Green Extraction and Characterization of Selected Bioactives and Essential Oil from Citrus Fruit Peel and Assessment of their Antioxidant Potential

A Thesis Submitted in the Partial Fulfillment for the Degree of Master of Philosophy in Chemistry

By

Quratul Ayn Zahara

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INSTITUTE OF CHEMISTRY
UNIVERSITY OF SARGODHA
SARGODHA, PUNJAB, PAKISTAN.

DEDICATION

I dedicate this thesis to
my beloved parents
&
Teachers
(Who always pray for my success)

ACKNOWLEDGEMENTS

All praises to The Allah Almighty who has created this world of knowledge for us. He is The Gracious, The Merciful. He bestowed man with intellectual power and understanding, and gave him spiritual insight, enabling him to discover his "Self" know his Creator through His wonders, and conquer nature. Next to all His Messenger Hazrat Muhammad (SAW)Who is an eternal torch of guidance and knowledge for whole mankind.

No research is ever the outcome of single individual's talent or efforts. I have seen and experienced the countless blessings showered on me by my parents, teachers and friends.

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Anyone missed in acknowledgement are also thankful. If I didn't mention someone's name here, it doesn't mean I do not acknowledge your support and help. Again thanks to all who have directly or indirectly helped me in the completion of thesis.

QuratulAynZahara

APPROVAL CERTIFICATE

It is to certify that this dissertation titled "*Green Extraction and Characterization of Selected Bioactives and Essential Oil from Citrus Fruit Peel and Assessment of their Antioxidant Potential*" is being submitted by Quratul Ayn Zahara to the Institute of Chemistry, University of Sargodha, Sargodha, Pakistan for the partial requirements of the degree of MASTER OF PHILOSPHY in Chemistry.

Supervisor:

Dr. Farooq Anwar

Professor
Institute of Chemistry
University of Sargodha, Sargodha

Director:

Dr. Farooq Anwar

Professor,
Institute of Chemistry,
University of Sargodha, Sargodha.

Director
Institute of Chemistry
University of Sargodha
Sargodha

DECLARATION

I, Dr. Farooq Anwar, Professor, Institute of Chemistry, University of Sargodha, Sargodha, Pakistan, declare that the work described in this thesis was carried out by Quratul Ayn Zahara under my supervision in the partial fulfillment of the requirements for the degree of "MASTER OF PHILOSPHY in Chemistry". I certify that the main content of this thesis accounts for research has not previously been submitted for a degree at any educational institution. Further, it is submitted that the material taken from other sources has been properly acknowledged.

Supervisor:

Dr. Farooq Anwar

Professor,
Institute of Chemistry,
University of Sargodha, Sargodha.

DECLARATION

I declare that the work described in this thesis was carried out by me under the supervision of Prof. Dr. Farooq Anwar, Professor, Institute of Chemistry, University of Sargodha, Sargodha, Pakistan, in the partial fulfillment of the requirements for the degree of "MASTER OF PHILOSPHY in Chemistry". I certify that the main content of this thesis accounts for my own research and has not previously been submitted for a degree at any educational institution. Further, it is submitted that the material taken from other sources has been properly acknowledged.

Quratul Ayn Zahara

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ABSTRACT

Citrus fruits are well known for their high nutritional value and multiple health benefits. The medicinal benefits and biological properties of citrus fruit and citrus by products are attributed to the existence of important functional /bioactive components (phenolic components, carotenoids and ascorbic acid) along with essential oil. Pakistan is one of the major citrus producing countries of the world. Processing of citrus fruits into different products or their consumption as such produce agro-waste such as peel, seed, and pulp which are usually wasted. The bioactive components, especially recovered from citrus peel can be potentially used for various food and pharmaceutical applications with the perspective of value-addition. Moreover, recently, there is greater focus on the use of some green solvents for isolation of bioactive components from plant materials, especially for uses in food and nutraceutical industry. The present research work has been planned for extraction of selected functional components from peel of different citrus fruits followed by evaluation of their antioxidant potential. Efforts have been made to devise green extraction protocol to extract/isolate functional components by involving the use of green solvents and ultrasound-assisted extraction technique. The bioactive extracts obtained have been evaluated for antioxidant attributes using different antioxidants assays (TPC, TFC, DPPH etc.). Advanced spectroscopic and chromatographic techniques have been used for characterization of functional components in the extracts and volatile oils isolated. The results of this study indicated that peels of *Citrus* species are rich source of phenolics, flavonoids and possess antioxidant potential making them suitable enough to be used in nutraceuticals as a source of natural antioxidants.

