increased oxidative stability in waste cooking oil biodiesel. The lowest tested dose i.e. 250 ppm extract, was found to enhance two folds oxidative stability in all tested biodiesels confirming all passion fruit extracts can be effectively used as green oxidation stabilizersin various biodiesel.

- Due to oxidation, fatty acid composition, and various environmental conditions, biodiesel becomes corrosive. However, using plant-based phytochemicals as green corrosion inhibitors for biodiesel can be a sustainable approach. For that,various phenolic acids (2-hydroxy- Propanoic acid, 2,4-Dithiapentane, Piperazine, Maltol), fatty acid (Palmitic acid, Myristic acid, and Stearic acid) and aromatic compounds (1,4 Butanediol) were identified from the rind and seed extracts of YPF and PPF using GC-MS. These extracts showed corrosion inhibition efficiency ranging from 24-74%. The rind extract of the YPF showed the best corrosion protective effect (71-74%) on mild steel surfaces for biodiesels (NBD, KBD, and WCOBD) in gravimetric analysis. This extract also drastically lessens the corrosion rate (0.04-0.06 mm/year) compared to the neat biodiesel (0.17-0.23 mm/year) samples. The presence of

corrosion products such as $FeCO_3$, FeO(OH), and Fe_2O_3on the metal surface dipped in neat biodiesel was confirmed by XRD analysis. However, minimal degradation products were noticed in the extract-doped samples due to the formation of protection by various phytochemicals of the extracts. The morphological SEM analysis also confirmed various pits and crack and corrosion inhibition. The change in compositional behavior because of the corrosion in the metal surface was analyzed through EDX analysis. This study confirmed the formation of protective layer on metal surface (due to increased surface carbon weight percentage and decreased oxygen weight percentage) because of the presence of different passion fruit extracts, thereby inhibiting corrosion of metal submerged in other biodiesel.

In summary, the thesis outlines various applications of waste products, such as the rind and seed of yellow passion fruit and purple passion fruit collected from Northeast India. The present research further encourages the waste-to-wealth concept for the economic and sustainable development of the nation.

7.2 Scope for future work

This study emphasizes the characterization of two passion fruit varieties found in Northeast India for their use in nutritional and therapeutic applications. Further, the scope of passion fruit extracts as a natural antioxidant additive for enhancing biodiesel oxidation quality and green corrosion inhibition was explored. However, this study can further be extended for a better understanding related to the following topics.

- More research on passion fruit waste is needed to identify value-added products and their applications in the health and energy sector.

- The extracted passion fruit seed oil could be analyzed for its application in the food, cosmetics, and pharmaceutical sectors.
- Comprehensive research is needed on the optimization and selectivity of SC-CO_2 extraction of flavonoids from passion fruit waste.

- The application of sustainable additives to absorb bio extracts in different biodiesel needs further investigation.

- Mechanism for adsorption of bio extracts on different metals need to be studied to understand their corrosion inhibition properties.

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Appendix

Appendix

Table A.1. Oil quality score (%) of different passion fruit seed oil.

	YPFS	PPFS	Best score	Reference
DPPH (mg/mL)	83.02	128.7	85.16	(Pereira et al., 2017)
ABTS (mg/mL)	65.33	117.5	130	(Pereira et al., 2017)
TPC (mg/g)	0.01	0.05	339000	(da Silva and Jorge, 2017)
TFC (mg/g)	0.06	0.05	103000	(de Santana et al., 2017)
UFA (%)	96.01	98.86	87.59	(Malacrida and Jorge, 2012b)
Acid Value (mg-KOH/g-sample)	36.6	73.33	6	FSSAI
Iodine value (g-I_2/100 g-sample)	122	126.3	104	FSSAI
Peroxide value (mg-eq/ 1000 g-sample)	45	43	10	(Alimentarius, 1999)

Note: Yellow Passion Fruit Seed Manipur (YPFS MP), Yellow Passion Fruit Seed Assam (YPFS AS), Purple Passion Fruit Seed Manipur (PPFS MP). Total Phenolic content (TPC), Total Flavonoid content (TFC), 2,2-diphenyl-1-picrylhydrazyl (DPPH), 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid (ABTS), Unsaturated fatty acid (UFA)

Table A.2. Preliminary Phytochemical Screening of YPFR and YPFS**.**

PhytochemicalName	Name of the test	YPFR				YPFS			
		EA	AT	ME	AQ	EA	AT	ME	AQ
Carbohydrates	Molisch test	+	+	+	-	++	+	+++	-
	Fehlings' test	+	++	++	+++	+	+	+++	-
	Benedicts test	+	+	+	+	+	+	+++	+++
	Tollens phloroglucinol	++	++	+++	+	+++	+	+	++
	Cobalt chloride test	+	+	+	+	++	+	-	+
	Barford test	-	+	-	-	-	-	-	-
	Iodin test	-	+	-	-	-	-	-	-
Glycosides	Keller-killiani test	+	+++	++	+	+	+	+	++
	Borntragers test	+	++	+++	+	+	++	+++	+
	Mod.Borntragers test	+	+	++	+	+	+	++	+
	Legal test	-	-	+++	+	-	-	++	+
	raymonds test	-	-	-	-	-	-	-	-
	Mercury test (3%aqs.)	-	-	-	-	-	-	-	+
	Foam test	-	-	+	+++	+	+	++	+++
	Alkaline test	-	-	-	-	-	-	-	-
Alkaloids	Dragendorff test	+	+	+++	+	+	+	+	+
	Mayer's test	+	-	-	-	-	+	-	-
	Hager's test	+	-	-	+	+	-	-	+
	Wargner's test	+	-	+++	++	+	+	+++	++
Tannins and phenolic compounds	5% $FeCl_3$	+	+	+	+	-	+	+	+
	Lead acetate	+	+	+	+	+	+	++	+
	Acitic acid sol.	-	-	-	-	-	-	-	-

	Dil.iodin solution	-	-	-	-	-	+	++	+
	Dil.HNO_3	++	++	+++	+	-	+	-	-
	Dil.$KMnO_4$	+	+	+++	++	-	-	-	-
Flavonoids	H_2SO_4 test	+++	+	++	+	-	-	+	+
	Lead acetate test	+	+	+	+	+	-	-	+
	NaoH & HCl test	+	+	+	+	+	-	++	+
	Zinc powder test	+	-	+	+	-	-	-	-
Proteins	Biuret test	-	+	+	-	-	-	-	-
	Millions test	++	+	+++	-	-	-	+	+
	Protein containing sulpher	+	+	+	-	-	-	+	-
	Abs.alcohol	-	-	+	-	-	-	-	-
	5% $CuSo_4$	++	+	-	-	+	+	+	+
	5% lead acetate	+	+	+	+	+	+	+	+
	5% (NH4)2So4 test	++	-	-	-	+	+	+	+
Amino Acids	Ninhydrin test	+	+	+++	++	-	+	+++	++
	Tyrosine test	+	++	++	+	+	+	++	+
	Cysteine test	-	-	-	-	-	-	-	-
Steriods	Salkowski reaction	-	-	-	+	+	+	-	+

Note: Qualitative presence (confirmed by extent of change in colour of the solution containing above reagents and plant extracts under suitable conditions) of phytochemicals in different extracts were denoted as; highly present (+++), moderately present (++), less present (+) and absent or not detected (-). EA, AT, ML and WR are ethyl acetate, acetone, methanol and water extracts respectively.

Table A.3. Preliminary Phytochemical Screening of PPFR and PPFS

PhytochemicalName	Name of the test	PPFR				PPFS			
		EA	AT	ME	AQ	EA	AT	ME	AQ
Carbohydrates	Molisch test	+++	+++	+++	+++	+++	+++	-	+++
	Fehlings' test	++	+++	+++	+++	+++	++	-	+++
	Benedicts test	++	++	+++	+++	+++	+++	-	+++
	Barford test	-	-	-	-	+	+	+	-
	Tollens phloroglucinol test	+++	+++	+++	+++	++	++	+	++
	Cobalt chloride test	++	-	++	+	+	-	+	++
	Iodin test	-	-	-	-	-	-	-	-
Glycosides	Keller-killiani test	+	+	+	+	+	++	+	+
	Borntragers test	-	-	-	-	+	++	-	_
	Mod.Borntragers test	-	-	-	-	-	-	-	_
	3% AQS mercury test	++	++	++	++	+++	+++	++	++
Alkaloids	Dragendorff test	++	++	++	++	+++	+++	+++	++
	Mayer's test	++	++	++	+++	+++	+++	+++	++
	Hager's test	+++	++	+++	++	+++	+++	++	++
	Wargner's test	+	++	++	++	+++	+++	+++	++
Tannins and phenolic compounds	5% $FeCl_3$	++	+++	+++	+++	-	++	+++	++
	Lead acetate	-	-	+	-	++	+++	+++	-
	Acitic acid sol.	+	+	+	++	-	+	+	++
	Dil.iodin solution	+	++	++	+++	+	++	++	+++
	Dil.HNO_3	+	+	+	+	-	+	++	++
	Dil.$KMnO_4$	+++	+++	+++	+++	-	+	+	+++

Flavonoids	H_2So_4 test	+	++	+++	++	+	+	++	+
	Lead acetate test	++	++	++	+++	-	-	-	+++
	NaoH & HCl test	++	++	++	+++	+++	+++	++	+++
	Zinc powder test	+	+	-	+	-	-	+	+
Proteins	Biuret test	+	+	+	-	-	-	+	-
	Millions test	++	++	++	+++	++	++	++	++
	Xanthoprotein test	+++	+++	++	+	++	++	+	+
	Protein containing sulpher	-	-	-	-	+++	+++	+++	+
	Abs.alcohol	-	-	-	-	-	-	-	-
	5% $CuSo_4$	++	+	+	+	++	+++	++	+
	5% lead acetate	+++	+++	+++	+++	++	+++	+++	+++
	5% $(NH_4)2So_4$ test	+	+	++	+	+++	+++	+++	+
	5% $HgCl_2$ sol.	+++	+++	+++	++	++	+++	+++	++
Amino Acids	Ninhydrin test	-	-	+	-	-	-	-	-
	Tyrosine test	-	+	++	+	+	+	++	++
	Cysteine test	-	-	-	-	-	+	+	-
Steroids	Salkowski reaction	+	+	+	+	+	++	++	+

Note: Qualitative presence (confirmed by extent of change in colour of the solution containing above reagents and plant extracts under suitable conditions) of phytochemicals in different extracts were denoted as; highly present (+++), moderately present (++), less present (+) and absent or not detected (-). EA, AT, ML and WR are ethyl acetate, acetone, methanol and water extracts respectively.

