Effective edible utilization and toxicological study of value-aided products from fruits residue

Thesis Submitted

by

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PROFORMA- 1

"Statement of Originality"

I ...**Ranjay Kumar Thakur**...registered on.....**04.01.2017**..... do hereby declare that this thesis entitled **"Effective edible utilization and toxicological study of value-aided products from fruits residue"** contains literature survey and original research work done by the undersigned candidate as part of Doctoral studies.

All information in this thesis have been obtained and presented in accordance with existing academic rules and ethical conduct. I declare that, as required by thesis rules and conduct, I have fully cited and referred all materials and results that are not original to this work.

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“Experimenting gives experience and experience develops understanding”

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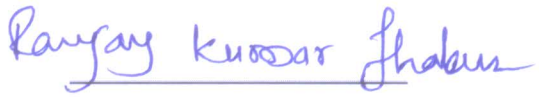

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Dedicated to

my beloved

family

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List of Symbol and Abbreviation

%	Percent
w/w	weight by weight
$	Dollar
pH	-log [H+]
C	Carbon
K	Potassium
Ca	Calcium
N	Normal
ml	milli litre
nm	nano meter
g	gram
mg	milligram
µl	microlitre
^{o}C	Degree centigrade
eV	electro volt
sec	seconds
LD_{50}	Lethal Dose 50%
LD_{25}	Lethal Dose 25%
LD_{75}	Lethal Dose 75%
MSW	Municipal Solid Wastes
FAO	Food and Agriculture Organization
UN	United Nation
WWF	World Wide Fund
p-Hydroxy	para-hydroxy
EAE	Enzyme Assisted Extraction
PEFE	Pulsed Electric Field Extraction
UAE	Ultrasound-assisted Extraction
PLE	Pressurised Liquid Extraction
GAE/g	Gallic Acid Equivalent per gram
ROS	Reactive Oxygen Species
DNA	Deoxyribo Nucleic Acid
HNE	Hydroxy nonenal

MDA	Malondialdehyde
RNA	Ribo Nucleic Acid
COVID	Corona Virus Disease
BHT	Butylated HydroxyToluene
UV	Ultra Violet
PVM	Percentage Volatile Matter
PMC	Percentage Moisture Content
PFC	Percentage Fixed Carbon
PAC	Percentage Ash Content
HHV	Higher Heating Value
WAC	Water Absorption Capacity
OAC	Oil Absorption Capacity
SP	Swelling Power
PS	Percentage Solubility
CV	Calorific Value
SEM	Scanning Electron Microscope
XRD	X-Ray Diffraction
EDS/EDX	Energy Dispersive X-Ray Spectroscopy
IIT-BHU	Indian Institute of Technology, Banaras Hindu University
Aβ	Amyloid beta
HPLC	High-Performance Liquid Chromatography
DMSO	Dimethyl Sulfoxide
n-butanol	normal-butanol
FeCl3	Ferric chloride
H2O	Water
NaOH	Sodium hydroxide
DPPH	2,2-Diphenyl-1-picrylhydrazyl
NIST	National Institute of Standards and Technology
GC-MS	Gas Chromatography-Mass Spectroscopy
BSA	Bovine Serum Albumin
HCl	Hydrogen Chloride
OD	Optical Density
UV-VIS	Ultra Violet- Visible

GAE	Gallic Acid Equivalents
$AlCl_3$	Aluminium chloride
mole L^{-1}	mole per litre
I_2	Iodine
TAA	Total antioxidant activity
TPC	Total phenol content
TAC	Total antioxidant capacity
TFC	Total flavaoid content
RT	Retention time
NCBI	National Center for Biotechnology Information
MIC	Minimum Inhibitory Concentration
TTC	Tetrazolium chloride
h	hour
rpm	revolution per minute
B. subtilis	*Bacillus subtilis*
P. aeruginosa	*Pseudomonas aeruginosa*
LB	Luria bertoni
CT	Calf thymus
TAE	Tris-acetate-EDTA
RBC	Red blood cell
PBS	Phosphate Buffeed Solution
SD	Standard Deviation
HPTLC	High Performance Thin Layer Chromatography
HSCC	High Speed Countercurrent Chromatography
TLC	Thin Layer Chromatography
PC	Positive Control
NC	Negetive Control
EE	Ethanol Extract
AE	Aqueous Extract
ME	Methanol Extract
PEE	Petroleum Ether Extract
BE	Butanol Extract
AB	Antibody

Chapter 1

Introduction

1.1 Fruit

In angiosperms, the seed-bearing fleshy structure known as fruit originates from the ovary of the flower and is typically characterized by a sweet or sour taste, making it edible in its raw state or as a processed product.

Fruits are often referred to as the "Treasure house of nature," and their importance in our diet has led to a steady increase in demand for these essential food items. Adoption of fruits has increased dramatically as dietary habits change and the world's population expands, especially in middle-income nations that are experiencing rapid economic expansion. (Schieber*et al*., 2001). Large areas of land are dedicated to fruit production to meet consumer demand, a practice that has been expanding globally. In 2011, the total fruit harvest amounted to 640 million tons, and it is projected to reach 870 million tons in 2018 (Romelle*et al*., 2016; FAO, 2021).

Fruits are an essential source of dietary fiber, vitamins (especially A and C), and various minerals that play a crucial role in preventing vitamin deficiencies. Increased devouring of fruits may mitigate the threat of acquiring a number of chronic diseases, according to numerous epidemiological studies. Including various fruits in a daily healthy diet is crucial because no fruit or vegetable can provide all the essential nutrients required for a healthy life. Earth offers a wide range of fruit varieties, each containing different beneficial compounds that can be processed and served in various ways to fulfil our body's nutritional requirements. Consuming at least five different types of vegetables and two kinds of fruit daily is essential for good health.Fruits are a significant source of essential nutrients, such as folic acid (folate), potassium, and polyphenols, which possess antioxidant and antimicrobial properties. Including fruits and vegetables in your diet can have a favorable impact on blood sugar levels, heart health, blood pressure, the risk of neural tube defects, protection against cell damage, and digestion issues. It also reduces the risk of heart disease and the possibility of having a heart attack. Due to their lower glycemic loads, fruits help prevent blood sugar spikes and increase satiety, making them beneficial for weight management (Bertoia*et al*., 2015).

With their nutritional richness and health-promoting properties, horticulture crops, particularly fruits, and vegetables, have become increasingly popular. As demographics change and nutritional preferences shift, more people are involved in producing and preparing these items, especially fruits and vegetables. Consumption of fruits yields residual waste that contributes significantly to the Municipal Solid Wastes (MSW).

1.2 Fruit wastes

Food waste is a significant global problem, and fruits and vegetables contribute to a substantial portion of this waste. According to research by FAO (UN International, 2022), approximately 14-17% of the food produced worldwide is wasted annually. More recent estimates by Tesco and WWF (World Wide Fund for Nature, 2022) suggest that 2.5 million tonnes of food are wasted each year, with over 40% of total food being uneaten globally. Fruits and vegetables account for a significant proportion of this food waste, with 46% of wastage attributed to residues from these food items. Throughout the production and supply chain of fruits and vegetables, various factors contribute to their wastage. Pests and diseases in cropped fields, ineffective harvesting, storage, and shipping techniques lead to a considerable amount of fruit losses. Additionally, shops, supermarkets, and homes also contribute to fruit waste, discarding approximately 35% of the fruits produced intentionally (The World Counts, 2022).

Some fruits are more likely to be discarded than others, with bananas being the most likely because of things like brown marks and minor bruising (Mattssonet al., 2018). Amazingly, 3.7 trillion apples are discarded annually worldwide, and large quantities of other common fruits, such as lemons, pineapples, watermelons, oranges, and bananas, contribute to the annual waste of 25-57 million tons (Leong *et al*., 2022).Fruit waste can be categorized into two types: solid waste, including peels, seeds, and stones, and liquid waste, which consists of wash water and juice generated during fruit processing. Fruit peels, which are frequently thrown away, make up a sizeable amount (15–60%) of a variety of fruit waste (Zhang *et al*., 2020). A considerable percentage of certain fruits, like bananas, mangoes, pineapples, and oranges, are also wasted due to consumption habits and the food industry's processing practices.In developed and developing cities, the consumption and processing of fruits lead to the generation of large quantities of fruit residues, including seeds, peels, leftovers, rinds, bagasse, pomace, and cut-offs of whole

fruits. This emphasizes the need for more effective strategies to manage and reduce fruit waste to address the environmental and economic implications of this global issue. The different type of fruit wastes and chemical components are described in the following section.

1.2.1 Chemical composition of various fruit residues

The residues (peels, seeds, pomace, stones, etc.) of numerous fruits, including banana, mango, apple, orange, pineapple, pomegranate, and watermelon, which constitute the significant proportions of these fruits (Table 1.1), contain the majority of the necessary nutrients in appreciable quantities and can be used as good ingredients in the production of food products with health benefits (Romelle et al., 2016).

Table 1.1 A proximate composition of the dry weight base (% dry weight) of various fruit wastes.

Fruits	Fruit Wastes	Moisture Content	Crude Protein	Crude Fat	Total Carbohydrate	Crude Fibre	Ash
Mango	Peels	3.94	5.00	4.72	63.80	15.43	3.24
	Stones	7.94	9.20	10.92	74.18	3.26	2.44
Apple	Peel	3.05	2.80	9.96	59.96	13.95	1.39
	Pomace	10.60	5.67	3.90	62.00	51.1	6.10
Orange	Peel	9.50	9.73	8.70	53.27	14.19	5.17
	Seeds	6.25	6.13	12.00	-	5.30	2.00
Grapes	Pomace	3.37	8.49	8.16	29.20	46.17	4.68
Pineapple	Peel	6.29	5.11	5.31	55.52	14.80	4.39
	Pomace	3.77	4.71	0.61	43.46	45.22	2.24
Pomegranate	Peel	5.54	3.46	3.36	59.98	17.63	6.07
	Pomace	6.98	9.11	0.61	58.52	20.66	4.12
Banana	Peel	50.50	5.30	1.60	6.65	19.20	8.80
Tomato	Peel	5.71	13.30	6.01	-	86.15	3.01
	Pomace	8.10	19.27	5.85	25.73	59.03	4.14
Watermelon	Peel	9.82	12.42	12.61	32.16	26.31	5.03
	Seeds	3.08	21.44	-	19.45	-	1.0

Adopted from Romelle*et al.,* 2016, Teshome*et al., 2023,* Inmaculada*et al.,* 2011, Valle *et al.,* 2006, Alsubhi*et al., 2022*, Oikeh*et al.,* 2013, Selani*et al.,* 2014, Zubairu*et al.,* 2014, Mutua*et al,* 2017, Hassan and Peh, 2018).

Mango: Depending on the mango fruit variety, biochemical composition differs. Mango fruit wastes are frequently high in carbohydrates, including glucose, fructose, and pectin. Additionally, the wastes contain a sizable amount of crude protein, fat, fiber, and ash. Ripe mangoes have greater moisture compared to unripe fruit. Mutua*et al*. (2017) found that the moisture content of mango stones varies significantly by region, while the protein content ranges from 6.74 to 10.20%, the fat content is close to 10.09%, and the ash content is minimal.

Apple: The main by-products of the apple juice industry, apple peels and pomace, are nutrient- and bioactive-rich. Apple pomace, a plentiful byproduct of pressing and crushing apples to make juice, is obtained. As seen in table 1.1, the apple fruit's peels and pomace both contain considerable amounts of dietary protein, fat, and ash in addition to an abundance of dietary fiber, carbohydrates, and ash. A lot of variation in apple pomace composition was observed, primarily due to apple cultivar, harvesting, and processing (Antonic*et al*., 2020).

Orange: Citrus fruits are the most produced fruits and have a tremendous economic worth. For the sweet fruit, which is peeled or chopped (to avoid the bitter rind) and eaten whole or processed to obtain orange juice, orange trees are frequently cultivated in tropical and subtropical climes. Between 50 and 65 percent of the weight of citrus fruits are made up of the peels. If not further treated, this by-product turns into a very dangerous waste that can seriously harm the environment. The orange fruit is particularly significant economically since all parts—peel, seeds, albedo, and flavedo—have an assortment of possible commercial applications. Orange fruit wastes contain a variety of potentially beneficial nutritional elements, as seen by the close composition of orange peel and seed extracts (Oikeh*et al.*, 2013).

Pomegranate: The pomegranate (Punicagranatum) is a food that is high in phytochemical components and is nutrition dense. Popular uses for pomegranates include fresh fruit, juice, beverages, culinary items, and extracts. According to several studies, phytochemicals have been discovered in the peel, juice, and seeds of pomegranate fruit as well as in other components of the pomegranate tree (Elfalleh*et al*., 2011). The by-product of making pomegranate juice, pomegranate pomace, contains a variety of highly

nutritious ingredients. Pomegranate fruit peel is an inedible byproduct of pomegranate juice production. According to Table 1.1, both pomegranate peels and pomace are regarded as good sources of crude fibers, ash, carbs, crude protein, and crude fat. Therefore, pomegranate fruit peels, pomace, and seeds should be used for food fortification and other purposes. (Rowayshed*et al.,* 2013).

Banana: One of the top ten most important crops is the banana, the biggest herbaceous plant in the world and the fourth most harvested crop worldwide. But wastage, primarily in the form of peels, makes about 40% of this banana production. Peel is a waste material that can be obtained in significant amounts; however, the applications of peel depend on the chemical compositions of the material. Furthermore, the nutritional value of banana peels may be higher. Due to the significant amount of water in peels, they are extremely perishable. Additionally, both the pulp and the peel of these fruits are rich in a variety of vitamins and minerals (Hassan and Peh, 2018). Banana peels contain considerable amounts of carbohydrates, dietary fiber, crude fat, protein, and nonvolatile minerals, as seen in Table 1.1. These nutrients are abundantly available, making it possible to utilize them in the development of products with added value.

In addition to the above mentioned fruits, additional fruits including grapes, pineapple, watermelon, tomatoes, etc. generate a significant number of wastes in various forms, such as peels, seeds, leftover pulp, and many more.Each one have a adverse effect on the ecosystem. However, these wastes contain significant amounts of valuable nutrients like vitamins, minerals, dietary fibre, carbohydrate, protein, and fat that can be reused by using various methods to increase dietary acceptability and improve health outcomes.

1.2.2 Fruit peels

Peel is the outermost protective covering of fruit which protects the inner fleshy part from adverse environmental effects and also from micro- and macro-organisms. In some fruits, it is also known as skin or rind. It may remain firmly attached to the inner fleshy part as in an apple, or berry or loosely attached as in banana, orange, and others. Fruit peels may have a neutral taste and be edible (orange, grapes) or bitter and inedible (banana, papaya). Peels of watermelon are called tough rind, outer layer, and inedible. The majority of the fruit delivery byproducts that are thrown away are peels (Ajila*et al*., 2010). About 15–

20% of the weight of the mango in fresh fruit is made up of peels (Ajila *et al.*, 2013), Nearly 30% of the total mass of the pineapple is made up of it (Aparecida *et al.*, 2016), in mosambi it is 38-48%, 35-40% in malta, 35-41 % in grapefruit and it is about 50% total weight of pomegranate (Ahmad *et al.*, 2016). The pomace of tomato consists of about 40% tomato skin during processing. 43% (w/w) of pomegranate is wasted as rid.

1.2.3 Fruit Seeds

Seeds are one of the most important parts of fruits. It is units of reproduction are generally oval in shape. A significant amount of the weight of fresh fruit is made up of seeds, which are extensively thrown as wastes during fruit processing. Different kinds and amounts of seeds are produced by diverse kinds of fruits which ultimately affects their viability. It is estimated that seeds constitute approx 13.5% of the total weight of fresh mango, it is 6.51% of diced papaya, and in sliced apples, it is about 10.91%ofg with pulp (Joshi *et al.*, 2012). About 60% of pomace constituted by their seeds are wasted during processing (Ruiz *et al.*, 2009). Tomato seeds have a significant nutritional value and they constitute about 32% of protein, 27% of total fat, and 18% fioffiberduetoth r high protein content it has been utilized as an animal feed supplement (Gebeyew*et al.*, 2015). 11% (w/w) part of pomegranate is discarded as seed residues. Litchi seeds are the major by-product produced during processing.

Table 1.2 Nature and percentage weight loss of various fruit wastes

Fruits	Nature of wastes	% Weight loss
Mango	Peel, stones	15-20
Apple	Peel, pomace, and seeds	12-47
Orange	Peel, rag, and seeds	3
Grapes	Stem, skin, and seeds	3-58
Pineapple	Crown, peels, core	80
Pomegranate	Rid and seeds	54
Guava	Peel, core, pulp leftover, and seeds	30
Banana	Peel	40
Tomato	Skin, core, and seeds	5-25
Litchi	Seeds and peels	30-40

(Gera and Kramer, 1969, Gupta and Joshi, 2000, Joshi *et al.*, 2012)

1.2.4 Residual Pulp

During the processing of various fruits, some part of pulp remains unused and it is thrown as waste along with peels, pulps, seeds, and others. For example, during the extraction of juice from apples, wastage of large amounts of pulp along with other residues is thrown as pomace (Cargnin and Gnoatto, 2017). In the case of various citrus fruitsand in berries (grapes,black berriesetc.), which is the largest source of juices,wastage of pulp in excessive quantities takes place. About 5-10% pulp or leftoverof juice remained useless and wastage as a residue during extraction of juice from mango. This residual pulp contains a huge amount of biologically active organic compounds and can be reutilized for different purposes by using suitable techniques.

1.2.5 Pomace

It is a residual part or by-product of fruits obtained after processing of fruit like pressing of grapes, or extraction of juice frapplesple and other citrus fruit. It mainly consists of seeds, peels, left-over pulp, st,alks etc. (Corrado *et al.*, 2023). These wastes have no value and are generally disposed by landfilling, composting or by incineration. However,thenutritional value of pomaces is very high as it constitutes pcarbohydratesbohydrate, lignin, pectin, phenolic compounds (antimicrobial, antioxidant) and various fermentative substrates (Corrado *et al.*, 2023). Key wastes after processing of apples is pomace (about 30% of fresh fruit),which contains 2-4% seeds , 90% peels, and 1% stem (Bhushan *et al.*, 2008). Excess quantity of pomace as peels and stone released the production of plum juice. High amounts of wastes as pomace (peels, flash, seeds, stones) are produced as by-products from sour cherry industries. Phytochemicals present in these wastes have anti-incendiary, antimicrobial and antioxidant behaviour (Yılmaz, *et al.*, 2019).

1.2.6 Stones

Mainly stone fruits are those which consist of pulp (fleshy mesocarp) protected by endocarp (wood-like) or stone. Epicarp (skin) is smooth and thin tiny hair like coatings. It mainly includes peach, apricot, cherry, plum etc. The pomace (residual parts) of processed stone fruits mainly consists of skin, seeds and left-over pulp, which contains high quantities of effective valuable bioactive compounds (Coman *et al.*, 2020). Besides the above mentioned fruit residual parts there is so many other fruit

parts like stems (apple, grapes) rind (pomegranate, mangosteem, passion fruit) kernel(mango, apricot, almonds, cherries) also discarded as residue after processing and became the part of municipal solid wastes. However, these all mentioned fruit residual wastes has plentiful amount of nutritional components, dietary fibres, polyphenols, antioxidants and others which promotes the health of consumers and also plays vital role as antimicrobial, anti-fungal, ant-tumour , anti-inflammatory and helps in prohibition of various abnormalities by keeping us safe and healthy.

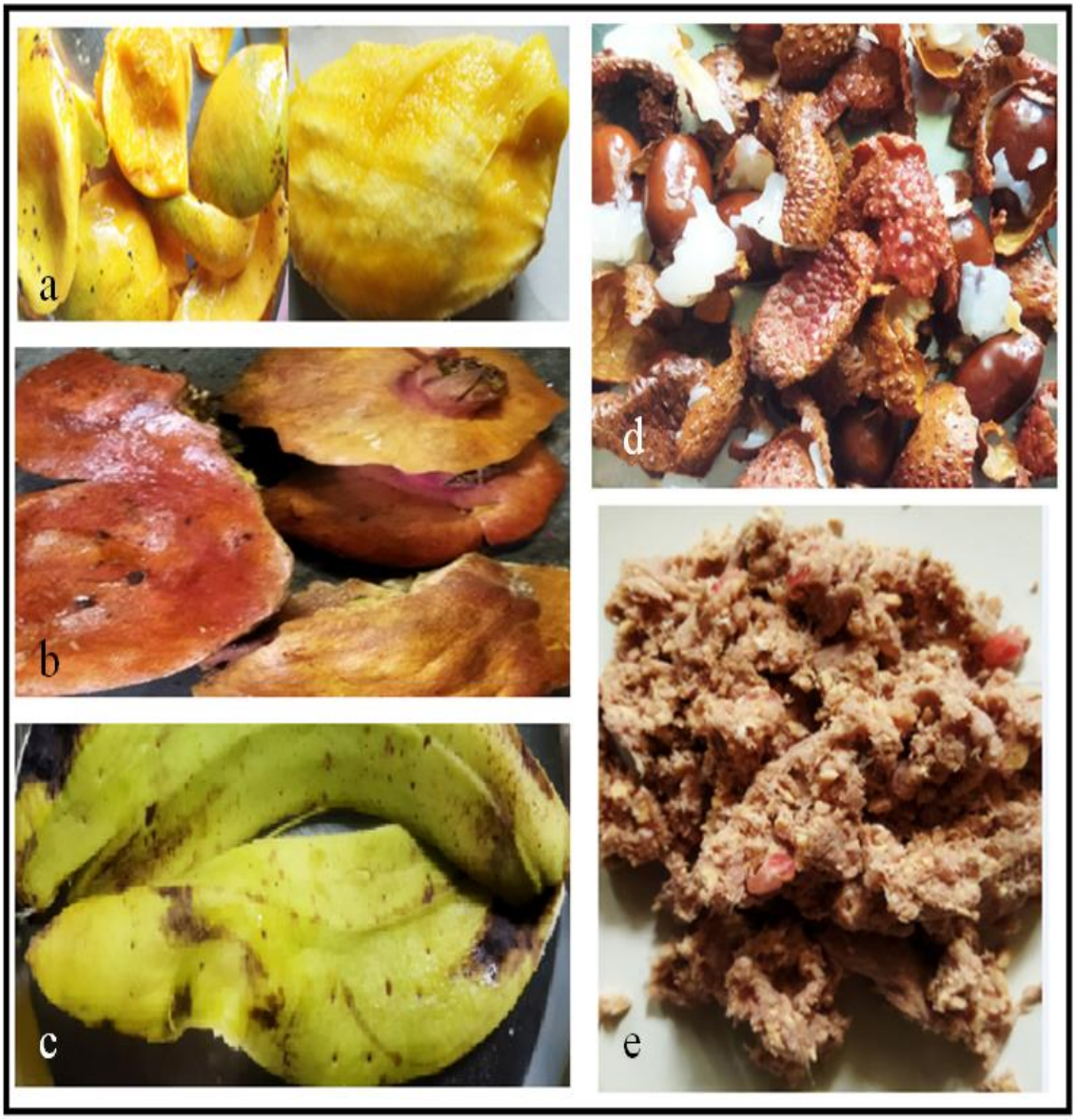


Figure 1.1 Fruits residual wastes.(a) Mango peel and stones; (b) Pomegranate peel; (c) Banana peel; (d) Litchi peel and seeds (e) Pomegranate pomace

1.3 Bioactive elements in fruit wastes

Fruit residues are a museum of vital organic compounds, which, nutritionally and functionally, plays important roles as far as health. These bioactive molecules are secondary metabolic products of plants. Practically it has been seen that the peels of different fruits like orange, lemon, grapes and other fruits contain more than 15% bioactive elements than their pulps (Gorinstein *et al.,* 2001a). Potentially the fruit residues contain large quantities of phytochemicals like polyphenols (flavonoids, flavanols. anthocyanins, isoflavanones, phenolic acids), terpenoids, carotenoid, ascorbic acid and others (Sagar *et al.,* 2018). Besides above mentioned phytochemicals the residues also contain plentiful amounts of nutritional compounds viz polysaccharides, proteins, fats, dietary fibres etc. It also carries a variety of compounds that are frequently employed in the food, cosmetic, and pharmaceutical sectors as pigmenting and flavouring additives (Sharma *et al.,* 2021). Selective phytochemicals and their properties are discuss below:

1.3.1 Phenolic Compound

There are several different important types of secondary metabolisms in plants, and phenolic compounds are one of the numerous. They come in a form that is distinct due to the aromatic rings' hydroxyl groups. These substances are classed as common phenols, flavonoids, phenolic acids (benzoic acid,cinnamic,p-hydroxy benzoic acid acid etc.), xanthones, steel bans, and lignans based on the structural components that link the rings to each other and the number of phenol rings. According to their structural features, each molecule has distinct modes of action, which identify and describe the antioxidant capabilities of the chemical. They primarily purify oxygen's reactive species, nitrogen or chlorine, or can chelate metal ions, interacting both at the initiation and increasing stages of the oxidation process.

The amount and location of hydroxyl groups as well as the types of substitutions on the aromatic rings largely influenced their activity, which was linked to the increased capacity of phenolic compounds to chelate metals. Anti-mutagenic, anti-carcinogenic, and anti-inflammatory qualities are additional biological activities that are all connected to the antioxidant venture, valuable for preventing and treating cancer (Fernanda *et al.,* 2018).

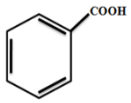
1.3.2 Carotenoids

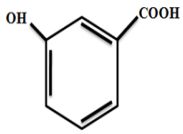
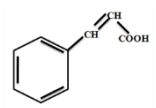
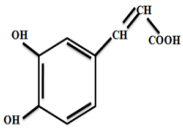
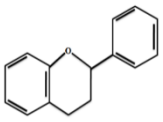
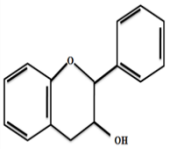
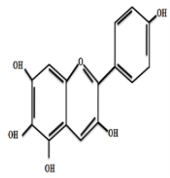
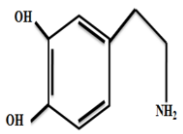
Carotenoids are widely present in fruits residues like peels, pomace, and seeds, have the potential to produce colour, and are used extensively in the food industry, cosmetics manufacturers, which has resulted in their enormous market demand. The term "carotenoids" generally refers to the bulk of the pigments that are naturally present in animal and plant bodies. This pigment, which is primarily fat-soluble and contains at least 700 distinct compounds, create the red, orange, and yellow colours. Numerous carotenoids are hydrocarbons with two terminal rings and 40 carbon atoms (Saini *et al.,* 2022). All photosynthetic species, such as algae, cyanobacteria, and plant species, along with some non-photosynthetic organisms like bacteria and fungi, can create carotenoids.

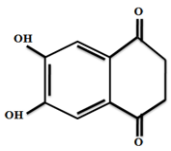
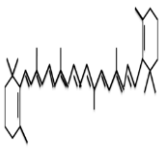
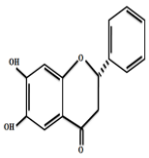
There are two distinct kinds of carotenoids.
(a) The primary class, α -carotene, which is the most prevalent. They are made of linear hydrocarbons and can undergo cyclization at either or both of the molecule's ends.
(b) The second class consists of the xanthophylls, which are also known as oxygenated carotenoids and include lutein, violaxanthin, neoxanthin, and zeaxanthin (Pavía and Concepción 2006).

They are mainly employed in the pharmaceutical, food, cosmetic, and animal feed sectors because of their colouring abilities. Because of their potential as provitamin A, they have been extensively employed in food fortification in addition to being used as a colourant. Their biological consequences additionally enhance the health of living things by strengthening the immune system, minimising the tendency of degenerative diseases, and having antioxidant, anti-obesity, and hypolipidemic properties. (Singh *et al.,* 2015).

Table 1.3 List of bioactive element in fruit residues

Bioactive Compounds	Structure	Fruit Wastes Source	Benefits	References
Benzoic Acid		Blueberry pomace extract, Apple peels, Pineapple peels, Orange pomace, Watermelon, Banana peel,Apricot	Cosmetics (perfumes, dye), Pharmaceuticals (drugs), Preservative, Antimicrobial	Kevser*et al., 2022*

P-Hydroxy Benzoic Acid		Pumpkin (seeds, peel flesh), Grape pomace, litchi peels	Antbacterial, used to in preparation of shampoos, moisturizers, shaving gels, spray tanning solutions,	Shivashankar a*et al*, 2013
Cinnamic Acid		Blackberry, Strawberry, Tomato seeds	Hepatoprotective, Antidiabetic, Neuroprotective	Pei *et al.,2016*, Azabouet*al., 2020*
Caffeic Acid		Grape pomace, Citrus fruit peel, Apple(seed, peel,pomace), Citrus peel, Tomato (peel, pulp)	Antioxidant property, Reduce risk of cancer, Diabetes, Anti-inflammation	Kevser *et al.*, 2022, Belović*et al.*, 2016
Flavanoid		Pumpkin (seeds, peel flesh), Grape pomace, Orange peel, banana peel	Antioxidant property,Preservatives, Antimicrobial, neuroprotective, Anticancer	Zhao *et al, 2018*, Camero *et al, 2018*, Omaima *et al., 2022*
Flavanol		Blueberry pomace extract, Mango peel, Apple peel, Tomato pomace, Banana peel	Antiinflammatory, Antioxidant, Anticancerous	Shukla *et al.*, 2019, Zaini*et al.*, 2022
Anthocyanine (Aurantidin)		Apple peels. Strawberry fruit peel, Blackberry, blue berry skin, Raspberyy pomace, Grape pomace, Pomegranate (seeds, peel), litchi peels	Colouring agent, Antimicrobial, Anticancerous, Antdiabetic	Wang *et al.*, 2019. Takikawa*et al.*, 2010, Pojer*et al.*, 2013
Catecholamines (Dopamine)		Banana peel, grape pomace, cherry seeds	Antioxidant, Antibacterial, Antibiotic, neutraceutical applications	Varzakas*et al., 2016.* Zaini*et al.*, 2022

Napthaquinone		Walnut	Antifungal, Antibacterial, Antiviral, Antitumour	Solar *et al., 2006*, Pinho *et al., 2012*
β-Carotenoid		Pumpkin (seeds, peel flesh), papaya peel, Melon peel, Apricot (peel, pomace), Cherry, Tomato peel	Antioxidant, Antiobesity, strengthening immune system	Kevser*et al.*, 2022, Szabo *et al.*, 2019, Mezzomo and Ferreira, 2016
Flavanone		Citrus fruit pomace	Antiobesity, Anticancerous, Antiinflammatory, Antioxidant,	Kim and Kim., 2017

1.3.3 Dietary fibre

Dietary fibres are chemically constituted of polysaccharides (homo- and hetero-) oligosaccharides, gums, lignin. It is the main constituent of fruits and vegetables, giving plants structural stability. (Ahmad and Khalid, 2018). It is majorly obtainable from fruit residual by-products of diverse kinds of fruits like mango, pineapple, grapes pomace, carrot, papaya, guava (Beres *et al.*, 2016) and other fruits wastes. It remains undigested in the intestine as the endogenous enzyme has no any action on this fibre and its consumption is beneficial as far as health is concerned. It mainly acts as an antioxidant, besides that it also regulates blood cholesterol level, used in treatment of cancer, heart diseases and diabetes (Subiria-Cueto *et al.*, 2021).

1.4 Extraction methods

Separating and concentrating vital molecules such phenolic compounds, carotenoids, and flavonoids is crucial for the bioactive compounds extraction components from food wastes, fruits, and vegetables. These bioactive substances are highly sought after for a collection of uses in the pharmaceutical,food, and cosmetic industries due to their potential health advantages. Bioactive chemicals can be extracted from food waste, which

minimises waste and benefits the environment while also giving these unwanted things a new use. Various extraction procedures are employed with the goal of releasing the desired bioactive components from the food matrix. (Azmir*et al.*, 2013).

Additionally, great sources of bioactive substances include fruits and vegetables. phenolic compounds like flavonoids and phenolic acids are common in these plant-based products. Carotenoids like beta-carotene and lycopene are what give fruits and vegetables their vibrant colours. Two extraction techniques are generally used to isolate these chemicals: (i) Traditional techniques include maceration, solvent extraction, soxhlet extraction, and percolation (ii) A few examples of novel extraction techniques are enzyme-assisted extraction (EAE), pulsed electric field extraction (PEFE), ultrasound-assisted extraction (UAE), and pressurised liquid extraction (PLE). (Nirmal*et al.*,2023).

Most effective and standard conventional technique for extraction of phytochemicals is the Soxhlet method, in which analytes are extracted from solids. Inside soxhlet apparatus, the solid material (from which bioactive components have been extracted) using specific solvent is heated and condensed. Later on concentrating the solution by evaporating the excess solvent. The solubility, nature of the solid materials, mass transfer, and last but not least the choice of solvent for the extraction of certain bioactive substances are all variables that affect how effective this method is. By implementing this technique, the total phenol content estimated from pineapple residues was 28.8 mg GAE/g dry basis by Alias and Abbas, 2017 and about 387.4 mg GAE/g by Reis *et al.*, 2016 from tamarind seeds.

After extraction, these bioactive compounds can be put to several uses. They can be utilised in the food sector as organiccolourants and antioxidants, as well as in functional foods and beverages, dietary supplements, and nutraceuticals. They also have potential health advantages, including as antioxidant and anti-inflammatory properties, which make them appealing for pharmaceutical and aesthetic uses.

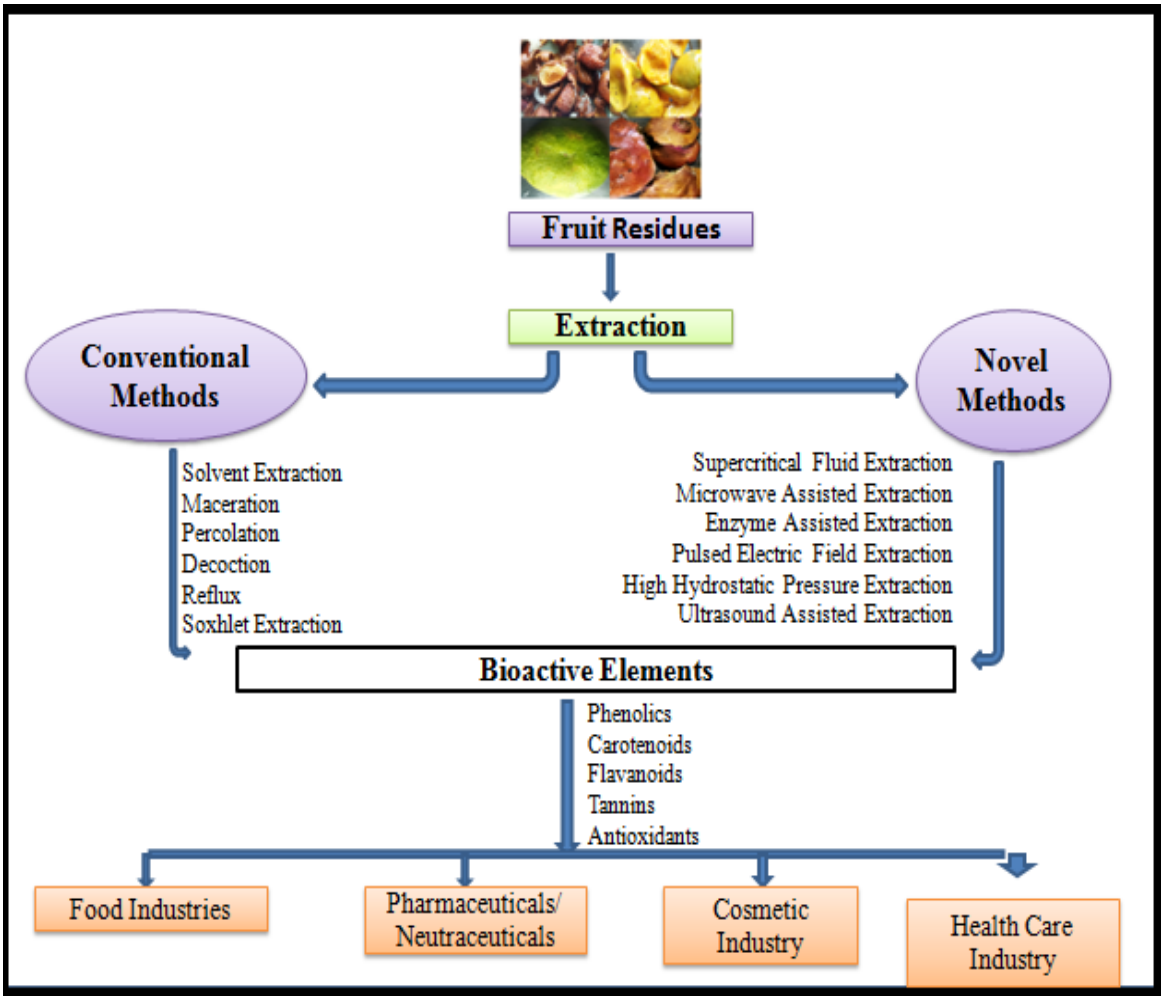


Figure 1.2 Schematic representation of the destiny of fruit wastes (Nirmal*et al.*, 2023)

1.5 Biovalorisation of fruit wastes

When fruits and vegetables are processed, substantial wastes or byproducts are produced, and they make up around 25% to 30% of the entire product category (Kumar *et al.*, 2020). There are difficulties in managing food waste and seeks to provide practical, long-lasting solutions that might serve as a manual for decision-makers in various departments of towns, districts, or cities. They are taking numerous actions to treat this food waste before any further processing and limit it as much as possible. Municipal solid waste contains a significant amount of biodegradable organic matter, such as fruit and vegetable peels, food scraps, and extracts. The requirement for these essential food products has been gradually increasing because of the importance of fruits and vegetables in our diets and human life, as well as the growing worldwide population and shifting dietary preferences.

To handle municipal solid waste materials, different regions, and countries have their distinctive waste management systems and regulations in place. Examples include waste segregation, recycling programs, composting, waste-to-energy plants, landfilling, incineration, open burning and other waste treatment procedures (Rifna and Misra, 2021). Municipal solid waste administration is critical for minimizing pollution, upholding resources, drastically reducing greenhouse gas emissions, and fostering sustainable development. Public awareness, responsible consumption, efficient garbage collection systems, and adequate waste elimination infrastructure are all required.

As the above mentioned methods for MSW are inadequate due to sort of available land and also lead to other serious issues like secondary wastes (methane gas, carbon di- & mono-oxide etc) regeneration occurs, better to recovered the valuable components from the waste by applying recycling process and utilized to produce value-added products (Leong and Chang, 2022), the process is called valorisation. As above discussed that the by-products (peels, seeds, pomace, kernel) from fruit processing industries are rich source of biological active components like antioxidants (flavonoid, benzoic acid and their derivative, dietary fibre etc.), enzymes, carbohydrate, protein and others (Trigo*et al.*, 2020). These bioactive compounds are potentially to develop functional food in food industries or pharmaceuticals or cosmetics (Coman *et al*., 2020). The active substances found in fruit residues (particularly in peels and seeds) can kill or inhibit pathogenic and deteriorating bacteria, hence increasing the longevity and enhancing the attribute of food goods. Presence of pectin is most common in residues and recovery and valorisation slightly difficult to generate significant feedstock (Calderón-Oliver and López-Hernández, 2020).