CHAPTER 1

INTRODUCTION

1.1 Phytochemicals/Metabolites:

Medicinal plants are obtained from a variety of plant groups and used as plant extracts, essential oils, or a combination of both [1]. Plant extracts such as bark, stem, root, flowers, fruits, rhizomes, and stem plants contain antibacterial and antifungal bioactive chemicals [2]. The active principle responsible for the biological activities of medicinal plants is more than one chemical [3].

Phytochemicals are chemical compounds created by plants [4], which are used by insects and other animals to help them withstand fungus, bacteria, and plant virus diseases. Phytochemicals are chemical molecules produced by plants that aid in the fight against fungal, bacteria, and virus inflammation, as well as protecting insects and other animals from weariness. The name comes from the Greek word phyton, which means "plant," and is supposed to provide health advantages [6]. Phytochemicals are plant-derived compounds [7].

1.1.1 Phytochemicals as a source of antioxidant agents:

Free radicals (atoms or molecules with an unpaired electron in their atomic orbital) are extremely reactive and unstable molecules that degrade the overall quality of food and cause a variety of unfavorable changes in the nutritional composition of foods. Free radicals such as superoxide anion, oxygen singlet, hydroxyl radical, hydrogen peroxide, nitric oxide radical, and peroxynitrite radical cause damage to biomolecules such as DNA, proteins, carbohydrates, and lipids. Despite the fact that free radicals play a crucial role in cellular homeostasis, an imbalance between free radical production and the cell's antioxidant defense system causes oxidative stress, which is responsible for biomolecule damage. The reactive oxygen species works as a signaling agent at low levels, but it becomes poisonous at high levels. Lipids, nucleic acids, and proteins are more commonly the principal targets of free radicals in the food system. Rancidity is a very prevalent oxidation of lipid biomolecules that has a major negative impact on nutritional quality, food flavor, and overall food quality, rendering foods unappealing. According to a recent study, lipid peroxidation products may

have significant health effects, including carcinogenic effects [8]. As a result, the usage of antioxidants, whether natural or synthetic, is recommended to avoid free radical damage.

An **antioxidant** is a chemical that slows down the oxidation process or neutralizes free radicals at lower concentrations than the substrate, decreasing the harmful effects. Antioxidants can interact with free radicals and stop the chain reaction that causes the key cellular organ to be damaged. In the food system, antioxidants such as vitamin E (a-tocopherol), vitamin C (ascorbic acid), b-carotene, butylhydroxyanisole (BHA), butylhydroxytoluene (BHT), propyl gallate (PG), and tertbutylated hydroquinone (TBHQ) are currently employed. Synthetic antioxidants such as BHA, BHT, and PG, on the other hand, have been reported to be poisonous, carcinogenic, and have low solubility [9, 10, 11, 12]. As a result, industries are exploring for natural antioxidants to replace synthetic antioxidants. Phytochemicals with antioxidant capacity found in food are of great interest because they protect against oxidative degradation and have a positive impact on human health [13]. Regular eating of fruits, vegetables, and whole grains, according to epidemiological and animal research, lowers the risk of chronic diseases linked to oxidative damage [14, 15]. Flavonoids, carotenoids, phenolic acids, alcohols, tocopherols, lignans, stilbenes, tannins, and ascorbic acid, which are traditionally used plant-based bioactive compounds (nonnutritive constituents usually occur in very small quantities), could be a preferred alternative to synthetic antioxidants that quench reactive oxygen species (ROS).

1.1.2 Types of metabolites:

Plant metabolism is divided into primary and secondary metabolic types. Vegetables include a wide range of organic substances as a result of primary and secondary plant metabolism. Carbohydrates, amino acids, fatty acids, and organic acids are primary metabolites that play a role in growth and development, respiration and photosynthesis, and hormone and protein synthesis. **Primary metabolites** are created by the same (or nearly the same) metabolic processes in all organisms belonging to major evolutionary groupings. Flavonoids, carotenoids, sterols, phenolic acids, alkaloids, and glucosinolates are **secondary metabolites** that affect the color of vegetables, defend plants from herbivores and pathogens, attract pollinators and seed-dispersing animals, and act as stress signal molecules [18, 19].