Table A.4. Retention time (min) of standards and samples.

Phytochemicals	Standards	RT Std	YPFR AT	YPFR ML	YPFS AT	YPFS ML	PPFR AT	PPFR ML	PPFS AT	PPFS ML
	Gallic Acid	3.623	3.727	3.708	3.72	3.718	3.72	3.718	3.724	3.734
	P-Coumaric Acid	3.992	ND	ND	ND	4.16	ND	4.034	ND	4.169
Phenolic Acid	Caffic Acid	4.873	ND	ND	ND	ND	ND	ND	ND	ND
	Ferulic Acid	5.062	ND	5.035	ND	ND	ND	5.009	ND	ND
	Quercetin	12.841	12.626	12.371	ND	ND	12.719	12.435	ND	ND
Flavonoids	Myercetin	9.273	9.476	9.462	ND	ND	9.473	9.422	9.308	9.931
	Kaemferol	18.6	0	18.244	ND	ND	ND	ND	ND	18.225
	Isorhmnetin	19.907	ND	ND	ND	ND	ND	ND	ND	ND

Note: ND= Not detected.

Table A.5. Study of zone of Inhibition of YPF and PPF extracts (mm).

Bacteria	YPFR AT	YPFR ML	YPFS AT	YPFS ML	PPFR AT	PPFR ML	PPFS AT	PPFS ML	Control	P&T (25µg/ml)
EC										
10*	ND	ND	ND	8.5±0.7	ND	ND	ND	ND	ND	
30*	ND	ND	3.5±0.7	13.5±0.7	ND	ND	ND	3	ND	20
50*	4.5±0.7	ND	5	15.5±0.7	ND	ND	ND	5	ND	
KP										
10*	ND	ND	ND	13±1.41	ND	ND	ND	ND	ND	
30*	ND	ND	ND	15.5±0.7	ND	ND	ND	ND	ND	23
50*	ND	ND	ND	18.5±0.7	ND	ND	ND	ND	ND	

EA										
10*	ND	ND	ND	ND	ND	ND	ND	5	ND	
30*	5.5±0.7	ND	ND	7.5±0.7	ND	ND	ND	8.5±0.7	ND	25
50*	9.5±0.7	ND	ND	10.5±0.7	ND	ND	ND	10.5±0.7	ND	
PA										
10*	ND	ND	ND	12.5±0.7	ND	ND	ND	ND	ND	
30*	4	ND	2	17.5±0.7	ND	ND	ND	ND	ND	25
50*	7	ND	5	20±0.7	ND	4±1.41	ND	ND	ND	
SA										
10*	ND	ND	ND	15	ND	ND	ND	ND	ND	
30*	ND	ND	ND	20.5±0.7	ND	ND	ND	ND	ND	36
50*	3.5±0.7	ND	ND	26±1.41	ND	ND	ND	ND	ND	
BS										
10*	ND	ND	5	7.5±0.7	ND	ND	ND	7.5±0.7	ND	
30*	5	ND	10.5±0.7	13	ND	ND	ND	10.5±0.7	ND	30
50*	8.5±0.7	ND	13.5±0.7	15.5±0.7	ND	ND	ND	13.5±0.7	ND	
SE										
10*	ND	ND	ND	22.5±0.7	ND	ND	ND	ND	ND	
30*	ND	ND	ND	30.5±0.7	ND	ND	ND	4.5±0.7	ND	50
50*	4	ND	4	35.5±0.7	ND	ND	ND	6.5±0.7	ND	
ML										
10*	ND	ND	ND	16.5±0.7	ND	ND	ND	ND	ND	
30*	5	7	ND	20	5	ND	ND	4.5±0.7	ND	30
50*	10	9	ND	24.5±0.7	10	ND	ND	8	ND	

Note: ND= Not detected, P&T= Piperelline and Tazobactam. EC= *Escherichia coli,* KP= *Klebsiella pneumonia,* EA= *Enterobacter aerogenes,* PA= *Pseudomonas aeruginosa,* SA=*Staphylococcus aureus,* BS= *Bacillus subtilis,* SE= *Staphylococcus epidermidis,* ML= *Micrococcus luteus.*
* = Different concentration of extracts used for the study (10 mg/mL, 30 mg/mL and 50 mg/mL)
All data are presented as mean ± SD of two different experiments in duplicate.

Table A.6. Extractive values of different fractions of Yellow passion fruit rind and seed.

Parameters	**YPFR**	**YPFS**
Amount of sample	130 g	50 g
Solvent Used	80% Methanol	80% Methanol
Total extract	55.8 g	7.22 g
Extract yield (%)	42.92 %	14.44 %
Extract subjected to Fractionation	50 g	6.5 g
Hexane Fraction yield	330 mg	50 mg
Dichloromethane fraction yield	700 mg	70 mg
Ethyl Acetate (Base) Fraction yield	150 mg	30 mg
Ethyl Acetate (Acid) Fraction yield	1.37 g	1.54 g
Butanolic Fraction Yield	2.73 g	2.68 g
Water Fraction Yield	44.72 g	2.28 g

Table A.7. Zone of inhibition by scirpusin B on different bacterial strains**.**

	Scirpusin B concentration		
Strains	2.5 mg/mL	5 mg/mL	10 mg/mL
E. coli	5.5 ± 0.71	7.5 ± 0.71	12 ± 1.41
K. pneumoniae	6	9.5 ± 0.71	11.5 ± 0.71
E. aerogenes	6.5 ± 2.12	8	11 ± 1.41
P. aeruginosa	5.5 ± 0.71	7.5 ± 0.71	10.5 ± 0.71
S. aureus	3.0 ± 1.41	6.5 ± 2.12	9.5 ± 0.71
B. subtilis	7.5 ± 0.71	10.5 ± 0.71	13
S. epidermidis	7.5 ± 0.71	8.5 ± 0.71	9.5 ± 0.71
M. luteus	8.5 ± 0.71	9.5 ± 0.71	12.5 ± 0.71

Note: Results are presented as mean ± SD of duplicate results of two individual experiments.

Table A.8. Comparative table of oxidative stability exhibited by different plant extracts with the present study.

Extract	Concn. (ppm)	Biodiesel	IP value (h)	Reference
Potato peel	100-250	*Mesea ferra* L.	5.9-7.1	(Devi et al., 2018)
Rosemary, Oregano and Basil	7000	Soybean	9-10	(Spacino et al., 2015)
β-carotene and Curcumin	500	Soybean	6	(De Sousa et al., 2014)
Quercetin, Basil, Oregano and Rosemary	200 mg/mL	Soybean	8-12	(de Sousa et al., 2021)
Thuja	500	Waste cooking oil	8	(Devi et al., 2019)
Green Tea	1000	Waste cooking oil	7	(Bharti and Singh, 2020)
Corn silk	1000	Neem	10	(Ali and El-Anany, 2017)
Indian gooseberry	1000	Karanja	14.2	(Singh et al., 2019)
YPFR	250-1000	Neem, Karanja and Waste cooking oil	5.02-16.05	Present study
YPFS	250-1000	Neem, Karanja and Waste cooking oil	6.27-16.22	Present study
PPFR	250-1000	Neem, Karanja and Waste cooking oil	4.86-16.52	Present study
PPFS	250-1000	Neem, Karanja and Waste cooking oil	8.04-20.84	Present study

Note: YPFR: Yellow passion fruit rind, YPFS: Yellow passion fruit seed, PPFR: Purple passion fruit rind and PPFS: Purple passion fruit seed.