The best method to utilise these ingredients is to create edible coatings that, thanks to their preservative (antioxidant and antibacterial) nature, extend product shelf life while maintaining the food's nutritional and functional properties at the lowest possible cost. (Zaragoza *et al.*, 2018).In processed (minimally) and foodstuffs (fresh), coatings also provoked variation in numerous areas like colour, inflexibility, sensory feature, antioxidant nature etc. (Ullah *et al.*, 2017).Fruit peels are suitable for use as an ingredient in coatings and films for food products because they contain phenolic compounds with high antioxidant qualities. (Kumar *et al.*, 2020).

1.6 Potential Health Benefits of Recovered Fruit Waste Bioactive Compounds

Exploratory investigations on the bioactivities of fruit byproducts' constituent parts are required to better utilise them. For researchers and manufacturers, scientific investigations on the quantities and applications of these active components are a useful source of information and assistance for the efficient extraction of these compounds. Fruit redues are known to be a great source of high-quality chemicals, such as bioactive chemicals including anti-inflammatory, antioxidant, antibacterial, and antimutagenic substances that are expected to have an impact on health of human due to their biological qualities. The physiological activity of the many fruit by-products is caused by the synergistic interaction of these diverse chemicals.

1.6.1 Antioxidant activity

As talked about earlier, natural product squanders (fruit residues) are a wealthy source of bioactive chemicals which will have antibacterial and antioxidant capabilities. These chemicals, which have a big impact on people's wellbeing and sickness anticipation, incorporate polyphenols, flavonoids, carotenoids, and vitamins.Massive amounts of oxidants (free radicals or reactive species), including hydroxyl free radical, singlet oxygen, hydrogen peroxide, lipid peroxides, and others, are produced by cellular metabolic processes such as breathing (Halliwell and Gutteridge, 2007). In living being, these oxidant cause raise within the level of ROS (Reactive Oxygen Species) or turn down resistance by antioxidant, eventually provoke the hurting of essential biological molecules (DNA, Protein) by making oxidative atmosphere inside the cells (Circu, M.L and Aw, 2010For instance, lipid peroxidation is the process of oxidising lipids under oxidative stress and produces 4-hydroxy-2-nonenal (4-HNE) and malondialdehyde (MDA) (Pillonet al., 2012). Numerous disorders, like heart disease, diabetes, cardiovascular,cancer or neurological abnormalities, atherosclerosis, etc., have been linked to oxidative stress and their occurrence. (Halliwell and Gutteridge, 2007).

Antioxidants are substances that decline free radicals or transition metal ion concentration by moderate down or delay oxidation handle (Zeb, 2020). Different anomalies such as cardiovascular illness, cancer can be controlled by the utilization of

nourishment items consolidated with these components by keeping up antioxidant status. Different explores have done and compared the antioxidant potential of fruits residues (peel, seeds, residual mash etc.). Among the various fruits, apples are known for stores of ample phenolic compounds uncommonly apple pomace. Duda-Chodak and his co-workers Tarko compared antioxidant highlights of diverse natural product build up and found that the apple peels (7925 mg Trolox x 100 g^{-1}d.w) has most extreme antioxidant activity besides by grapes (6944 mg Trolox x 100 g^{-1}d.w) and after that seeds of orange (Duda-Chodak and Tarko, 2007). Most utilized and developed natural products gather has place citrus family which create 50-60% of add up to by-product produced and report from distinctive processing industry tells that the polyphenol concentration in by-product of citrus fruit is much more noteworthy than their consumable portion (Sharma *et al.,* 2017).Papaya peels contained a total phenolic content that was 1.2 times greater than papaya seeds due to their stronger antioxidant activity. (Ng *et al.,* 2012).The likelihood of pomegranate peels having greater antioxidant activity than the edible components was further explained by Li, et al. in their study. Various ponder detailed that the peels of few fruits constitute higher volume of antioxidant substance than their mash.

Fruit waste bioactive peptides are important since it has been established that they have a role in preventing oxidative stress. Date seed protein hydrolysates, for instance, showed substantial antioxidant movement by increasing the hydroxyl radical from 12.3 to 43.8% (Ambigaipalanet al., 2015). Furthermore, in protein hydrolysates cherry seed made with thermolysin (degree of hydrolysis , 55.5%) successfully reduced the rate of peroxidation of lipid by 30% and 80%, respectively, in in-vitro studies (García*et al.,* 2015).

These studies demonstrate the importance and sustainability of fruit residues as sources for acquiring antioxidant compounds that may have beneficial effects on health. We'll talk more about this subject in the following chapter.

1.6.2 Antimicrobial properties

Antimicrobial agents are substances that have an impact on the eradication or concealment of the growth of destructive or decaying microscopic organisms. Given that microbial growth and activity are frequently influencing variables for both food product

integrity and security, the significance of these agents is clear. The fruit residues,antibacterial potential is influenced by a various factors, including the type of residue involved, just like antioxidant activity. Different fruits have diverse bioactivity potentials as a result of variances in the chemical makeup of specific portions. In contrast to the seed extract, pomegranate peel extracts shown remarkable antioxidant action against *Bacillus cereus* and*Staphylococcus aureus*. The different types and concentrations of bioactive chemicals that were found in the two tissues were responsible for this (Kanatt et al., 2010). Plant parts such as peels, fruits, seeds, leaves, and other parts are known to have antibacterial elements. The article provides examples of numerous bioactive substances, such as phenolic compounds, saponins, aldehydes, phytosterols, and terpenoids that can be discovered in fruit remnants. Since these compounds contain anti-inflammatory, antifungal, antiviral, immunoregulatory, and antimicrobial characteristics, scientists are paying more and more attention to them. (Gunwantrao*et al.*, 2016, Górniak*et al.*, 2019).

Phenolic chemicals' antibacterial property is mostly linked to harm to the membrane's structure of microbes (Yi *et al*, 2010, Adnan*et al.*, 2017). Due to variations in the design of cell membranes of bacteria, gram-negativeand gram-positive bacteria have distinct reactions to specific phenolic compounds. (Steinmann *et al.*, 2012, Yoda and Hu, 2004). Because they are less fond of negatively charged lipopolysaccharides, some phenolic compounds have a lesser attraction for Gram-negative bacteria. Resveratrol and pinosylvin both had antibacterial effects on Gram-positive bacteria. The 1,1-dimethylallyl ester of caffeine was bactericidal to Gram-negative bacteria. The lipid bilayer is disrupted, the external and internal membranes become more permeable, the ion transport mechanisms are changed, the lipid membranes aggregate, and The effects of phenolic chemicals on membranesofbacterial cell have an impact on the membrane fluidity (Yi *et al*, 2010, Wu,*et al*, 2013, Phan*et al*, 2014). A polyphenol called epigallocatechin-3-gallate (EGCG), which is usually found in grape seeds, can prevent gram-positive and gram-negative bacteria like B. subtilis, Campylobacter jejuni, S. aureus, S. typhimurium, and others from adhering to epithelial cells and reduce their capacity to cause haemolysis. (Nakayama *et al*., 2015; Steinmann *et al*., 2012). Procyanidins have the ability to modulate the metabolic energy systems, leading to a decrease in metabolism and inhibition of bacterial growth (Li et al., 2017). Similarly, tannins are associated with

inducing structural and morphological changes in the cell wall of S. aureus by suppressing genes involved in protein synthesis and RNA. Polyphenols, including myricetin, bacalein, pyrogallic acid, resveratrol, epigallocatechin, punicalagin, tannic acid, castalagin, , geraniin, prodelphinidin, and theaflavin, also play roles in controlling the metabolic system and suppressing genes, contributing to their antibacterial effects. Various phenolic compounds have demonstrated antibacterial properties, and studies have shown that extracts from pineapple and orange peel exhibit a significant zone of inhibition, effectively combating strains of pathogenic bacteria like Klebsiella pneumonia, Bacillus subtillis and Pseudomonas aeruginosa (Gunwantrao et al., 2016). Furthermore, the antibacterial potential of by-products from fruits such as banana, mango, cloudberry, raspberry, and others has been investigated against S. aureus development and activity (Mordi et al., 2016, and Mutua et al., 2017). Mango kernel and banana peel extracts, enriched with phytochemicals like flavonoids, terpenes, coumarins, and tannins, display potent antibacterial and antifungal effects (Mutua *et al.*,207).

1.6.3 Other health benefits

Fruits and vegetable by-products contain bioactive compounds that offer various physiological benefits beyond their primary purposes, including anti-inflammatory, anti-melanosis, anti-carcinogenic, and cardioprotective effects, among others. These traits warrant detailed exploration. For instance, punicalagin and ellagic acid, two bioactive derivatives found in pomegranate, have demonstrated anti-cancer, anti-aging properties and anti-inflammatory (Wu and Tian, 2017). By-products ofcitrus fruit are renowned for their anti-inflammatory and anti-cancer properties, attributed to their abundant biological active components like flavanones, anthocyanins and flavones (Panwar et al., 2021). The presence of terpenes, esters, and citral aldehydes, major constituents of citrus oil, further contribute to their effectiveness in therapeutic approaches for cancer-related conditions. (Palazzolo*et al.*, 2013).

1.7 Toxicological study of bioactive compound

In recent times, consumers have become increasingly aware of the positive impact of a well-balanced diet on their health, leading to a heightened focus on natural bioactive

compounds (Guiné et al., 2020). Various agro-food waste categories, like fruits, vegetables, roots, tubers, fish,meat, cereals, dairy,and oilseeds, contain different bioactive compounds (Campos et al., 2020). This growing interest has resulted in a wide array of functional foods, supplements, and nutraceuticals available worldwide, with the market for dietary supplements, food antioxidants, and nutraceuticals projected to reach approximately $210 billion by 2026 (COVID-19 Impact Analysis, 2021).

However, the use of bioactive compounds from food waste in a circular economy context requires clear legal definitions, as each country has unique laws regarding their utilization. Transitioning from traditional methods of disposal (animal feeding, composting, and anaerobic digestion) to modern approaches (incorporation into the food industry) is essential for sustainable resource utilization (Osorio et al., 2021).

The introduction of novel foods or ingredients necessitates adherence to safety regulations to protect public health (Coppens ana da Silva, 2006). Extracting valuable components from food waste presents several challenges, including biological instability, potential pathogenic contaminants, high water activity, rapid auto-oxidation, and the presence of active enzymes (Devkota et al., 2017). Extracts from food waste may also contain pollutants like pesticides, mycotoxins, microbiological contaminants, heavy metals, and biogenic amines, which can pose health risks. Considering these biological hazards is crucial when determining the suitability of food waste for extracting valuable components to avoid potential health issues.

Studies have been conducted on the safety parameters of agathisflavone, a biflavonoid consisting of two apigenin units, including its half-lethal dose and its effects on histopathological,hematological, biochemical, physiologicaland behavioral, aspects when orally administered. Citrus flavonoids, also known as citroflavonoids, have shown promising biological activity related to various health conditions, including cardiovascular disease, viral and bacterial infections, diabetes, and certain consequences of these conditions (Gandhi et al., 2020, Ciumărnean et al., 2020). These substances have also established antioxidant, estrogenic, and anticancer actions (Rodríguez et al., 2020, Tronina Rodríguez et al., 2020). Despite sharing many characteristics with drugs, the safety profiles of these compounds still require more comprehensive study.

1.8 Biocompatibility

One of the key characteristics of biomolecules is biocompatibility, which is described as "the ability of a biomolecule to perform its desired function, without eliciting any undesirable local or systemic effects on the recipient or beneficiary, or the ability of molecules to perform with an appropriate host response in a particular situation." Biomolecules must adhere to fundamental biocompatibility standards. To elicit the proper biological response, they must not cause any toxic effect, poisonous, thrombogenic, carcinogenic, antigenic, or mutagenic (Helmus*et al., 2008*). Bioactive compounds called phytochemicals are derived from live organisms like plants and the non-pathogenic bacteria that occupy them. With their anti-inflammation, anti-maturing, anti-disease, and cell reinforcement characteristics, they are beneficial to human well-being. The biocompatibility of bioactive components recovered from wastes is taken into account in this study.

1.9 Industrial application of fruit waste extracts

Efficient management of food waste is crucial for addressing global sustainability challenges, reducing environmental impact, and ensuring food security. Food waste utilization management entails using food waste in a useful and sustainable way, reducing the amount that is disposed of, and maximizing the value of it through a variety of uses. Food waste is a major problem that affects the entire food supply chain and is prevalent throughout the world. According to recent estimates, in every year food produced for human is about t 1.3 billion tonnes globally is wasted (Gustavsson*et al.*, 2011). However, it is essential to emphasize that efforts are being made to utilize a portion of this waste effectively.Currently, food waste utilization management encompasses a range of applications. In the agricultural sector, food waste can be used as organic fertilizers, enhancing soil quality and reducing the need for chemical fertilizers. Food waste is also utilized in the production of animal feed, contributing to sustainable livestock farming practices. Additionally, the generation of biogas through anaerobic digestion provides a renewable energy source for electricity and heat production, further reducing reliance on fossil fuels (Monfared*et al.*, 2020).

1.9.1 Application in the food industry

Currently, industrialized agricultural output, such as food industry waste, and agricultural waste itself produce significant volumes of organic waste. The industrialization process generates large quantities of co-products with distinct chemical and physical-chemical properties, making their retention challenging. These byproducts have typically been used as compost or animal feed. However, due to their potentially valuable compounds, several highly beneficial substances can likely be found within them. With proper conversion processes, these compounds can be transformed into products suitable for use as ingredients in creating new food products. several by-products from the food corporations, such as fruits, skins, seeds, and membrane remnants, contain abundant sources of bioactive substances. These include dietary fiber, minerals, terpenes, organic acids, carotenoids, vitamins, phenolic acids, and others. Numerous studies, (Zhang *et al.*, 2020; Gomez-Mejia *et al.*, 2019; Mahato*et al.*, 2018; Multariet al., 2020; Singh et al., 2020) have linked these bioactive compounds to a range of health benefits, such as anti-inflammatory, anti-hypertensive, anti-bacterial, antioxidant properties, and neuroprotective effects.(Mahato*et al.*, 2018).As a result, the food sector is becoming more interested in the production of various products using by-products from agro-industrial waste. Bioactive substances that are extracted from fruit leftovers are employed in the food business in a variety of ways, including food preservation, food fortification, as enzymes, flavorings, colors, and packaging aids.

Food Fortification: Fruit pomace, a significant by-product derived from fruit processing, offers a cost-effective solution as a bulking agent in food items, effectively replacing some sugar, oil, or flour. By incorporating fruit pomace, food products benefit from improved emulsion stability and retention of water and oil, thereby enhancing overall functionality (Iqbal et al., 2021). Moreover, the addition of fruit pomace imparts a distinct flavor and aroma to baked goods, skillfully combining fruity and baked characteristics in the final products. Notably, researchers have achieved success in creating high-fiberextruded snacks and baked by including apple pomace (30% w/w) without causing any chemical composition alterations to the snacks (Reis et al., 2014).

Similarly, the introduction of powder ofmango peel, up to 20% (w/w) in soft dough cookies, resulted in improved soluble dietary fiber content and hardness while minimizing spreading. When the powder of mango peel was increased to 30% (w/w) in cookies, their

nutritional value was enhanced with no compromising sensory or textural attributes (Bandyopadhyay et al., 2014). Likewise, incorporating pomace of red grape in pork burgers led to improved coloracceptability and stability by reducing oxidation of lipid. However, excessive substitution of fruit pomace, around 6%, caused decreased cohesiveness and hardness in the burgers (Younis et al., 2015).

Citrus co-products, rich in dietary fiber, possess the unique ability to retain water, oil, and other liquids, swell, froth, and emulsify, making them valuable as fat substitutes in meat products and for enhancing nutritional fiber content.

Food Preservation: Grape seed extracts, known for their natural antioxidant properties, have demonstrated efficacy compare to the synthetic antioxidant butylated hydroxytoluene (BHT) in preventingoxidation of lipid in processed, cooked, and refrigerated meat of chicken for up to 14 days. Incorporating extracts of grape into vacuum packaging has been shown to improve the stability of lipid of cooked chicken (Shirahigue et al., 2010). Additionally, various studies have emphasized the potent antibacterial effects of grape extracts against foodborne pathogens, lactic acid bacteria, and wine-rotting yeasts (Brown et al., 2009, Baydar et al., 2004, Jayaprakash et al., 2003).Mango seed ethanolic extracts have also displayed diverse antibacterial activities, particularly against Gram-negative bacteria (Kabuki et al., 2000). Similarly, the antibacterial potential of different extracts of mango peel was evaluated against both Gram-positive Staphylococcus aureus and Gram-negative Pseudomonas fluorescens, revealing varying levels of antibacterial activity against both strains.Citrus peels contain an array of nutrients with beneficial and antibacterial properties, depending on their composition. These peels encompass secondary metabolites such as terpenoids, coumarins, carotenoids, flavonoids,andfuranocoumarins, particularly polyethoxylated flavones and flavanones (Ahmad et al., 2006). The use of citrus oil in conjunction with mild heat treatment in apple and orange juices has been found to influence the control of spoilage bacteria (de Souza et al., 2019).

Enzyme: In various traditional practices, papaya leaves and other plant parts and extracts have been utilized to tenderize meat and serve in processes like cheese and curd making, soy sauce production, brewing, and baking. Plants contain various enzymes like cellulase,

amylase, protease, and lipase. These plant-derived enzymes can be directly consumed without the need for any processing, retaining their functionality (Verma et al., 2022).

Food Packaging: In recent years, significant advancements have been made in the packaging industry, particularly in the food sector. The advent of industrial revolutions and smart packaging concepts has introduced novel possibilities for food packaging. These packaging innovations can be categorized into two main types: intelligent packaging and active packaging.In smart packaging, the base material is infused with one or more active agents that possess specific qualities like antimicrobial, antioxidant, pH-sensitive, etc. Incorporating active components into the packaging material allows for the release of these agents during storage, potentially elongating the shelf life of the packaged product. Plant extracts, known to contain various active ingredients, are commonly used for their antioxidant and antibacterial properties in packaging. Certain plants, rich in anthocyanins, hold promise as potential candidates for developing pH-sensitive packaging materials. This chapter largely focuses on exploring the diverse applications of plant extracts in the packaging industry for various purposes (Oladzadabbasabadi *et ai.*, 2022).

Colouring agent: Carotenoids, anthocyanins, chlorophylls, and porphyrins are the primary families of naturally occurring coloring pigments and are widely present in plants and plant components. These colorants are utilized to treat many chronic diseases and have enormous pharmacological and health potentials in addition to serving as colorants. From plant tissues, several natural colorants are isolated and utilized commercially as food coloring. Consumers today require food that is both secure and healthful. The best option is to replace colors with natural pigments rather than synthetic dyes, which have several negative health impacts (Meena*et al.*, 2022).

1.9.2 Application in pharmaceutical industry

Food waste is a significant global issue, with millions of tons of food being discarded every year. However, this waste can be repurposed and utilized in various industries, including the pharmaceutical sector. Incorporating food waste can provide sustainable options while minimising environmental impact. The pharmaceutical sector frequently uses renewable resources to produce pharmaceuticals. Here are some applications of food waste in the pharmaceutical industry:

Antioxidant Extraction: Examples of food waste that contains bioactive ingredients with antioxidant properties include fruit peels and seeds. These compounds can be extracted and used in theindustry of pharmacy due to their potential health benefits. For instance, citrous peels contain flavonoids and polyphenols that have antioxidant and anti-inflammatory properties. Numerous studies have looked into the potential applications of these compounds obtained from citrous peels in the creation of pharmaceuticals. (Rodríguez-Rodríguez *et al.*, 2020).

Drug Delivery Systems: Materials from food waste can potentially be used to create medicine delivery devices. To improve the durability, bioavailability, and controlled release of medications, chitosan, a polysaccharide produced from shrimp and crab shells, can be utilised to encapsulate them (Iqbal et al., 2020). Targeted medication delivery and wound healing are just two pharmaceutical applications where chitosan-based drug delivery systems have shown promise.

Antibacterial Agents: Food waste, such as grape pomace and olive leaves, contains natural antimicrobial compounds that can be utilized as alternatives to synthetic antibacterial agents. Extracts from these sources have demonstrated antimicrobial activity against various pathogens (Gouvinhas*et al.*, 2019). The creation of new antimicrobial medications or topical infections treatments may benefit from the inclusion of these extracts in pharmaceutical formulations.

Excipients and Binders: Food waste materials can serve as excipients or binders in the production of pharmaceutical tablets. For example, cellulose derivatives derived from agricultural by-products like wheat bran or rice husk have been used as binders in tablet formulations (Liu *et al.*, 2019). Utilizing food waste in this manner reduces the need for synthetic binders and enhances the sustainability of pharmaceutical manufacturing processes.

Incorporating food waste in the pharmaceutical industry offers several advantages, including cost-effectiveness, sustainability, and the potential to create value-added products from waste streams. However, Further investigation and development are

needed to improve extraction techniques, assess the efficacy and safety of chemicals obtained from food waste, and guarantee regulatory compliance.

1.9.3Application in cosmetics industry

The cosmetics industry is constantly seeking innovative and sustainable solutions to address environmental concerns. One area that has gained attention is the utilization of food waste in cosmetic products. Food waste, which refers to any discarded food material, can be repurposed and incorporated into cosmetic formulations, offering a sustainable and eco-friendly approach to product development. Here, we will explore some of the applications of food waste in the cosmetics industry, supported by relevant references.

Fruit and Vegetable Extracts: Due to their high concentration of antioxidants, vitamins, and minerals, extracts from fruits and vegetables, such as citrous peels, tomato skins, and grape seeds, have demonstrated potential in cosmetic compositions. These extracts can be used in skincare products for their anti-aging, brightening, and soothing properties (Abidin*et al.*, 2020; Cheong *et al.*, 2018).

Coffee Grounds: Coffee grounds, a common food waste, have found applications in cosmetic products. The caffeine content in coffee grounds can help reduce puffiness and dark circles in eye creams. Additionally, the texture of coffee grounds can act as a natural exfoliant in body scrubs, promoting smooth and rejuvenated skin (Pakade*et al.*, 2020).

Olive Oil Waste: Olive oil production generates significant waste in the form of olive pomace. However, this waste can be utilized in the cosmetics industry. Extracts derived from olive pomace have demonstrated moisturizing, anti-inflammatory, and anti-aging properties, making them suitable for incorporation into skincare and haircare products (Mellou*et al.*, 2020; López-Corbala*et al.*, 2019).

Algal Extracts: Proteins, lipids, vitamins, and minerals are abundant in algae. Various species of algae, including those derived from food waste, have been used in the cosmetics industry. Algal extracts have shown potential as natural moisturizers, emollients, and antioxidants in skincare and haircare products (Ratih*et al.*, 2021; Gómez-Lorente*et al.*, 2020).

Rice Bran: The by-product of milling rice called rice bran is frequently thrown away. However, it includes beneficial elements including phytic acid, ferulic acid, and antioxidants. These ingredients have been used in cosmetic compositions because of their benefits on skin radiance, anti-aging, and UV defence (Vitale *et al.*, 2020; Jeong*et al.*, 2019).

These examples highlight the diverse range of food waste materials that can be repurposed in the cosmetics industry. Cosmetic companies can reduce waste production and encourage sustainability in their product development processes by utilising food waste. However, it is crucial to take the necessary testing and quality control steps to guarantee the stability and safety of these chemicals.

1.9.4 Other industrial application

Food waste is a significant issue on a worldwide scale since every year, almost one-third of all food produced for human use is wasted. However, food waste can be made into a useful resource with a variety of uses in other small-scale companies. This not only helps reduce environmental impact but also provides economic opportunities. Let's explore some of the applications of food waste in other industries, supported by references and citations.

Animal Feed: The conversion of food waste into animal feed offers a sustainable substitute for current feed supplies. According to a study by Yoruk and Gul, using food waste as a feed additive for cattle has the potential to increase feed conversion rates and lower production costs. (Yorukand Gul, 2021).

Agriculture and Horticulture: Food leftovers can be used as animal feed or composted to create a nutrient-rich product. Food waste composting boosts water retention, enhances soil quality, and lessens the demand for commercial fertilisers. Additionally, food scraps can be fed to animals to reduce the requirement for regular feed sources. Compost made from food waste has been shown to increase soil fertility and crop growth, according to a study. (Qi *et al.*, 2017).

Bioenergy Production: Food waste can be converted into bioenergy through anaerobic digestion or fermentation processes. This may produce biogas, which is a renewable fuel source or a source of biogas that can be used to create power. The feasibility of utilizing food waste for biogas production, offering a sustainable solution to both waste management and energy production (Dolfing*et al.* 2020).

Organic Fertilizers: Composting is a process that turns food waste into organic fertiliser. These fertilisers add vital nutrients to the soil, promoting crop growth and yield. The potential of food waste composting as a sustainable method of waste management and soil fertility improvement was highlighted in a study by Kumar and Suthar in 2019.

Bioplastics and Packaging: Fruit peels and vegetable leftovers, for example, can be utilised to create bioplastics and sustainable packaging. Compared to conventional petroleum-based plastics, these materials are more environmentally friendly and biodegradable. Utilising food waste to create biodegradable packaging materials encourages the development of a more sustainable and circular economy. (Auras *et al.*, 2019).

Textile Industry: Food waste can also find application in the textile industry. For instance, fruit waste can be used to produce natural dyes for fabric coloring. Pomegranate peel waste was used in a study to produce natural colours, showing that it has the potential to replace synthetic dyes sustainably. (Ouchbani*et al.*, 2020).
By harnessing the potential of food waste in these minor industries, we can mitigate environmental pollution, reduce resource depletion, and create economic value from what would otherwise be discarded, these programmes aid in the growth of a more circular and sustainable economy.

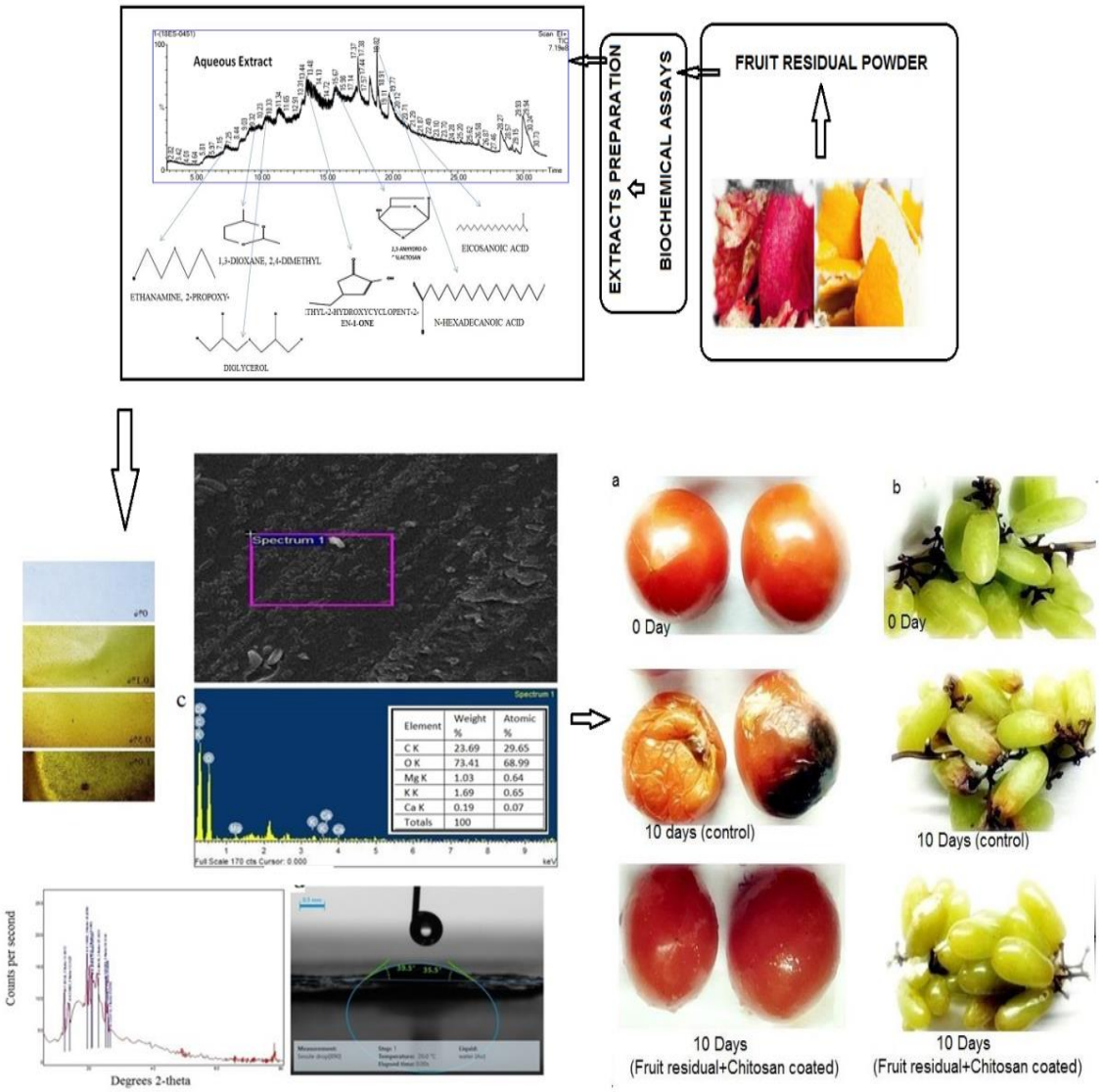
Municipal Solid Wastes (MSW) primarily consist of organic wastes. Recovering the bioactive components by biovalorization and fully utilising them in the food, pharmaceutical, and cosmetics industries, as detailed in earlier sections of this chapter, is the most advantageous method for treating such wastes.

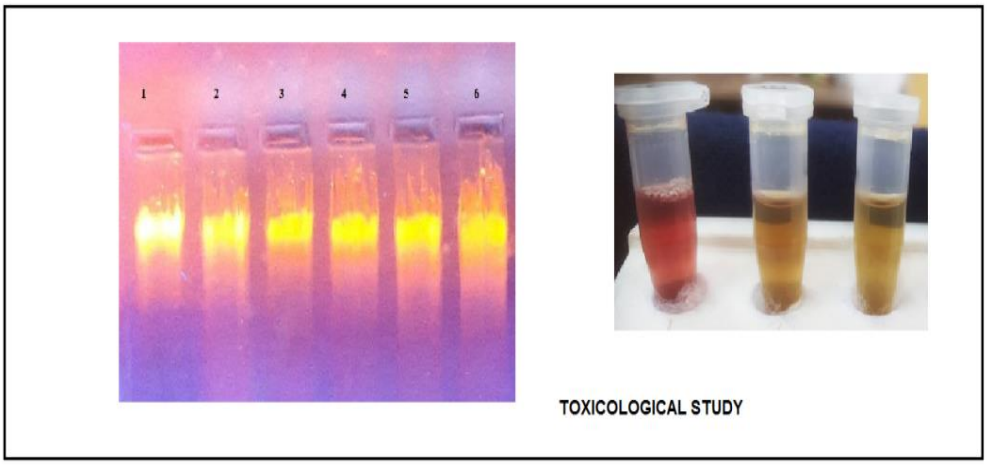
Objectives of the Work

The current study was an effort to study bioactive components in fruit residues (mostly peels, pomace, and seeds) and to determine how best to use them in edible packaging.The following characteristics served as the foundation for the works' major goals:

1. Sample preparation from fruit residual wastes for phytochemical extraction
2. Phytochemicals profiling in fruit residual extracts by GC–MS and thereafter, evaluation of antioxidant and antimicrobial properties.
3. In-vitro Biocompatibility study of fruit extracts evaluated by hemolysis assay using goat erythrocytes.
4. Fruit and vegetable coverings based on aqueous extracts of chitosan.
5. Selective bioactive components from extracts made from fruit residual powder are partially isolated and purified.

Graphical representation of Outline of Work





Chapter 2

Fruit Residual Wastes Powder Preparation, Morphological and Physiochemical Characterization

2.1 Introductions

The enormous importance of fruits and vegetables in our diets and daily lives has led to a rise in the popularity of food products made with fruit. This demand surge can be attributed to population growth and shifts in dietary preferences (Schieber*et al.*, 2001, Vilariño*et al.,* 2017). However, the processing of fruits in food industries generates substantial amounts of fruit residues, including peels, leftover pulp, and fibers (Nurliyana*et al.,* 2010). Unfortunately, these peels are often discarded and contribute to environmental pollution, posing health risks to both humans and ecosystems (Goswami*et al.*, 2017). Approximately 25-35% of a fruit's total weight consists of its peel, leading to a considerable waste volume, especially in urban areas with significant fruit and vegetable manufacturing. The disposal of fruit waste has become a significant concern in municipal solid waste management, where land filling and incineration are the primary methods of waste disposal. However, There is a growing market for food products made from fruit because fruits and vegetables are so important to our diet and daily lives (Qdais*et al.*, 2010, Buekens and Huang, 1998). To address these environmental challenges, it is crucial to find cost-effective and eco-friendly ways of managing agricultural and food industry waste. A highly beneficial approach is the extraction and utilization of bio-active constituents from these waste materials in the food, pharmaceutical, and cosmetics industries, which can have positive effects on the environment and the economy (Makris*et al.*, 2007).

In this investigation, fruit leftover wastes were used as experimental materials. The fruit residues were collected and processed into powder under ambient environmental conditions, and their physico-chemical and morphological characteristics were thoroughly analyzed in this chapter. Chapter 3 will delvered into the detailed biochemical analysis of extracts prepared from this fruit powder. Measurements of the sample powder's volatile matter, fixed carbon, moisture content, fat,ash content, higher heating value (HHV) and , pH, were made proximally. Additionally, various properties such as absorption capacity

of oil and water, percentage solubility, and Swelling Power (SP) were determined. These properties are valuable in developing different value-added products for the pharmaceutical, cosmetics, and food processing industries. SEM (Scanning Electron Microscopy) was employed to study the powder's homogeneity, morphology of surface, geometric shape, and size, providing important insights for processing, handling, and manufacturing in industries aiming to develop value-added goods from the sample powder.

2.2 Materials and Methods

2.2.1 Processing and accumulation of fruit residual wastes

The fresh fruit residues procured from various locations which include fruit juice centre as well as residential in the district of PurbaMedinipur, West Bengal, India. Figure 2.1 shows the sites from which samples were collected. The collected samples were washed uniformly in running tap water, followed by rinsing in sterile double distilled water to eliminate dirt and dust from the skin. By using a mechanical grinder the samples were made into small pieces and uniformly exposed for the sun drying by spreading finely on a tray until it became completely dried and crispy. The sun dried peels were powdered in a mechanical grinder into fine powder and then passed through a sieve having fine pores to get homogeneous particle size. Using an analytical balance and the following formula, the powder yield was determined:

Yield of powder = $W_1 - W_2$

where, W_1 is weight of primary fruit peel and W_2 is weight of powder

The powder was kept in air tight containers in dark storage to retain color and for prevention of moisture sorption by the powder. This prepared powder was used for further experiments in this dissertation.

2.2.2 Physico-chemical characterization of powder

The volatile matter, fixed carbon, moisture content, fat, ash content, pH, and higher heating value (HHV) of the powder were all determined using proximate analysis. These analyses aid in determining the powder's quality (Yang et al., 2018). IIT-BHU in India

used a ZEISS scanning electron microscope to study the powdermorphology. The exclusive JEOL software was used to obtain the SEM pictures. Fruit leftover powder is subjected to XRD(X-Ray Diffraction) examination to find out the crystalline structure of the sample's particles. EDS was used to find out he chemical composition of a sample on both a qualitative (element type) and quantitative (element concentration %) level.
Proximate analysis procedure of powder described in detail below:

Percentage Moisture Content: The Percentage Moisture Content of powder was evaluated to determine moisture content following standard procedure (Dutta and Singh, 2011). Briefly, weight of powder was measured before and after the water elimination by evaporation by moisture analyzer (Kern-MLS). Percentage moisture in the sample was determined using the formula,

$$\mathbf{MC\ (\%)\ =\ \frac{W_1 - W_2}{W_2}\ x\ 100}$$

where, W_1= Initial Weight of the sample (g) and W_2= Final Weight of the sample (g)

Volatile Matter Content: A known mass crucible was filled with 2g of the mixed fruit powder sample, which was then baked at 105℃ for an hour. Oven-dried sample is currently being held for 10 minutes at 550 ºC in a muffle furnace. The sample was then put in a desiccator to cool. The equation was used to compute the percentage of volatile matter are follows:

$$\mathbf{VM\ (\%)\ =\ \frac{W_1 - W_2}{W2}\ x\ 100}$$

Where W_1 is the sample's weight after being dried in an oven (g), and W_2 is the weight of sample's following being heated to 550 ℃ (g).

Ash Content of powder: A Muffle furnace was used to measure the amount of ash present. Sample powder (10-15 g) was taken in a porcelain bowl and kept inside the Muffle furnace at 550ºC for 3 hours and then cooled the sample, keeping the crucible bowl in a desiccator. The weight difference method calculated the % of total ash present in the food sample.

$$\mathbf{PAC} = \frac{\mathbf{W_4 - W_1}}{\mathbf{W_2 - W_1}} \mathbf{x\ 100}$$

Before heating:

Weight of crucible = W_1 gm

Weight of crucible with food sample = W_2 gm

Weight of powder sample (W_3) = W_2-W_1 gm (W_2> W_1)

After completion of heating:

Weight of crucible = W_4 gm

Then, weight of residue (ash) = W_4- W_1 gm

Fixed Carbon of powder: The fixed carbon (in%) has been established using the conventional methodology (Akowuah*et al.*,2012).

$$\mathbf{FC\ (\%) = 100 - (VM + MC + AC)}$$

where, VM= Volatile Matter, MC= Moisture Content and AC= Ash Content

Percentage of Fat: Total fat in the powder sample was extracted using Soxhlet techniques (Reis et al., 2016). The Soxhlet apparatus was filled with powder (3 g), which was added in an extraction thimble. In the round bottom flask the solvent mainly Petroleum benzene (Non-Polar) was taken and apparatus was arranged along with condenser properly. As the condensed liquid (boiled solvent) drops on the thimble containing the sample, it causes dissolution of organic matter and is filtered out from the sample. As the liquid level rises the solution flows through siphon and comes back in a round bottom flask and completes one cycle. This cycle was run 5-6 times. The organic substance is left behind when the solvent in the boiling flask is distilled off. Now on the weight difference basis calculate the percentage of fat in the sample.

$$\mathbf{Fat\ (\%) = \frac{W_3 - W_1}{W_2} X100}$$

Where, W_1 = Weight of Empty Flask in g

W_2 = Weight of Powder sample in g

W_3 = Weight of Flask (g) with Fat (after drying)

Calorific Value (Higher Heating Value): The CV(Calorific Value)or HHV (Higher Heating Value) was calculated using the bomb calorimeter method. 2 g of powder sample

was burnt completely in oxides. During the heating process water and calorimeter gained that liberated heat. As a result, the heat gained by the water and calorimeter was equivalent to the total heat lost during the burning of the powder.The formula below was used to calculate theCalorificValue or Higher Heating Value using the measured data. (Obi *et a.*, 2013).

$$\textbf{HHV or CV (Kcal/Kg)} = \frac{\textbf{BF x } \Delta\textbf{t - 2.3 length of wire}}{\textbf{W}}$$

Where**,** BF = Burn Factor is constant having value 13,257.32

Δt = temperature change $(t_2 - t_1)$; t_2 = final temperature

and t_1 = initial temperature

W = Weight of Powder sample taken (g)

Estimation of pH : After properly calibrating the device with buffer solutions of pH 4, 7, and 10 pH of the solutions was determined with a digital pH meter. In a nutshell, 1 hour was given for 5 g of the powder sample to dissolve in 100 ml of distilled water in a flask. Then the appropriate amount of clear aqueous solution was transferred from the flask into the beaker and measurement of solution was recorded.