1.1.2.1 Phenolics and polyphenolic compounds:

Phenolic compounds are one of the several phytochemical classes with health-promoting properties and functions. Secondary metabolites, phenolic compounds are widely dispersed in the kingdom of plants and have complex structures and functions. It is thought to be the most important and abundant class of chemicals in the kingdom of plants [20, 21, 22].

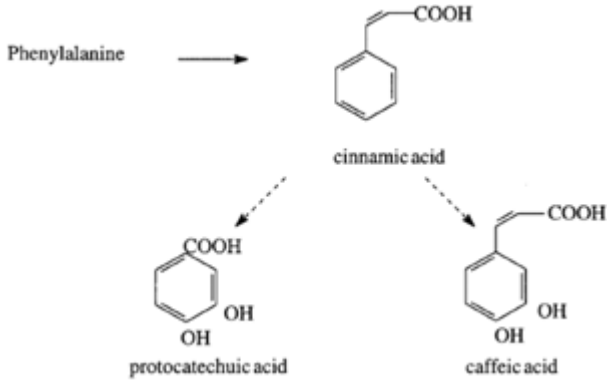
Water-soluble compounds (phenolic acids, flavonoids, phenylpropanoids, and quinones) and water-insoluble compounds (phenolic acids, flavonoids, phenylpropanoids, and quinones) are the two types of phenolic compounds (condensed tannins, lignins, and cell-wall bound hydroxycinnamic acids). Because its solubility and digestibility are most important for successful consumption within the gastrointestinal system and various physiological operations, this classification is crucial due to the nutritional content or ingredients. When insoluble phenolic compounds are unable to be digested, they are excreted entirely or partially in the feces, whereas soluble compounds can be taken into the circulation as metabolites through the intestines. Phenolic acids (hydroxybenzoic acids and hydroxycinnamic acids), flavonoids (flavones, flavanols, flavanones, and isoflavones), tannins, stilbenes, and lignans are some of the subtypes [23, 24, 25, 26, 27].

Secondary plant metabolites, such as phenolic chemicals, are found in practically all plant materials, including plant-based foods. These chemicals are believed to be present in both human and animal diets [28]. The bulk of natural antioxidants are phenolic compounds, with tocopherols, flavonoids, and phenolic acids being the most prominent classes.

1.1.2.2 Phenolic acids:

Phenolic acids can be found in practically all plants and plant-derived foods, and they make up a large part of the human diet. Depending on preferences and dietary habits, the average phenolic acid consumption in the human diet has been reported to be in the range of 200 mg/day [29]. The recent focus on phenolic acids derives from their potential preventive effect against oxidative damage disorders such as coronary heart disease, stroke, and malignancies [30] and antiglaucoma [31, 32, 33, 34, 35] through the ingestion of fruits and vegetables. In both free and bound forms, phenolic acids account for roughly 30% of the dietary phenolic

contained in plants [36]. The latter is more common, and it can be found as esters, glycosides, and insoluble-bound complexes [37]. Phenolic acids are hydroxy derivatives of aromatic carboxylic acids that originate from the benzoic or cinnamic acid groups. P-hydroxybenzoic acid, 3,4-dihydroxybenzoic acid, vanillic acid, syringic acid, p-coumaric acid, caffeic acid, ferulic acid, sinapic acid, chlorogenic acid, and rosmarinic acid are among the many phenolic acids found in plants [32]. Rosmarinic acid, an ester of caffeic acid and 3,4-dihydroxyphenyl lactic acid, was found to be a powerful inhibitor of several metabolic enzymes, including carbonic anhydrase, glutathione S-transferase, lactoperoxidase, acetylcholinesterase, and butyrylcholinesterase [38, 39]. Cinnamic acid derivatives are more active antioxidants than benzoic acid derivatives [40].