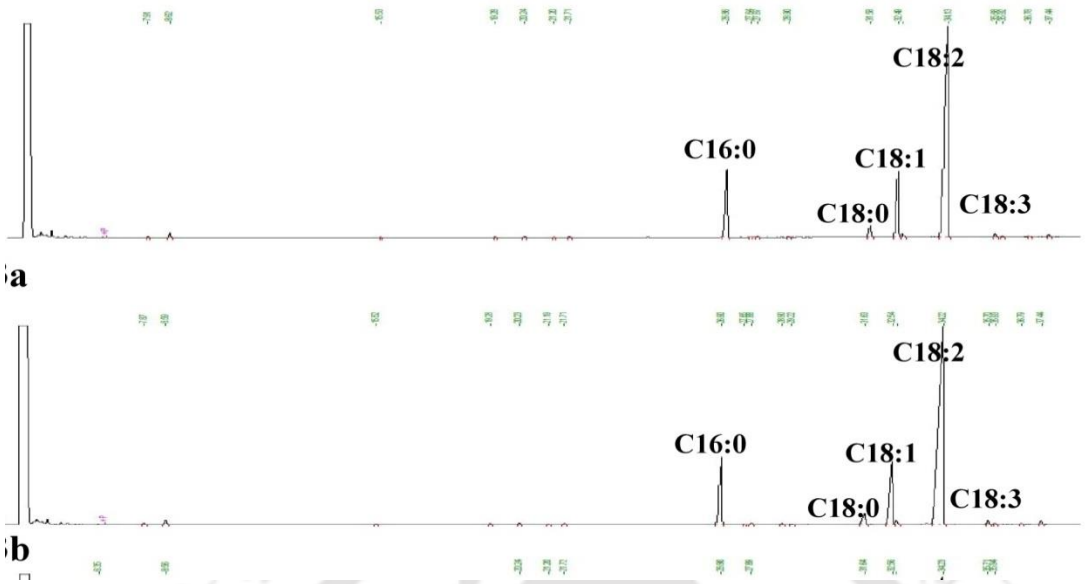


Figure A.1. Gas chromatograms of fatty acid methyl esters from YPFS (a) and PPFS (b) oil.

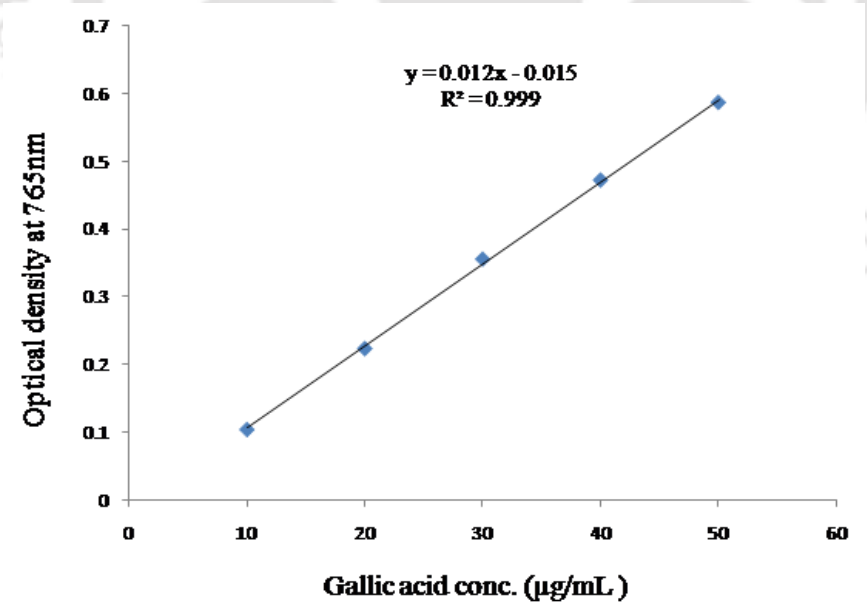


Figure A.2. Standard plot of Gallic acid from 10 to 50 µg/mL for the determination of Total phenolic content.

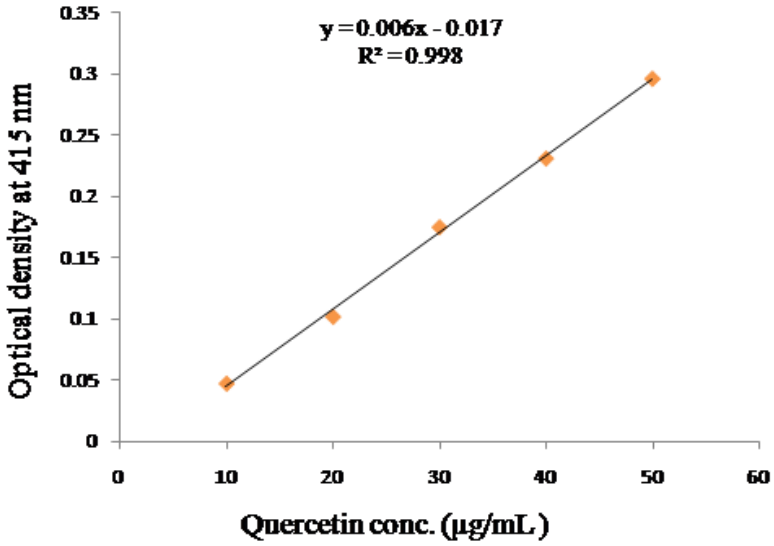


Figure A.3. Standard plot of Quercetin from 10 to 50 µg/mL for the determination of Total flavonoid content.

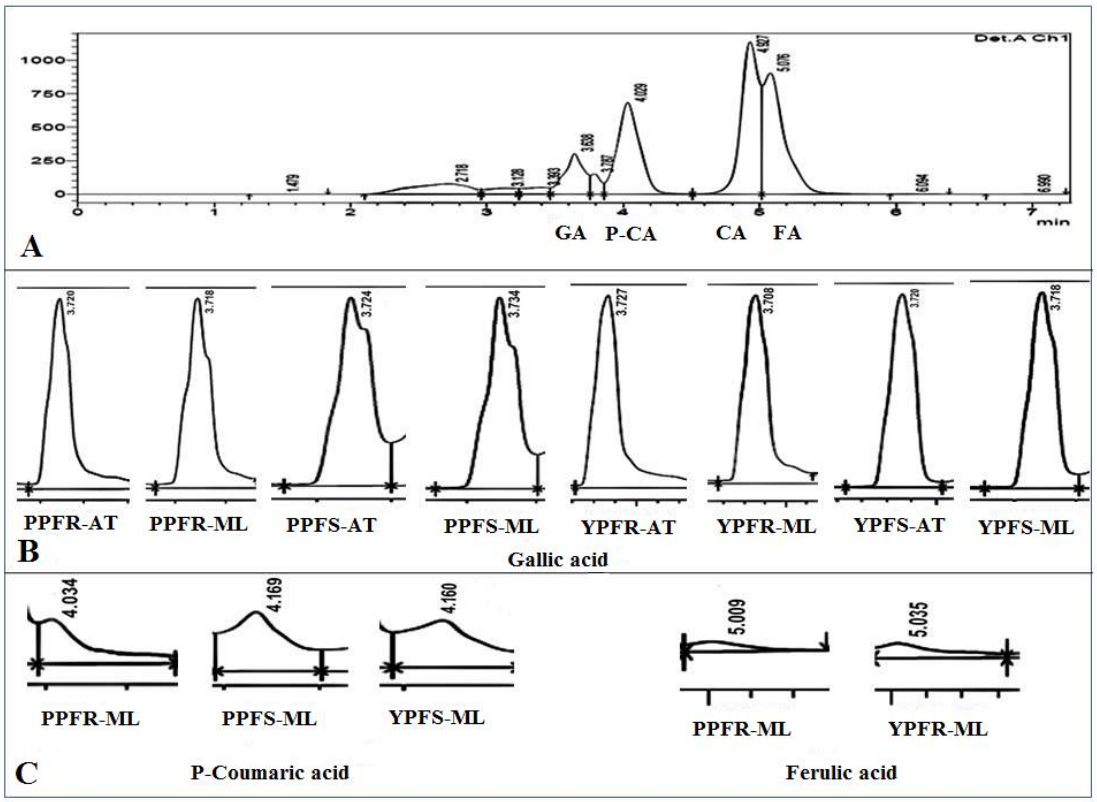


Figure A.4. HPLC chromatogram for identification of phenolic acids. HPLC profile of standards gallic acid (GA), p-coumaric acid (P-CA), caffaic acid (CA) and ferulic acid (FA) have been depicted in panel A. (B) Identification of gallic acid in PPFR-AT, PPFR-ML, PPFS-AT, PPFS-ML, YPFR-AT, YPFR-ML, YPFS-AT and YPFS-ML. (C) Identification of p-coumaric acid in PPFR-ML, PPFS-ML and YPFS-ML and identification of ferulic acid in PPFR-ML and YPFR-ML.