Estimation of functional properties of powder: Utilising accepted techniques, the powder's functional characteristics, including its ability to absorb water (WAC), oil (OAC), swell (SP), and be dissolved, were evaluated. The results were presented as percentages. Both WAC and OAC were determined with minor modifications based on the method by Beuchat, 1977. To determine WAC, One gramme of the powder sample was dissolved in 10 ml of distilled water using an analytical balance, and the water was then graded to 100 ml. The suspension was shaken vigorously for an hour on lab shakers, followed by a 10-minute centrifugation at 2500 rpm. The OAC was calculated using the same procedure, however oil was utilised in place of distilled water. The expelled fluid's volume was then quantified using a technique developed by Sousulski et al. in 1976.
WAC and OAC were calculated using the provided formulas below:

$$\textbf{WAC (\%)} = \frac{\textbf{W}_2}{} \textbf{X100}$$

$$W_1$$

Where, W_1 is the weight of sample in gram taken

W_2 is the weight of absorbed distilled water in gram

$$\textbf{OAC (\%)} = \frac{W_3}{W_1} \textbf{ X100}$$

Where, W_1 is the weight of sample in gram taken

W_3 is the weight of absorbed oil in gram

Standard techniques were used to measure the Swelling Power (SP) and Percentage Solubility (PS) (Oladale and Aina, 2007). One gram of the powder sample was dispersed in 10 ml of distilled water, which was then placed in a graduated 50 ml centrifuge tube. A magnetic stirrer was used to properly mix the suspension as it was heated at 800^{o}C for 20 minutes. The supernatant from this combined mixture was discarded after centrifuging it for 30 minutes at 2500 rpm. Using the following formula, the sediment (paste/swollen sample) was weighed to determine the powder's Swelling Power (SP).

$$\textbf{Swelling Power (SP in \%)} = \frac{\textbf{Weight of mass sediment (g)}}{\textbf{Weight of dry powder taken (g)}} \textbf{ X100}$$

For determination of Percentage Solubility (PS) the paste was dried in air. The weight of dried sediment is measured by using analytical balance and then Percentage Solubility is calculated by using formula given below

$$\textbf{\% Solubility} = \frac{\textbf{Weight of dried residue (g)}}{\textbf{Weight of powder sample taken (g)}} \textbf{ X100}$$

2.2.3 Sensory evaluation and organoleptic properties

The fruit peel powder was also analysed by considering different attributes of sensory quality like flavor, texture, appearance, aroma etc on a 9 point scale (Singh and Mishra, 2022). For that purpose different panel each consists of five members are served with the prepared powder for rating by considering above mentioned quality ranging from 9 (Like extremely), 8 (Like very much), 7(Like Moderately), 6(Like slightly), 5(Neither like nor Dislike), 4(Dislike slightly), 3(Dislike moderately), 2(Dislike very much), 1(Dislike extremely)

2.2.4Morphological analysis of powder

The fine powder of fruit peels was studied using a Scanning Electron Microscope (SEM) by a ZEISS-Scanning Electron Microscope (SEM) to get the size, homogeneity and morphological features of particles in the sample. Images were captured by the proprietary JEOL software (Sathya*et al*., 2014). Energy Dispersive X-Ray Spectroscopy (EDS) was performed to study for the minerals present in the powder sample.

Scanning Electron Microscope study (SEM): The fine powder of fruit peels was studied using a Scanning Electron Microscope (SEM) by a ZEISS-Scanning Electron Microscope (SEM) to get the size, homogeneity and morphological features of particles in the sample. Images were captured by the proprietary JEOL software (Sathya*et al.*, 2014).

EDX study: Energy Dispersive X-Ray Spectroscopy (EDX) is an analytical technique for the qualitative (type of element) and quantitative percentage (of concentration of each element) chemical characterization of a given sample. EDX micro analysis was performed to detect the presence of various elements in peels powder.

XRD study: X-Ray Diffraction (XRD) is an important research aspect for the crystalline structure of the compound. In this study it was analyzed for the presence of different minerals and morphological variations in peels powder. The most common application of X-ray powder diffraction is for the detection of unidentified crystalline materials. (e.g. minerals, inorganic compounds).

2.2.5 Statistical Analysis

Investigation of mixed fruit powder components is mainly studied to know about their functional properties and proximate amounts to make certain about utilization of the powder extract for different purposes. Utilizing a triplicate data collection, all values were shown in mean and standard deviation.

2.3 Results and Discussion

In figure 2.1, the sites from where fruit residual wastes were collected and proceeded for experimental sample preparation. Eleven towns from Purbamedinipur (West Bengal)

were chosen for the sample collection. The towns were nearer to Haldia. Samples were collected from fruit juice centres and residential areas. Before the sample collection, detailed information of tropical fruits which are mainly available throughout the year and during different seasons were recorded. Table 2.1 exhibits the available fruits during different seasons in this district.

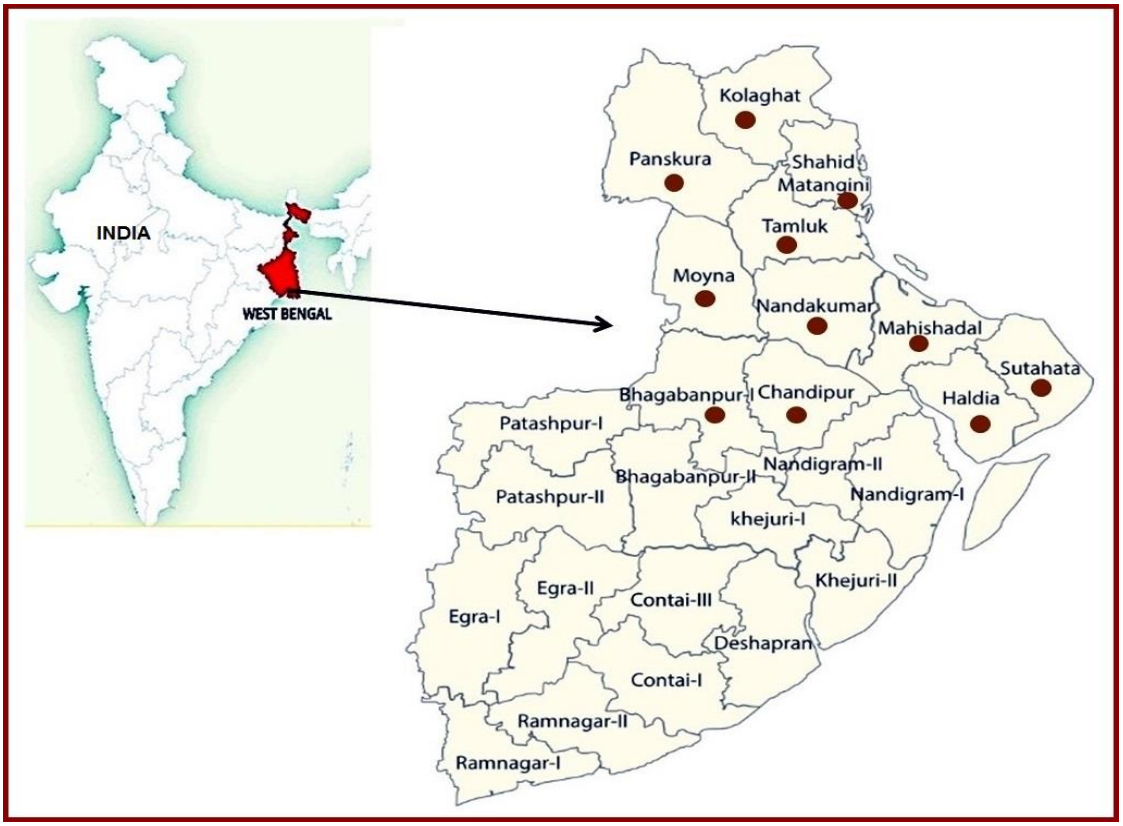


Figure 2.1 Sample collection sites in PurbaMedinipur in West Bengal. Sites where samples for this study were taken are represented by orange dots.

Based on the initial survey, fruit residual wastes were collected during different seasons and powder prepared. Finally, equal volumes of each powder sample were mixed and stored in container(airtight) for use in further experiments. By using the waste from fruit in isolation of phytochemicals for value-added goods, this dissertation aims to minimize MSW and reduce waste from fruit. This will help in proper handling of MSW which might have added up to become a sensitive environmental issue.

Table 2.1 Seasonal fruits available in the district of PurbaMedinipur, West Bengal (India).

Summer	**Monsoon**	**Winter**
Watermelon	Pear	Orange
Lychee	Pomegranate	Sweet Lime
Mango	Apple	Guava
Lime	Banana	Grapes
Apple	Papaya	Apple
Banana		Banana
Papaya		Papaya

2.3.1 Physico-chemical characterization of Fruit waste powder

It was observed that one kilogram of fruit produces approximately 213.34g of peels which is about 21.33 percent of the total weight of fruit (Table 2.2). After completely sun drying the fresh peel keeping it crispy the weight is decreased to about 31.12g which is approximately 14.56 percent of total weight of fresh peels. This decrease in peel quantity from fresh to dry was mostly caused by the presence of fruit remnants with a high moisture content. After grinding in mechanical grinder properly and sieving the powder weight is reduced to 30.07g which is approx 14.1 percent of the weight of fresh peels and it is 0.46 percent less than total dry weight of peels. Overall weight difference between fresh peel and powdered peel was 183.27g and 1.05g respectively. Based on the result it was observed that after consuming one kilogram of fruit, total yield recovery is about 14 percent which quite big amount. The reutilization of these powders in different sector can reduce the municipal wastes and cause great contribution to health and environmental safety.

Table 2.2 Yield of powder from mixed fruit peels

Parameter	Wt. of fresh peel (g)	Wt. of sun dried peel (g)	Wt. of powder (g)
Value	213.34 ± 1.84	31.12 ± 1.26	30.07 ± 1.21

2.3.2 Proximate analysis of powder

Proximate analysis, including measurements of the percentages of Percentagemoisture content (PMC), Percentage volatile matter (PVM), Percentage ash content (PAC), Percentagefixed carbon (PFC), fat percentage, higher heating value (HHV), and pH, were performed on peel powder using conventional and easy methods by standard analytical procedures. Peel powder has been identified as a possible source of beneficial food components.

The moisture content of peels powder was found 4.2 which are quite low and acceptable(Table 2.3). The lower value of moisture content has potential to inhibit various enzymatic activities and chemical degradation. Additionally, the low moisture level limits the development of germs, extending the shelf life of items. Table 2.3's findings revealed that the average proportion of volatile matter (PVM) in powdered fruit peels is almost 70.8 percent. The quick burning, ease of ignition, and increase in flame length with low heating values are all effects of the powder's high volatile matter content. Fruit peel powder's percentage ash concentration was found to be 3.86%, as indicated in table 2.3. Low ash content offers higher heating value and higher calorific value because ash content makes an effect on burning rate because of heat transfer minimization. Presence of ash in peels powder indicates that it contains considerable amounts of valuable minerals which account its nutritional value and may warrant its consideration for use in food formulation. Table 2.3 showed the percentage of fixed carbon in powder was about 21.11%. Value of this fixed carbon in powder indicates its greater calorific value, lower the moisture contents and its better the quality of powder as far as concerned shelf life and quality.Percentage fat in powder was analysed up to 2.1% as shown in Table 2.3. The low value of fat recommended the peels powder as a food supplement for those people who suffered from lipid induced disorder or having cardiac problems. Lower value of fat reduces the chances of rancidity so it also enhances the shelf life of powder. Higher Heating Value used to indicate the heat energy amount present in a powder. Table 2.3 results indicate the average calorific value of powder produced is 5030.18 Kcal/kg which indicates its better quality as far as nutrition is concerned. pH of food and food products act as one of the important parameters as far as shelf life and quality. Table 2.3's results reveal that the powder's pH was determined to be 3.5. The low pH values indicate that the powder is acidic in nature which is essential to keep it in native form for a longer time.

Table 2.3 Proximate examination of fruit waste fine powder

Parameters	**Amount**
PMC	4.2
PVM	70.8
PAC	3.86
PFC	21.11
Fat (%)	2.1
HHV (KCal/kg)	5030.18
pH	3.5

2.3.3 Determination of functional properties of powder

The typical methods used to calculate the powder's percentage solubility, swelling power, and water and oil absorption capacities are presented in Table 2.4. The powder sample's water absorption capacity (WAC), as indicated by the results in Table 2.4, was about 3.2 %. Powder's ability to absorb water means that it will quickly turn into dough and may be effortlessly blended to create a product with additional value. It also measures whether the protein is incorporated with the aqueous food formulations and therefore implies that utilization of the powder necessitates the use of more liquid, and this may find application in food industries. OAC of peels powder is about 2.4 %. This value of OAC indicates that the powder is as good as oil absorbent and lipophilic in nature. It is potentially useful for structural interaction in food and ultimately enhancing the retention of flavour, increasing shelf life and improvement of palatability.The value of swelling power the time required by powder to reach its wetness. Result shows the Swelling Capacity of powder is around 4.4 %. Essential features of swelling influenced by the presence of fat and protein in powder. Since it can absorb water and swell, therefore, it can be used as a thickener in liquid and semi-liquid foods to improve the consistency in food formulations. The molecular size and weight of the powder greatly influenced the solubility. Percentage Solubility for prepared powder samples was observed as 63.5 % shown in Table 2.4. The high value of solubility of powder indicates that it is easily digestible and suitable for formulations of different foods.

Table 2.4 Fruit peel powder's practical characteristics

Parameter	Value (%)
WAC	3.2±0.23
OAC	2.4±0.19
SP	4.4±0.29
PS	63.5±0.33

2.3.4 Sensory evaluation and organoleptic properties

Results for Sensory and organoleptic properties like Flavour, Texture, Appearance, Aroma etc on a 9 point scale by different panels are displayed in Table 2.5. The average score for flavour and aroma is 8.6. This might be due to the presence of peels of citrus fruit. Average value for the texture was 7.4 and for the appearance were 7.2.

Table 2.5 Sensory evaluation and organoleptic properties

Pannel Members	SCORE			
	Flavour	Texture	Appearance	Aroma
Panel Members 1	9	7	8	9
Panel Members 2	9	8	7	8
Panel Members 3	7	8	7	9
Panel Members4	9	6	8	9
Panel Members 5	9	8	6	8
Mean Value	8.6	7.4	7.2	8.6

2.3.5 Scanning Electron Microscope study (SEM)

In Figure 2.2, A-D, show the morphological nature of powder at magnification 200X, 1000X, 2000X and 5000X. The purpose of SEM analysis was to observe the exact morphological appearance of powder. Results show that the peel powder depicts an uneven geometry of present particles at magnification 200X due to amorphous nature of the powder sample, but as we increase the magnification the smoothness of surfaces increases and it shows very clear smooth and bigger surface particles at 5000X of magnification.

2.3.6 EDX study

Energy Dispersive X-Ray Spectroscopy of the powder in Figure 2.3 shows highest percentage of carbon (C) and the least amount of potassium (K)

2.3.7 XRD study

As a result of the atoms' random orientation in amorphous materials, incoming X-ray light is spread randomly and produces broad peaks. Contrarily, when X-ray radiation interacts with crystalline materials' lattice planes, it scatters specifically in some directions and produces tight, high-intensity peaks. Dairy powder crystals show typical peaks at 2 = 17.2° and 21.7° (Figure 2.4).

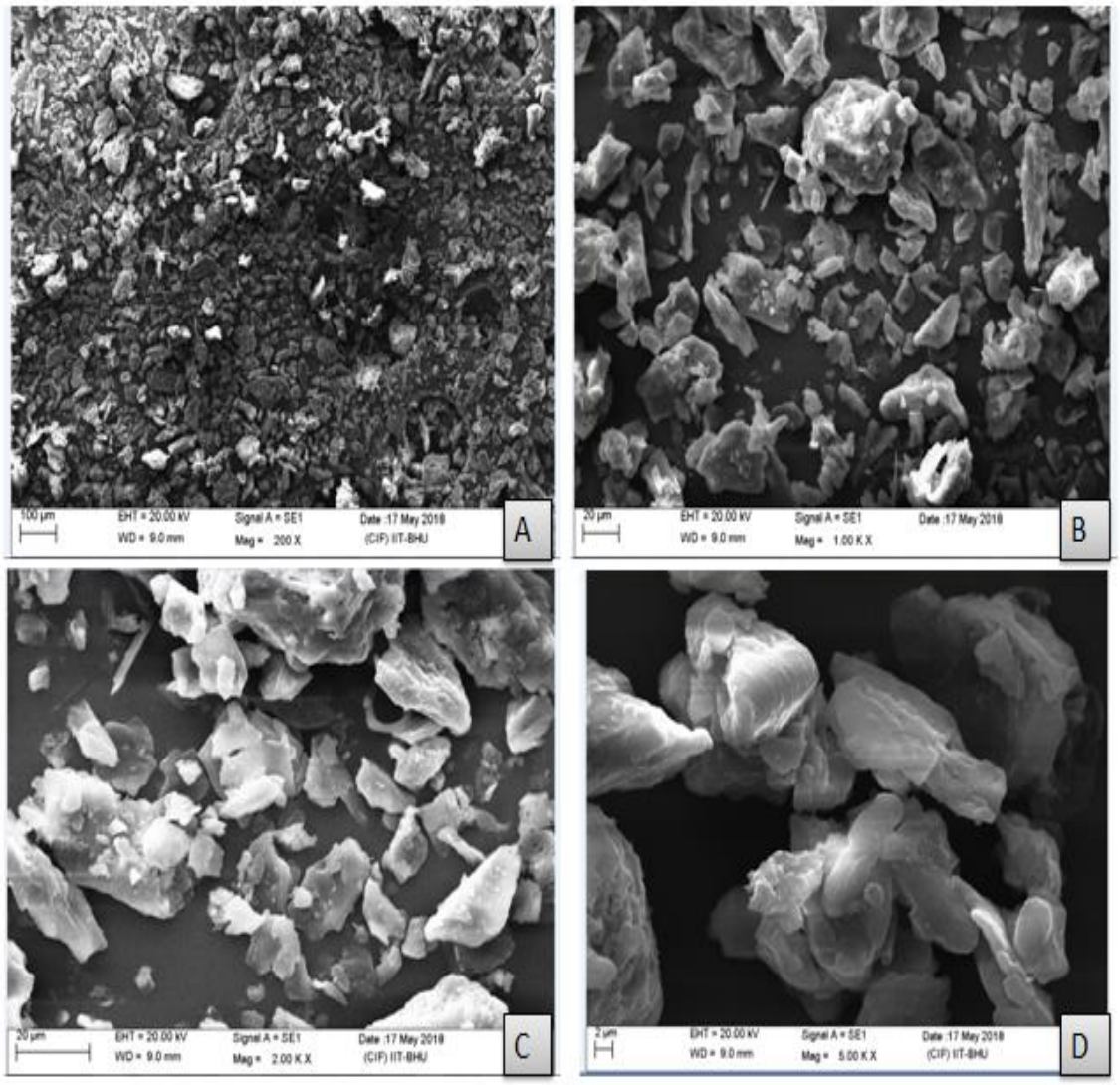


Figure 2.2 SEM images of fruit waste powders at different magnifications (A) 200x (B) 1000x (C) 2000x (D) 5000x

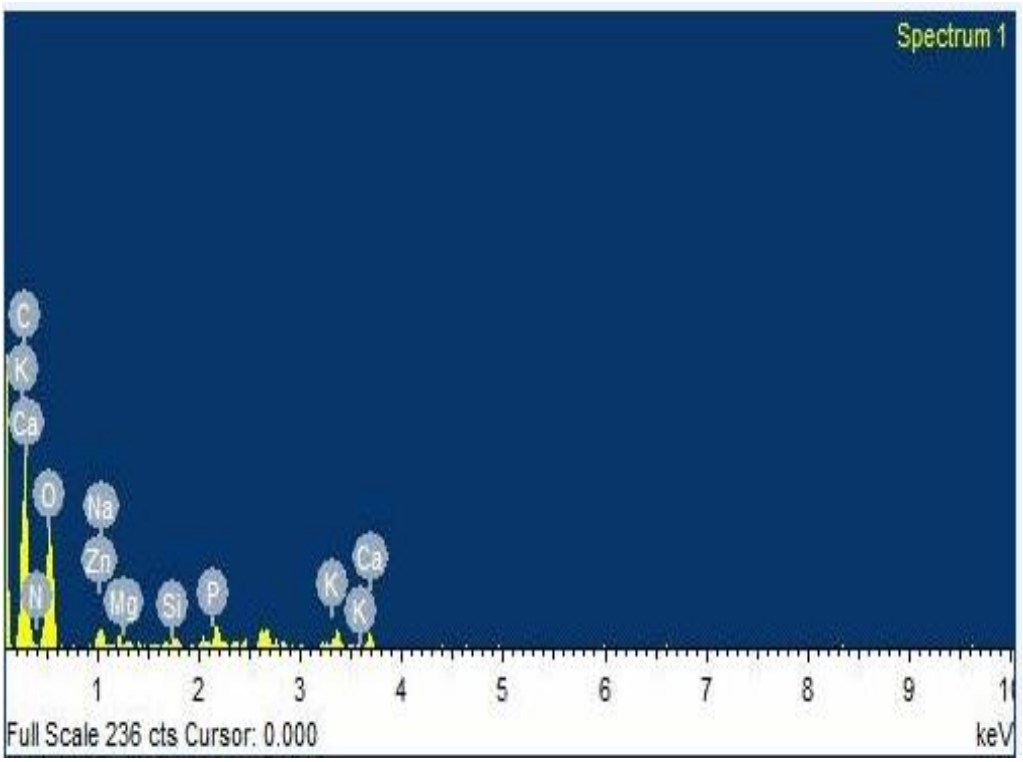


Figure 2.3EDX of Fruit Peels Powder

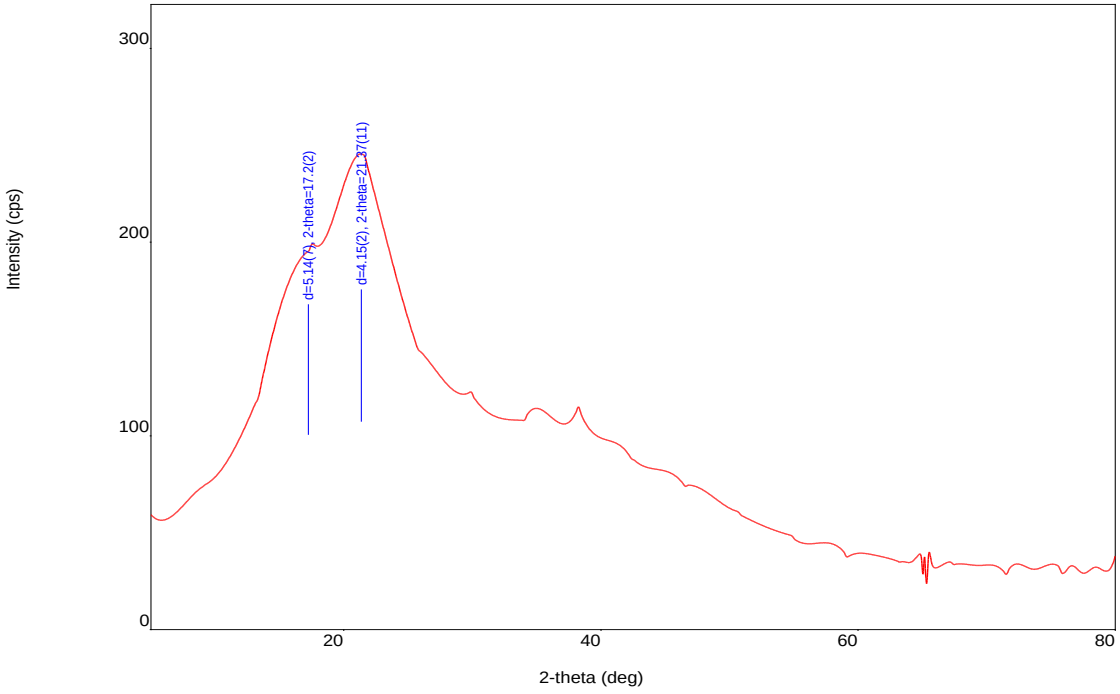


Figure 2.4 X-Ray Diffraction (XRD) of fruit peels powder

2.4. Conclusion

The main conclusion of this study was that, despite the fact that fruit leftovers have beneficial properties and can be used for a variety of purposes, such as the food processing industry for food formulations, antioxidant-rich cosmetics, and herbal drugs, they are typically thrown away as waste after the pulp has been consumed. A sizable portion of crude fat, ash, moisture, and little carbohydrate were present. The results of the proximate analysis show that dried mixed fruit powder is an excellent source of nutrients.

Chapter 3

Identification of Phytochemicals using GC-MS in Five Fruit Extracts residual Wastes Powder

3.1 Introduction

Fruits and vegetables play a crucial role in our diet, and due to shifting dietary habits and the expanding global population, their demand has continuously increased. (Schieber*et al.*, 2001, Vilariño*et al.*, 2017). According to the FAO (Food and Agriculture Organization), fruits and vegetables account for up to 60% of losses and waste among all types of foods. The processing of these food items generates significant amounts of organic waste, comprising approximately 25% to 30% of the total product group. Unfortunately, this organic waste has become a major contributor to municipal solid waste (MSW), leading to environmental concerns (Salehiyoun*et al.*, 2019). Fruit and vegetable wastes are excellent sources of beneficial bioactive substances such carotenoids, polyphenols, dietary fibres, vitamins, enzymes, and oils. This includes seeds, skins, rinds, and pomace. These bioactive substances have uses in a number of industries, including textiles, pharmaceuticals, food, and health. Utilizing waste to produce bioactive components is an essential step toward sustainable development. Bioactive compounds found in food wastes have potential health benefits, and they are studied for their roles in preventing and protecting against diseases like cancer and heart disease. Antioxidants found in these compounds help eradicate free radicals in the body, including those involved in amyloid beta (Aβ) aggregation (Koulakiotis*et al.*, 2020). These compounds occur in small quantities in foods and provide extra nutritional benefits. Various extraction techniques are used to analyze and determine these bioactive compounds, and solvent extraction methods, employing solvents like methanol, ethanol, butanol, water, and petroleum ether, have been found effective (Abubakar and Haque, 2020). Flavonoids are among the most abundant bioactive compounds found in fruit waste powder, and their dietary intake is recommended to prevent and reduce the risk of chronic diseases (Paul *et al.*, 2002, David *et al.*, 2010). Flavonoids have molecular pathways that reduce natural antioxidant activity, decrease cell growth and angiogenesis, and alter transcription factors and related kinases. (Panche*et al.*, 2016). Fruit peels, in particular, contain higher

amounts of polyphenols and flavonoids compared to the fruit flesh. Anthocyanin, flavonol, kaempferol, and xanthone glycoside are also found in the peels of many fruits (Chinnici*et al*., 2004; Berardini*et al*., 2005).

This dissertation aims to analyze the biochemical components present in each extract of fruit waste powder using standard protocols. This includes quantitative and qualitative analysis of bioactive components and the phytochemicals present in water, ethanol, methanol, petroleum ether, and butanol extracts, using GC-MS as an analytical technique.

3.2 Materials and Methods

This study employed a powder made from fruit waste. Following the preparation of extracts using various solvents (water, methanol, ethanol, butanol, and petroleum ether), several tests were used to determine the presence of significant nutritional and bioactive components. All of the solvents utilized in this were HPLC grades. Chemicals and reagents were purchased from Himedia and Sigma.

3.2.1 Soxhlet method of extracts preparation

The extraction procedure was carried out according to Redfern and co-workers with minor modifications (Redfern *et al*., 2014). 100 mg of dried powder was filled in the extraction thimble and positioned inside the Soxhlet apparatus. In the round bottom flask 500 ml of each selected solvents were taken separately and apparatus was arranged along with condenser properly. As the condensed liquid (boiled solvent) drops on the thimble containing the sample, it causes dissolution of organic matter and is filtered out from the sample. The solution runs through the syphon as the liquid level increases, returns to the flask with a flat bottom, and completes one cycle. This step was repeated 5-6 times. After the extraction process, it was filtered using filter paper (Whatman No 1) and thereafter, allowed to evaporate to dryness using a vacuum evaporator. The weight of dried extracts was recorded and the stock solution of 10 mg/mL was made using 1.0 % DMSO. The extracts were designated as aqueous extract, ethanolic extract,methanol extract,petroleum ether extract and butanol extract.

3.2.2 Phytochemicals analysis of extract

Qualitative Phytochemicals Screening: Estimation of phytochemicals is a basic step to check the medicinal values of plants. Presence of phytochemicals such as steroids, amino

acids, flavonoids, reducing sugars etc were determined based on some standard methods (Devmurari *et al.*, 2010, Dutta and Singh, 2011,Trease and Evans, 1989).

Amino acids: For the amino acid analysis, 0.5ml of each extract from the sample was extracted, and it was then mixed with 0.5ml of methanol. Then, a few drops of ninhyrin reagent (0.25% w/v) were added to this solution. The ninhyrin reagent was made by combining 30 mg ninhyrin with 10ml of n-butanol and 0.3ml of 98% acetic acid. For a short while, the solution was heated in a water bath. The existence of amino acids was established by the appearance of a purple tint.

Anthraquinones: To conduct this test, 1 ml of the sample's extract was evaporated and then dissolved in 2 ml of chloroform. Additionally, 2ml of cold ammonia was added to it. The presence of anthraquinones was identified by red or orange coloration.

Phytosterol: Extract from the sample diluted in chloroform after 2 ml of it had been evaporated. By the side of the test tube, 2ml of sulfuric acid (concentrated) was added to it. The presence of phytosterol was shown by the red colour of the ring.

Tannin: Methanol was added to 0.5ml of each extract. The solution also contained 0.5ml of lead acetate that had been diluted to 1% in methanol. The appearance of a brownish yellow precipitate indicated the presence of tannin.

Cardiac glycosides: Each extract sample's was diluted in one mililitre of glacial acetic acid after 0.5ml of each extract was evaporated. After adding one mililitre of concentrated sulphuric acid at the side of the test tube, 1 drop of 10% ferric chloride was added to the mixture. Green colour is visible in the top layer, which was the acetic layer, and a brown ring may be seen at the interface. This established whether cardiac glycosides were present.

Reducing sugar: To conduct this test, 0.5 millilitres of each extract were obtained, and 1 millilitre each of Fehling's A solution and B solution was added. Water was used to boil the mixture. Precipitation that was brick red indicated the presence of reducing sugar.

Phenols: To 2ml of each extract, add 3-4 drops of ferric chloride (2 gm $FeCl_3$ + 12.5 ml H_2O), mix vigorously, and let stand for a few minutes. The existence of phenols was established by the appearance of the blue blade hue.

Flavanoids: Each 2 ml sample of the extract is given a few drops of the NaOH solution. After a while, a strong yellow colour that fades quickly showed the existence of flavanoids.

Alkaloids: For this test, 2 ml of each sample are obtained, and they are combined with Mayer's reagent, which is a solution of potassium iodide (5.00 g) and mercuric chloride (1.36 g) in 100 mililitre of distilled water. After standing for a little while, a yellow precipitate indicated the presence of alkaloids.

Tri-terpenoids:1 mililitre of chloroform was added to 0.5 mililitre of each extract. Then it received 2 ml of concentrated sulphuric acid and 1 ml of cold, cooled acetic anhydride. Tri-terpenoids were detected when a reddish violet tint developed.

Resins:1 ml of petroleum ether is then used to dissolve 0.5 ml of each extract after it has evaporated. 2% Copper acetate (made in water) in a volume of 2 ml was then added. briskly shaken, then given time to separate. The bottom layer's appearance of green hue indicated the presence of resins.

Quantitative Phytochemicals Estimation: To determine the medicinal and dietary value of plant specimens, it is crucial to dissect plant extracts to profile the many phytochemicals present in plant tissues. The five extracts from fruit powder prepared using different solvent system was subjected to biochemical analysis to quantity the nutritional components (proteins, carbohydrates and vitamins); antioxidants compounds (total phenols and flavonoid contents) and potential in scavenging free radicals (by DPPH model) (Devmurari *et al*., 2010,; Dutta and Singh, 2011). Standard methodologies including GC-MS studies were followed to evaluate the important bioactive compounds present in each extract. Characterization of each identified extract component was investigated by the National Institute of Standards and Technology (NIST) library (Mallard *et al*., 2018)

Total protein content estimation: To assess the nutritional value of the plant extract, the total protein content was analysed. The Lowry technique was used to conduct the test (Lowry, 1951). 80 µl of distilled water were mixed with 20 µlof the extract. After that, 500 µlof the Protein's reagent was added, and it was incubated for 5 minutes. Following that, 50 µl of FolinCiocalteu reagent was added, and the mixture was again incubated for 20 minutes in the dark. As a reference protein, bovine serum albumin (BSA) was utilised. Using a UV-VIS spectrophotometer, the produced color's absorbance at 660 nm was measured. Protein concentration was calculated in g/mg using the usual plot.

Carbohydrate estimation: Anthrone reagent was used to calculate the total amount of carbohydrates. In a boiling water bath for three hours, sample extract (1 ml) was hydrolyzed with 5 ml of a 2.5 N HCl solution. The reactant is cooled to room temperature once the hydrolysis process is finished, and then neutralised with solid sodium carbonate till the effervescence appears. A 100ml volume was created, and it was centrifuged. For analysis, the supernatant was used. 1 ml of the hydrolyzed sample was combined with 4 ml of the anthrones reagents, which was then in a water bath heated for 8 minutes. The sample's absorbance at 630 nm was measured after cooling it down. OD630nm measurements of various glucose concentrations were put on a standard graph, and the quantity of carbohydrates (g/mg) present was determined from the graph.

Total phenol content estimation: The Folin-Ciocalteau spectrometric technique was used to quantify the phenolic content of the plant extract with minor modification (McDonald et al., 2001). 100 µl of each extract were mixed with 1 ml of water. The FolinCiocalteu reagent was then added to each set in an amount of 0.5 ml. The mixture was mixed briskly and allowed to sit at room temperature for 3 minutes. One millilitre of 20% Na_2CO_3 was added after incubation. Using a UV-VIS spectrophotometer, the mixture was vortexed and heated for five minutes at 95 degrees Celsius in an incubator. The absorbance of the produced colour was then measured. Gallic acid was used to plot an industry-standard curve. In terms of GAE(gallic acid equivalents) per gm of powder weight in g/mg, total phenolic content (TPC) was stated.

Total Flavonoid Content estimation: The aluminium-chloride colorimetric technique was used to measure the flavonoid content (Prieto *et al*., 1999). The following ingredients were added progressively and well blended: 0.5 mililire of each extract, 1.5 mililire methanol, 0.1 ml of $AlCl_3$ (10%), 0.1 ml of potassium acetate solution, and 2.8 mililire of distilled water. Similar steps were taken to create a blank sample, but instead of using $AlCl_3$, they used distilled water. The test solution was shaken ferociously. At 440 nm, absorbance was measured following 30minute incubation. At 440 nm, a standard calibration plot was produced using quercetin quantities that were known. The calibration plot was used to compute the flavonoid concentrations (g/mg) in the test samples, which were then reported as mg quercetin equivalent/g of the sample.

Vitamin C content estimation:A 250 ml conical flask was filled with 20 mililire of sample solution, 150 mililire of distilled water, 3 ml of starch indicator solution, and 20 ml more. Iodine solution containing 0.005mole L^{-1} was then used to titrate the sample solution. The first enduring evidence of the starch-iodide complex's dark blue-black color was found at the titration's endpoint. The Standard solution (Ascorbic acid, 10mg in 20ml distilled water) followed a similar process. Until we had findings that agreed with each other, the titration was repeated with additional aliquots of sample solution.
Concentration of Vitamine C is calculated by using formula:

$$\frac{\textbf{Avg. value of } I_2 \textbf{ solution used for standard solution}}{\textbf{10 mg}} = \frac{\textbf{Avg. value of } I_2 \textbf{ solution used for sample solution}}{\textbf{X mg}}$$

Total Antioxidant Activity estimation: Total antioxidant activity (TAA) is calculated by converting each extract's molybdenum (VI) to molybdenum (V) and then developing a green solution (Raghavendra*et al*., 2010). 1 ml of the reagent solution (0.6 M sulphuric acid, 4 mM ammonium molybdateand 28 mM sodium phosphate) was combined with 0.1 ml of the extract from each solvent. Each tube was sealed, then incubated for 90 minutes at 95°C before being cooled to ambient temperature (25°C). The same conditions as the samples were used to incubate a blank that included 1 ml of reagent solutions mixed with the proper quantity of water. Measured the sample's absorbance at 695 nm in comparison

to a control. Ascorbic acid equivalents (g/mg) were used to indicate the amount of water-soluble antioxidant activity.

DPPH radical scavenging capacity determination: Each extract's ability to scavenge the stable DPPH free radical was measured using a slightly modified version of Blois' 1958 methodology. Briefly stated, 1 ml of 0.1 mM methanolic DPPH solution and 4 ml of each extract (10–50 g/ml) were combined. After thoroughly blending, the reaction mixture was let to remain at ambient temperature for about 20 minutes. At 517 nm, the samples' altered absorbance was gauged. The inhibition percentage, which was determined using the formula used to express the radical scavenging activity are as follows:

$$\textbf{Percentage Inhibition Activity} = \frac{\textbf{A-B}}{\textbf{A}} \textbf{ X 100}$$

Where, A = blank solution absorbance and B = test solution absorbance.

Gas chromatography-mass spectrometry (GC–MS) analysis: The study's packed fused silica column, the Elite-5MS (5% biphenyl, 95% dimethylpolysiloxane, 30 m, 0.25 mm ID, 250 m df), was employed in the Clarus 680 GC. Helium was used as the carrier gas, flowing at a constant rate of 1 ml/min, to separate the components. The injector temperature during the chromatographic run was 260°C. When the 1 liter sample of extract was placed in the apparatus, the oven's temperature was as follows: After maintaining a temperature of 60 °C for two minutes, 300 °C was attained for six minutes at a rate of 10 °C per minute. 240 °C for the transfer line, 240 °C for the ion source, and 70 eV for the electron were the mass detector's operating conditions.