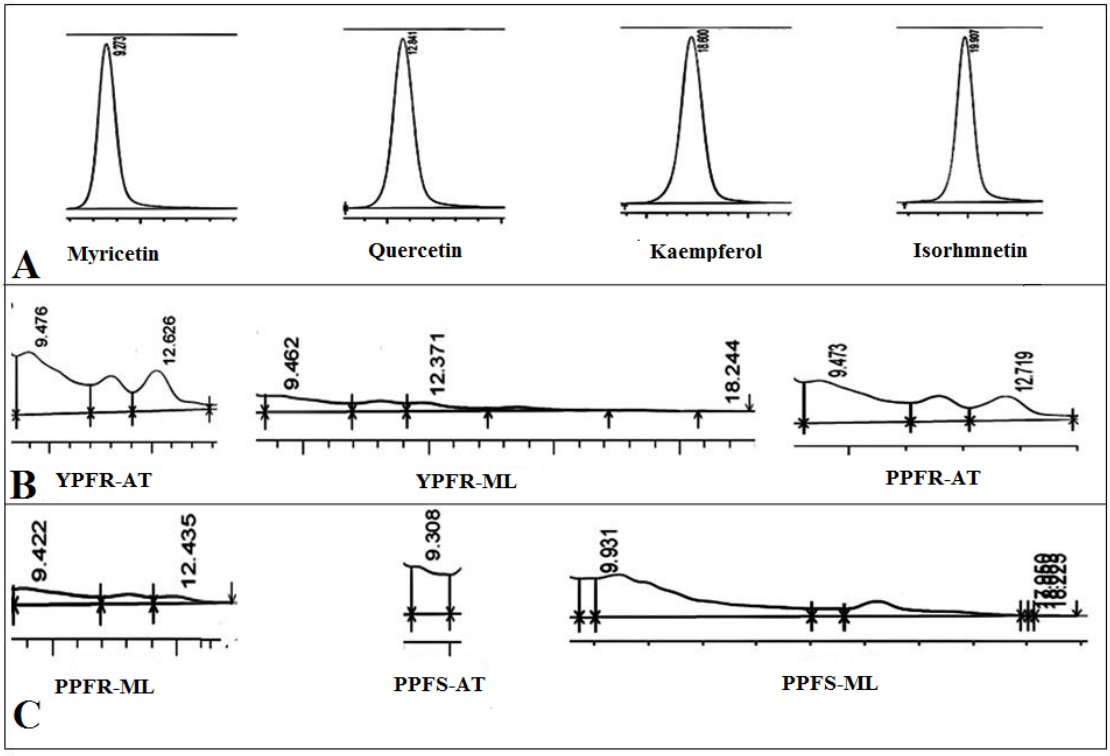


Figure A.5. HPLC chromatogram for identification of flavonoids. HPLC profile of standards myrecetin, quercetin, kaemferol and isorhamnetin has been in panel A. (B) and (C) identification of flavonoid compounds in YPFR-AT, YPFR-ML, PPFR-AT and PPFR-ML, PPFS-AT, PPFS-ML respectively.

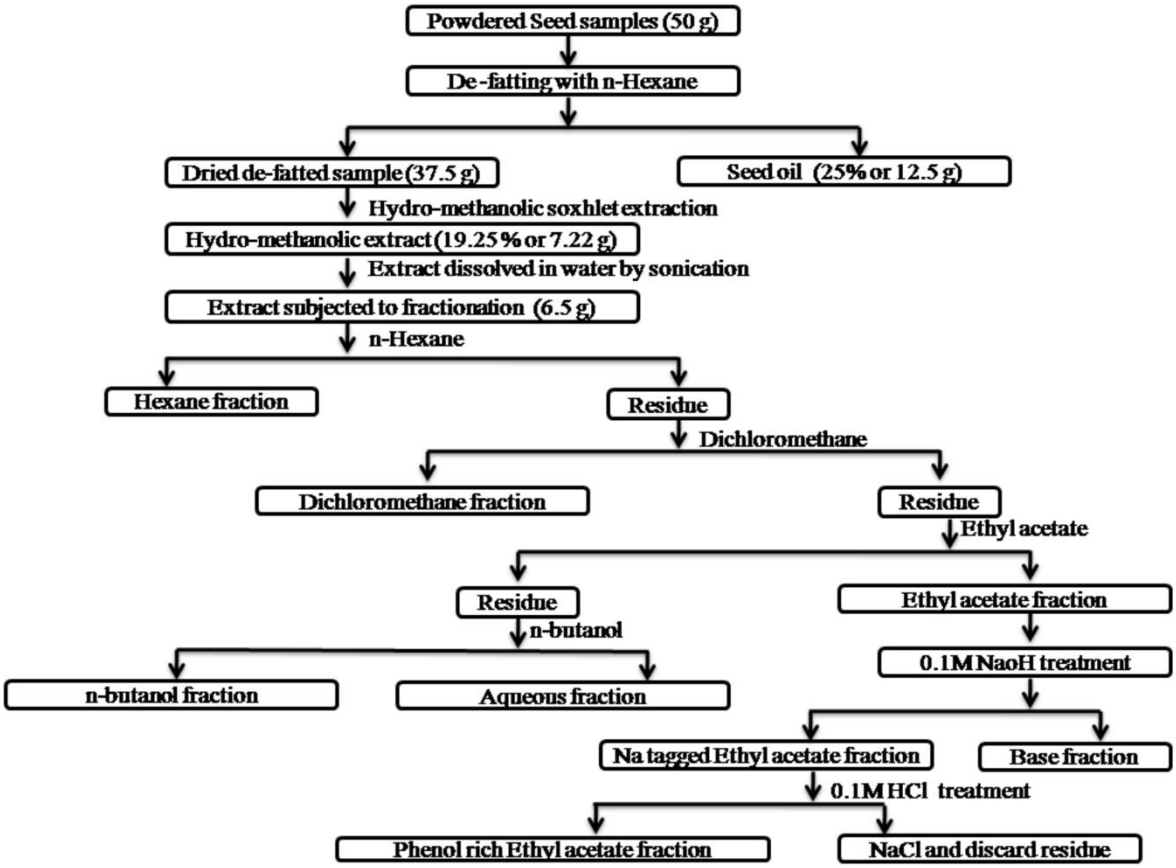
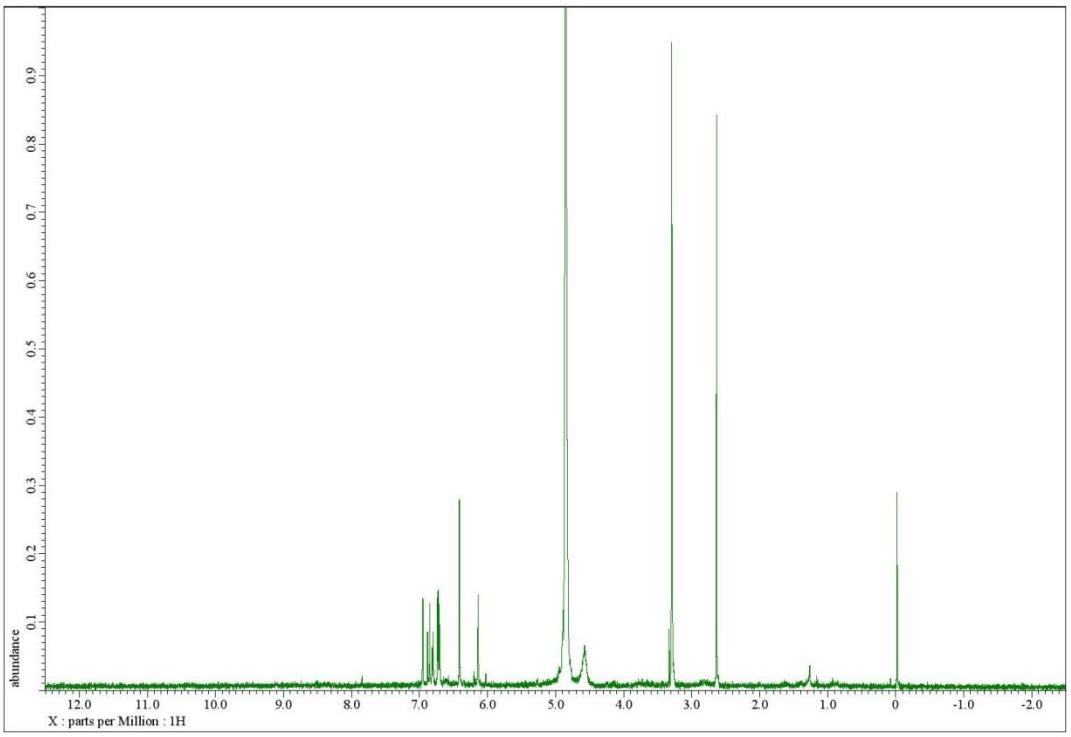


Figure A.6. Schematic diagram of extraction and fractionation of Yellow passion fruit seed.

(i)



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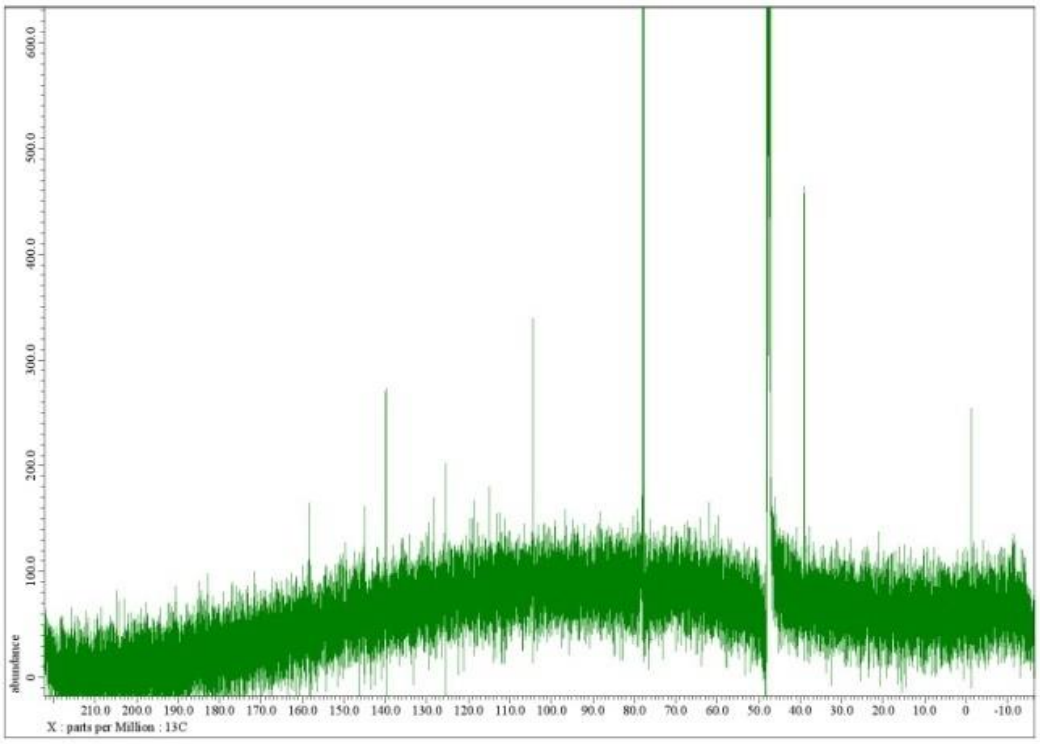

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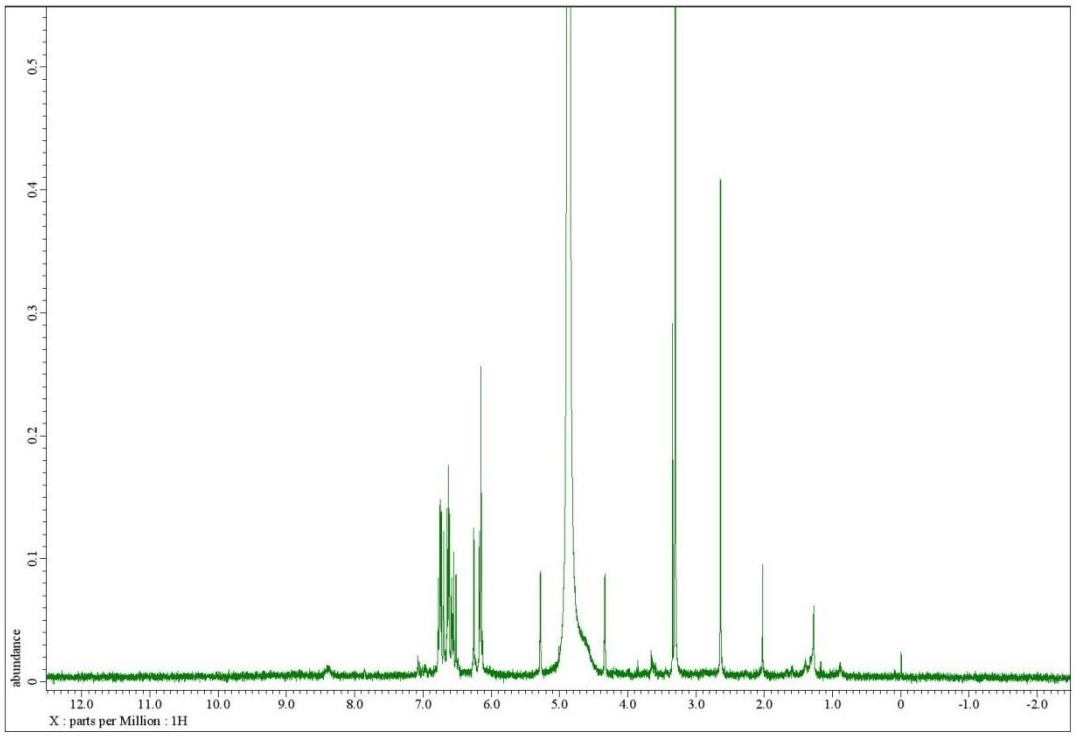
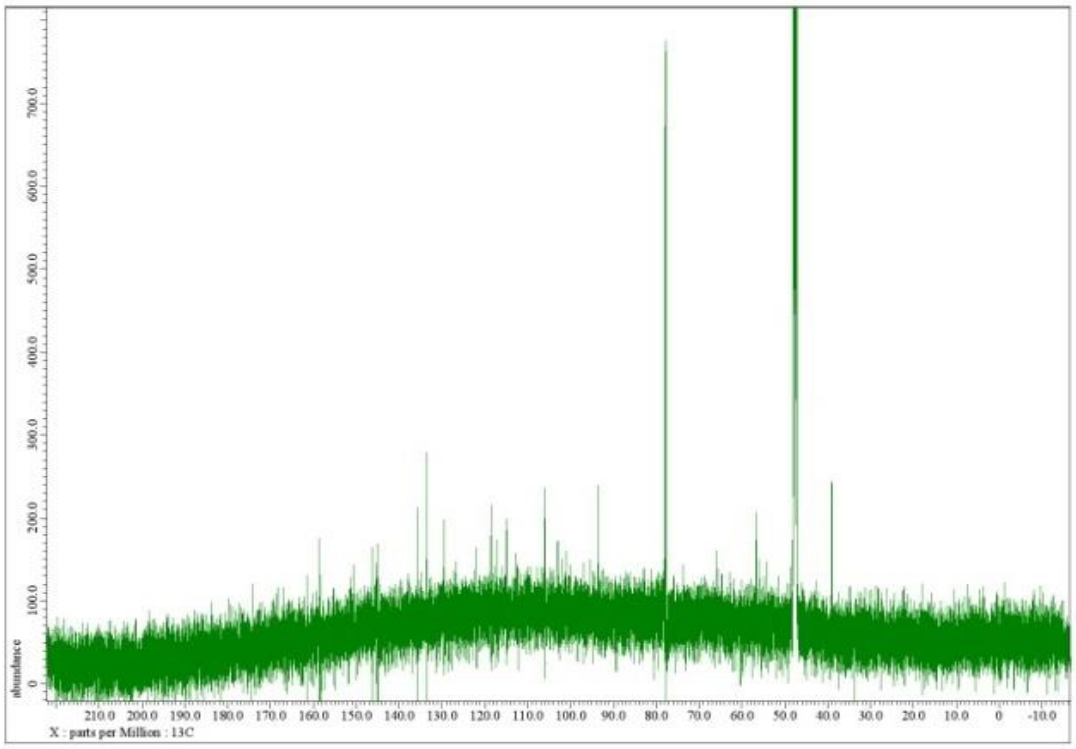
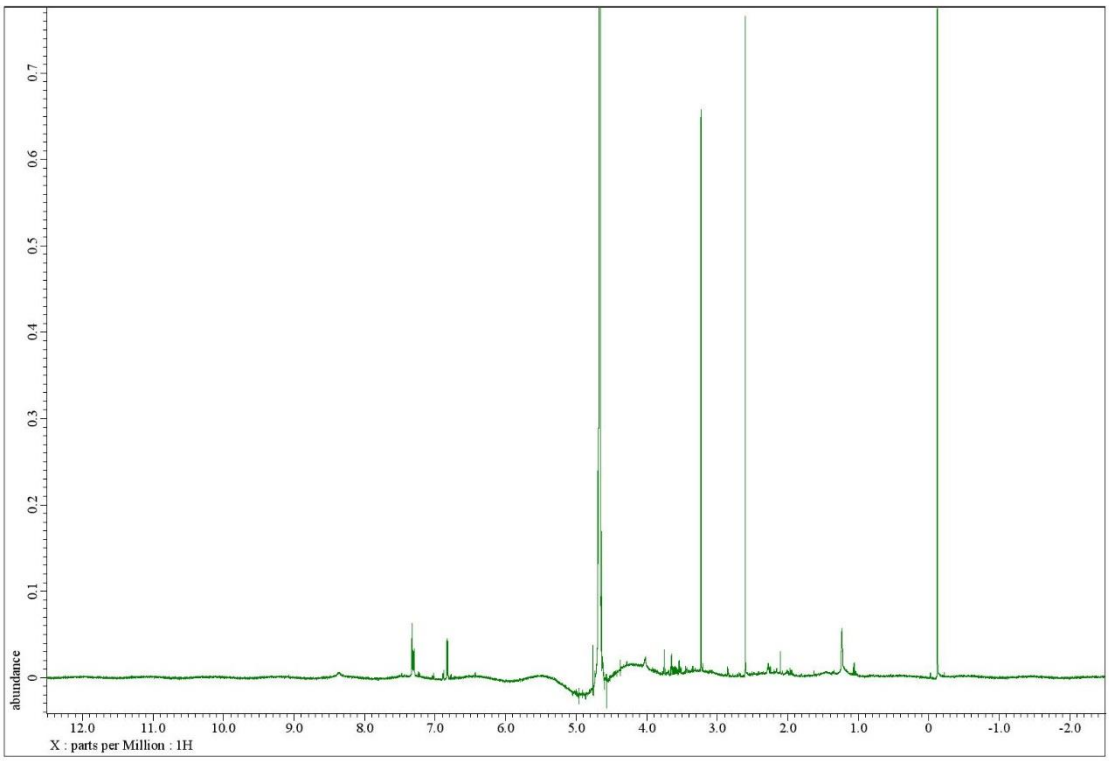