3.2.3 Statistical Analysis

The main goal of mixed fruit extract analysis is to identify specific bioactive components, and then to determine their pharmacological and neutraceutical capabilities for use in a variety of industries to create products with added value. Using a triple data collection, the mean and standard deviation of each value were reported.

3.3 Results and Discussions

The fruit wastes extracts were used for screening and identification of commonly reported phytochemicals. The results showed the presence of phytosterols,amino acids, anthraquinones, cardiac glycosides,tannin,flavonoids andreducing sugar. The presence of such important bioactive components in significant amounts from fruit waste demonstrates that MSW organic fraction mainly vegetables and fruit wastes are a treasure house of important phytochemicals (Chockchaisawasdee and Stathopoulos, 2017). These phytochemicals are reported to have important application in pharmaceutical, cosmetics and food industries

3.3.1 Determination of the yield of extract

Extract yield was determined using fine analytical balance on weight difference basis and found that each extract shows sufficient yield but the yield was maximum in water (as shown in Table 3.1).

Table 3.1 Phytochemicals yield of five extracts

Extract	Yeild (mg/g)
Methanol extract	245±6.4
Ethanol extract	26.3±0.7
Petroleum ether extract	3.2±0.4
Butanol extract	21.3±0.4
Water extract	337±5.7

3.3.2 Qualitative Phytochemical Analysis

The sample of each extract was used for determination of commonly reported phytochemicals. The results are given in Table 3.2which showed the presence of amino acids, anthraquinones, phytosterols, tannin, cardiac glycosides, reducing sugar, phenols, flavanoids, alkaloids, tri-terpenoids and resins. The presence of such important bioactive components in significant amounts isolated from fruit waste demonstrates that MSW containing fruit wastes can be reduced by utilizing these wastes to isolate important

phytochemicals (Chockchaisawasdee and Stathopoulos, 2017). These phytochemicals are reported to have important applications in food industries, pharmaceuticals and cosmetics. (Mohamed*et al*., 2018).

Table 3.2 Phytochemicals identification of solvent extracts-

Phytochemicals	**Water extract**	**Ethanol extract**	**Methanol extract**	**Petroleum ether extract**	**Butanol extract**
Amino acids	++++	++	+	+	++
Anthraquinones	++	+++	+	+++	-
Phytosterol	+++++	++	++	+	+
Tannin	++++	++	+	+	++
Cardiac glycosides	+++	++	++	+	+
Reducing sugar	+++++	++	++	+	++
Phenols	+++	+	+	+	++
Flavanoids	+++	++	++	++	+
Alkaloids	+++	++	+	+	++
Tri-terpenoids	++	+	+	++	+
Resins	++	+	+	-	+

+ indicates degree of abundance in the extract

3.3.3 Quantitative Phytochemical Analysis

The results of quantitative analysis of various phytoconstituents of ethanol, methanol, petroleum ether and butanol extracts of fruit peels powder are shown in Table 3.3. The total protein content was estimated for each of the extracts using the standard plot of BSA solution (y= 0.0034x+0.0044; R^2=0.9989). The total carbohydrate content is calculated from the standard curve of glucose (y=0.0012+0.867, R^2=0.9945). The total phenolic content was estimated from the standard curve of gallic acid (y= 0.014x-0.036, R^2=0.9031). The total flavonoid content was evaluated on the standard curve of quercetin (y=0.0001x+0.011; R^2=0.988). The total Vitamin C of each extract is estimated by an oxidation-reduction method using ascorbic acid as standard. The result showed that the fruit peels are rich in important phytochemicals which are related to antioxidant and antimicrobial properties. The total antioxidant capacity is significantly high in the all

extract, which demonstrates good free radical scavenging activity. In this study, DPPH• is used as a source of stable free radical. DPPH• is widely used to test the potential of any chemical compound or herbal extracts in eradicating free radicals (Dash *et al.*, 2005). The LD_{50} value, a measure of the extract concentration that was required for 50 % inhibition of the free radical DPPH•, was determined and was recorded for each of the extracts. Hence, dietary compounds rich in antioxidants are considered as food supplements to prevent free radical damage to cellular biomolecules like DNA, proteins and lipids. Equally, the phytomolecules be exploited for food preservation and storage.

Table 3.3 Total protein content (µg/mg), total carbohydrate content (µg/mg), total phenol content (TPC in µg/mg), total antioxidant capacity (TAC in µg/mg) , total flavanoid content (TFC in µg/mg), Vitamin C in µg/mg and LD50 in µg for radical scavenging of each extract.

Extract	Total Protein Content	Total Carbohydrate Content	TPC	TAC	TFC	Vitamine C	LD50 of DPPH
Water	16.04± 1.6	12.32 ±1.21	44.11±0.82	190.73±1.74	121.1±1.21	1.51±0.32	10
Ethanol	10.67±0.51	11.72 ± 0.84	38.22±0.81	160.22±1.33	111.21±1.01	1.01±0.43	70
Methanol	9.12± 0.76	10.41 ± 0.82	33.12±1.21	180.22±1.09	118.33±0.98	1.11±0.88	56
Petroleum Ether	6.32± 0.19	6.45 ± 0.54	28.87±0.76	175.34±.88	120.22±1.11	1.38±0.67	62
Butanol	10.13±0.23	11.28± 0.34	36.34±1.33	156.23±0.97	109.11±0.76	1.21±0.48	43

3.3.4 GC-MS analysis of extracts

Extraction and characterization of phytochemicals having antimicrobial and antioxidant properties from plants have resulted in the relief of many medical complications caused by pathogens and pollutants. GC–MS have been widely used for identification and characterization of various bioactive compounds present in plants (Ralte*et al.*, 2022, Singh, and Vellapandian, 2022, Ferdous*et al.*, 2017,Ezhilan and Neelamegam, 2012). In this study, fruit wastes powder extracts (aqueous, ethanolic, methanolic, petroleum ether and butanolic) were analyzed by GC-MS for identification of various bioactive compounds present in five extracts. Bioactive compounds in each extract are identified and were shown in Figure 3.1-3.5. Apart from a few common compounds each extract has

a different compound identified in them. The National Institute of Standards and Technology (NIST) library (NIST Chemistry Web Book, 2008) identified the vital bioactive molecules in the extract (Mallard *et al.*, 2008). The GC-MS data of the four extracts were analyzed in detail and were individually presented below.

Aqueous Extract: The aqueous fruit peel extract's GCchromatogram revealed 13 peaks(Figure 3.1). The presence of 13 phytochemicals with specific functional groups, or chemical components, is indicated by the peaks at various RT-value. The thirteen bioactive chemicals were characterised and identified using the NIST library(Table 3.4). The substances found are N-Hexadecanoic acid, Ethanamine, 2-propoxy-, 1,3-Dioxane, 2,4-Dimethyl, Diglycerol, L-Talose,6-Deoxy-3-C-Methyl2-O-Methyl-, 7-ene-1,3-diol butaneboronate, 2-Amino-octadec-4-Ethyl-2-hydroxycyclopent-2-en-1-one, 2,3-Anhydro-D-Galactosan, Heptacosanoicacid,methyl ester,1-Tridecyne, Eicosanoic acid, 3-Deoxy-D-mannitol, and 14-Heptadecenal. N-Hexadecanoic acid is one of the compounds found in the aqueous extract that have been shown to have antioxidant and antibacterial activities. Some can use ethanamine as a dye intermediary. Diglycerol is used in cosmetics, food additives, and other products (NCBI, 2005). According to Narasimhamurthyet al., 2020, 2-propoxy-, 1,3-Dioxane, 2,4-Dimethyl possesses anti-inflammatory and anti-diabetic characteristics. Along with their powerful antibacterial and anticancer properties (Whyte *et al.*, 2013), 4-Ethyl-2-hydroxycyclopent-2-en-1-one suppresses and destroys biofilm (Guchhaitet al., 2002). Eicosanoic acid, commonly known as arachidic acid, is essential for maintaining vascular permeability, cell and organelle integrity(Beck *et al.*, 1998), and emulsifying properties in the cosmetic industry (NCBI, 2004). In the food sector, 3-Deoxy-D-mannitol is employed as a stabilizer and thickening, flavoring agent, lubricant and releasing agent, and nutritive sweetener (Pigman, and Horton, 1957). Several scholars during his investigation recognized 14-Heptadecenal as an aroma-imparting chemical (Chakraborty et al., 2022).The structure of each molecule in the aqueous extracts of fruit wastes as determined by MS is given in appendix.

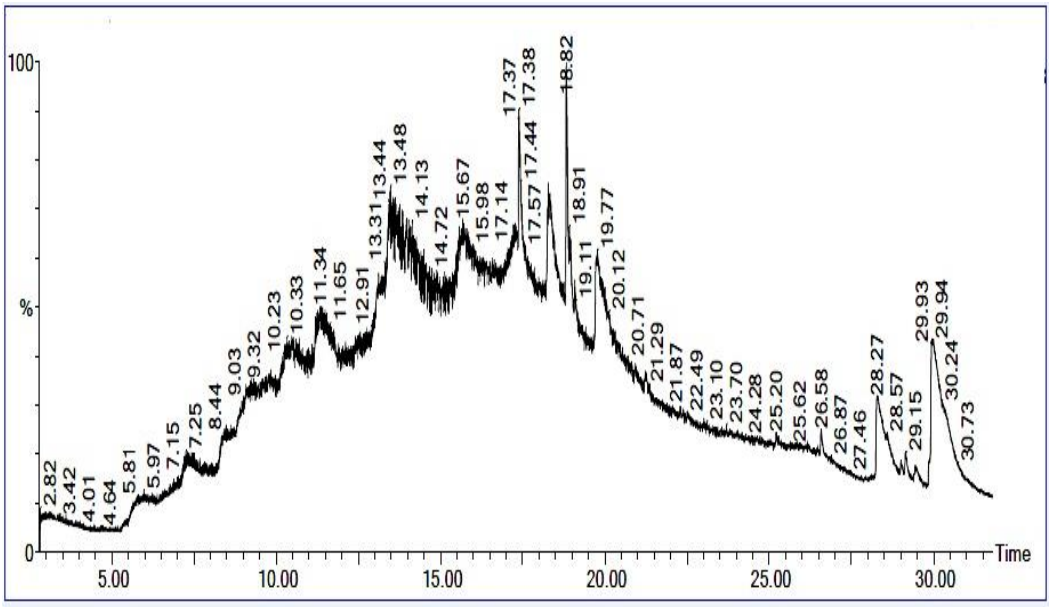


Figure 3.1GC chromatogram of aqueous extract prepared from fruit residual wastes.

Table 3.4 Bioactive components identified by GC-MS analysis of aqueous extracts prepared from fruit wastes powder.

Sl.No.	Bioactive Components	RT-Value	%Area
1	N-Hexadecanoic acid	18.275	12.374
2	Ethanamine, 2-propoxy-	7.355	3.034
3	1,3-Dioxane, 2,4-Dimethyl	9.83	3.034
4	Diglycerol	10.471	3.499
5	L-Talose, 6-Deoxy-3-C-Methyl2-O-Methyl-	11.377	1.99
6	2-Amino-octadec-7-ene-1,3-diol butaneboronate	12.56	0.891
7	4-Ethyl-2-hydroxycyclopent-2-en-1-one	13.628	2.123
8	2,3-Anhydro-D-Galactosan	15.673	16.06
9	Heptacosanoic acid, methyl ester	17.384	20.432
10	1-Tridecyne	18.82	3.896
11	Eicosanoic acid	18.915	2.581
12	3-Deoxy-D-mannitol	19.085	2.907
13	14-Heptadecenal	28.274	5.499

Ethanol Extract:GC chromatogram analysis of the ethanolic extract of fruit peels (Fig. 3.2) showed 12 peaks. The peaks at different RT-values indicate the presence of 12 phytochemicals having specific functional groups i.e., chemical constituents. The twelve bioactive molecules were characterized and identified by the NIST library (Table 3.5). The compounds identified are N-Hexadecanoic acid; Sulfurous acid, 2-Propyl tetradecyl ester; 9,12 Octadecadienoic acid(z,z); Vitamine-E; 9,19-Cyclolanost-24-en-3-ol, Acetate (3.Beta.);7 Hexadecenal, (Z); Squalene; Tetratriacontane; Pregnan-16-one, 20-((3-hydroxy-6 Methyl)oxacyclohex-3-yl)methyl; Tetradecane, 1-chloro-3,3- Bis-tert-butylsulfanyl-2-fluoro acrylonitrile and Oxirane, tetradecyl. MS analyzed spectrum of each molecule in the ethanol extracts of fruit wastes are shown inappendix.

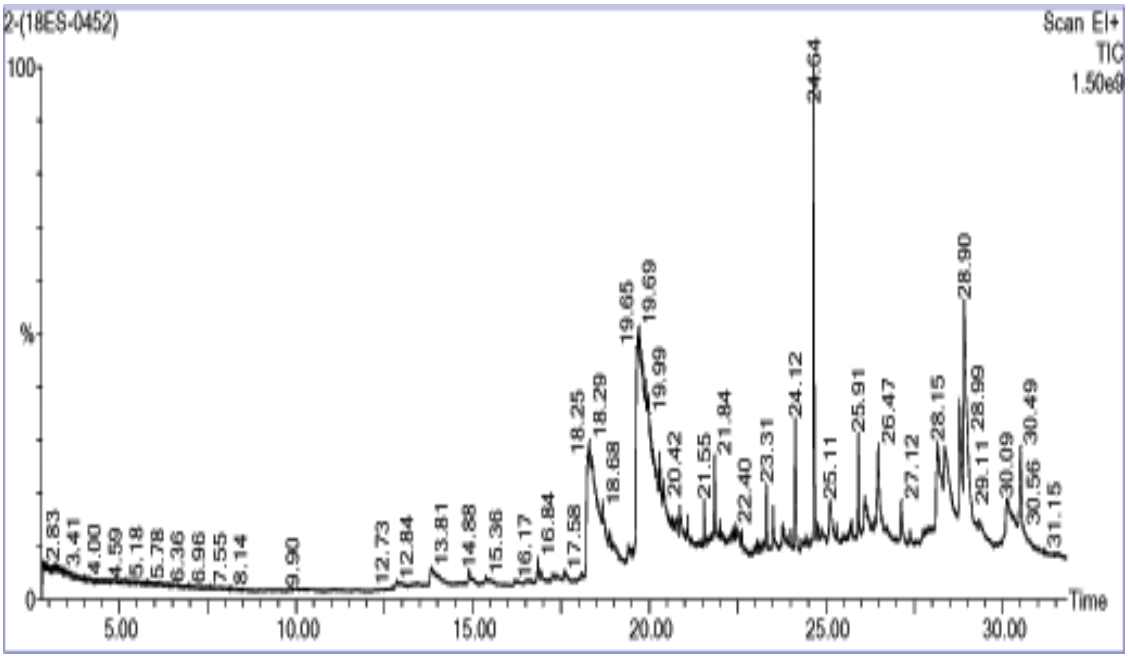


Figure 3.2GC chromatogram of ethanol extract prepared from fruit residual wastes.

Compounds identified in ethanolic extract reported to have antioxidant and antimicrobial properties (N-Hexadecanoic acid). Few also have anti-inflammatory action in the biological system (squalene). The squalene acts as a defense molecule against certain pathogens causing human and animal diseases (Ezhilan and Neelamegam, 2012). The traditional Siddha medicine (Vajra kandimaathirai) treats COVID as well as other forms of fevers and inflammatory illnesses. (Shiva *et al.,* 2022).GC-MS analysis of the drug revealed its formulation. The bioactive components are 1H-Imidazole, 4,5-dihydro-2-(phenylmethyl), and9,12-Octadecadienoic acid (Z,Z)- a 9-Octadecenoic acid-(E) along

with mercury(NCBI, 2022; 1). 9,12-Octadecadienoic acid and Tetradecaneare used as flavoring agents in food (NCBI, 2022; 1 and 2). But Tetradecane was reported a scarcinogen and tumor inducer in mice by enhancing the mitosis cell division of murine spleen lymphocytes (NCBI, 2022; 2). Oxirane is used as a chemical intermediate in fabric, foods and cosmetic industries. It is also widely used for synthetic waxes (NCBI, 2022; 3). Vitamin E is used as a nutrient supplement due to its diverse functions in the biological system. It helps in scavenging or retardation of oxidation reactions in cells (NCBI, 2022; 4).The structure of each molecule in the ethanol extracts of fruit wastes as determined by MS is given in appendix.

Table 3.5 Bioactive components identified by GC-MS analysis of ethanol extracts prepared from fruit wastes powder.

Sl.No.	Bioactive Components	RT-Value	%Area
1	N-Hexadecanoic acid	18.295	15.776
2	Sulfurous acid, 2-Propyl tetradecyl ester	18.67	2.105
3	9,12-Octadecadienoic acid (z,z)	19.705	38.91
4	Vitamine-E	28.148	5.664
5	9,19-Cyclolanost-24-en-3-ol, Acetate (3.Beta.)	28.769	2.628
6	7-Hexadecenal, (Z)	20.39	2.708
7	Squalene	24.122	1.936
8	Tetratriacontane	30.094	4.683
9	Pregnan-16-one, 20-((3-hydroxy-6-Methyl)oxacyclohex-3-yl)methyl	28.349	5.362
10	Tetradecane, 1-chloro-	24.642	5.367
11	3,3-Bis-tert-butylsulfanyl-2-fluoro-acrylonitrile	30.489	2.984
12	Oxirane, tetradecyl-	20.28	2.639

Methanol Extract:GC chromatogram analysis of the methanolic extract of fruit peels (Figure3.3) showed thirteen peaks among which few are more prominent than others. The peaks at different RT indicate the presence of thirteen bioactive molecules of specific chemical constituents. The thirteen bioactive molecules were characterized and identified

by the NIST library (Table 3.6). The compounds identified are N-Hexadecanoic acid; Sulfurous acid, 2 Propyl tetradecyl ester; 9,12-Octadecadienoic acid (z,z;) Vitamine-E; 9,19-Cyclolergost-24 en-3-ol, Acetate (3.Beta.); 7-Hexadecenal, (Z); Squalene; Tetratriacontane; Phenol,3,5 bis(1,1-dimethylethyl)-; Heneicosane, 11-(1-ethylpropyl)-; Ergosta-7,22-dien-3-ol,acetate, (3.beta.,5.alpha.)-; 2-methyl- Z,Z-3,13-Octadecadienol and Octadecanoic acid, 2-oxo, methylester. MS analyzed spectrum of each molecule in the methanol extracts of fruit wastes are shown in Fig 13b. Plant extract having 9,19-Cyclolanost-24-en-3-ol, Acetate (3.Beta) reported to have invitro antibacterial activities (Madureira*et al.,* 2003). Phenol,3,5-bis(1,1 dimethylethyl) used as a food additive because of its antioxidant property (NCBI, 2022; 5). Octadecanoic acid or stearic acid used as a food flavouring agent (NCBI, 2022; 6).The structure of each molecule in the aqueous methanol of fruit wastes as determined by MS is given in appendix**.**

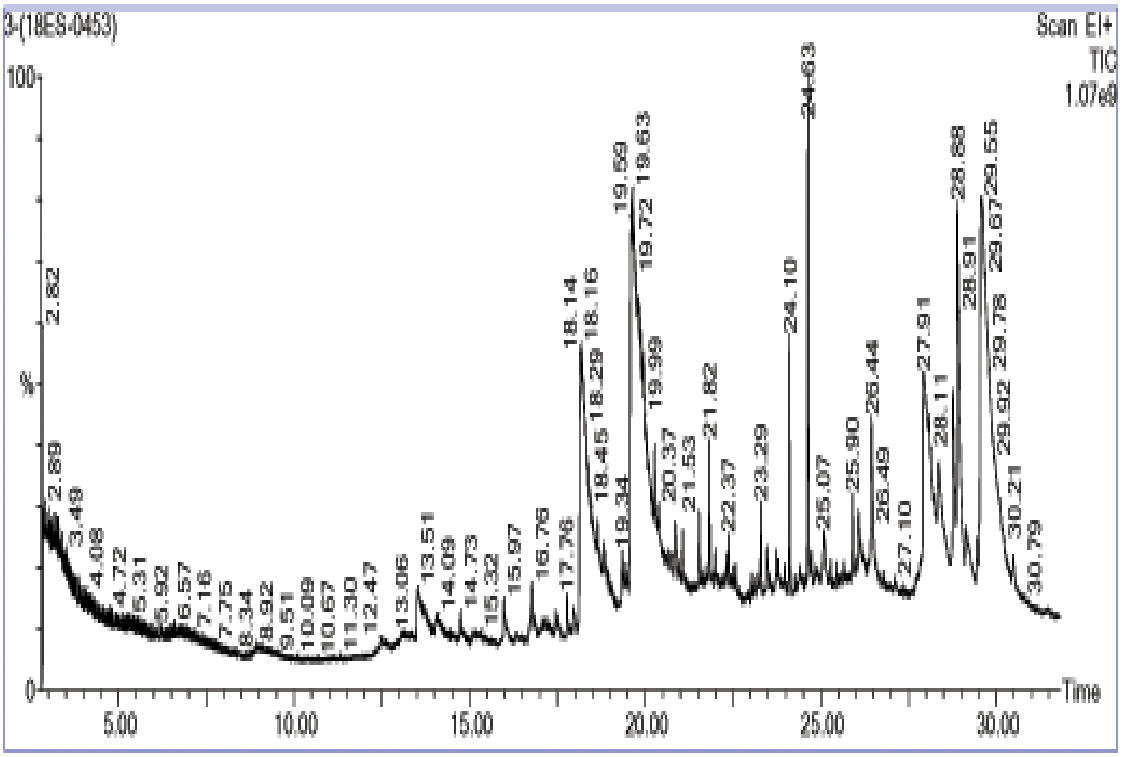


Figure 3.3GC chromatogram of methanol extract prepared from fruit residual wastes.

Table 3.6 Bioactive components identified by GC-MS analysis of methanol extracts prepared from fruit wastes powder.

Sl.No.	Bioactive Compounds	%Area	RT-Value
1	N-Hexadecanoic acid	15.776	18.159
2	Sulfurous acid, 2-Propyl tetradecyl ester	0.987	18.64
3	9,12-Octadecadienoic acid (z,z)	23.422	19.63
4	Vitamine-E	10.377	27.913
5	9,19-Cyclolanost-24-en-3-ol, Acetate (3.Beta.)	23.804	29.579
6	7-Hexadecenal, (Z)	1.008	20.37
7	Squalene	1.225	24.102
8	Tetratriacontane	2.861	24.627
9	Phenol,3,5-bis(1,1-dimethylethyl)-	1.009	13.648
10	Heneicosane, 11-(1-ethylpropyl)-	2.109	16.759
11	Ergosta-7,22-dien-3-ol,acetate, (3.beta.,5.alpha.)-	0.908	28.353
12	2-methyl-Z,Z-3,13-Octadecadienol	1.18	19.945
13	Octadecanoic acid, 2-oxo ,methylester	1.087	20.265

Petroleum ether extract: GCchromatogram analysis of the petroleum ether extract of fruit peels (Figure3.4) showed 10 peaks among which few are more prominent. The peaks at different RT indicate the presence of 10 phytochemicals of specific chemical constitutents. The ten bioactive molecules were characterized and identified by the NIST library (Table 3.7). The compounds identified are N-Hexadecanoic acid; Sulfurous acid, 2-Propyl tetradecyl ester; 9,12- Octadecadienoic acid (z,z); Vitamine-E; 9,19-Cyclolanost-24-en-3-ol, Acetate (3.Beta.); Phenol, 2,4-bis(1,1-Dimethylethyl)-; Tetrateracontane; Silane, Dimethyl di(3,7-dimethyloct-3-yloxy); Beta.,.Beta.-Carotene, 5,6-Dihydro-5,6-dihydroxy- and 1 Methylene-2B-Hydroxymethyl-3,3- Dimethyl -4B-(3-Methylbut-2-enyl)-. MS analyzed spectrum of each molecule in the petroleum ether extracts of fruit wastes are shown in Fig. 14b.The structure of each molecule in the petroleum ether extracts of fruit wastes as determined by MS is given in appendix**.**

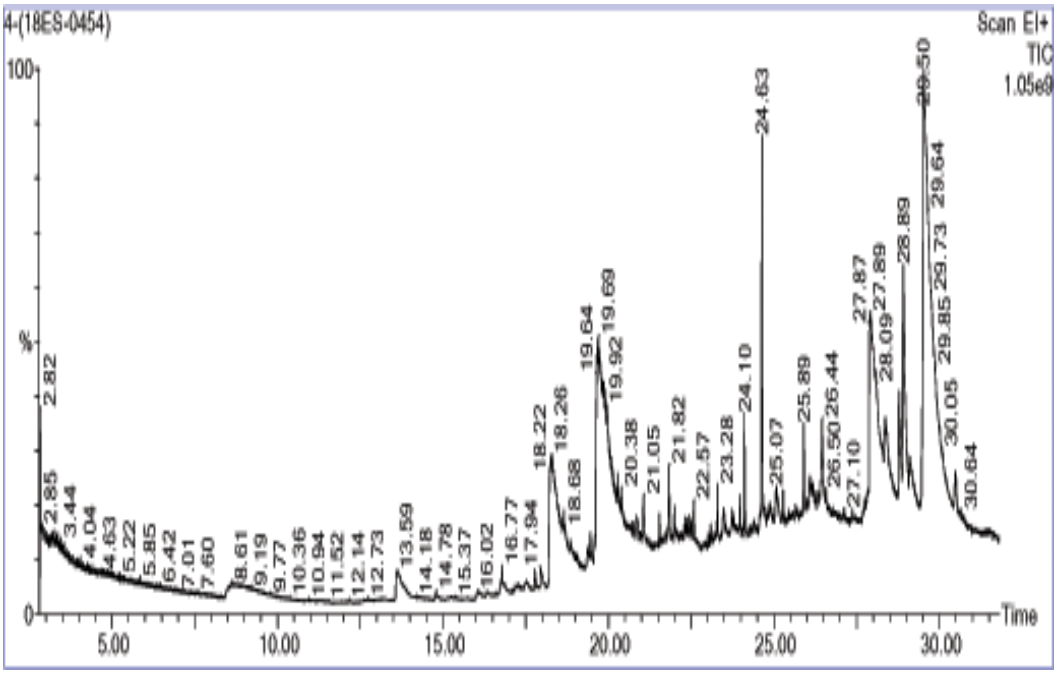


Figure 3.4GC chromatogram of petroleum ether extract prepared from fruit residual wastes.

Table 3.7 Bioactive components identified by GC-MS analysis of petroleum ether extracts prepared from fruit wastes powder.

Sl.No.	Bioactive Components	RT-Value	%Area
1	N-Hexadecanoic acid	18.275	9.741
2	Sulfurous acid, 2-Propyl tetradecyl ester	18.65	1.566
3	9,12-Octadecadienoic acid (z,z)	19.69	19.501
4	Vitamine-E	26.443	1.469
5	9,19-Cyclolanost-24-en-3-ol, Acetate (3.Beta.)	29.514	36.961
6	Phenol, 2,4-bis(1,1-Dimethylethyl)-	13.613	2.102
7	Tetrateracontane	24.627	2.821
8	Silane, Dimethyldi(3,7-dimethyloct-3-yloxy)	27.903	15.96
9	.Beta.,.Beta.-Carotene, 5,6-Dihydro-5,6-dihydroxy-	28.359	3.961
10	1-Methylene-2B-Hydroxymethyl-3,3-Dimethyl-4B-(3-Methylbut-2-enyl)-	28.774	1.569

Butanol extract: GC chromatogram analysis of the butanolic extract of fruit peels (Figure 3.5) showed 14 peaks among which few are more prominent. The peaks at

different RT indicate the presence of 14 phytochemicals of specific chemical constituents. The fourteen bioactive molecules were characterized and identified by the NIST library (Table 3.8). The compounds identified are N-Hexadecanoic acid; 9,19-Cyclolanost 24-en-3-ol, Acetate (3.Beta.); Pregnan-16-one, 20-((3-hydroxy-6-Methyl)oxacyclohex-3 yl)methyl; 1- Butanol; Butane, 1-Chloro-; Hexadecanoiv acid, 1,1-Dimethylethyl ester; 11 Tridecen-1-ol; Z-8- Methyl-9-tetradecenoiv acid; Oleic acid; 1-Octadecyne; Heptacosane, 1 chloro; 2,4-Dimethyl-7- oxo-4,7-Dihydro-trizolo(3,2) Ctriazine; 4,22-Stigmastadiene-3-one and Hexadecane , 1,16- Dichloro.Oleic acid is having Flavoring Agents in food additives (NCBI, 2022; 7), inhibitors of fatty acid and cholesterol biosynthesis (Gabriele *et al.,* 2010). It is used for formulations of many therapeutic or cosmetic products. It has special properties by which percutaneous absorption of cosmetics and drugs get enhanced (Ruiz *et al*., 2010). Therefore, oleic acid is used for the preparation of effective drug delivery systems. 4, 22-Stigmastadiene-3-one having antioxidant activity, metal chelating and ferric reducing power assays. Its antiproliferation activity is not yet proved (Liu *et al*., 2014).

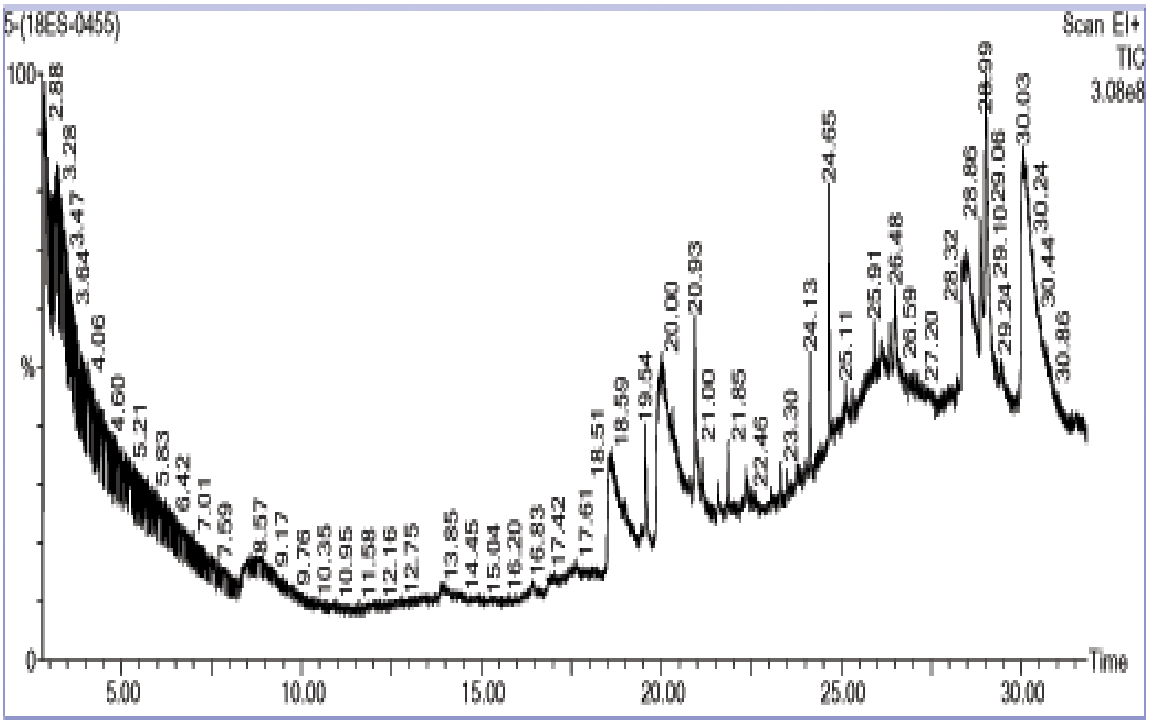


Figure 3.5GC chromatogram of butanol extract prepared from fruit butanol wastes.

Table 3.8 Bioactive components identified by GC-MS analysis of butanol extracts prepared from fruit wastes powder.

Sl.No.	Compounds	RT-Value	%Area
1	N-Hexadecanoic acid	18.595	8.039
2	9,19-Cyclolanost-24-en-3-ol, Acetate (3.Beta.)	28.994	9.206
3	Pregnan-16-one, 20-((3-hydroxy-6-Methyl)oxacyclohex-3-yl)methyl	28.453	13.464
4	1-Butanol	3.344	2.026
5	Butane, 1-Chloro-	3.424	2.072
6	Hexadecanoiv acid, 1,1-Dimethylethyl ester	19.545	1.752
7	11-Tridecen-1-ol	19.995	18.434
8	Z-8-Methyl-9-tetradecenoiv acid	20.31	3.24
9	Oleic acid	20.415	1.251
10	1-Octadecyne	20.926	2.514
11	Heptacosane, 1-chloro	24.647	2.386
12	2,4-Dimethyl-7-oxo-4,7-Dihydro-trizolo(3,2-C)triazine	26.483	2.096
13	4,22-Stigmastadiene-3-one	28.859	3.133
14	Hexadecane, 1,16-Dichloro	30.034	28.727

A total of 45 bioactive substances are discovered by GC-MS (Table 3.9), the majority of which are secondary metabolites of plants. N-Hexadecanoic acid typical bioactive chemicals, were found in all five extracts when the five were compared. Along with the GC chromatogram, the chemical structure of the detected bioactive chemicals in each separate extract from fruit peel powder is shown.

Table 3.9 Phytomolecules identified by GC-MS study of five extracts prepared from fruit wastes powder.

Sl.No.	Compounds	Aqueous	Ethanol	Methanol	Pet-Ether	Butanol
1	N-Hexadecanoic acid	√	√	√	√	√
2	Sulfurous acid, 2-Propyl tetradecyl ester	X	√	√	√	X

3	9,12-Octadecadienoic acid (z,z)	X	√	√	√	X
4	Vitamine-E	X	√	√	√	X
5	9,19-Cyclolanost-24-en-3-ol, Acetate (3.Beta.)	X	√	√	√	√
6	7-Hexadecenal, (Z)	X	√	√	X	X
7	Squalene	X	√	√	X	X
8	Tetratriacontane	X	√	√	X	X
9	Pregnan-16-one, 20-((3-hydroxy-6-Methyl)oxacyclohex-3-yl)methyl	X	√	X	X	√
10	Ethanamine, 2-propoxy-	√	X	X	X	X
11	1,3-Dioxane, 2,4-Dimethyl	√	X	X	X	X
12	Diglycerol	√	X	X	X	X
13	L-Talose, 6-Deoxy-3-C-Methyl2-O-Methyl-	√	X	X	X	X
14	2-Amino-octadec-7-ene-1,3-diol butaneboronate	√	X	X	X	X
15	4-Ethyl-2-hydroxycyclopent-2-en-1-one	√	X	X	X	X
16	2,3-Anhydro-D-Galactosan	√	X	X	X	X
17	Heptacosanoic acid, methyl ester	√	X	X	X	X
18	1-Tridecyne	√	X	X	X	X
19	Eicosanoic acid	√	X	X	X	X
20	3-Deoxy-D-mannitol	√	X	X	X	X
21	14-Heptadecenal	√	X	X	X	X
22	Tetradecane, 1-chloro-	X	√	X	X	X

23	3,3-Bis-tert-butylsulfanyl-2-fluoro-acrylonitrile	X	√	X	X	X
24	Oxirane, tetradecyl-	X	√	X	X	X
25	Phenol,3,5-bis(1,1-dimethylethyl)-	X	X	√	X	X
26	Heneicosane, 11-(1-ethylpropyl)-	X	X	√	X	X
27	Ergosta-7,22-dien-3-ol,acetate, (3.beta.,5.alpha.)-	X	X	√	X	X
28	2-methyl-Z,Z-3,13-Octadecadienol	X	X	√	X	X
29	Octadecanoic acid, 2-oxo ,methylester	X	X	√	X	X
30	Phenol, 2,4-bis(1,1-Dimethylethyl)-	X	X	X	√	X
31	Tetrateracontane	X	X	√	√	X
32	Silane, Dimethyldi(3,7-dimethyloct-3-yloxy)	X	X	X	√	X
33	.Beta.,.Beta.-Carotene, 5,6-Dihydro-5,6-dihydroxy-	X	X	X	√	X
34	1-Methylene-2B-Hydroxymethyl-3,3-Dimethyl-4B-(3-Methylbut-2-enyl)-	X	X	X	√	X
35	1-Butanol	X	X	X	X	√
36	Butane, 1-Chloro-	X	X	X	X	√
37	Hexadecanoiv acid, 1,1-Dimethylethyl ester	X	X	X	X	√
38	11-Tridecen-1-ol	X	X	X	X	√

39	Z-8-Methyl-9-tetradecenoiv acid	X	X	X	X	√
40	Oleic acid	X	X	X	X	√
41	1-Octadecyne	X	X	X		√
42	Heptacosane, 1-chloro	X	X	X	X	√
43	2,4-Dimethyl-7-oxo-4,7-Dihydro-trizolo(3,2-C)triazine	X	X	X	X	√
44	4,22-Stigmastadiene-3-one	X	X	X	X	√
45	Hexadecane, 1,16-Dichloro	X	X	X	X	√

√: Compound detected, X: Compound not detected in the extract

3.4 Conclusion

The mixed fruit leftovers were gathered from the market, and a qualitative and quantitative screening of the five extracts (water, ethanol, methanol, petroleum ether, and butanol) indicated some potent and significant phytochemicals. By using GC-MS to identify a total of forty five phytochemicals, N-Hexadecanoic was found to be a common bioactive component across all extracts. The prospect of employing the wastes as functional meals to prevent or treat some major diseases is increased by the presence of a number of bioactive compounds with considerable pharmacological and neutraceutical value.

Chapter-4

Cytotoxicity study of five extracts prepared from fruit residual wastes against Gram positive and negative bacteria

4.1 Introduction

Food waste is a pressing global issue with far-reaching impacts on society, the environment, and the economy. Utilizing food waste offers several economic benefits, as diverting it from landfills and processing it through methods like anaerobic digestion and composting can yield valuable products such as animal feed, biofuels, compost, and biogas. These applications not only reduce disposal costs but also create opportunities for income generation and new job prospects (Parfitt*et al*., 2010). Fruit and vegetable waste, containing valuable compounds like phenolic compounds and dietary fiber, has caught the attention of the food industry for potential utilization. Incorporating food waste into various applications not only reduces waste but also fosters the development of novel and sustainable food products. Additionally, this waste can find uses in the pharmaceutical industry, offering sustainable alternatives and reducing environmental impact. The emergence of multidrug-resistant strains in pathogenic bacteria has become a significant public health concern, as it limits the effectiveness of antimicrobial drugs in treating infectious diseases (Boucher *et al*., 2009). As a potential solution, various plant sources have been explored as significant reservoirs of organic antibacterial compounds (Iwu*et al*., 1999). Fruit leftovers contain secondary metabolites like flavonoids, phenolic compounds, alkaloids, and tannins, which have shown in vitro antibacterial activity (Duraipandiyan*et al*., 2006). Considering the promising potential of fruit wastes as sources for antimicrobial treatments, the purpose of this study was to examine the in vitro antibacterial activity of extracts from mixed fruit residues against common microbial pathogens.