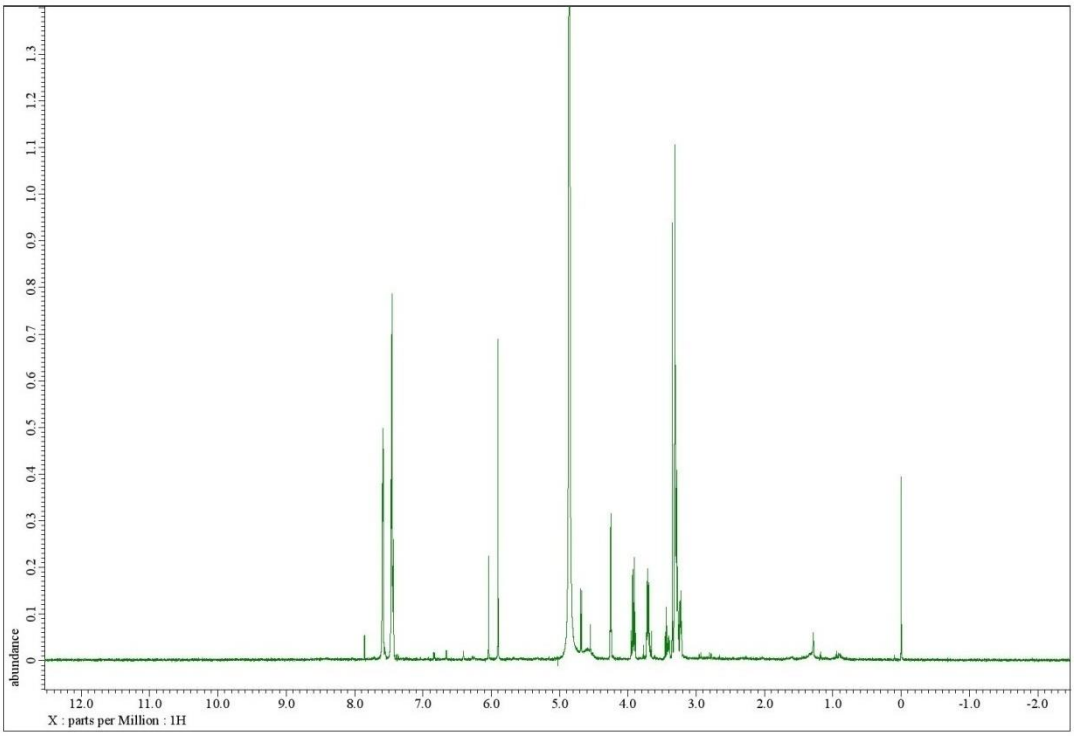
(iv)



(v)



(vi)



(vii)

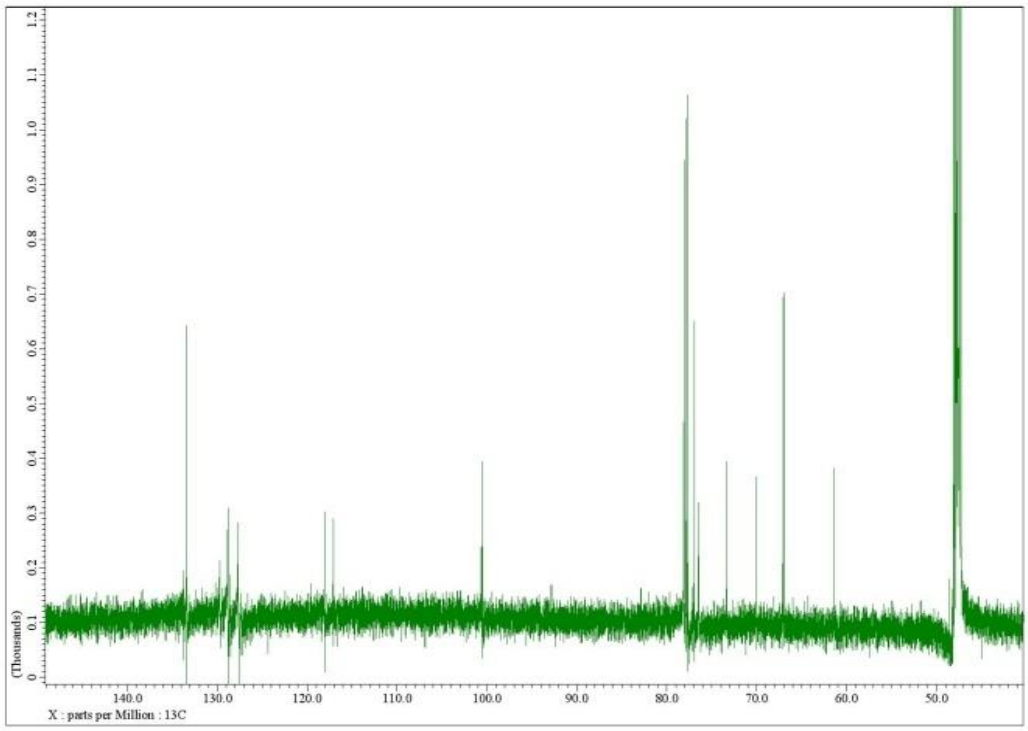
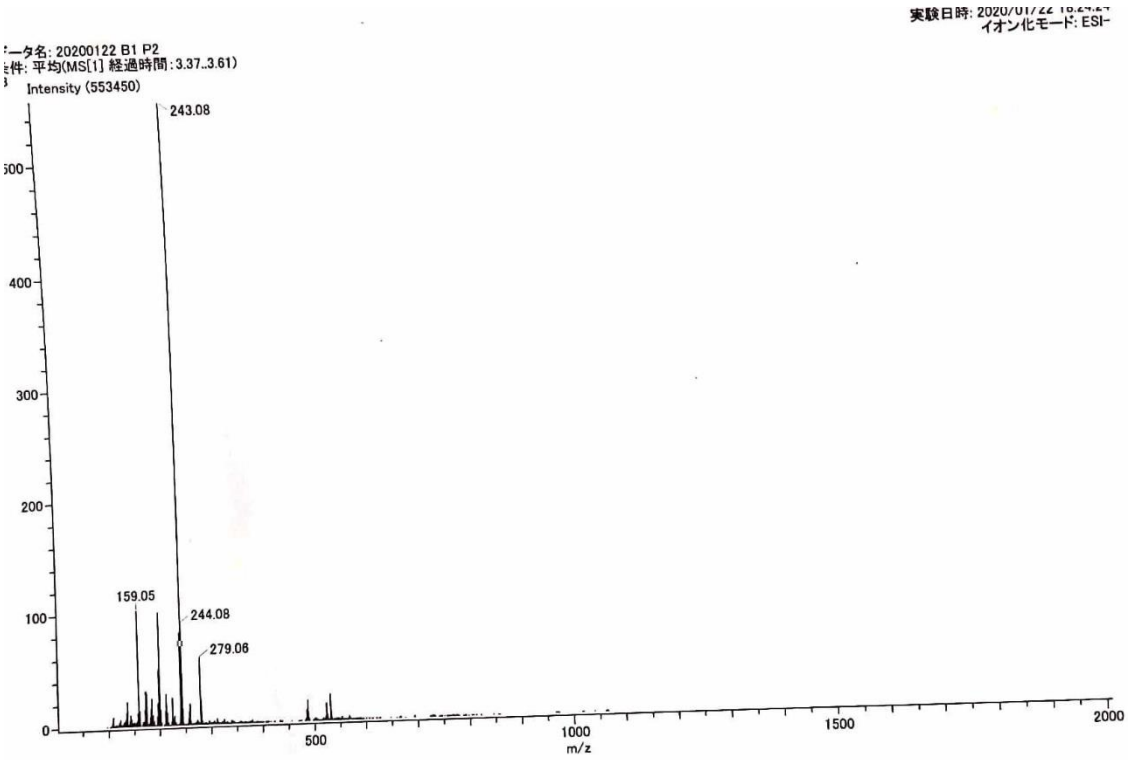


Figure A.7. NMR spectrums of the purified compounds. (i) ^{1}H NMR of Piceatannol, (ii) ^{13}C NMR of Piceatannol, (iii) ^{1}H NMR of Scirpusin B, (iv) ^{13}C NMR of Scirpusin B,(v) ^{1}H NMR of Protocatechuic acid, (vi) ^{1}H NMR of Prunasin and (vii) ^{13}C NMR of Prunasin.

(i)



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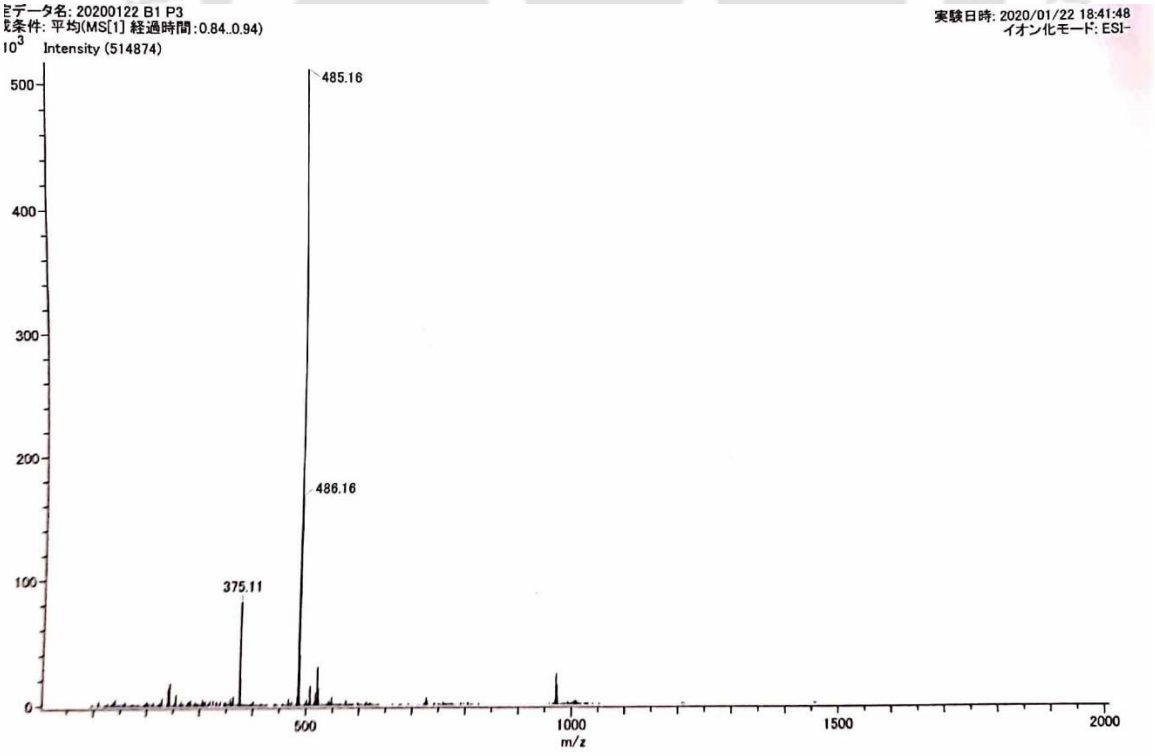
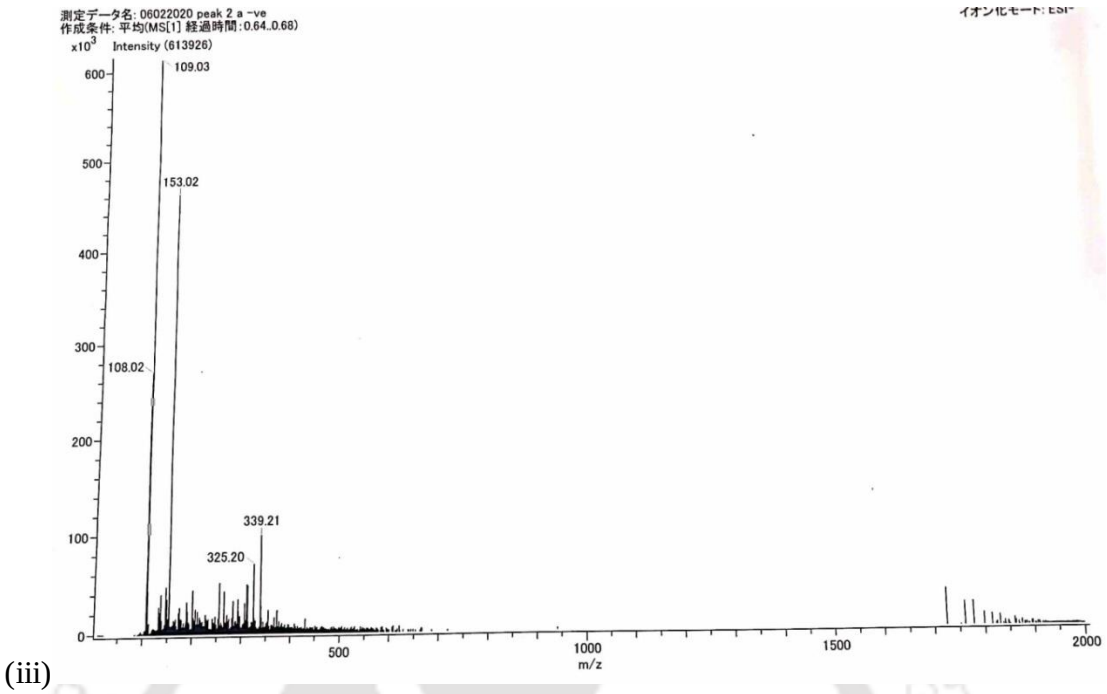
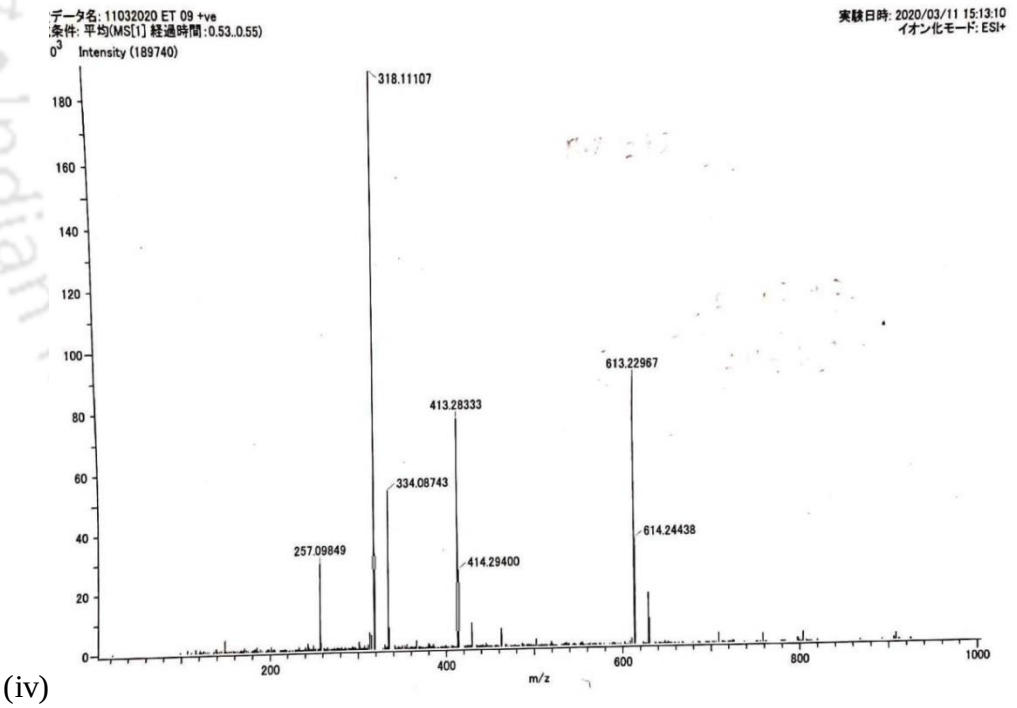




(iii)



(iv)

Figure A.8. Mass spectrum of the purified compounds. (i) ESI^-MS of Piceatannol, (ii) ESI^-MS of Scirpusin B, (iii) ESI^-MS of Protocatechuic acid and (iv) ESI^+MS of Prunasin.

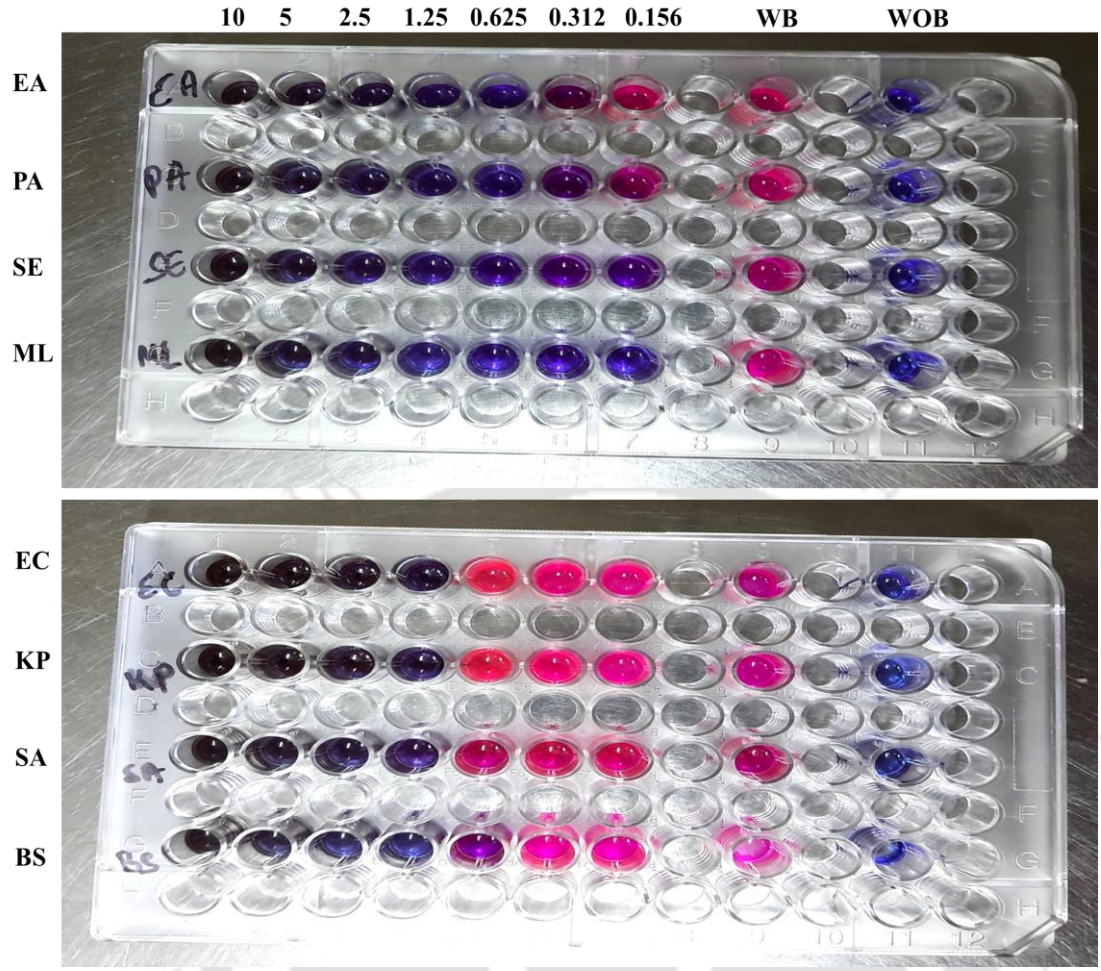


Figure A.9. Minimum inhibitory concentration analysis of scirpusin B on different bacterial strains. The concentration (10 to 0.156) of scirpusin B used in different wells were represented in mg/mL. Different bacterial strains used for the analysis were EA (*Enterobacter aerogenes*), PA (*Pseudomonas aeruginosa*), SE (*Staphylococcus epidermidis*), ML (*Micrococcus luteus*), EC (*Escherichia coli*), KP (*Klebsiella pneumoniae*), SA (*Staphylococcus aureus*) and BS (*Bacillus subtilis*). WB stands for with bacteria where WOB stands for without bacteria (in both these cases, scirpusin B was not added).