4.2 Materials and Methods

Extracts of mixed fruit residues were tested for their in vitro antimicrobial activity against the most common microbial pathogens, both Gramme-positive (*Bacillus subtilis*) and Gramme-negative (*Pseudomonas aeruginosa*), by determining MIC (Minimum Inhibitory Concentration), Agar-well diffusion method, and their Anti-motility activities.

4.2.1 Minimum Inhibitory Concentration (MIC)

MIC value of five solvent extracts against both Gram negative (*Pseudomonas aeruginosa*) and Gram positive (*Bacillus subtilis)* bacteria was determined to check antimicrobial effect of each extracts. The methodology using Tetrazolium/formazan (TTC), a growth indicator was followed (Singh and Sahareen, 2017). Briefly, fresh bacterial suspension (1 x 10^5 cells/ml) was added to each experimental set, followed by addition of extract (0-100 mg/ml) from stock solution and incubated at 37 °C overnight in an incubator with constant shaking (130 rpm). After termination of incubation period of 16 h, addition of TTC (5 mg/ml) was done to each set and further incubated for 1 h. The value of MIC (in µg/ml) was determined by reading the absorbance at 480 nm by Shimadzu UV spectrophotometer (PharmaSpac, UV 1700) of the red coloured developed after addition of TTC to each set. Decrease in absorbance death of cells because of extract treatment.

4.2.2 Agar-well diffusion method

Individual extracts' antibacterial activity was tested using the agar diffusion technique (Goswami*et al.*, 2015). The 90 mm-diameter nutritional agar plates were equally dotted with 100 µl of bacterial suspensions using a sterilized spreader. Equal amounts of variously concentrated extracts (0, 25, 50, 75, and 100 mg/mL) were added to wells that were 6 mm in diameter. The plates were then incubated for a further 24 hours at 37^{o}C. Then, for each extract, millimeter-scale growth inhibitions surrounding the wells were recorded. The antibiotic ceftriaxone was subsequently applied using the same procedure as the extract had been.

The main goal of mixed fruit extract analysis is to identify specific bioactive components, and then to determine their pharmacological and neutraceutical capabilities for use in a variety of industries to create products with added value. Using a triple data collection, the mean and standard deviation of each value were reported.

4.2.3 Haemolysis assay using goat erythrocytes

Using a hemolytic assay, the hemocompatibility of each extracts, aside from water extract(which will be discussed in chapter 5), on goat erythrocytes, was evaluated. To evaluate the extract's capability to hemolyze blood, fresh goat blood was procured from a

nearby butcher shop. The sample was centrifuged at 5000 rpm for five minutes to remove the plasma and serum. Blood cells were grown for 1 hour at 37 °C after being combined with PBS (pH 7.4) and various extract concentrations (2–10 mg/ml). PBS and 0.1% Triton X-100 served as the experiments' negative and positive controls, respectively. After the incubation period was complete, the cells were centrifuged, and the absorbance of the supernatant containing the lysed erythrocytes was measured at 540 nm.
The proportion of hemolysis was calculated using the calculation shown below:

% Haemolysis = $[a_t\text{-}a_c/a_{100}\text{-}a_c]$ x100

where a_c is the absorbance of the supernatant from controls (normal saline), and a_t is the absorbance of the supernatant from samples incubated with the particles. The absorbance of the supernatant of positive controls after being treated with 0.1% Triton X-100, which completely lyses RBCs, is a_{100}.

4.2.4 Statistical Analysis

Understanding how efficient the bioactive ingredients in mixed fruit extract are against pathogenic bacteria is the major objective of an analysis of antimicrobial activities. Increased microbial efficiency is crucial for extending product shelf life. It is now simpler to make a judgment regarding the use of these extracts to create lucrative products based on the information acquired. The mean and standard deviation of each number were reported using a triple data collection.

4.3 Result and Discussion

4.3.1 Evaluation of extracts antimicrobial property

Testing was done on *B. subtilis* and *P. aeruginosa* against all five extracts. On this test bacterium, the extract revealed a potential cytotoxic impact. Growth inhibition around each well that has been loaded with a varied amount of each extract is shown in Figure 16-21. Each well's clear zone serves as evidence of extract activity that is dose dependant.

Water Extract: The aqueous extract's effectiveness against microbes was assessed by observing the presence of an inhibition zone, measuring its diameter, and determining the minimum inhibitory concentration (MIC) values. Experimental results indicated that the extract exhibited activity against both gram-negative bacteria (*P. aeruginosa*) and gram-

positive bacteria (*B. subtilis*) as reported in previous study (Rakholiya*et al.*, 2014). The MIC values were 2 for *B. subtilis* and 5 for *P. aeruginosa* (refer to Table 4.1). The largest inhibition zone diameter observed was 24.33 ± 0.5 mm for *P. aeruginosa*MZ269380 and 26 ± 0.1 mm for *B.subtilis* (Figure4.1).Zone of inhibition is significant and dosage dependent.

This antimicrobial activity is recognized to the presence of various bioactive elements in the extract that inhibit microbial growth. Among the identified phytochemicals listed in Table-3.9 of Chapter-3, some compounds like Ethanamine, 2-propoxy-, 1,3-Dioxane, 2,4-Dimethyl, Eicosanoic acid, 4-Ethyl-2-hydroxycyclopent-2-en-1-one (Adelin*et al.*, 2017, Daniela *et al.*, 2009, Ahmedzade*et al.*, 2003) have been previously reported as antimicrobial agents that act against both gram-negative and gram-positive pathogenic microbes.

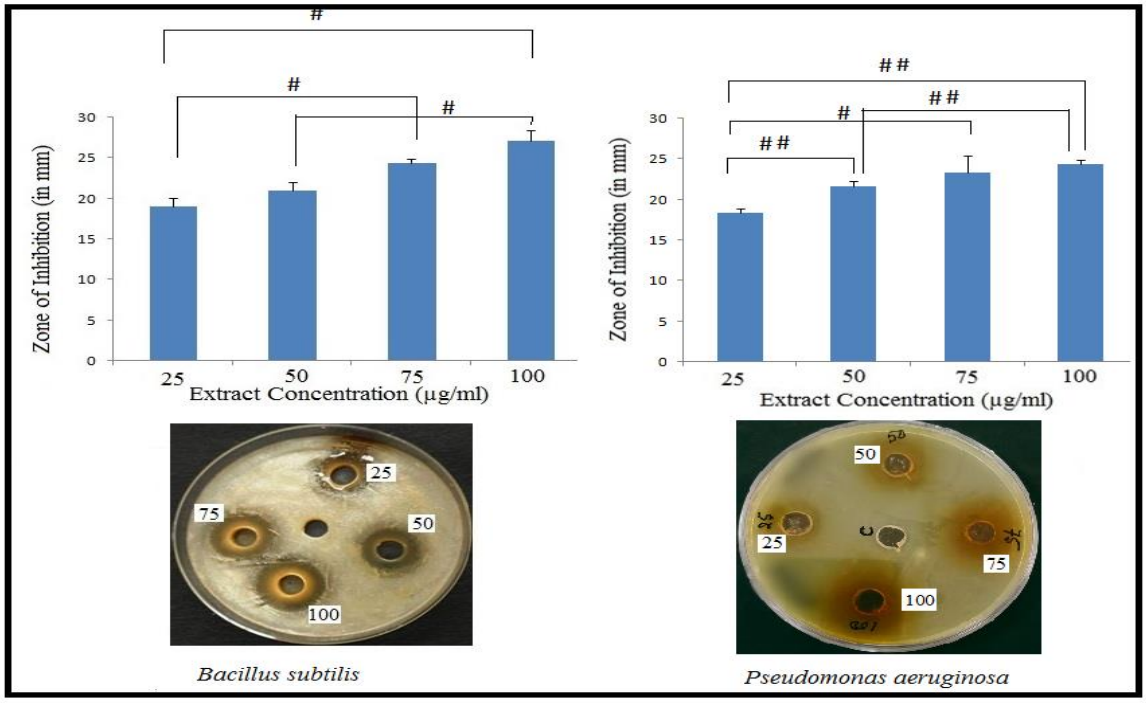


Figure 4.1 Zone of inhibition by fruit residue aqueous extract against *Bacillus subtilis* and *Pseudomonas aeruginosa*. Connecting line over bars signify significant at 0.05 and 0.01 level of significance (as analysed by student T test). # = significant ; ## highly significant.

Ethanol Extract: It has been revealed that ethanolic extract has a substantial MIC value and strongly inhibits both both gram-negative and gram-positive bacteria as reported in previous literatures (Hanafy*et al.*, 2021). *B. subtilis*had a MIC of 12.5, while *P. aeruginosa* had a MIC of 20 (Table 4.1). This showed that cytotoxic effects on gram

negative bacteria (here *P. aeruginosa*) having less effects compared to gram positive bacteria. *B. subtilis*exhibits high cytotoxic effects. Inhibition zones against the two bacteria had diameters of 24 ± 0.17 mm and 17 ±1 mm, respectively (Figure 4.2). Zone of inhibition is dose dependent and significant. Both MIC and agar diffusion assays result corroborate. It has been reported that the phytochemicals in ethanolic extract identified through GC-MS (Chapter 3, Table 3.9) such as N-Hexadecanoic acid (Palmitic acid), 9,12-Octadecadienoic acid (z,z), 9,19-Cyclolanost-24-en-3-ol, Acetate (3.Beta.)

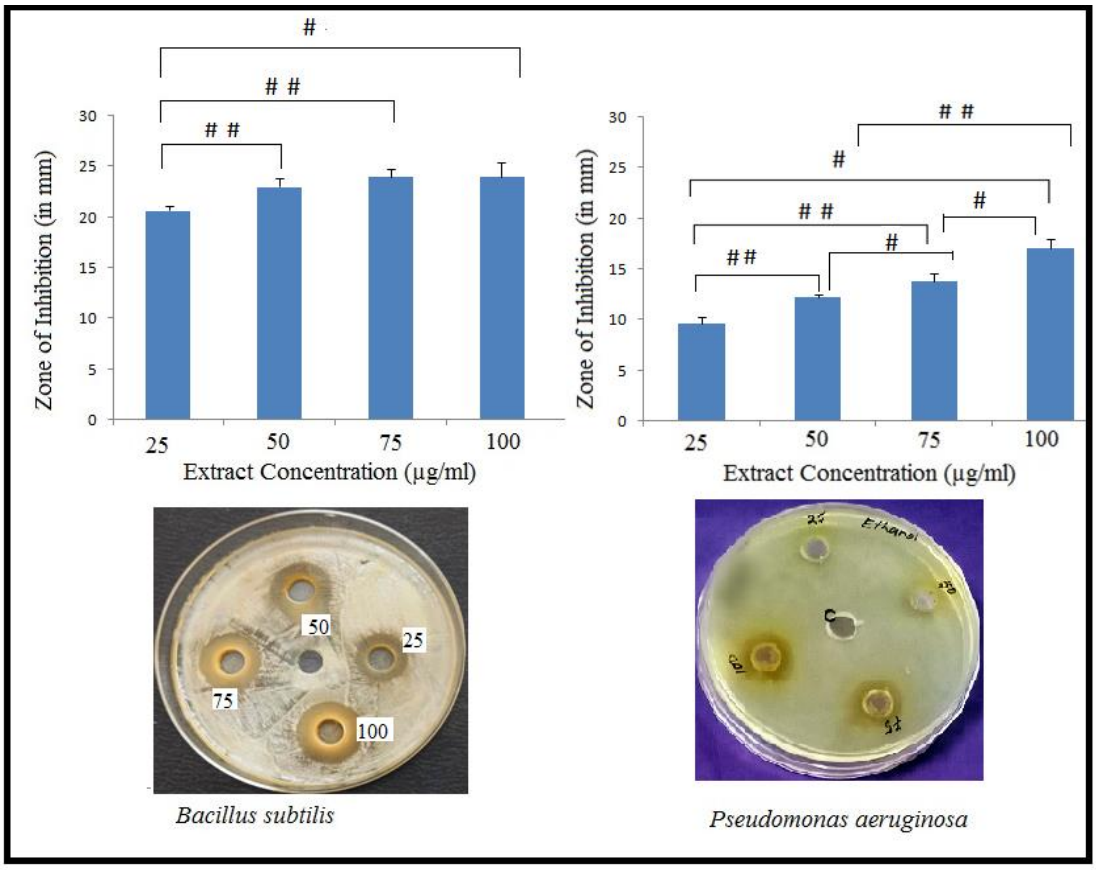


Figure 4.2 Zone of inhibition by fruit residue ethanolic extract against *B. subtilis* and *P. aeruginosa.* Connecting line over bars signify significant at 0.05 and 0.01 level of significance (as analysed by student T test). # = significant ; ## highly significant.

Methanol Extract: The MIC value and development of the inhibition zone of the methanolic extract show that it functions as the most powerful antibacterial agents supporting the previous literature (Hanafy*et al.*, 2021). During examination, the maximum diameter of the formed inhibition zone was observed to be 17 ± 6 mm for *P. aeruginosa*MZ269380 and 26 ± 2 mm for *B. subtilis*(Figure4.3). Zone of inhibition is large and dosage dependent. The MIC value for *Bacillus subtilis* was determined to be

19.5 and for *P. aeruginosa* to be 20 shown in Table 4.1. The presence of biologically active chemicals, which are stated in chapter 3 (Table 3.9), is primarily responsible for the extract's antimicrobial behavior. Squalene, one of the bioactive components, exhibits antibacterial and antifungal properties (Momoh and Oshin, 2023). Other physiologically active substances that are already stated in the ethanolic extract, such as 9,12-Octadecadienoic acid (z,z), N-Hexadecanoic acid (Palmitic acid), 9,19-Cyclolanost-24-en-3-ol, and acetate (3.Beta.), also acts against bacteria.

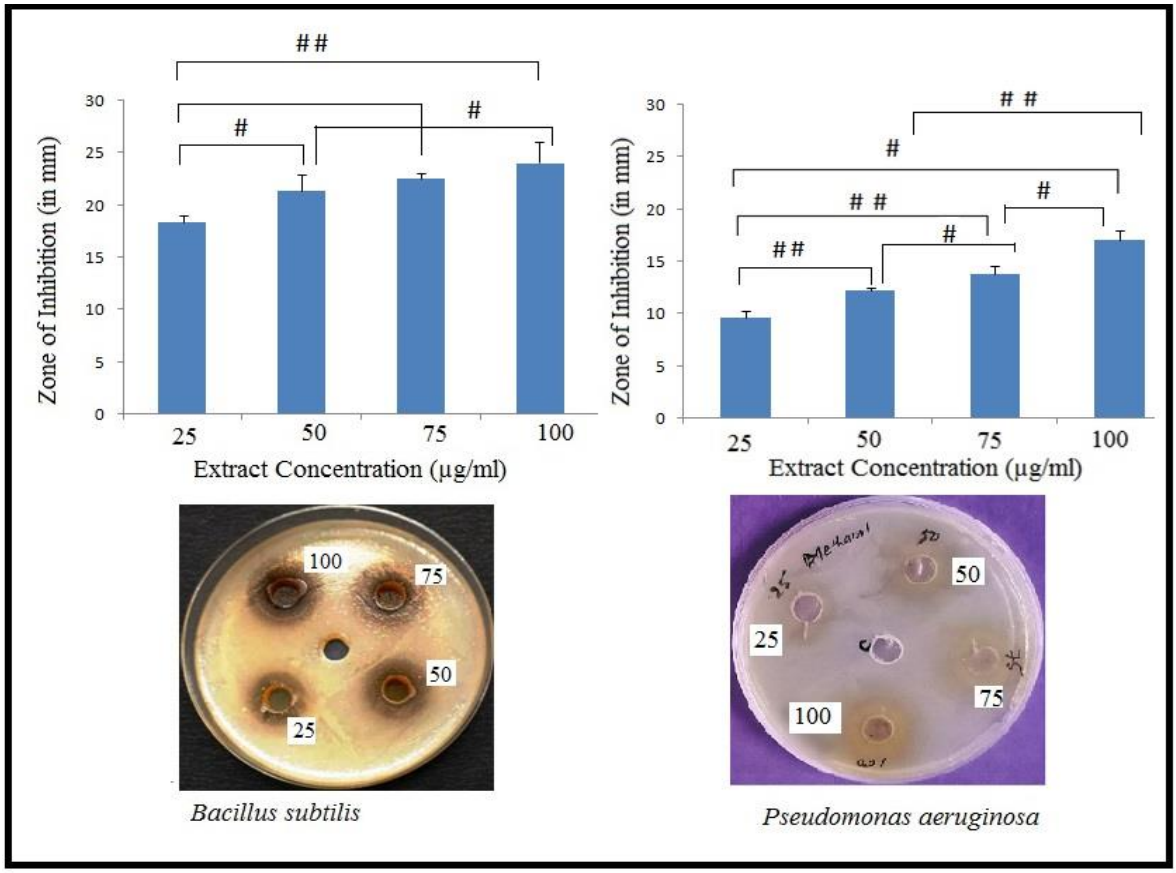


Figure 4.3 Zone of inhibition by fruit residue methanolicextract against *B. subtilis* and *P. aeruginosa*.Connecting line over bars signify significant at 0.05 and 0.01 level of significance (as analysed by student T test). # = significant ; ## highly significant.

Petroleum Ether extract: A distinct zone of inhibition for both *B. subtilis*(maximum 23 ± 0.2 mm) and *P. aeruginosa*(maximum 12.6 ± 0.5 mm) was found in the experimental results depicted in Figure 4.4. Zone of inhibition is a substantial and dose-dependent phenomenon. As shown in Table 4.1, the MIC against *B. subtilis*was 24.5 and against *P. aeruginosa*was 30.5. The extract's antimicrobial action against both gram positive and negative bacteria is principally caused by the presence of a biologically active component

as reported in different literature (Rahman*et al.*,2019), which is highlighted in the GC-MS data. Phenol, 2,4-bis(1,1 Dimethylethyl), is a naturally occurring antibacterial agent (Ren *et al*., 2019). Bioactive compounds with antibacterial and antifungal effects include 9,12-Octadecadienoic acid (z,z), N-Hexadecanoic acid, 9,19-Cyclolanost-24-en-3-ol, Acetate (3.Beta), and others.

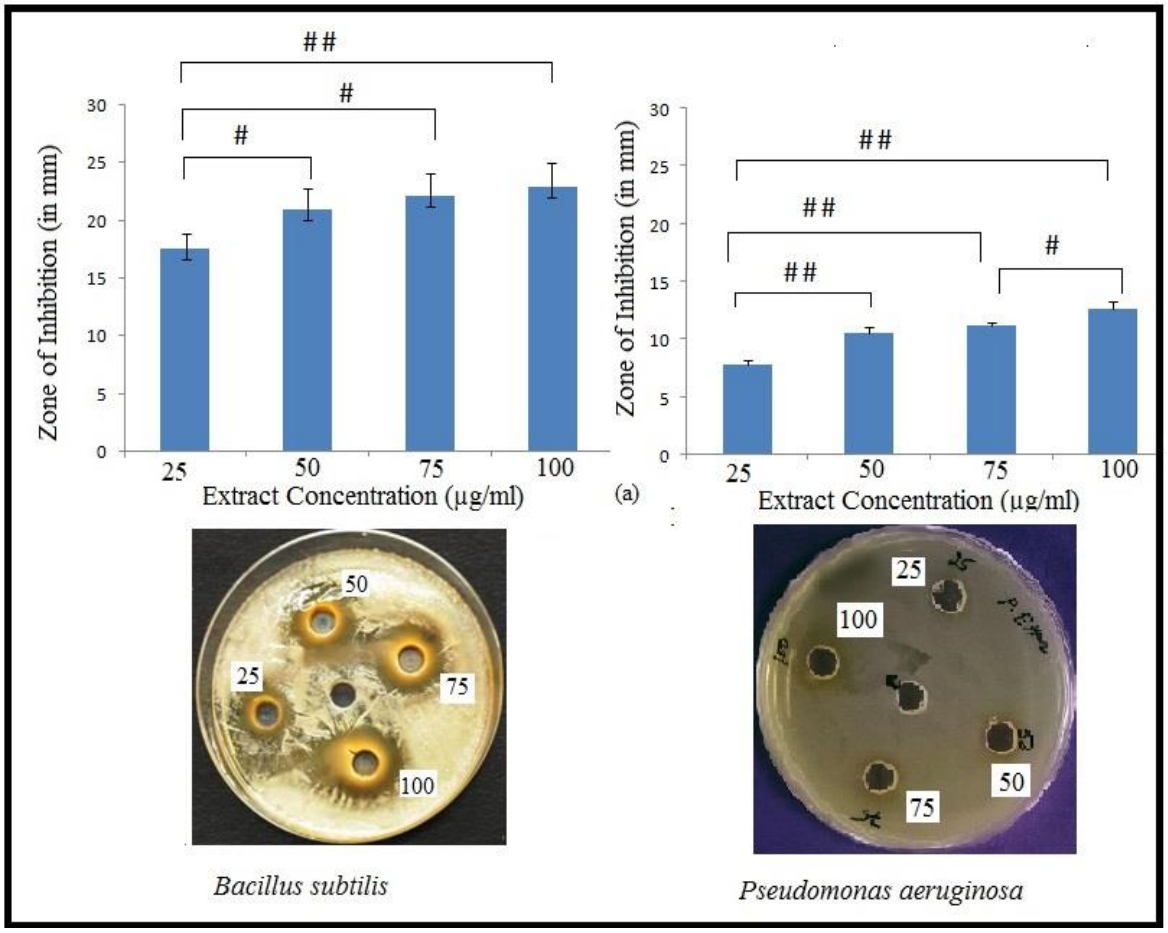


Figure 4.4 Zone of inhibition by fruit residue petroleum etherextract against *B. subtilis* and *P. aeruginosa*.Connecting line over bars signify significant at 0.05 and 0.01 level of significance (as analysed by student T test). # = significant ; ## highly significant.

Butanol Extract: The development of the butanolic extract's inhibition zone and MIC value show that it functions as one of the most potent antibacterial agents. Figure 4.5 illustrates the maximum diameter of the formed inhibition zone for *P. aeruginosa*and *B. subtilis*was 16 ± 1mm and 23 ± 1.2 mm respectively, supported by various literature (Rahman*et al.*,2019) as seen during analysis and significant dose-dependent zone of inhibition observed and the MIC values for both bacteria were determined to be 12 and 15, respectively (Table 4.1). The presence of biologically active chemicals, which are

indicated in chapter 3 (Table 3.9), is the major factor in the extract's antimicrobial behavior. N-Hexadecanoic acid, 9,19-Cyclolanost-24-en-3-ol, Acetate (3.Beta), Hexadecanoiv acid, 1,1-Dimethylethyl ester, and other compounds found in the butanolic extract are antimicrobial in nature and are in charge of eradicating or preventing the growth of germs.

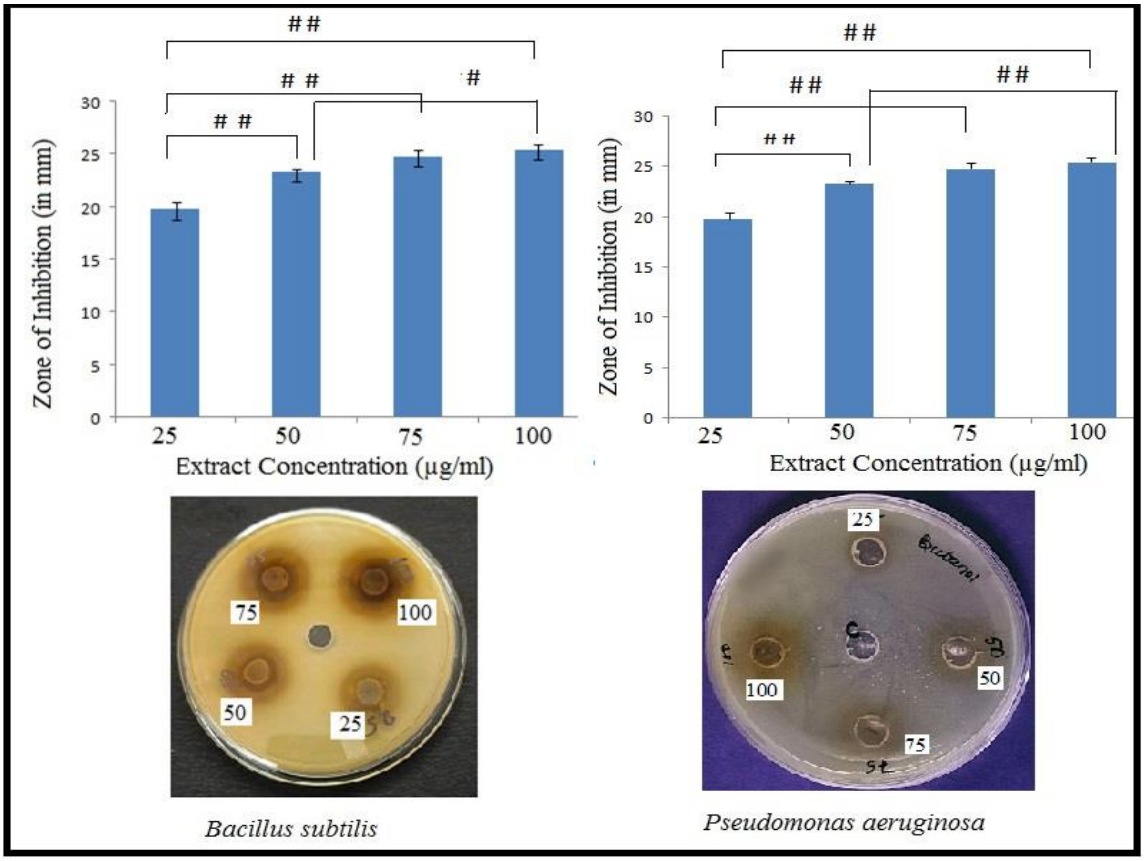


Figure 4.5 Zone of inhibition by fruit residue butanolextract against *B. subtilis* and *P. aeruginosa*.Connecting line over bars signify significant at 0.05 and 0.01 level of significance (as analysed by student T test). # = significant ; ## highly significant.

Antibiotic: An antibacterial Cefraiaxone, a member of the cephalosporin antibiotic class of drugs, is used to treat a range of bacterial infections. It functions by halting bacterial growth. As display in Table 4.1 after observation, the MIC values for *P. aeruginosa* and *B. subtilis*were 1.5 and 0.45, respectively, and the zone of inhibition diameter for *P. aeruginosa*was 25 ± 0.3 mm and for *Bacillus subtilis* was 27 ± 0.6 mm (Figure4.6). Significant and dose-dependent zone of inhibition observed.The primary goal of this experiment is to compare the efficacy and efficiency of extract from mixed fruit wastes

against gram positive and gram negative bacteria when used in conjunction with a certain antibiotic.

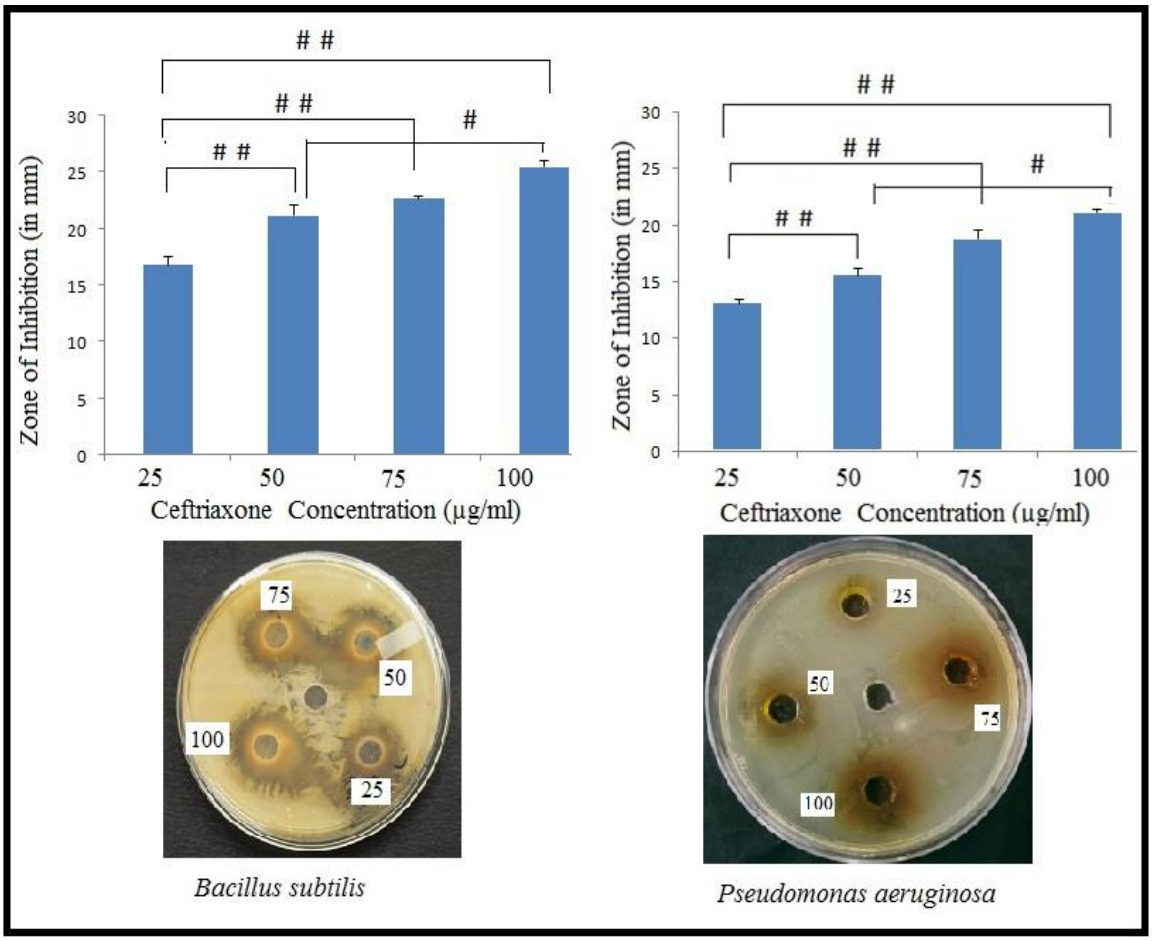


Figure 4.6 Zone of Inhibition by Antibiotic (Cefraiaxone) against *B. subtilis*and *P. aeruginosa*.Connecting line over bars signify significant at 0.05 and 0.01 level of significance (as analysed by student T test). # = significant ; ## highly significant.

Table-4.1:MIC (Minimum Inhibitory Concentration)values (mg/mL)

Extracts/Antibiotic	**MIC (mg/mL)**	
	Bacillus subtils	*Pseudomonas aeruginosa*
Water	2	5
Ethanol	12.5	20
Methanol	19.5	20
Petroleum ether	24.5	30.5
Butanol	12	15
Ceftriaxone	0.45	1.5

Water, butanol, and ethanol demonstrated more pronounced antimicrobial activity in comparison to the other two extracts (methanol and petroleum ether) of five fruit waste extracts that were examined for possible antibacterial activity against test pathogens. A high concentration of phytochemicals with antibacterial activity may be the cause of the extracts' strong antibacterial activity in water, ethanol, and butanol. Antimicrobial properties have been reported for N-Hexadecanoic acid (Aparna*et al.,* 2012; Zahara*et al.,* 2022), Pregnan-16-one, 20-[[3-hydroxy-6-Methyl]oxocyclohex-3 yl]methyl; 9,12-Octadecadienoic acid (z, z), Oleic acid, Vitamin E, 11-Tridecen-1-ol, and a few others (Casillas-Vargas *et al*.,2021). Unsaturated fatty acids have remarkable cytotoxic effects on pathogenic bacteria, including *P. aeruginosa, Neisseria gonorrhoeae, Bacillus subtilis, Porphyromonasgingivalis, Fusobacteriumnucleatum* and even *Helicobacter pylori* (Casillas-Vargas *et al*.,2021; Wright and Reynolds, 2007).Oleic acid, an unsaturated fatty acid, inhibits FabI (a bacterial enzyme that converts enoyl-acyl carrier protein to reductase). Numerous antibacterial medications have the potential to target FabI (Heath and Rock, 2004).The primary antioxidant in biological membranes is thought to be vitamin E. Vitamin E is a potential adjunct antibiotic treatment option for communicable diseases brought on by dreadful drug-resistant bacteria (*P. aeruginosa, Burkholderiacenocepacia*, and *methicillin-resistant Staphylococcus aureus*). Vitamin E helps in decreasing the MIC value of antibiotics when applied in combination.Vitamin E acts as a lipocalin antibiotic binding inhibitor, which Naguib and colleagues claim can be utilized to boost the effectiveness of killing (Naguib and Valvano, 2018). Vitamin E may therefore be employed as an antibiotic adjuvant for the treatment of infectious diseases, including multidrug-resistant bacteria, as it increased the effective concentration of drugs around bacterial cells.The remaining two extracts in this study, petroleum ether and methanol, had MIC values that were significantly higher than the previous three (water, ethanol, and butanol) against any of the tested bacteria. Petroleum ether had MIC values of 24.5 for *B. subtilis*and 30.5 for *P. aeruginosa* and methanol had MIC values of 19.5 (*B. subtilis)* and 20 (*P. aeruginosa)*. These extracts might not have any antimicrobial compounds or just have a small amount of antibacterial phytochemicals.

4.3.2 Haemolysis assay using goat erythrocytes

Because ruptured RBCs were minor for the concentration of extract evaluated at MIC values of each extract, the hemolytic percentage of extracts was determined to be promising (Figure 4.7). For ethanol, methanol, petroleum ether, and butanol, respectively, the % hemolysis was 1.59, 1.44, 1.82, and 1.91. Antibiotic exhibits 7.2% hemolysis at its MIC value.

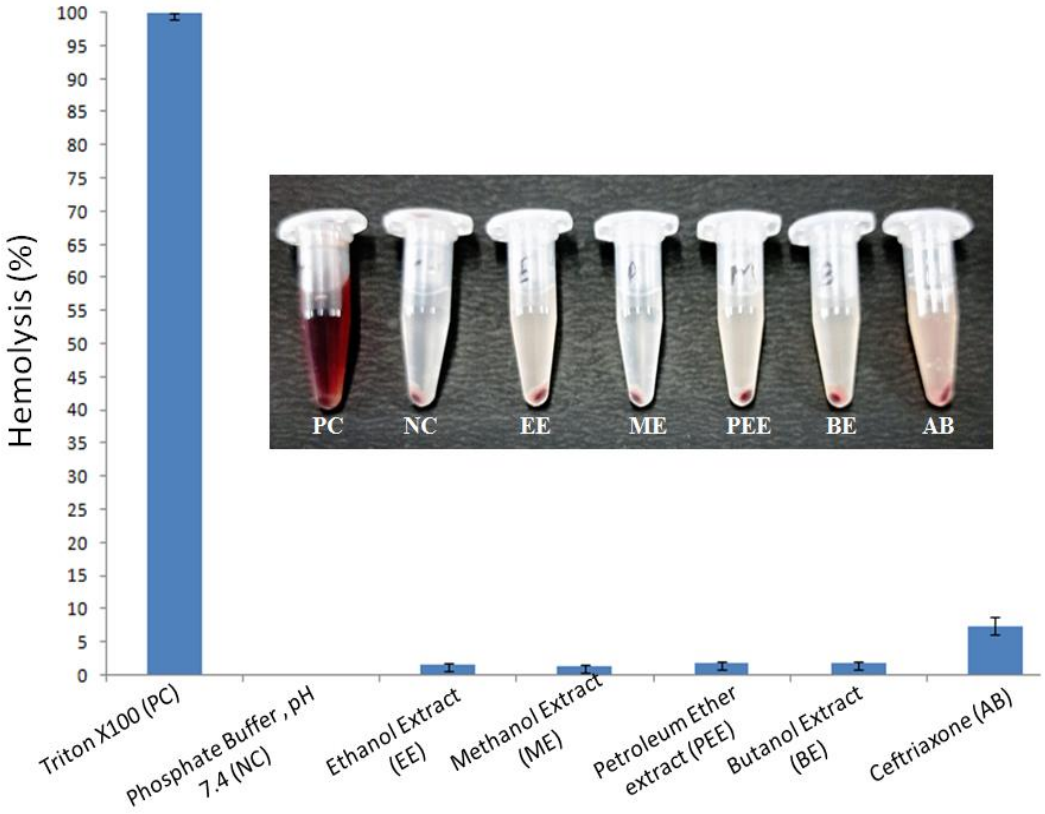


Figure 4.7 Hemolysis assay on goat's RBCs treated with four extracts along with standard antibiotic (Ceftrixone) at the respective MIC values. Phosphate buffer, pH 7.4 and Triton x 100 used as negative and positive controls respectively. Inset shows image of goat's RBCs hemolysis.

4.4 Conclusion

Upon investigation, it was found that the extract derived from mixed fruit residues displayed strong antimicrobial properties and showed significant potential in inhibiting the growth of various harmful bacteria, including both gram-positive and gram-negative types. The presence of inhibition zones, along with their respective diameters and MIC values, indicated that the aqueous extract exhibited greater antibacterial activity compared

to the other four extracts. Additionally, each extract demonstrated more effectiveness against gram-positive bacteria (*B. subtilis*) than gram-negative bacteria (*P. aeruginosa*MZ269380).

Given their remarkable antimicrobial efficacy, these extracts hold promise for diverse applications, especially in the food processing industry for the production of value-added edible products. In comparison to the commonly used antibiotic Ceftriaxone, which belongs to the cephalosporin antibiotic class, the antimicrobial efficiency of the extract was nearly satisfactory.

Chapter 5

Biovalorization of fruit wastes for the designof biodegradable antimicrobial chitosan-based fruit coatings

5.1 Introduction

Globally, food waste causes serious environmental and economic issues. However, creative strategies are being created to make use of food waste. Recent studies have concentrated on the extraction of bioactive components from food waste, which has demonstrated remarkable potential for a variety of uses in a variety of industries. In order to emphasize its potential for resource recovery, economic contribution, value creation, and sustainable practices (Kumar *et al*., 2021, Vargas-González and Rojas-Contreras. 2021).Food waste generates bioactive compounds with positive health effects that are employed as natural additives, functional ingredients, and preservatives in the food business as well as in the pharmaceutical and nutraceutical sectors for therapeutic purposes (González-Montoya *et al*., 2020). Food waste and bioactive ingredients can be used to create innovative products with positive effects on the economy and health. According to the principles of the circular economy, sustainable waste management techniques lessen environmental damage and conserve resources (Ahamed*et al*., 2022, Khanna andChakraborty, 2022).

The ability of plant-derived metabolites to act as hydrogen donors, reducing agents, free radical scavengers, and interact with metal ions confers antioxidant capabilities (Rice-Evans *et al.,* 1997). Fruits are given more significance among plant species since they can be consumed on a variety of occasions, are inexpensive, and are locally abundant. Furthermore, due to the high nutrient content of fruits, it is strongly advised for a variety of health difficulties as regular fruit consumption has a favorable link with the decline in cancer and other chronic diseases. Unexpectedly, the peel of a fruit was superior to the fruit because it contains significantly more anti-inflammatory and antibacterial molecules as well as antioxidant components than the other fruit parts (Lim *et al*., 2006, Ghasemi*et al.,* 2009). Studies comparing the peels to the fruit flesh have revealed a higher amount of polyphenols and flavonoids in the peels. Additionally, fruit peels were where phytochemicals like anthocyanin, flavonol, kaempferol, and xanthone

glycoside were first identified in fruits (Berardini*et al.,* 2005, Chinnici*et al.,* 2004).A packaging technique with an antimicrobial preservative sheet that incorporates isolated bioactive molecules or phytochemicals with high antioxidant content has been created and published. The inadequacies of the synthetic coating in terms of the shelf life of the built-in antibacterial agents of packaging films would be addressed by such a biopolymer-based film. Due to their limited partial breakdown by environmental bacteria, the widespread use of synthetic polymers like plastic in food packaging and storage leads to bioaccumulation of synthetic compounds in the environment (Cha and Chinnan, 2004). Microplastics are produced when plastic is widely used and applied in a variety of industries, including the storage and packaging of food (Sobhani*et al.,* 2020). These microplastics eventually cause food to become contaminated.

This study's objective was to utilization of phytochemicals found in aqueous extracts of fruit wastes having antioxidant and antimicrobial properties for development of chitosan based coatings. Biocompatible nature of coating formulations was tested by haemolysis assay using goat erythrocytes.

5.2 Materials and Methodology

The study evaluated moisture, volatile matter, ash, lipids, fixed carbon, HHV, and pH in waste fresh fruit from PurbaMedinipur, West Bengal, utilizing proximate analysis, X-ray diffraction, and EDS (as detailed in chapter 2). The powder was used for extract preparation and coating formulation.

5.2.1 Formulation combining aqueous extract and chitosan

Film casting: To test the formulations' efficacy at preventing the growth of microorganisms on the fruits and enhancing self-life after harvest, fruit coating formulations based on extract-chitosan were developed (Hongxia*et al.*, 2018). Glacial acetic acid (0.5%) was mixed all night long with chitosan (2.0%) that had been temporarily dissolved in a magnetic stirrer (REMI, India). The solution's pH was adjusted to 7.4 before adding water extract (0.1-1%) to it. The solutions were then given glycerol (0.3 g/g chitosan) as a plasticizer, and the mixture was constantly agitated for an additional 10 minutes to achieve homogeneity. The mixture of coatings (chitosan extract and pure chitosan) was maintained in storage for use on fruits.

For physicochemical examination, thin films of the extract and chitosan mixture were made. The semi-solid slurry (10 ml) was put into a glass petri dish to cast the film without the introduction of air bubbles. Then the casted film was left to cool to room temperature (24 °C) and harden. It took some time for the mixture to settle and form a thin covering.

Developed films characterization: One crucial aspect of food packaging is surface color of the packaging film, which not only influences consumer acceptance but also aids in food preservation by blocking the passage of light rays. In accordance with accepted procedures, manual evaluation was done to determine the film's color and its ability to block offlight (Basumatary*et al.,* 2018).The film samples were examined using SEM, thickness measurement, contact angle, and EDX to learn more about the chemical and physical characteristics of the films.

Estimation of the thin film's total water-soluble solid content: According to Souza and colleagues (Souza *et al.,* 2017), the total water soluble solid of the film was estimated. The cast films were cut into squares of 2 x 2 cm^2. Using an analytical fine balance, each square is weighed, and the starting weight (w_1) is noted. The cut films were then dried for 12 hours in a hot air oven at 72 °C. Dry weight (w_2) was recorded when the drying process was complete. Following that, pre-dried film fragments were soaked in 50 ml distilled water in a covered glass beaker for 2 days at room temperature (23± 2°C).The film fragments were taken out of the beaker when the incubation period was over and their surfaces were dried using filter paper. To calculate the final dry mass (w_3), the film fragments were once more kept in the oven at 72°C for additional 12 hours. The following formula was used to calculate film solubility:

$$\text{Solubility \%} = (w_2 - w_3)/ w_2 \times 100$$

A study was conducted to determine whether any soluble phytochemicals with antioxidant and antibacterial characteristics were released from the formed chitosan films. These biomolecules included proteins, phenols, flavanoids, carbohydrates, and antioxidants. The experiment's film components were examined for this investigation. For one day at room temperature, the film pieces (average weight 1g) were immersed in PBS

(pH 7.4). The PBS in which the films were immersed was tested for the biomolecules that were released. According to established protocols, each biocomponent was estimated.

5.2.2 Antimicrobial study of water extract developed from fruit wastes

The Tetrazolium/Formazan as a growth indicator was employed in the study to evaluate the antibacterial efficacy of extracts against *P. aeruginosa* MZ269380(Singh and Sahareen, 2017, Goswami*et al*., 2015). The minimum inhibitory concentration (MIC)was estimated (Chapter 4, section 4.2.1). From the data, MIC_{25} (sublethal), MIC_{50} and MIC_{75} (acute lethal) concentrations of extract was determined for further experiments. The overnight treated bacteria with concentration MIC_{25} (sublethal), MIC_{50} and MIC_{75} (acute lethal) were spread on the nutrient plates with sterile glass spreader and incubated for overnight at 37°C. After the completion of incubation period, colonies density formed in each plate were determined and compared with colonies formed in absence of extract.

Anti-motility activities: Swarming motility assay was performed in LB medium to determine the susceptibility of bacteria against the extract (O'May and Tufenkji, 2011). Each extract treated bacterial cells at concentration IC_{25} (sublethal), IC_{50} and IC_{75} (acute lethal) were added to the centre of petridish on motility agar medium (0.6% agar and 0.5% glucose) using a sterile pipette tip. The growth of swarming halos was evaluated after overnight incubation at room temperature (23±2^{o}C). After the completion of incubation period, diameter of bacterial growth in each plate were determined and compared with growth in absence of extract.

Exposure of thin film to environmental conditions: In this study, thin film prepared with varying concentration of aqueous extractswere tested for its antimicrobial property for three days at room temperature (23±2 °C) by exposing them in air. Daily observations were made to check the growth of microbes and mouldson them. And periodically the films were photographed.

5.2.3Coatings of fruits (tomatoes and grapes)

In this investigation, fresh tomatoes and grapes from the Haldia (West Bengal) neighbourhood market were used. Grapes and tomatoes were adequately cleaned with tap water before being repeatedly submerged in sterile water. After being uniformly coated

with the prepared coating, the item was air dried for a short while. Manually, this procedure is repeated ten times.Dipping control sets into sterile, distillation-sterilized water sets them. All experimental set samples underwent an overnight drying process at ambient temperature (25 ± 3 °C). The coated samples as well as the control samples were then left at room temperature. Every day, the samples were examined to check for any changes in sensory perception and microbial development.

5.2.4Toxicological study of water extracts on CT DNA and erythrocytes of goats

Water extract impacts on calf thymus genomic DNA: According to Dutta and Singh, 2011, the extract's biocompatibility was evaluated using genomic DNA. Aqueous extract in various concentrations (2–10 mg/mL) and 5 g of calf thymus (CT) DNA were combined, and the mixture was incubated for 1 hour at 37 °C. Following treatment, the DNA was electrophoresed in a 0.9% agarose gel using a 1 TAE buffer (40 mM tris-acetate, 1 mM EDTA), ethidium bromide staining (50 g/ml), and a gel documentation system to monitor the results. Analysis was done on the fragmentation that resulted and the suppression of fragmentation.

RBC haemolysis test: A hemolytic experiment was used to determine the hemocompatibility of aquous extract on goat erythrocytes. To evaluate the aqueous extract's potential to hemolyze blood, fresh goat blood was procured from a local butcher shop. Plasma and serum were recovered from the sample after centrifuging it at 5000 rpm for five minutes. Blood cells were grown for 1 hour at 37 °C after being mixed with various amounts of aqueous extract (2-10 mg/ml) in PBS (pH 7.4). 0.1% TritonX-100 and PBS were used as positive andnegativecontrols, respectively. After the incubation time, the cells were centrifuged, andat 540 nmthe supernatant absorbance containing the lysed erythrocytes was measured.

The following equation was used to calculate the percentage of hemolysis:

$$\% \text{ Haemolysis} = [a_t\text{-}a_c/a_{100}\text{-}a_c]x100$$

where a_c is the absorbance of the supernatant from controls (normal saline), and a_t is the absorbance of the supernatant from samples incubated with the particles. The absorbance of the supernatant of positive controls after being treated with 0.1% Triton X-100, which completely lyses RBCs, is a_{100}.

5.2.5 Statistical data analysis

Student's T-test and ANOVA were used to examine the experimental results. The data in this study were expressed as Mean ± SD (standard deviation). There were minimum three distinct experiments conducted.

5.3 Result and Discussion

This study investigates at the possibility of using fruit waste to produce value-added products. Here, aqueous extract prepared from fruit wastes was used in formulations for chitosan based coatings for fruits. The aim was to check antimicrobial growth, biodegradable nature of coatings for sustainable uses. Food processing facilities can get benefit from the powder's low moisture content and high ash content, which prevent microbial growth (Xie et al., 2020). The extract includes sucrose, a key ingredient in the synthesis of proteins and lipids as well as a precursor to vitamin C, and phytochemicals having antibacterial and antioxidant activities. The culinary, pharmaceutical, and cosmetic industries all have a lot of potential for the extracts. The DPPH•method measures the capacity of chemical compounds or herbal extracts to scavenge free radicals, which were the subject of Chapter 3's study. Therefore, water extracts prepared from fruit residual wastes have immense antioxidant and antimicrobial properties based on its phytochemicals contents being used in preparation of coatings which will increase shelf life and maintain their nutraceutical properties.

5.3.1Biodegradable aqueous extract-chitosan based edible coating

In this study, chitosan is chosen above other polymers for coating fruits due to its non-toxic and antibacterial qualities. To test its effectiveness in extending the shelf life of test fruit samples, extract made from fruit waste in water was added to the chitosan-based covering for fruits (tomatoes and grapes).

Film casting Coatings had been created with the consumer in mind, who prefers practical food packaging films with colour, lustre, and uses in food storage and preservation. The prepared coatings were applied to a spotless Petri-dish and cast into a thin layer (Figure 5.1a-b) to evaluate the characteristics of the coatings. The thin film had a yellow to dark yellow colour and contained the extract.

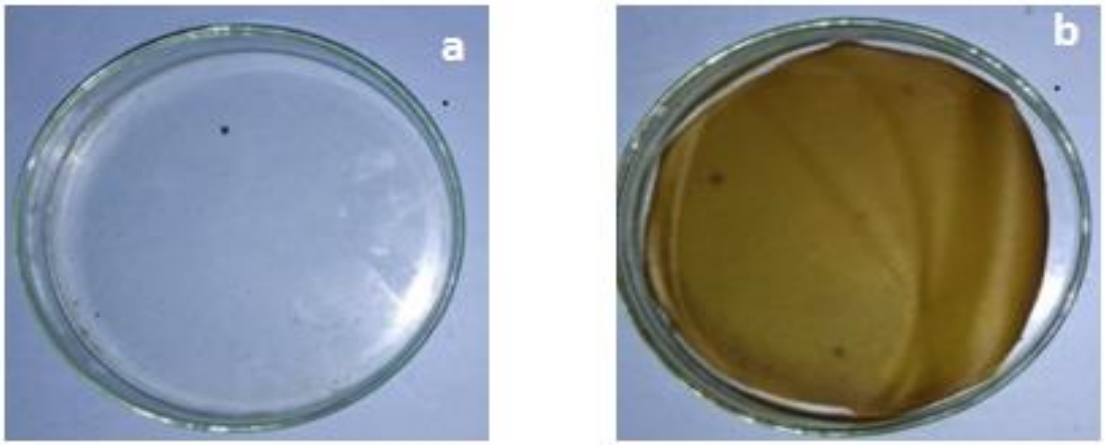


Figure 5.1: Thin film **c**asting by formulatedextract-chitosan solution a)thin film only contain chitosan, and b)thin film with extract-chitosan formulation

Developed films characterization: Increased preservation of color, flavor, and nutritional standards of packaged food will help to prevent complete penetration of visible and ultraviolet light through the films of various shades of color into the packed food. The film had a moisture content of 16.47±2.07 mg and a thickness range of 82–118 μm (Table 5.1). When evaluating the functional qualities of films intended for use in a variety of applications, the evenness of the film's thickness is a crucial factor.The surface morphology of produced films studied with the use of SEM examination (Figure 5.2a). A broad view of the film's structure is presented by the SEM research. SEM pictures can be used to study a uniformly smooth film surface with a minimal amount of air pockets and fissures. It is possible to see fibrous structures with dispersed clusters in the micrographs of the film. Minerals composed of Calcium, Carbon, Potassium, Magnesium, and minor amounts of Copper and Sodium were also found to be prevalent in these phases (Figure 5.2 b–c).The film's contact angle is shown in Figure 5.2d. Measurement of hydrophobicity is a crucial factor in the assessment of thin films. The contact angle was lowered when functional bio-components were added, in this case fine fruit residue

powder. The chitosan-based film had an average contact angle of 35.48±5.57^0 (Table 5.1).

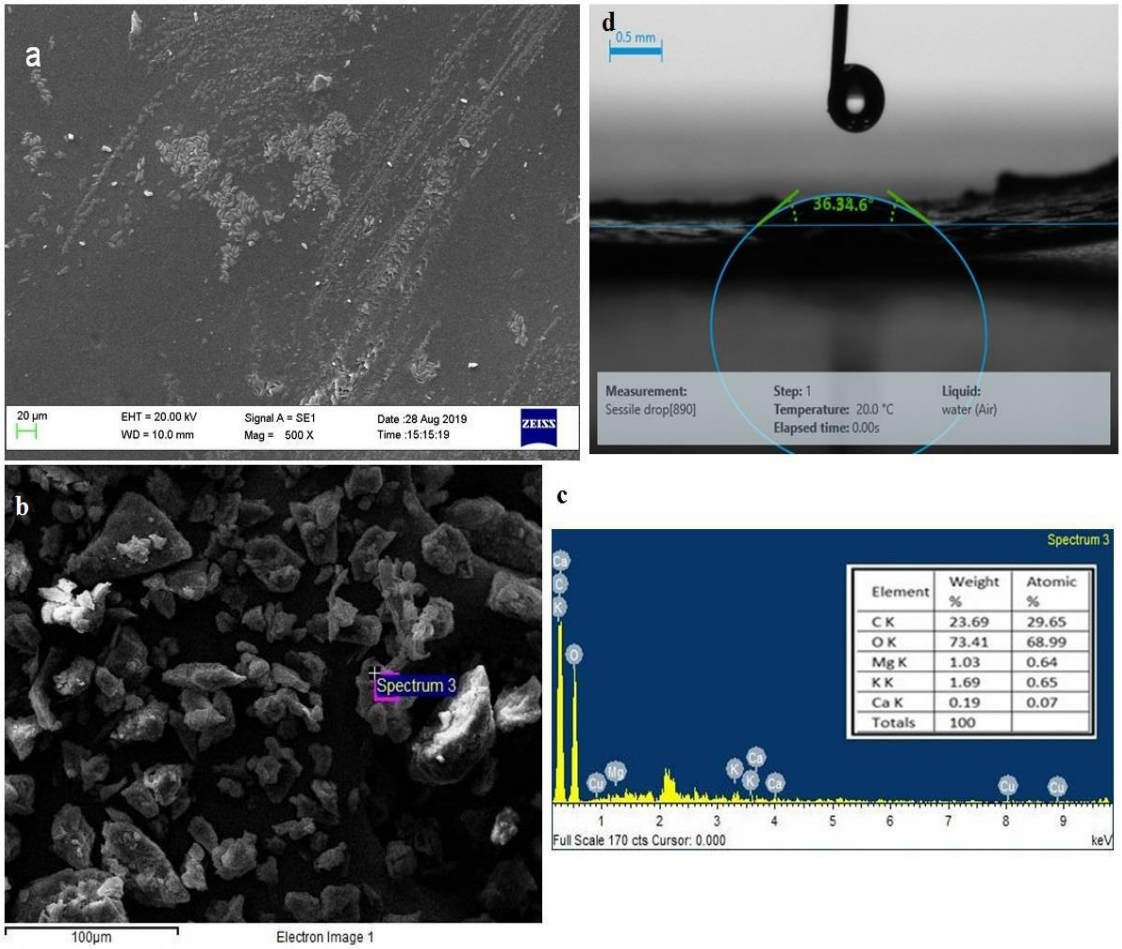


Figure 5.2 Characterization of thin film based on aqueous extract of fruit powder chitosan formulation. (a) SEM image, (b) SEM for identification of minerals, (c) EDX exhibiting various minerals and inset showing minerals percentage and (d) Contact angle measurement of thin film.

5.3.2 Film solubility assay and Biomass Released

The thin films cast in chitosan, either with or without aqueous extract, had their level of solubility in an aqueous environment evaluated. In 2 days of incubation, chitosan-free film loses 61% of its weight, but extract-incorporated chitosan film gains 68% (Table 5.1). The film's weight decrease proved that cast films are biodegradable.

Chitosan-Extract formulation used for fruit coating is investigated to determine the amount of biomass released in PBS during a specific period of time. Before being immersed in PBS, the coating formulation that had been cast as a thin film was divided into manageable squares and weighted. Protein (15 mg/g), phenol (14 mg/g), flavonoids (40 mg/g), carbohydrates (33 mg/g), and total antioxidants (33 mg/g) made up the biomass released from the coatings in PBS (shown in Table 5.1).

The outcome showed that large levels of essential phytomolecules with antibacterial and antioxidant activities were present. Therefore, these compounds could be used to cover fruits with an antibacterial barrier.

Table 5.1Characteristic features of developed chitosan based thin

Parameters	**Observations**
Colour	Yellow to dark yellow
Thickness (μm)	82-118
Moisture Content (mg)	16.47 ± 2.07
Contact angle (degree)	35.48 ± 5.57
Total water soluble solid %	68
Solubility (%)	91
Biomolecules Released	
i. Protein	15 ± 1 mg/g
ii. Phenol	14 ± 1.15 mg/g
iii. Flavanoids	40 ± 1.15 mg/g
iv. Carbohydrates	33 ± 0.5 mg/g
v. Antioxidants	33 ± 1.2 mg/g

5.3.3 Antimicrobial assay

AE treated *P. aeruginosa* at concentration (LD_{25}, LD_{50} and LD_{75}) showed growth andmobility retardation. Pseudomonas sp show a distinct mode of motility (swarming motility)with the help of their flagella. The growth of swarming halos was evaluated after

overnightincubation at room temperature (23±2 ºC) and the result showed positive retardation ofbacterial mobility at the mentioned concentrations (Figure 5.3). Extracts from plants and purebio-components have been reported to have parallel outcomes for the motility of test bacteria.Colonies forming the potential of bacteria on nutrient plates was assayed with *P. aeruginosa*MZ269380, treated with extract (LD_{25}, LD_{50} and LD_{75}) for 16 hours and spread on the nutrientplates. The result showed a gradual decrease in the number of colonies directly proportionalto the concentration of extracted treated bacteria. Bioactive components from cranberry andpomegranate rich in proanthocyanidins and ellagitannins, ellagitanninscatechin andepicatechin respectively, inhibit swarming motility.

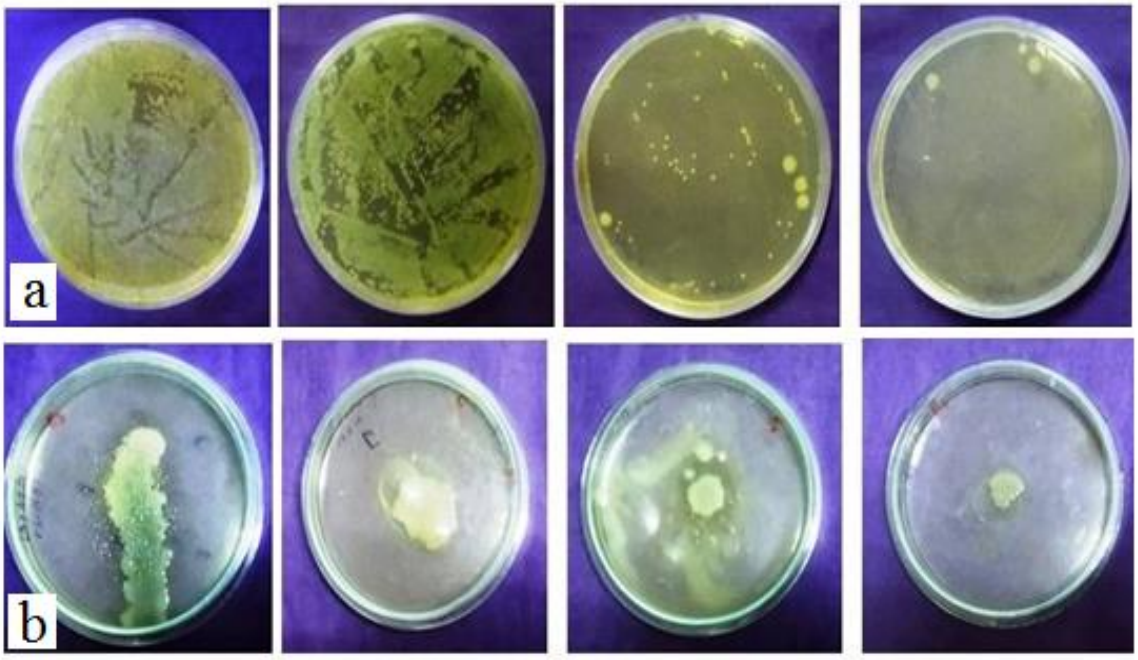


Figure 5.3 Antimicrobial effects of aqueous extract on *P. aeruginosa*MZ269380 bacterial isolate. (a) Cytotoxic effects of extract concentrations (LD_{25}, LD_{50} and LD_{75}) showing dose-dependent fashion; (b) anti-motility activity of extract.

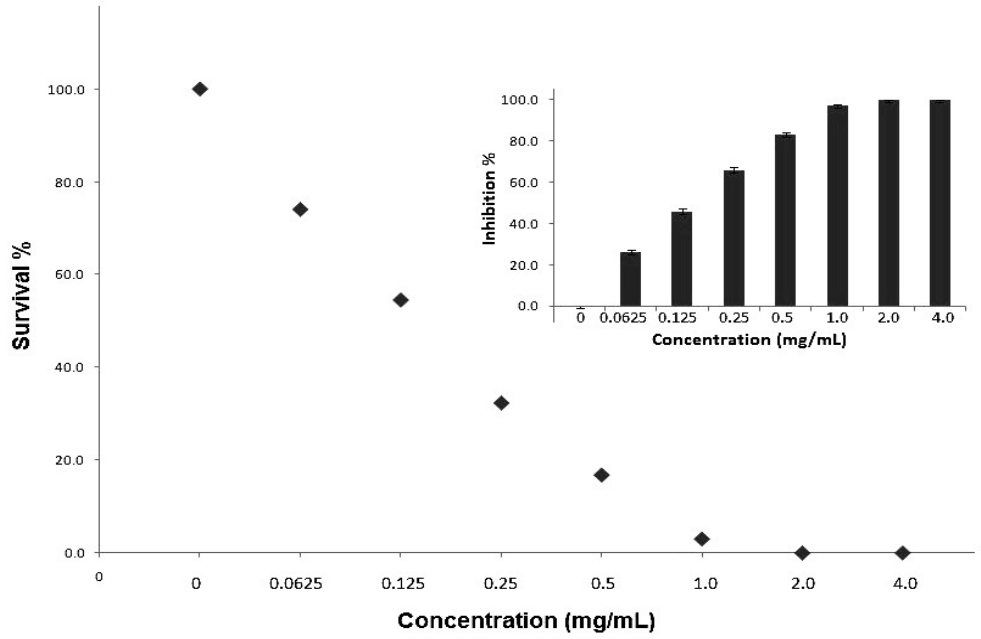


Figure 5.4 Cytotoxcity effect of water extract on *P. aeruginosa* MZ269380. Inset shows growth inhibition % of test bacteria by water extract.

Figure 5.4 shows the water extract's cytotoxic action on bacteria in a dose-dependent manner. 2 mg/mL was the MIC value that was noted. On this test bacterium, the extract revealed a potential cytotoxic impact. The fact that test bacteria's growth was inhibited around each well that had a varied amount of water extract implies that the substance has a strong antibacterial capability. Each well's clear zone serves as evidence of extract activity that is dose dependant. With the exception of 75 and 100 g/mL, Student's T test analysis revealed significant differences in the outcomes among the treatment combinations at 95%confidence.

5.3.4 Exposure of thin film to environmental conditions

Researches in development of biocompatible and bio-edible thin films for usage in food packaging are highly accelerated in recent years due to adverse effect of chemical based packaging. In this study, thin film of chitosan supplemented with different concentration of water extracts was made and tested for its antimicrobial property for three days at room temperature (23±2 °C) exposed to air. Figure 5.4 shows, dose dependent inhibition of microbial growth on the film. Film having 1 % fruit extract inhibited maximum microbial growth compared to other concentration, where appearances of microbes of various types

are seen. Film without fruit powder showed profuse growth of microbes of different types. Fruit extract prepared from waste part is effective in maintaining the microbe free film by preventing microbial growth. Such thin film can be used in food packaging which will helps in prevention of contamination with microbes under prevailing environmental factors at room temperature and thus avoid food spoilage (Singh and Sahreen, 2017). Waste from fruit industries are being used in isolation of value-added products and are utilized development of biodegradable thin films, having applications in packaging of food (Nascimento et al., 2012; Ramachandraiah et al., 2017).

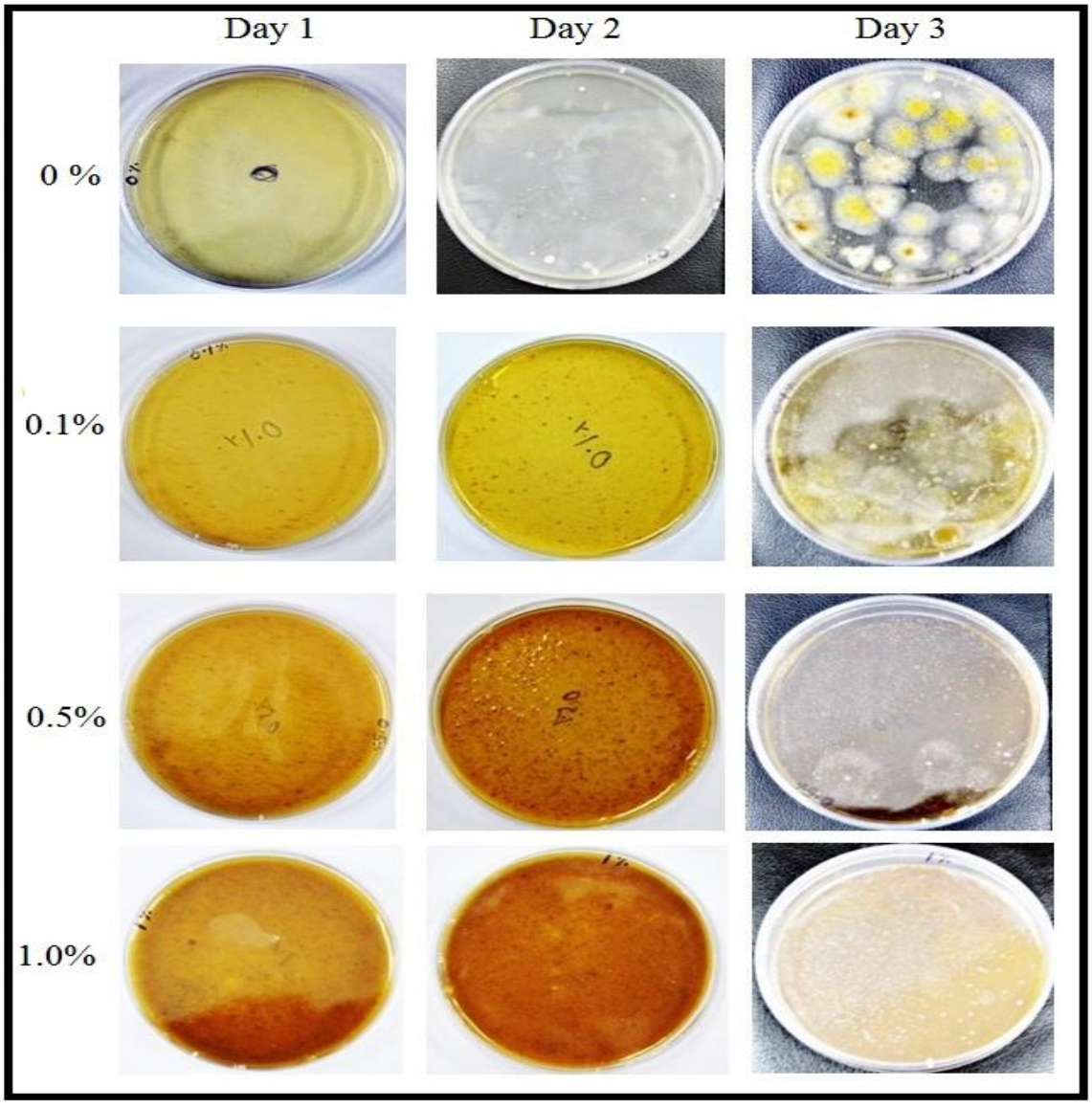


Figure 5.5 Chitosan based thin film supplemented with water extract of fruit wastes exposed to air in ambient environmental conditions for three days.

5.3.5 Coating of tomatoes and grapes

Food items can occasionally become unhealthy due to infestations of bacteria, fungus, and molds, including psychrophile bacteria, while being stored. This spoiling causes post-harvest loss (Singh andSahareen, 2017). The powder made from the fruit leftovers contained significant phytochemicals with high antibacterial and antioxidant activities that belonged to several chemical groups. To test the effectiveness of coatings on tomatoes and grapes, fruit waste extract was used in the formulation of fruit coatings. Fruits treated with sterile distilled water displayed changes in shape, hardness, and texture. Additionally, the peel's color altered, and microorganisms can be observed (Figure 5.5b). Extract-formulated coats, on the other hand, exhibited no such modifications and continue to be as fresh as they were 10 days ago (Figure. 5.5a). The outcomes show that adding extract to chitosan coatings can extend fruit's shelf life.

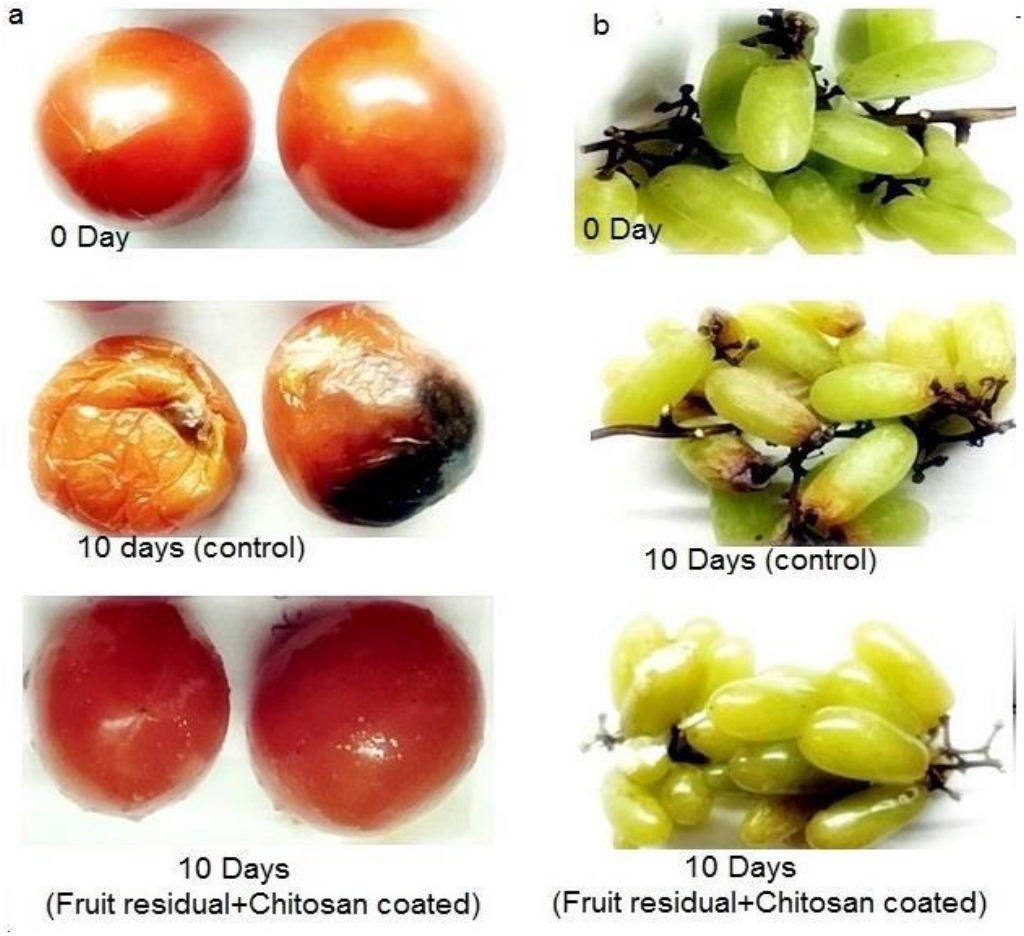


Figure 5.6 Coating of fruits with the formulation consisting of aqueous extract (1%) and chitosan (2%).

Because of food-born microbes, the freshness and shelf life of fruits after harvest may be significantly shortened during the duration of storage. This study looked at the effectiveness of coatings against microbial development on tomatoes and grapes for 10 days under ambient climatic conditions. Figure 5.6 depicts the development of bacteria on negative controls (samples dipped in sterile water) as well as the minimal development of bacteria from samples coated with an extract-chitosan formulation and solely chitosan. Even Nevertheless, samples coated just with chitosan showed less bacterial growth than samples only dipped in water, which showed more pronounced bacterial growth. These formulations can be used to coat fruits and vegetables for long-term storage and can also be made into thin films that can be used in food packaging. These formulations will help prevent microbial contamination under the current environmental conditions at room temperature, preventing food spoilage (An *et al.,* 2000, Yang *et al.,* 2018).

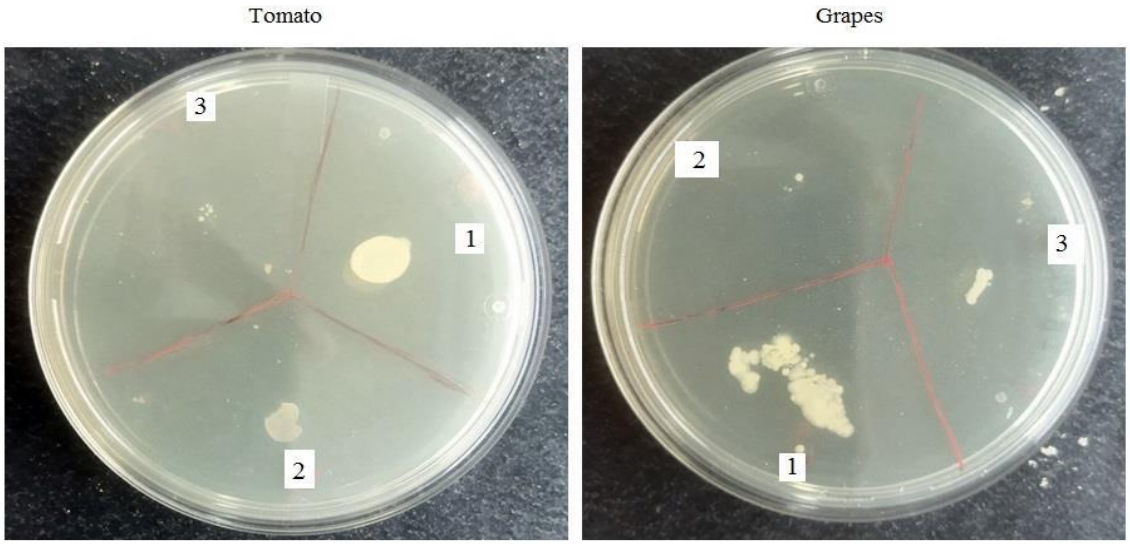


Figure 5.7 Microbial growths from the fruit and vegetable samples coated with chitosan were cultured on nutrient plates for 10 days at room temperature (1, fruits dipped in sterile water, 2, fruits coated with simply chitosan, and 3, fruits coated with extract-chitosan formulation).

The antibacterial activity of coatings will be further increased through green manufacturing of metallic nano-particles using phytochemicals derived from plant sources, which aids in the freshness of coated fruits for a longer period of time. Using plant extracts with antibacterial properties, many scientists are synthesizing nano-particles (Ameena*et al.,* 2022, Devi *et al.*,2019). Therefore, the creation of a smart packing system

will aid in the extension of the post-harvest period and the fruit's shelf life by the insertion of nano-particles coated with phytochemicals.

5.3.6Toxicological study of extract to check biocompatibility

Biocompatibility is the capacity of biomolecules to execute their intended function without generating adverse effects on the receiver or beneficiary.. Biomolecules must adhere to fundamental standards, avoiding toxic effects. Bioactive compounds are beneficial for human well-being due to their, anti-maturing, anti-inflammatory, anti-disease, and cell reinforcement properties.

Influence of aqueous extract on Calf Thymus DNA: A fruit extract-treated CT DNA profile is shown on an agarose gel in Figure 5.7. Untreated DNA in lane 1 displays a high molecular weight and no signs of fragmentation. The CT DNA was of this type up until lane 5, and the DNA loss in lane 6 is negligible. DNA damage is brought on by extracts with high metal ion concentrations. At the MIC value (2 mg/mL), DNA damage was nonexistent. By essentially scavenging ion-generated free radicals, phytochemicals found in plants have a protective function against DNA damage caused by free radicals (Dutta and Singh, 2011).

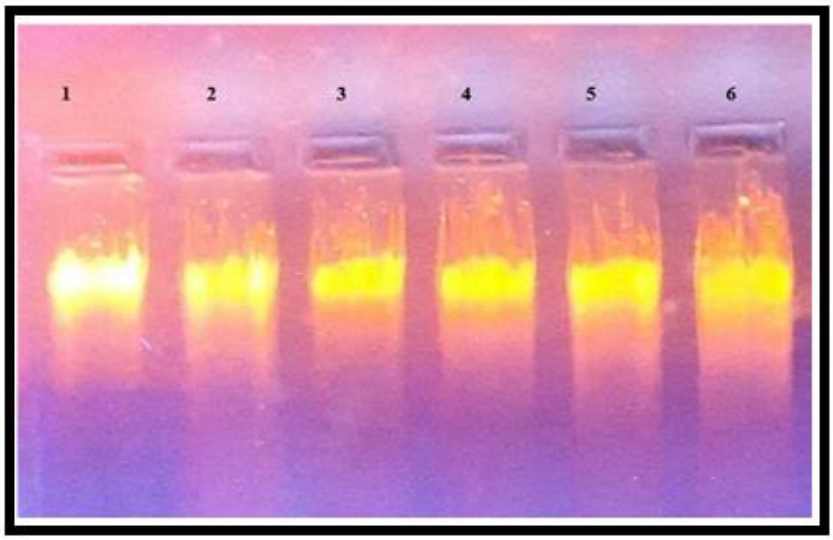


Figure 5.8 Electrophoretic mobility monitoring of the effects of a fruit waste extract on CT-DNA. After electrophoresis of agarose gel stained with ethidium bromide, DNA profiles were observed under UV light (Lane 1, CT DNA only; Lanes 2–6, CT-DNA with 2–4, 6–8, and 10 mg/mL, respectively).