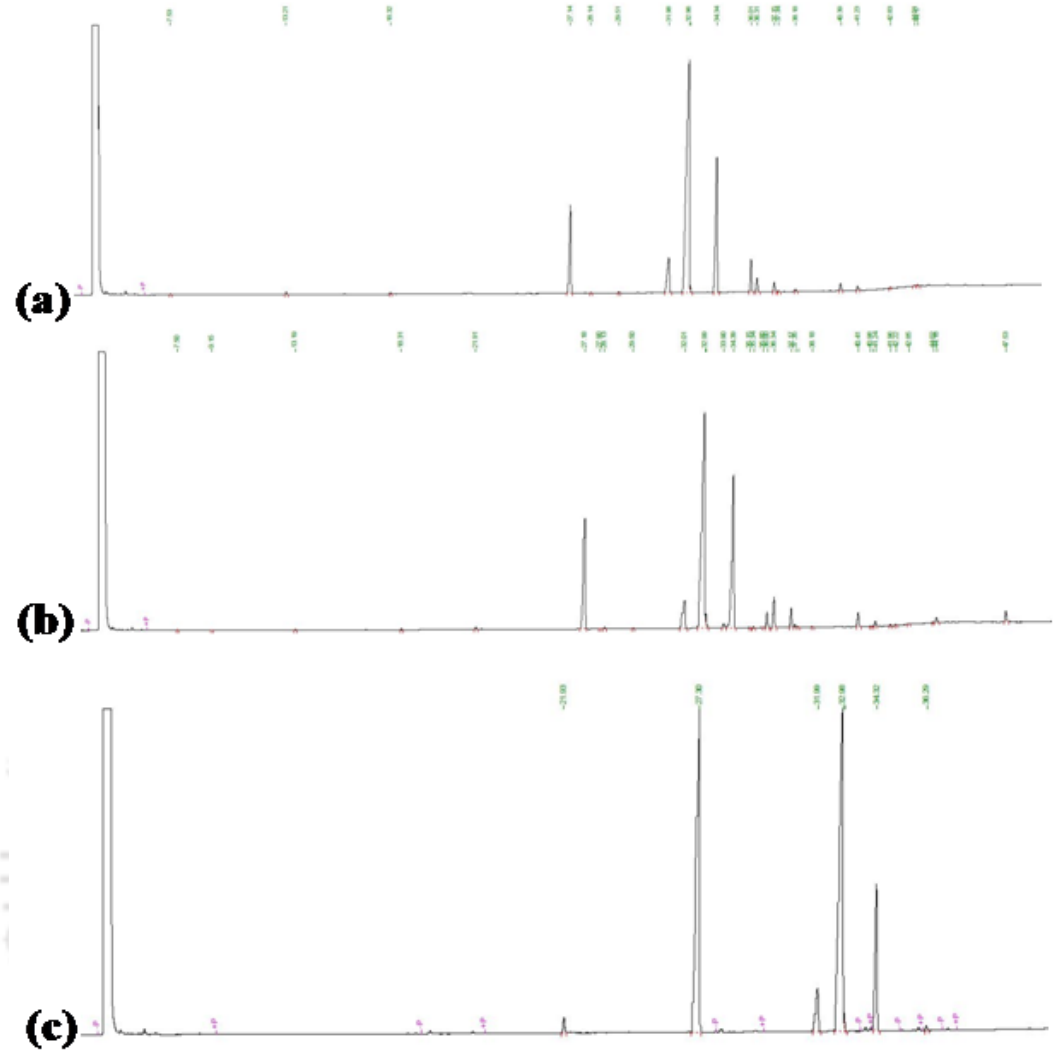


Figure A.10. Gas chromatograms of (a); neem biodiesel, (b); karanja biodiesel and (c) waste cooking oil biodiesel.

Research Output

Research output

Publications from the PhD thesis

1. **Sukumar Purohit,** Chitta Ranjan Barik, Dipsikha Kalita Lingaraj Sahoo, Vaibhav V. Goud "Exploration of nutritional, antioxidant and antibacterial properties of unutilized rind and seed of passion fruit from northeast India" *Journal of Food Measurement and Characterization*, 15, 3153–3167, 2021

2. **Sukumar Purohit,** Dipsikha Kalita, Chitta Ranjan Barik, Lingaraj Sahoo, Vaibhav V. Goud "Evaluation of thermophysical, biochemical and antibacterial properties of unconventional vegetable oil from northeast India" *Materials Science for Energy Technologie*s, 4, 81–90, 2021

3. **Sukumar Purohit,** Sosmita Girisa, Yuto Ocheie, A. B. kunnumakkara, Lingaraj Sahoo, Emiko Yanase, Vaibhav V. Goud **"**Valorization of waste *Passiflora edulis Var. flavicarpa* seeds for isolation of bioactive stilbene and evaluating its potential against carbohydrate digestive enzymes, pathogenic bacteria and oral squamous cell carcinoma**" (Manuscript under review)**

4. **Sukumar Purohit,** Kakali Borah, Lingaraj Sahoo, Vaibhav V. Goud "A systematic review on compositional & bioactive attributes, economic importance, renewable energy & environmental utilization of Passion fruit: A multifunctional fruit" (**Manuscript submitted**)

5. **Sukumar Purohit,** Dipesh Kumar, Lingaraj Sahoo, Vaibhav V. Goud **"**Improving sustainability by enhancing oxidative stability and controlling the corrosive effect of different biodiesel via antioxidant rich passion fruit extracts**" (Manuscript submitted)**

Conference proceeding publication from the PhD thesis:

1. **Sukumar Purohit,** Emiko Yanase, Lingaraj Sahoo and Vaibhav V. Goud "Assessment of biological activities of various phytochemicals isolated from passion fruit bagasse". **Northeast research conclave**, **ISBN 978-981-19-9703-7**

Other collaborative publications

1. Shubham Jain[#]**,** **Sukumar Purohit[#],** Dipesh Kumar, Vaibhav V Goud "Passion fruit seed extract as an antioxidant additive for biodiesel; shelf life and consumption kinetics**"** *Fuel*, 289, 119906, 2021 ([#] equal contribution)

2. Abebe Moges, Chitta Ranjan Barik, **Sukumar Purohit**, Vaibhav V. Goud "Dietary and bioactive properties of the berries and leaves from the underutilized Hippophae salicifolia D. Don grown in Northeast India" *Food Science and Biotechnology*, 30,1555–1569, 2021

3. Vikas Kumar, Jyoti Rawat , Ravichandra C. Patil, Chitta Ranjan Barik, **Sukumar Purohit** , Haardik Jaiswal, Nishchal Fartyal, Vaibhav V. Goud, Ajay S. Kalamdhad "Exploring the functional significance of novel cellulolytic bacteria for the anaerobic digestion of rice straw" *Renewable Energy*, 169, 485-497, 2021

4. Robinson Timung, Chitta Ranjan Barik, **Sukumar Purohit**, and Vaibhav V. Goud "Composition and anti-bacterial activity analysis of Citronella oil obtained by hydro distillation: Process optimization study". *Industrial Crops and Products*, 94; 178–188, 2016

5. Jyoti Rawat, Vikas Kumar, **Sukumar Purohit**, Vaibhav V Gaud, Veena Pande "A novel (M/HA) nano-composite enhances growth and fatty acids profiling in freshwater microalgal strains for efficient biodiesel production (manuscript under preparation)

Achievements

1. Selected as special research student for the six-month research program at GIFU University, Japan, from the period of 01.10.2019 to 31.03.2020.

2. Best paper award (Springer nature) at Northeast Research Conclaveheld from May 20-22, 2022 at IIT Guwahati.

Conference presentations

1. **Sukumar Purohit,** Emiko Yanase, Lingaraj Sahoo and Vaibhav V. Goud "Assessment of biological activities of various phytochemicals isolated from passion fruit bagasse" Northeast Research Conclave, May 20-22, 2022, IIT Guwahati, Assam, India

2. **Sukumar Purohit,** Chitta Ranjan Barik, Lingaraj Sahoo, Vaibhav V. Goud "Green Synthesis of silver nanoparticles using Passion fruit extract for its antibacterial activity" *Technological Innovations for Integration of Food and Health* (TiiFH 2019): A focus on North-Eastern India, February 14-16, 2019, Tezpur University, Assam, India.

3. **Sukumar Purohit,** Chitta Ranjan Barik, Lingaraj Sahoo, Vaibhav. V. Goud "Antibacterial activity of Passion fruit alcoholic extract against various pathogenic bacteria*" 3rd International Conference on Nutraceuticals and*

Chronic Diseases, September 14-16, 2018. Swami Rama Himalayan University, Dehradun, Uttarakhand, India.