RBC hemolysis test: It was observed in fruit wastewater extract can distinguish between eukaryotic and prokaryotic blood cells using an in vitro hemolytic experiment on goat RBC (Table 5.2). Evaluation of the hemolysis assay using an aqueous extract having concentrations between 0-5 mg/ml. In contrast to the positive control (0.1%) Triton X-100 at a concentration of 2 mg/mL (MIC value), the extract only slightly (1.7%) increased goat RBC hemolysis. Because of this, the bioactive components in water extract showed evidence of biocompatibility with eukaryotic cells.

Table 5.2 Haemolysis assay for evaluation of biocompatibility of water extract. Here, RBCs of goats were treated with different concentrations (0-5 mg/ml) extracts for 1 hour at 37ºC. Triton X 100 and PBS were used respectively as the positive control having 100% hemolysisand a negative control.

Water Extract(mg/mL)	**Hemolysis (%)**
0	0.05±0.045
1	1.05±0.080
2	1.7±0.05
3	2.05±0.063
4	2.95±0.057
5	3.46±0.087

5.4 Conclusion

Chitosan-based fruit covering AE of fruit wastes has been developed to inhibit food-borne microorganisms and extend post-harvest storage time. The coatings show significant antioxidant and antibacterial capabilities, reducing bacterial growth and maintaining color and texture. The coating also prevents moisture evaporation, improving the tolerance of tomatoes and grapes to microorganisms. Biopolymer antimicrobial packaging reduces environmental flow, prevents leakage, and minimizes food stability. Using fruit residue waste instead of standard films reduces manufacturing costs and food byproduct value.

Chapter 6

Partial purification and future scope of Bioactive Components from fruit residual extract

6.1 Introduction

Fruit waste typically contains significant levels of beneficial phytochemicals, such as polyphenols, carotenoids, sugars, and fibre, as well as proteins, lipid and carbohydrates. A sample must have an enriched concentration of a specific ingredient, for example, in order to extract that component or class of components(Mahato*et al.,* 2019).Two extraction techniques are generally used to isolate these chemicals: (i) Traditional techniques include maceration, solvent extraction, soxhlet extraction, and percolation (ii). According to Nirmal et al. (2023), new extraction techniques include SFE (supercritical fluid extraction), MAE (microwave-assisted extraction), EAE (enzyme-assisted extraction), PEFE (pulsed electric field extraction), UAE (ultrasound-assisted extraction), and PLE pressurized liquid extraction).

These chemicals were isolated from various fruit wastes. Purification and isolation procedures must be used to boost the concentration of every type of phytochemicals. Column chromatography, HPTLC (High-performance thin-layer chromatography), high-speed countercurrent chromatography (HSCC), and HPLC (high-performance liquid chromatography) are all components of the fundamental purification procedure. Methanol, n-butanol, ethyl acetate, water, chloroform, ethanol, and other commonly used solvents are also available. The most common method for detecting the bulk of phytochemical compounds from fruit components is water. UV-visible and mass spectroscopy is the main detection techniques used to locate and quantify the bioactive compounds recovered from mixed fruit waste(Mahato*et al.,* 2019).It is essential to use a variety of analytical techniques to study the phytochemical elements of the extract. Extract preparation from fruit waste powder, followed by HPTLC, and GC-MS were used in the current work to partial purification ofselected phytochemicals in fruit extracts. In this study, most common bioactive compound (N-Hexadecanoic acid) which is present in all five extracts and few more were successfully identified from the TLC eluted sample.

6.2 Materials and Methods

Fruit residue powder was extracted with water, ethanol, methanol, petroleum ether, and butanol by individually raising the polarity of each solvent. After the solvent extracts were dried, a preliminary phytochemical analysis using GC-MS was carried out, as detailed in chapter 3.

For partial purification of biological elements, HPTLC fractionation of extracts following by elution and finally GC-MS analysis were done (Figure 6.1)

6.2.1 HPTLC analysis of the fruit residual extract

The most sophisticated type of TLC is known as HPTLC (High-performance thin-layer chromatography), which uses chromatographic layers with the highest possible efficiency in separation along with cutting-edge equipment for each step of the process, including accurate sample application, standardized reproducible chromatogram development, and software controlled evaluation. A whole idea called HPTLC encompasses both the use of widely accepted methodologies for qualitative and quantitative research that are founded on scientific truths. HPTLC was conducted in duplicate using various injection volumes.A solvent mixture of chloroform, methyl alcohol, and water (75:25:2.5 v/v) was used for the separation of compounds in silica plate.

Instruments details:

Sampler: Linomat 5, S/N: 260659
Scanner: TLC Scanner 4, S/N: 260596
Software: Server DESKTOP- UOBI8P, Version 3.0.20196.1
TLC Plates: Merck, HPTLC Silica Gel 60 F254
Dimension: 100 × 100 mm
Mobile Phase: Chloroform: Methanol: Water (75:25:2.5) v/v

6.2.2 GC-MS analysis

After receiving the HPTLC results, methanolicextract was selected for GC-MS to identify the specific components. The specific bands formed by methanolic extract were eluted and dissolved in methanol and incubated in cold room for overnight to get phytochemicals get dissolved in methanol from the silica particles. The spots were selected based on the prominent nature of the bands. Thereafter, the samples were

centrifuged and clear soup collected into fresh tubes. The collected soups were evaporated to dryness and finally GC-MS analysis was done.

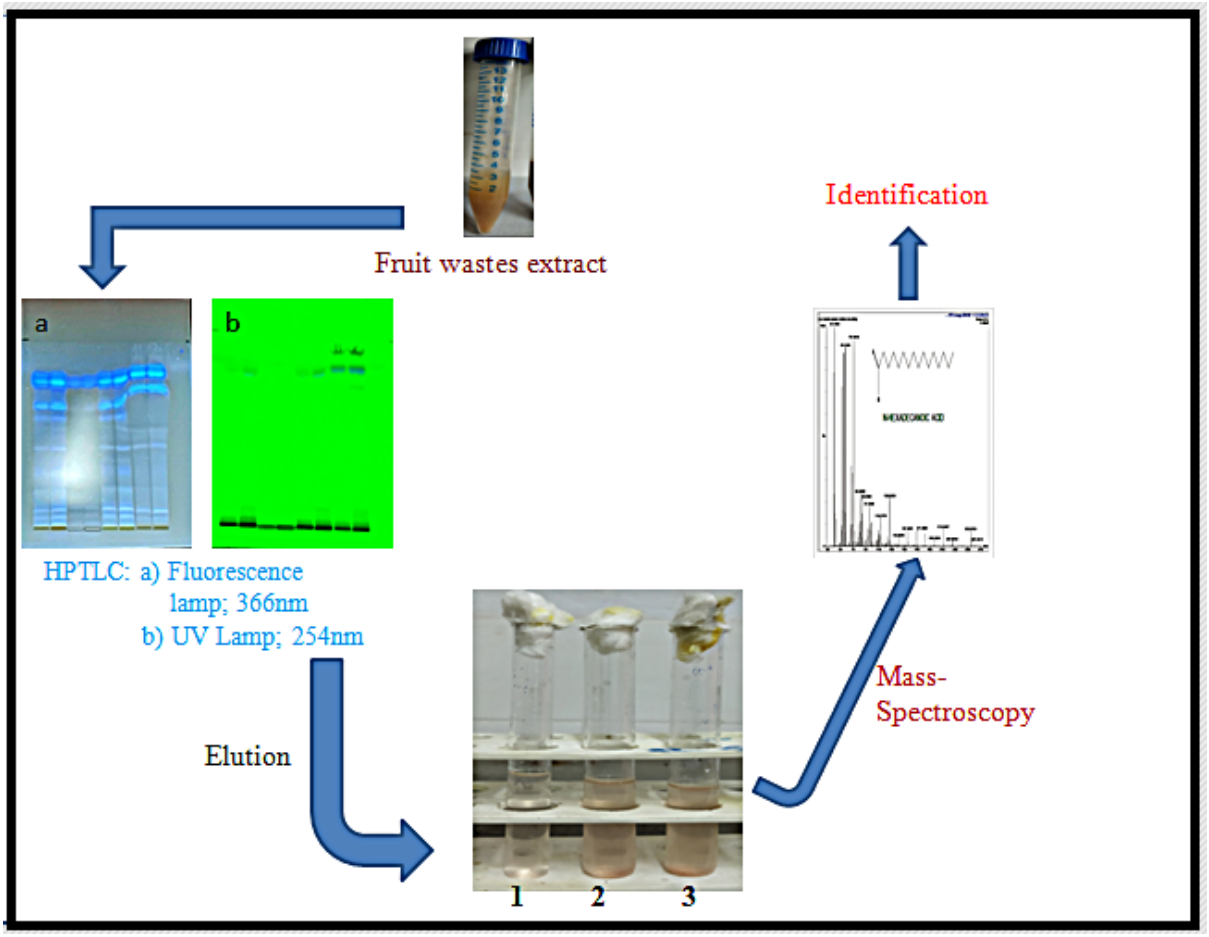


Figure 6.1 Schematicdiagram for partial purification and identification of bioactive componenets from fruit wastes extract.

6.3 Results and Discussion

6.3.1 HPTLC examination of the fruit residual extract

Fruit waste extract was analyzed using HPTLC. Results were obtained using a solvent mixture of chloroform, methyl alcohol, and water (75:25:2.5 v/v). After scanning and visualizing with fluorescence light at 366 nm and a UV lamp at 245 nm (after spraying anisaldehyde sulphuric acid reagent), the chromatogram displayed percentage area and R_f-value. The selected fractionsweeeluted because it was detected in significant amounts in the plate after the fractionation.

The methanolic extract was chosen for further examination because isolation of most common compound (N-Hexadecanoic acid) was the target which is readily get dissolved in methanol solvent (Ghosh*et al*, 2015).

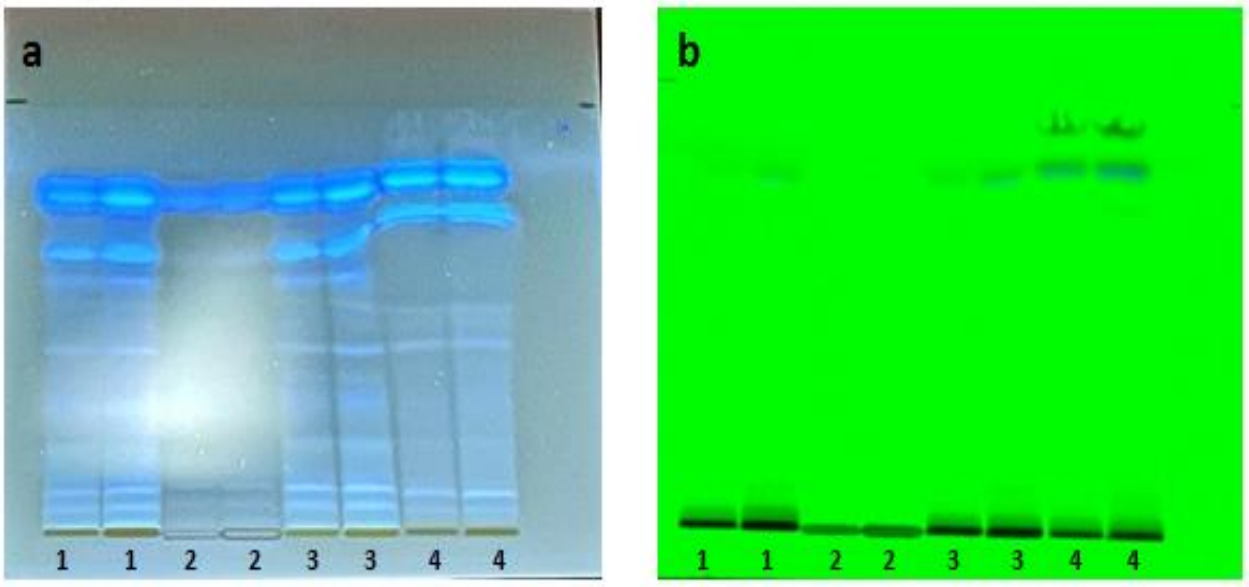


Figure 6.2HPTLC chromatograms of column fractions, a) Plate seen with fluorescence lamp at 366nm b) Plate seen with UV Lamp at 245 nm. Column i) Ethanol Extract ii) Petroleum Ether Extract iii) Methanol Extract and iv) Water. Mobile phase: Chloroform:Metanol: Water (75:25:2.5) v/v

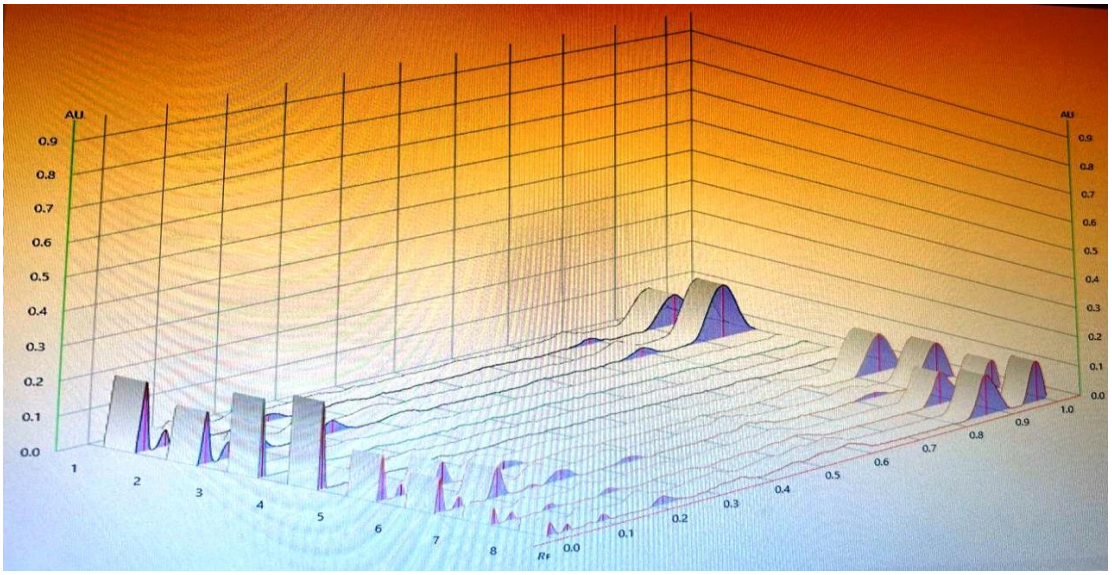


Figure 6.3: Integration Curve of column fractions

6.3.2 GC-MS analysis

GC-MS chromatogram study of the elucidated methanolic extract of fruit peels extract(Figure 6.4) showed peaks of different retention times among which few are more distinct and prominent at different RT than others of specific chemical constituents. The prominent bioactive molecules were characterized and identified by the NIST library. The compounds identified are N-Hexadecanoic acid methyl ester; 8-Octadecanoic acid, methyl ester;methyl ester, γ-Linolenic acid and phytol. MS analyzed spectrum and structure of each molecule in the methanol extracts of fruit wastes are shown in Figure 6.5-6.8.

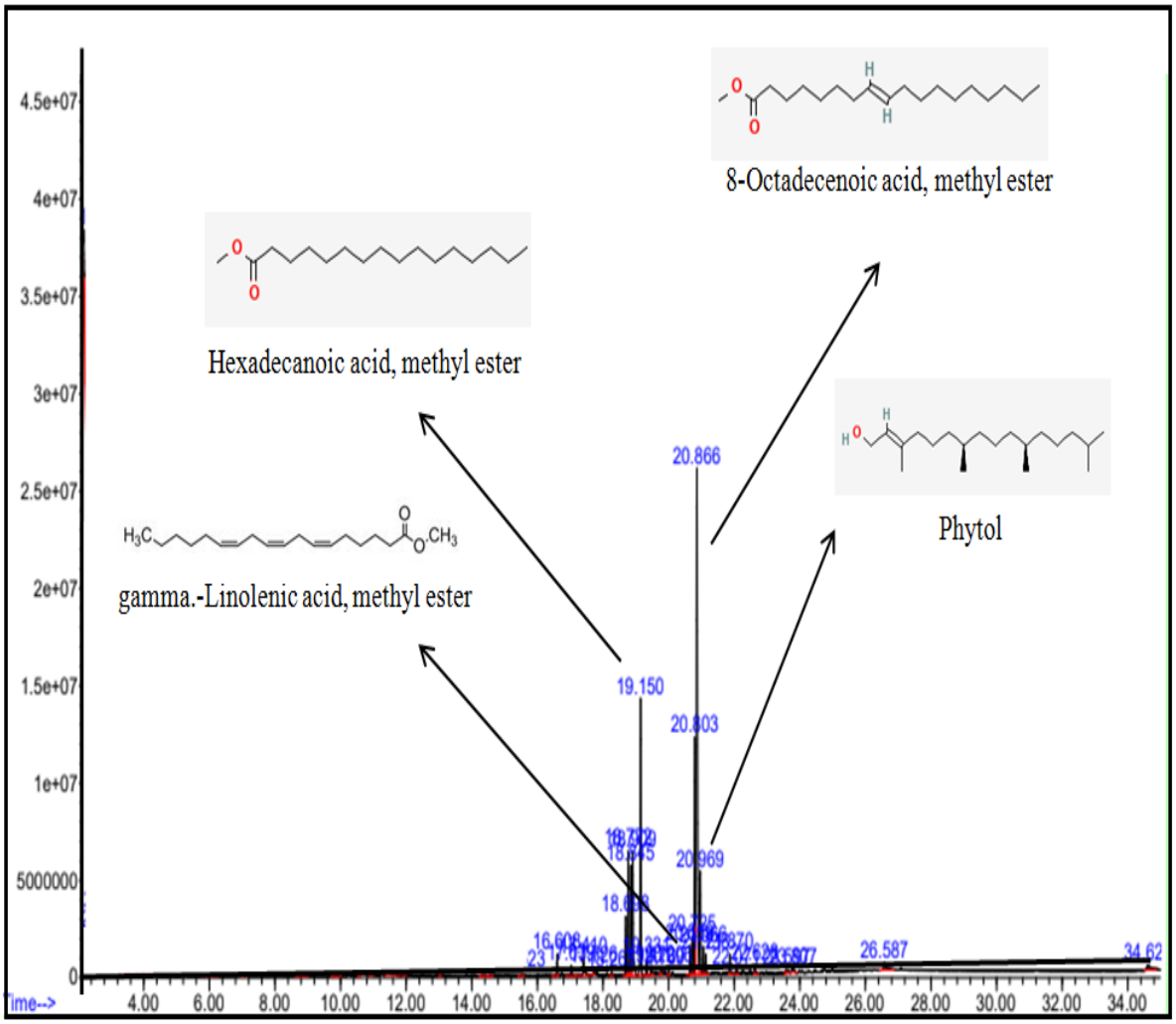


Figure 6.4 GC-MS analysis of methanolic fruit wastes extract after elucidation from TLC plate

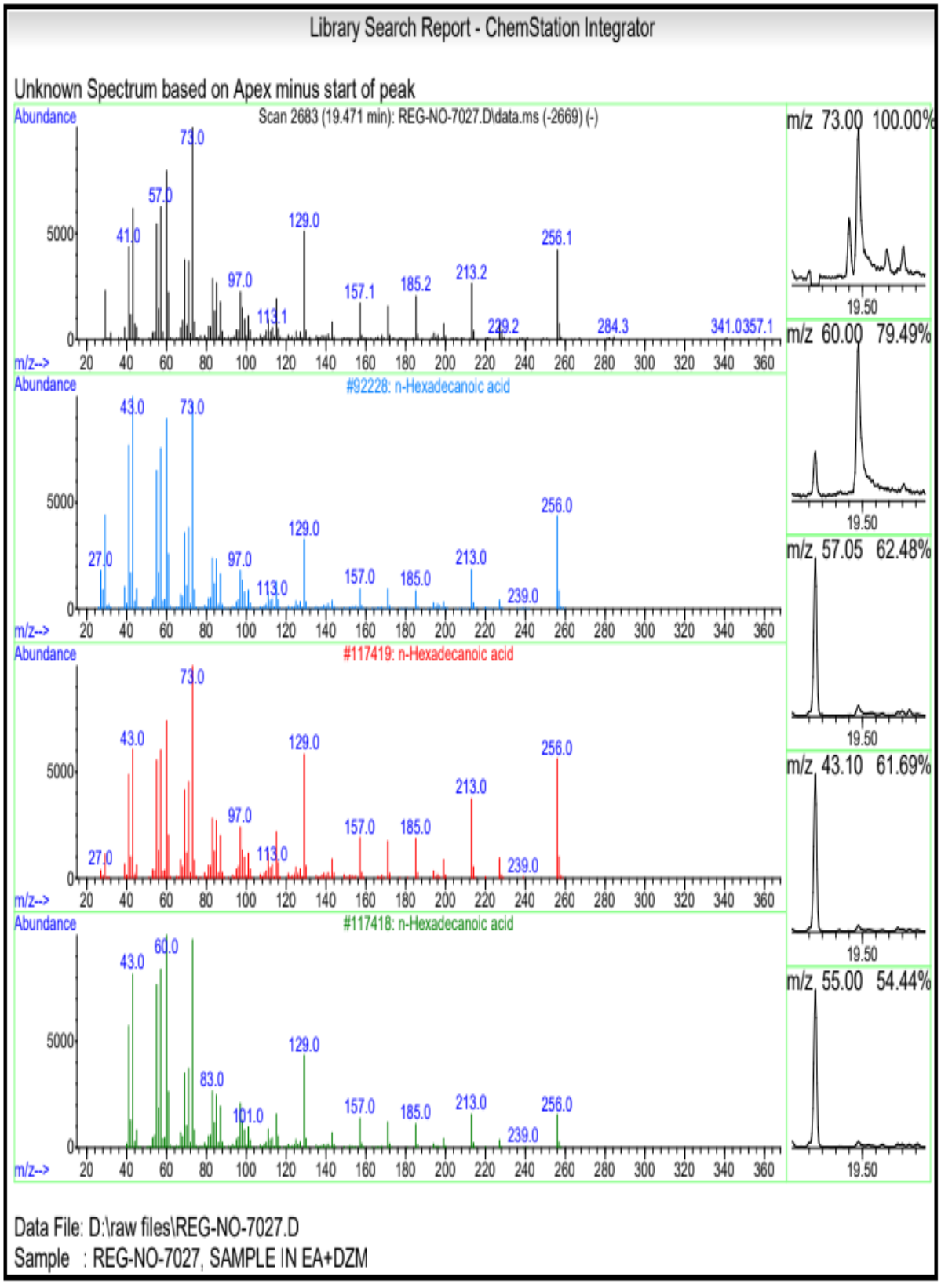


Figure 6.5 MS analysis of n-Hexadeconic acid in methanolic extract

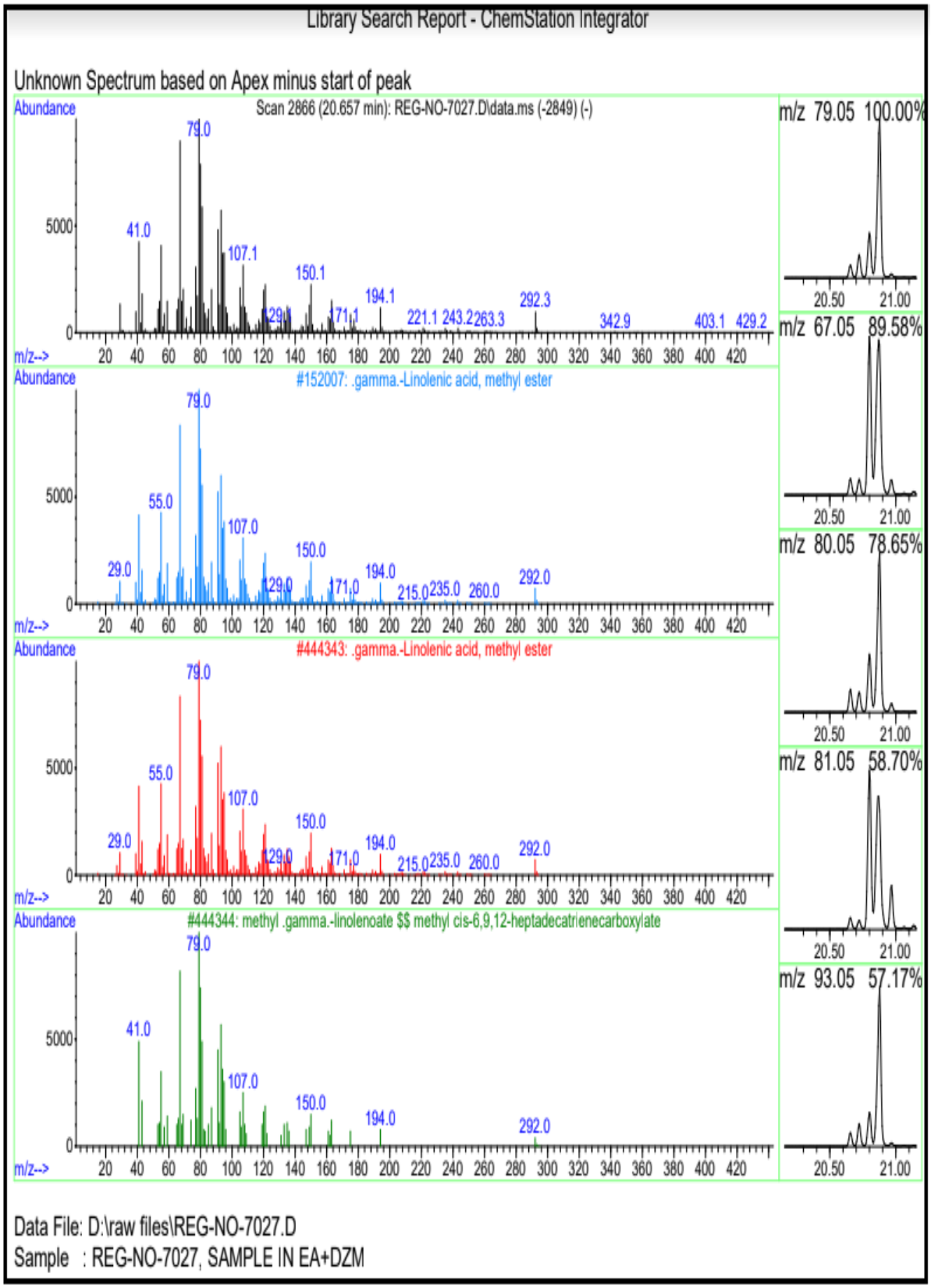


Figure 6.6 MS analysis of γ Linolenic acid, methyl ester in methanolic extract

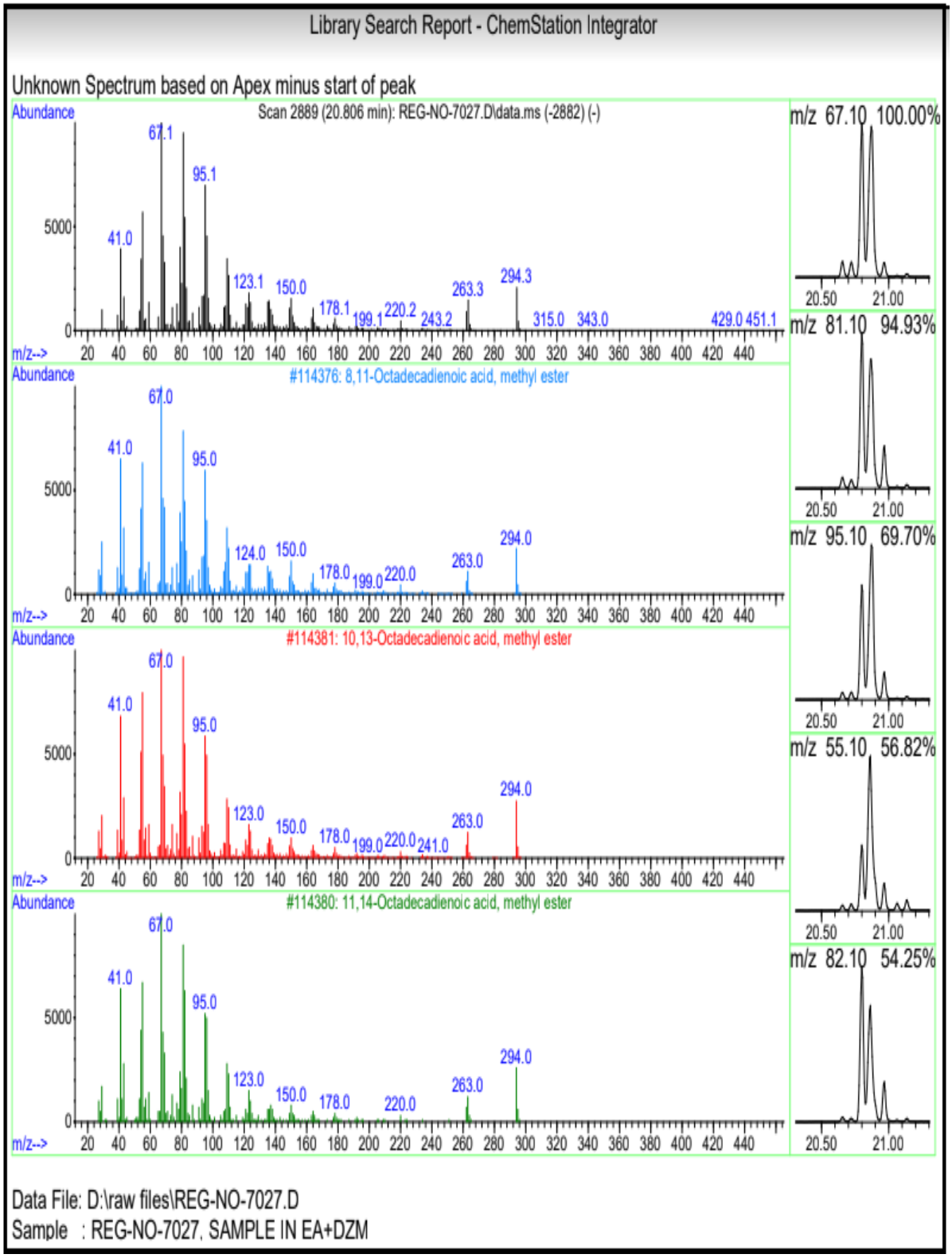


Figure 6.7 MS analysis of octadeconic acid, methyl ester in methanolic extract

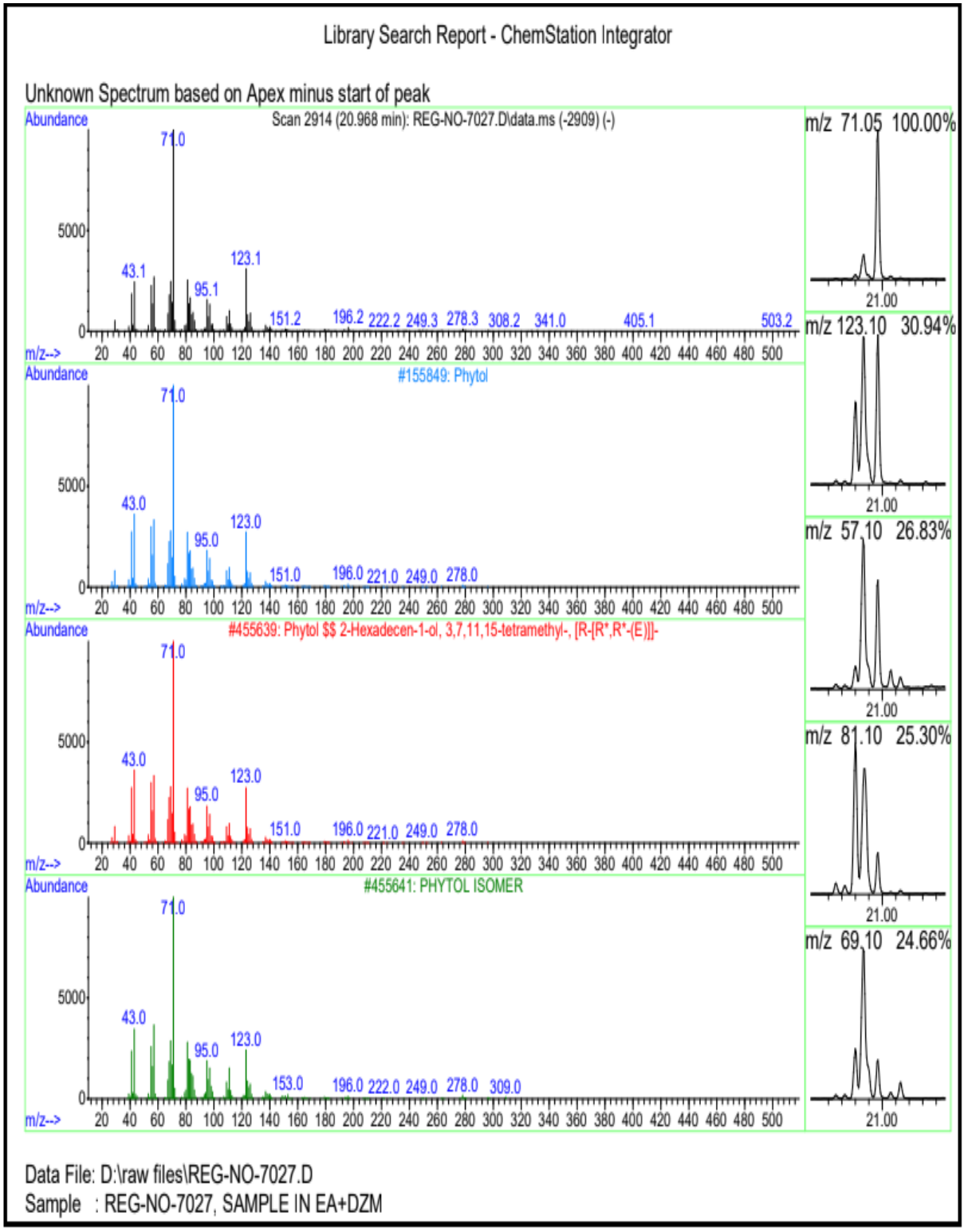


Figure 6.8 MS analysis of Phytol, methyl ester in methanolic extract

The results indicated that the selected bands from TLC plate showed presence of N-Hexadecanoic acid methyl ester; methyl ester, 8-Octadecanoic acid; γ-Linolenic acid, methyl ester, and phytol. N-Hexadecanoic acid is one of the compounds found in

allextracts that have been shown to have antioxidant and antibacterial activities(Ganeshan*et al*., 2022; Shaaban*et al*. 2021). 9,12-Octadecadienoic acid is used as a flavoringagent in food (NCBI, 2022; 1 and 2). Details of these compounds are discussed in Chapter 3. Further studies on the selected bands may be taken up to isolate the pure form of these bioactive compounds and exploit them in various applications in the cosmetics, medicine, and food industries.

6.4 **Conclusion**

In this work, a solvent mixture of water, methyl alcohol, and chloroform was used to examine the fruit waste extract using HPTLC. The methanolic extract was chosen for further investigation because it may include N-Hexadecanoic acid, a typical substance in significant amounts. The study of the GC-MS chromatogram revealed discrete peaks with various retention durations, which revealed important bioactive compounds. N-Hexadecanoic acid methyl ester, 8-Octadecanoic acid, -Linolenic acid, and phytol, which offers more potential for usage in the pharmaceutical,food, and cosmetic industries, were all characterized by the NIST library.

Summary

India, the top fruit producer in the world, has been looking into the possibilities of mixed fruit peels for diverse uses. Organic waste is being produced as a result of the rising demand for fruits and vegetables in the diet, which adds to municipal solid trash and environmental problems. Carotenoids, polyphenols, and vitamins are among the beneficial components found in fruit and vegetable waste. Fruit waste has a big impact on the economy, the environment, and society. Anaerobic digestion and composting techniques can produce beneficial substances for the food sector as well as economic advantages. Although there are serious environmental and financial problems associated with food waste. Beneficial phytochemicals like polyphenols, carotenoids, sugars, fiber, and carbohydrates can be found in fruit wastes and these can be exploited for various human uses. The phytochemicals which are reported to be present in the fruit wastes are being used in various commodities such as food items and packing and personal care products.

Chapter 2's findings demonstrated that fruit leftovers have many positive attributes and can be applied in a variety of industries including the food processing industry for food formulations, antioxidant-rich cosmetics, and herbal medications. Fruit residual wastes were collected and made into fine powder after primary processing. The powder's homogeneity, surface morphology, geometric shape, and to examined sizes scanning electron microscopy (SEM) were used andproximate analysis showed its physic-chemical nature. Results obtained from these analyses confirmed that the powder has high potential in utilization for various uses such as food packaging, and extraction of bioactive compounds and can also be made biochar for soil amendment.

In chapter 3, extraction and identification of phytochemicals from fruit wastes powder are described in details. The analysis of phytochemicals five extracts (aqueous, ethanol, methanol, petroleum ether and butanol) by GC-MS in this dissertation reveals the presence of protein, carbohydrates, phenolic compounds, and flavonoids. The study found 45 bioactive substances in the extracts, which are mostly plant secondary metabolites, as detailed in chapter 3. These substances' antioxidant, antibacterial, and antifungal qualities make them ideal candidate for treating a variety of illnesses. Due to their antioxidant qualities, these dietary components are considered food supplements.

For sustainable development and health advantages, it is crucial to use waste to create bioactive components.

Bacillus subtilis and *Pseudomonas aeruginosa*, two typical microbial pathogens, have been investigated for in-vitro antibacterial activity against fruit residue extracts (Chapter 4). Antimicrobial potential of five extracts prepared from fruit wastes showed high cytotoxic nature. Among the extracts tested, water, butanol, and ethanol shown high antibacterial action than methanol and petroleum ether extracts.

Aqueous extract-chitosan coatings with antibacterial and antioxidant characteristics can be made from fruit waste. The aqueous extract, which had a low MIC value, demonstrated substantial antioxidant properties. As discussed in chapter 5, chitosan (2%)-based coatings with 1% water extract were formulated to develop a fruit coating which demonstrateda notable reduction of microbial proliferation and fruit degradability. Plant-derived metabolites have antioxidant properties because they can function as hydrogen donors, reducing agents, free radical scavengers, and interact with metal ions. Fruit peels have high concentrations of anti-inflammatory, antibacterial, and antioxidant compounds in comparisonto other fruit portions.A method for packaging that uses isolated phytochemicals or phytochemicals with a high antioxidant content and an antimicrobial preservative sheet has been developed and published.

Maceration,soxhlet extraction, solvent extraction, and percolation are traditional extraction methods. New extraction methods include supercritical fluid extraction, microwave-assisted extraction, enzyme-assisted extraction, pulsed electric field extraction, ultrasound-assisted extraction, and pressurized liquid extraction. Each type or class of phytochemicals has a higher concentration following purification and isolation processes. The primary purification process requires to use ofHPTLC (high-performance thin-layer chromatography),column chromatography,HPLC (high-performance liquid chromatography), and HSCC (high-speed countercurrent chromatography). The primary methods for detecting bioactive chemicals recovered from mixed fruit waste are UV-visible and mass spectroscopy. Numerous phytochemicals in fruit extracts were identified using preliminary phytochemical, HPTLC, and GC-MS analyses, as reported in chapter 6.

Conclusion

The study found that fruit leftovers have potential applications in various industries, including food processing, antioxidant-rich cosmetics, and herbal drugs. However, they are often discarded as waste after pulp consumption. The mixed fruit powder is an excellent source of nutrients, and the extracts from mixed fruit residues show strong antimicrobial properties. The extracts have potential for use in functional foods and disease prevention. Chitosan-based fruit covering aqueous extract of fruit wastes has been developed to inhibit food-borne microorganisms and extend post-harvest storage time. These coatings show significant antioxidant and antibacterial capabilities, reducing bacterial growth and maintaining color and texture. Biopolymer antimicrobial packaging reduces environmental flow, leakage, and food stability, while using fruit residue waste instead of standard films reduces manufacturing costs and food by-product value.

Appendix

MS of Identified Bioactive elements and struture of selective componenets

Figure 1 MS of Water Extract

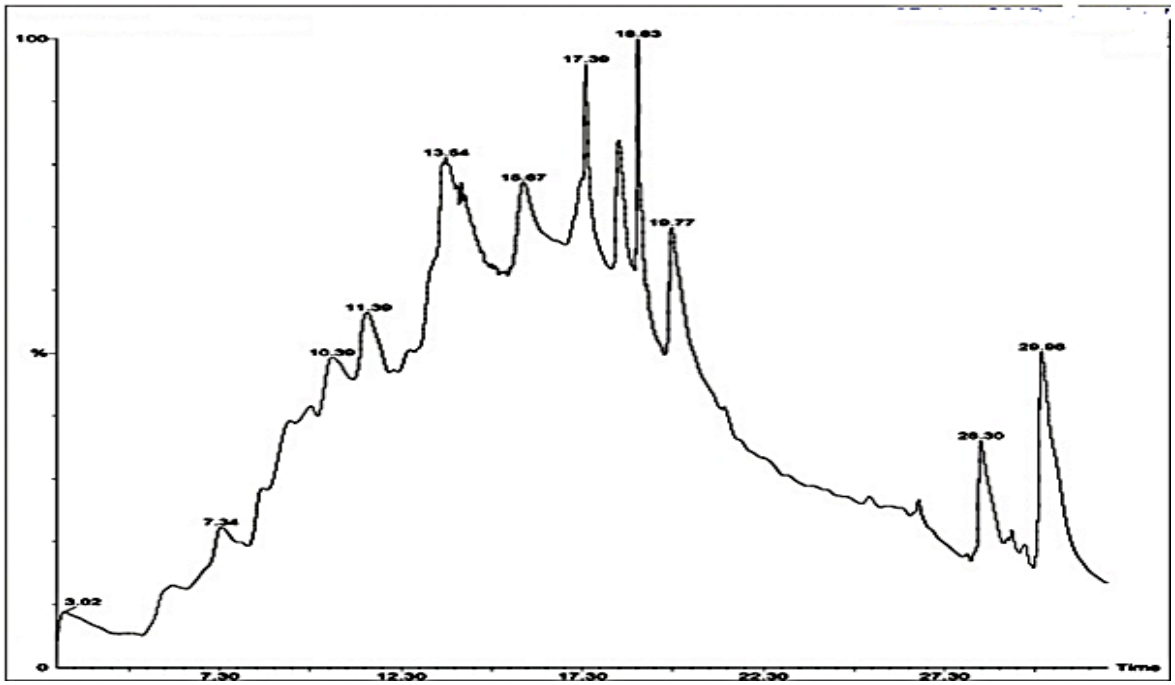
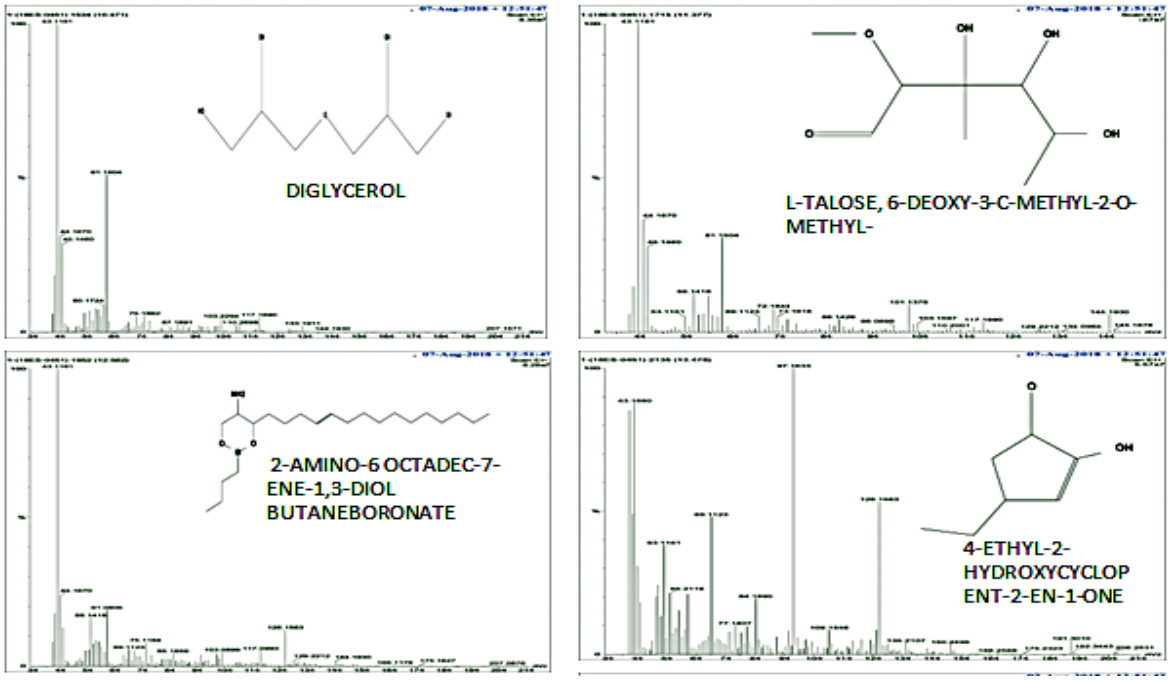


Figure 2 Chemical structure of selective Bioactive elements in aqueous extract



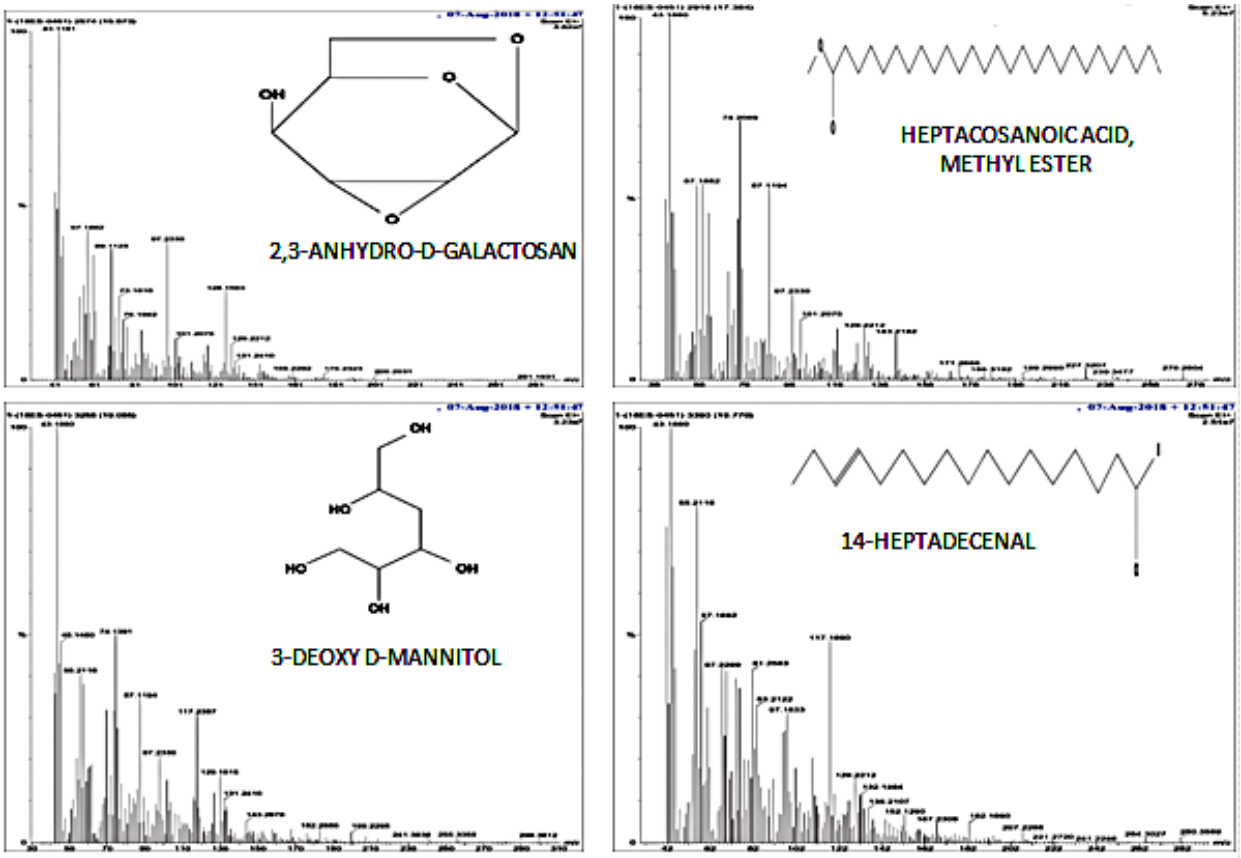


Figure 3 MS of Ethanol Extract

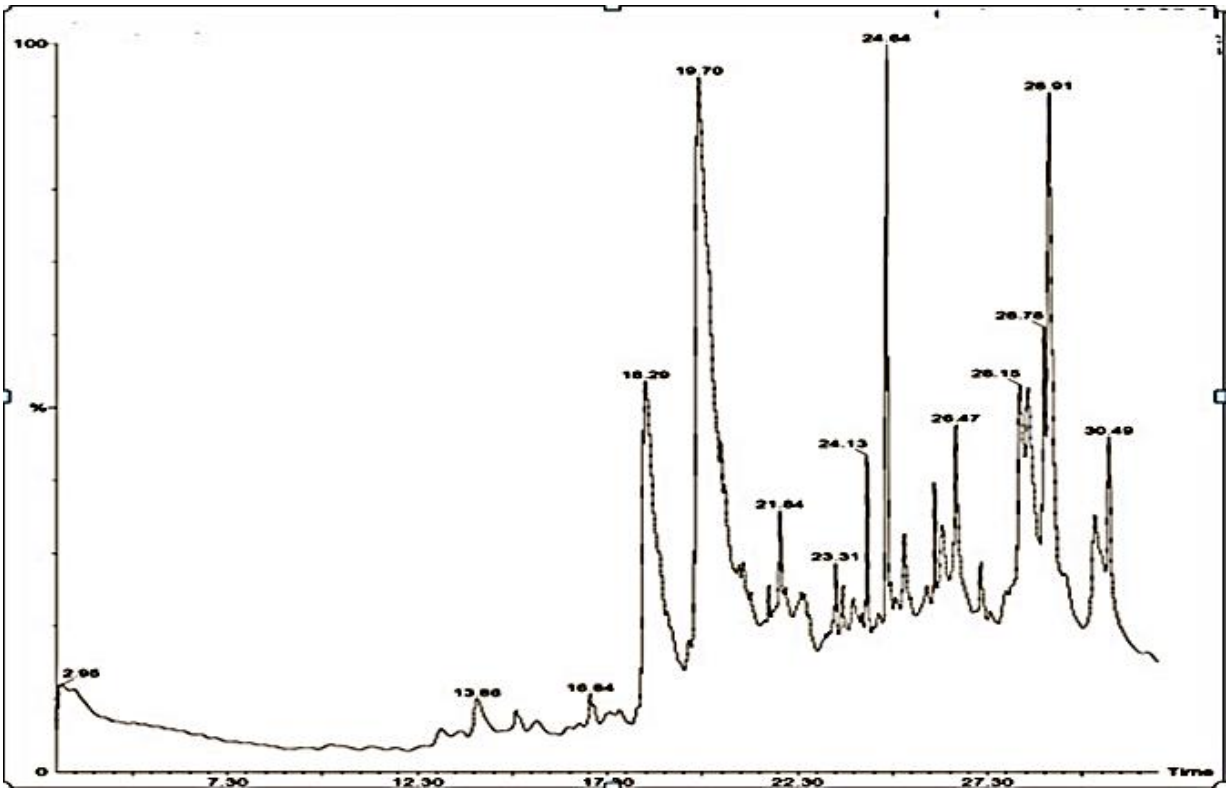


Figure 4 Chemical structure of selective Bioactive elements in ethanolic extract

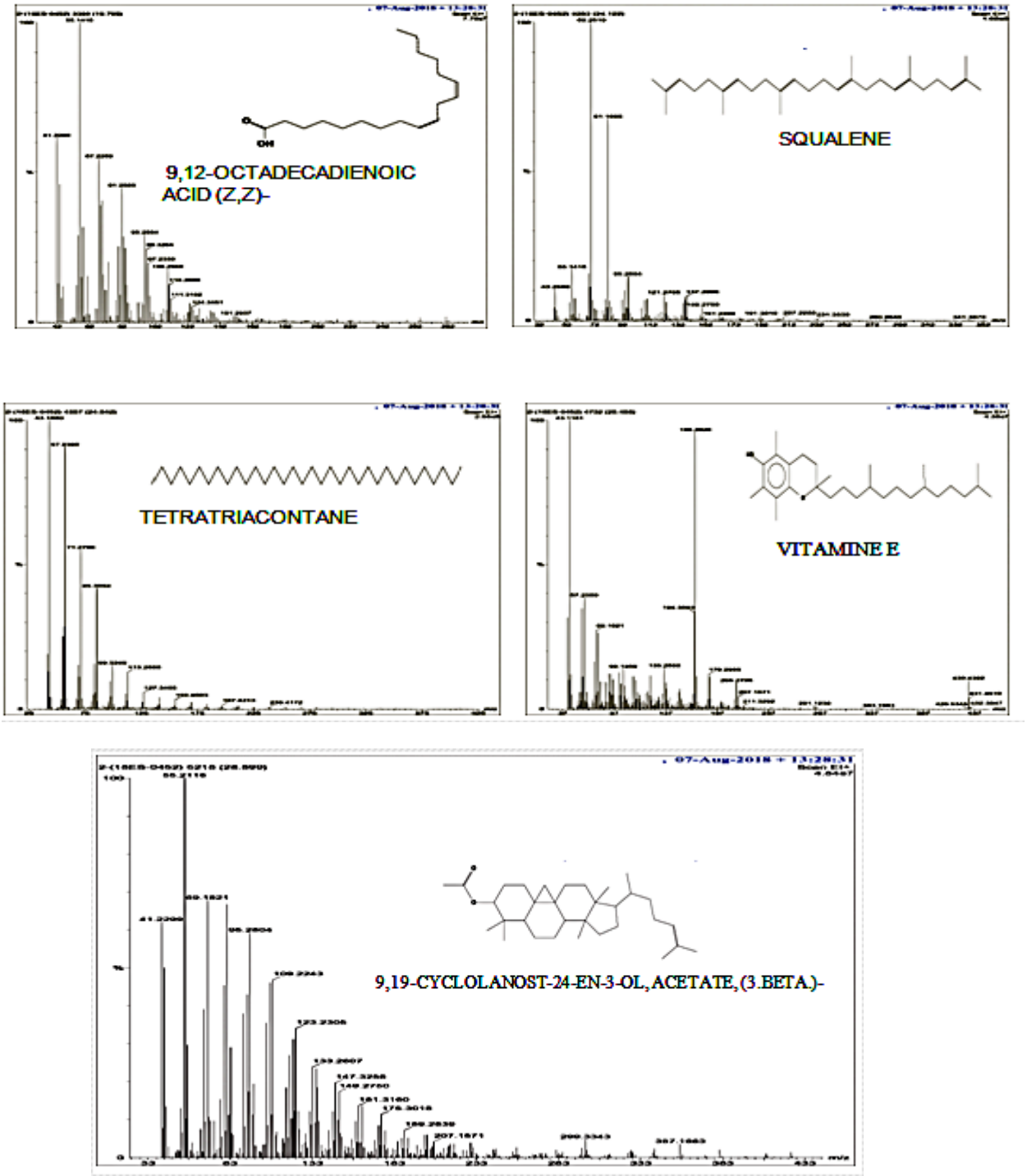


Figure 5 MS of Methanol Extract

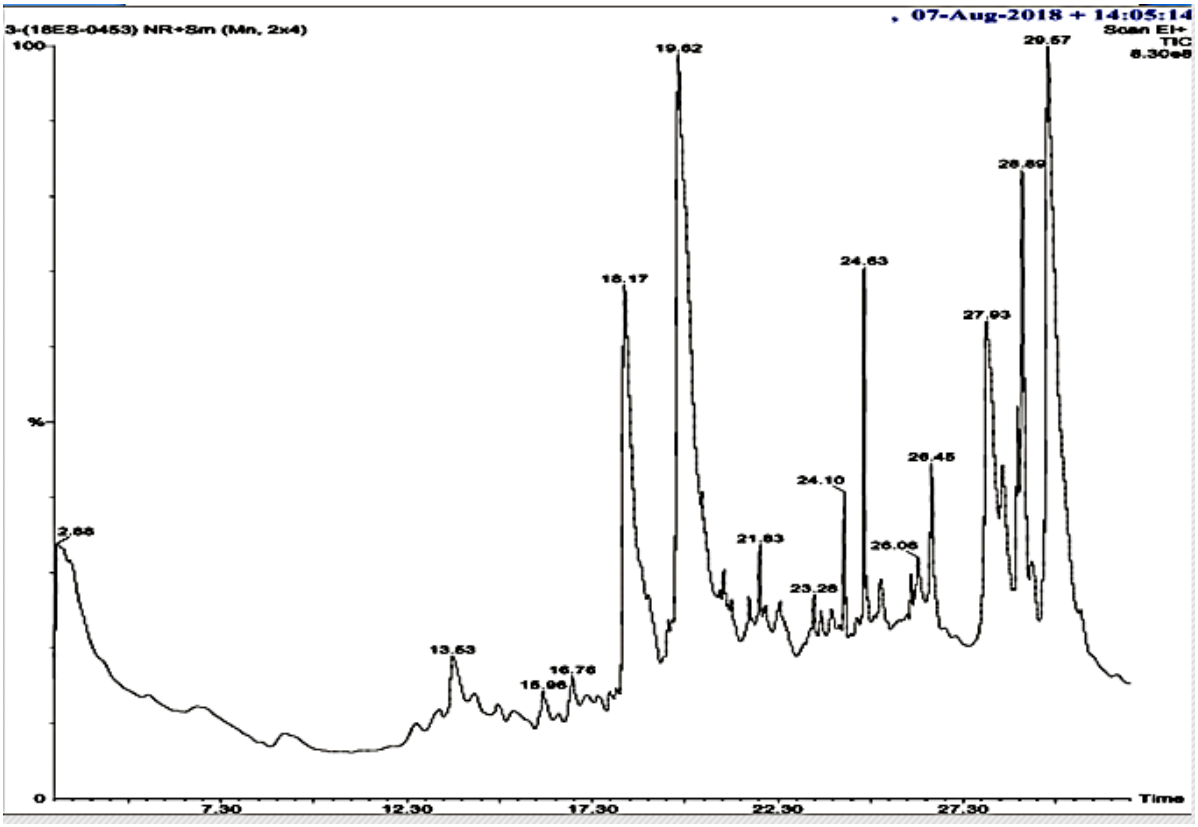
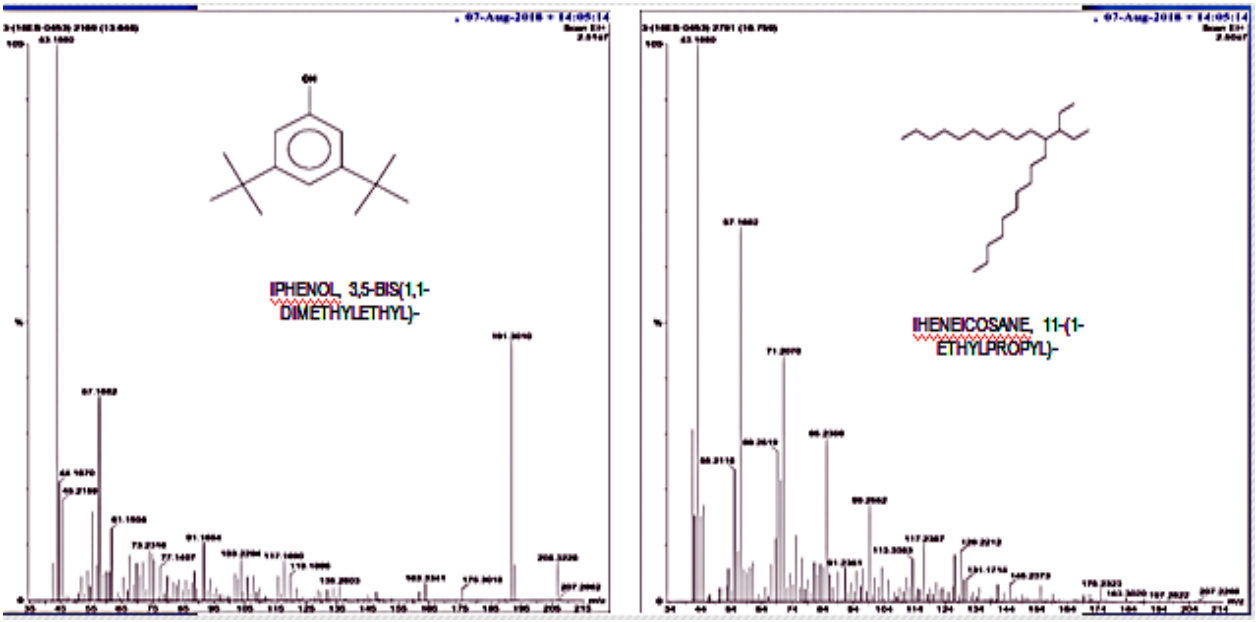


Figure 6 Chemical structure of selective Bioactive elements in methanolic extract



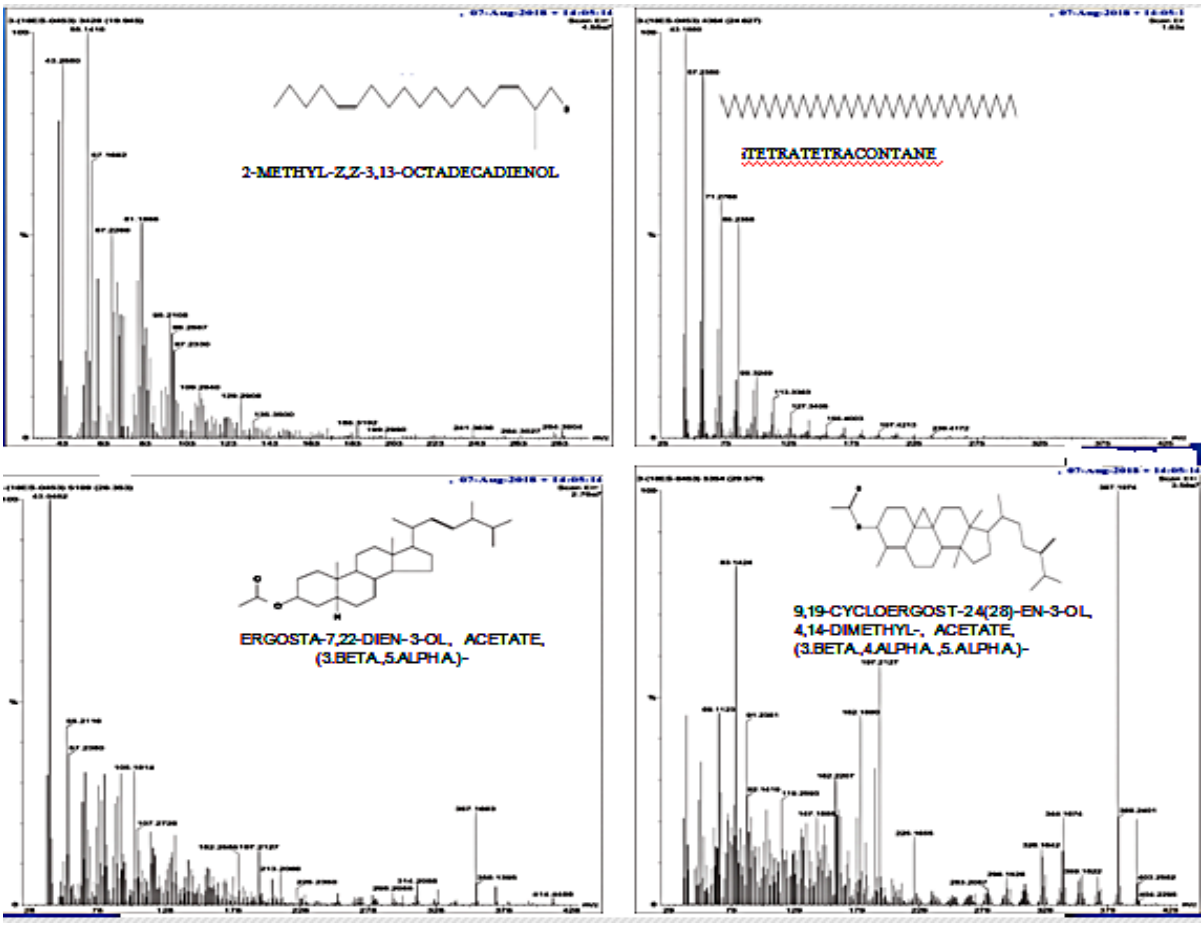


Figure 7 MS of petroleum ether extract

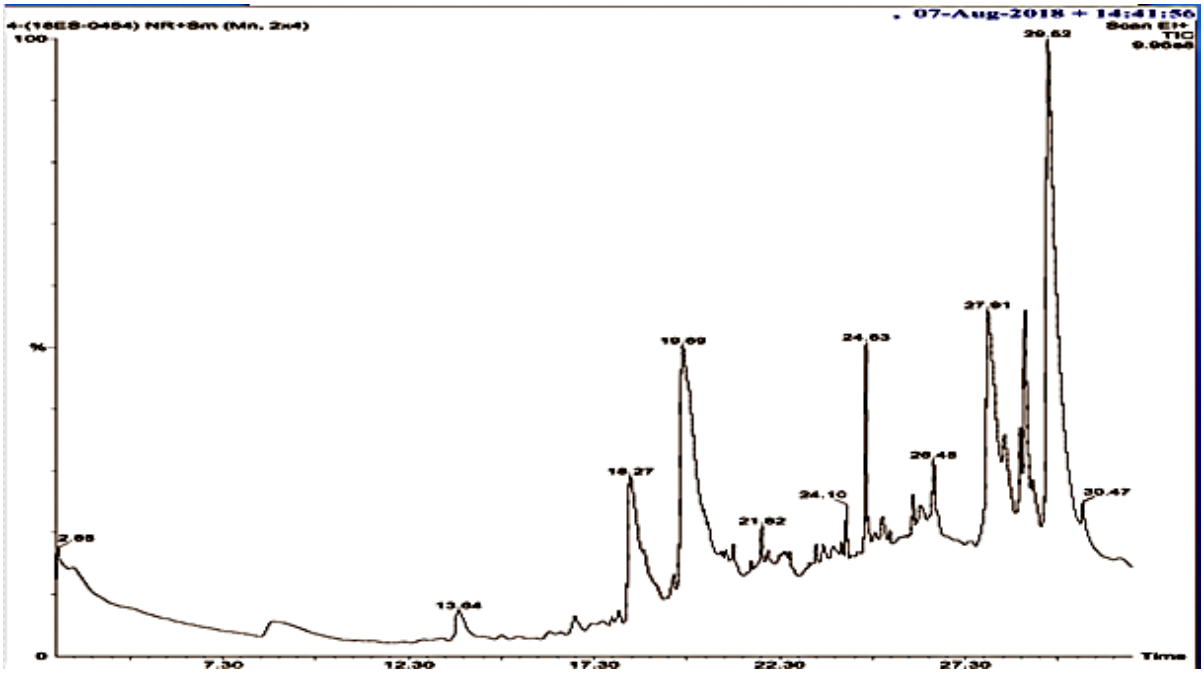


Figure 8 Chemical structure of selective Bioactive elements in petroleum ether extract

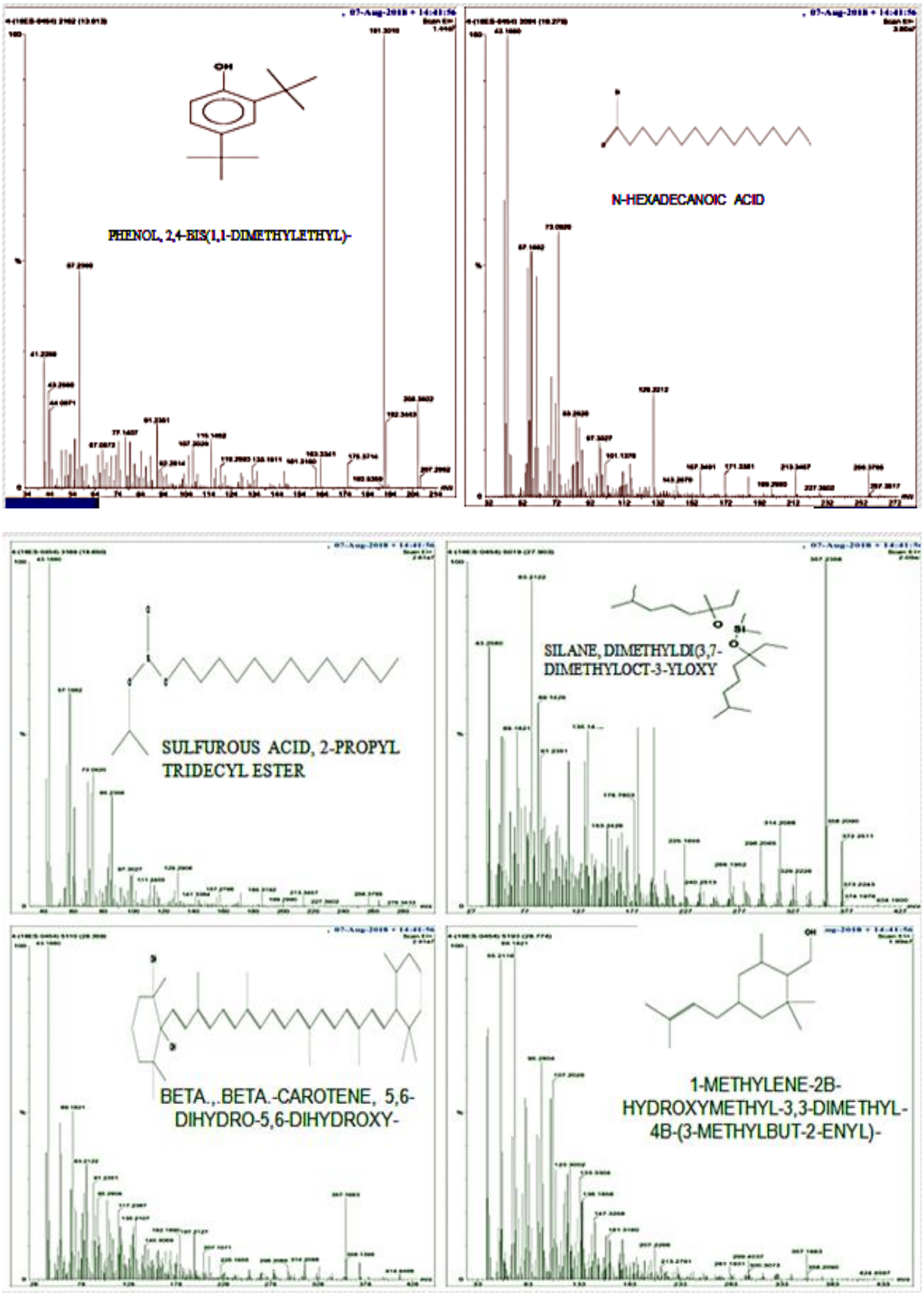
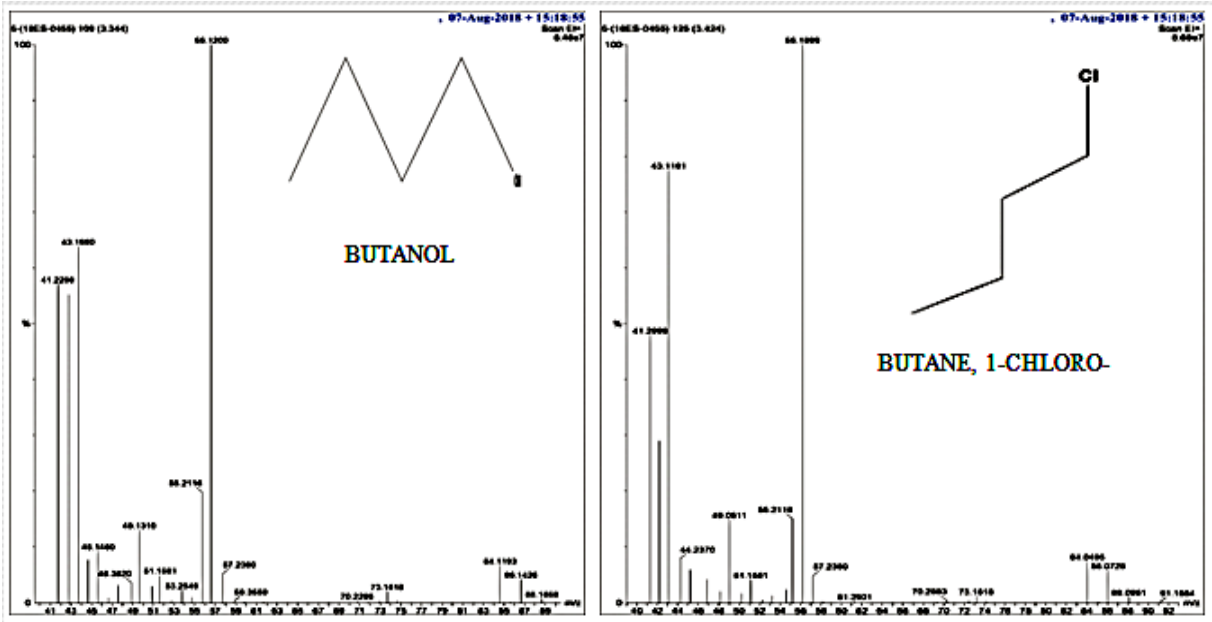
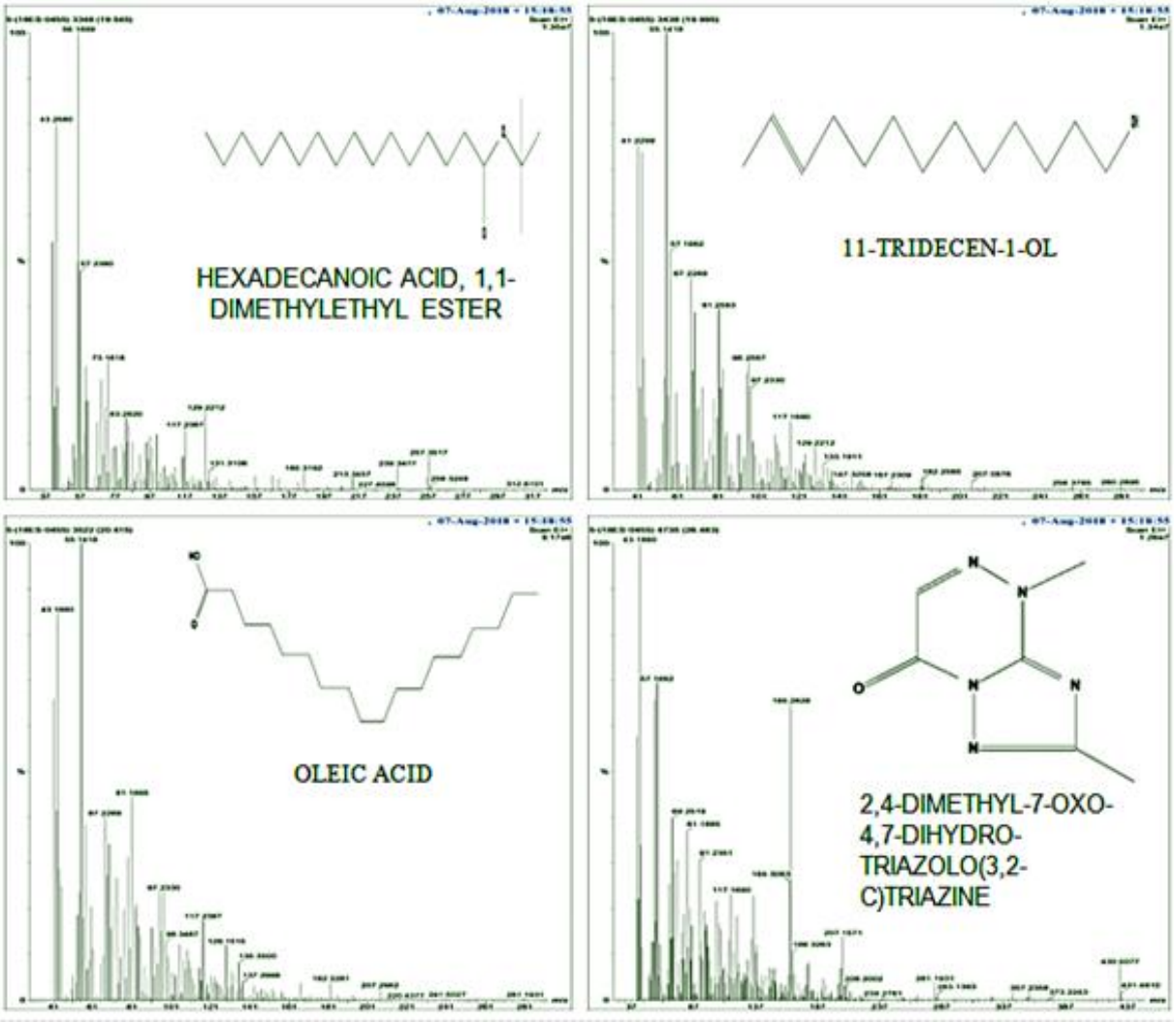


Figure 9 MS of Butanol extract

Figure 10 Chemical structure of selective Bioactive elements in butanolic extract




HEXADECANOIC ACID, 1,1-
DIMETHYLETHYL ESTER
11-TRIDECEN-1-OL
OLEIC ACID
2,4-DIMETHYL-7-OXO-
4,7-DIHYDRO-
TRIAZOLO(3,2-
C)TRIAZINE

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