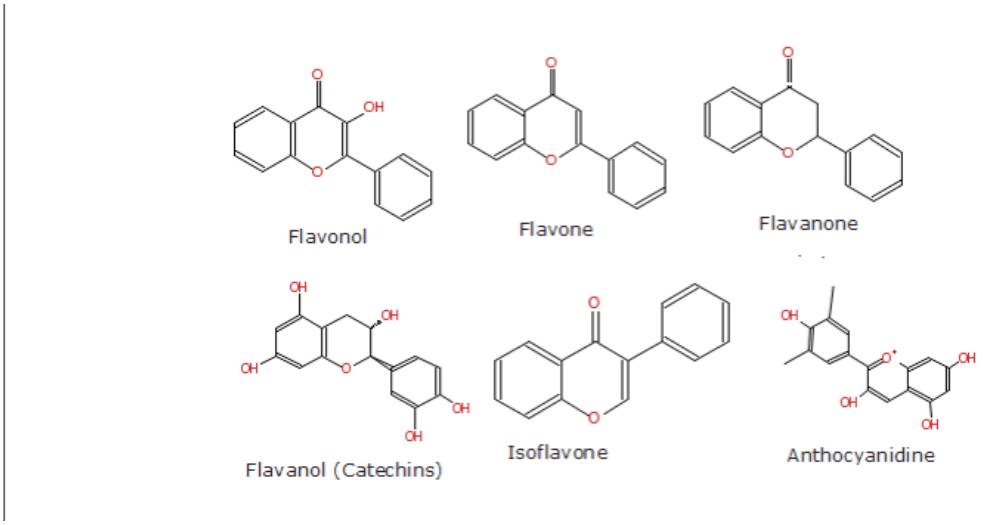


Possible biosynthetic formation of the phenolic acids. [41]

1.1.2.3 Flavonoids:

Flavonoids are found in all types of higher plant tissues, including flavones, flavonols, isoflavones, flavonones, and chalcones [42]. Flavones and flavonols can be found in practically every plant, especially in the leaves and petals, with flavonols being more common than flavones. Apigenin, chrysin, luteolin, datiscetin, myricetin, quercetin, kaemferol, and morin are some of the most common flavonoids. In addition, the majority of flavonoids found in plants are glycosides [43]. Flavonoids' potential to reduce lipid peroxidation in natural lipid products and model lipids is extensively documented and well understood [44]. Flavonoids have been shown to act as antioxidants by scavenging radicals such lipid peroxyl radicals [45], superoxide anion radicals [46], and hydroxyl radicals [47], as well as singlet oxygen quenchers [48] and metal ion chelators [49]. A flavonoid molecule must match the following characteristics for maximum radical scavenging activity: 3′,4′-

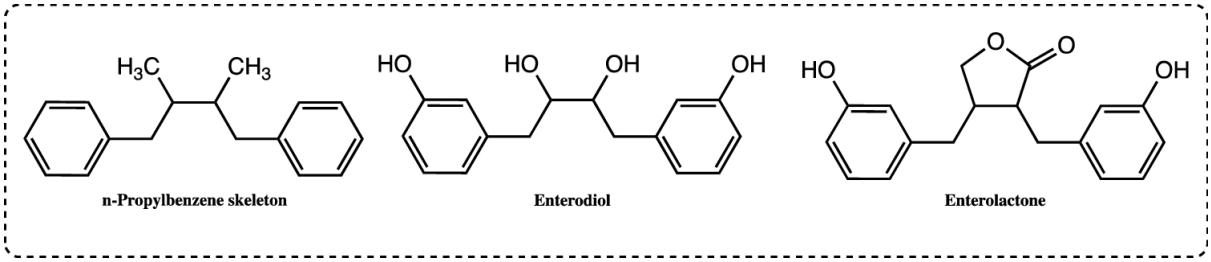
dihydroxy structure in the B-ring, 2,3-double bond in conjunction with a 4-oxo group in the C-ring, presence of a 3-hydroxyl group in the C-ring, and a 5-hydroxyl group in the A-ring [50]. Free hydroxyl groups in flavonoids operate as free radical scavengers, and many hydroxyl groups, especially in the B-ring, increase their antioxidant activity. The key active sites in disrupting the oxidation chain are the hydroxyls in ring B [51].



Flavonoids of various types [52].

1.1.2.4 Lignans:

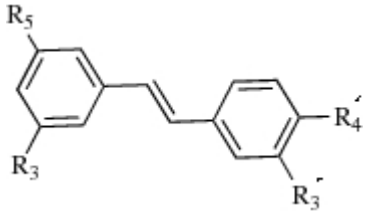
Lignans are a vast group of naturally occurring polyphenols that are generated from the shikimic acid biosynthetic pathway. They are widespread in the plant kingdom and human dietary sources, and they have a wide range of biological roles. Lignans can be found in a variety of plant parts, including roots, stems, rhizomes, leaves, fruits, flowers, seeds, resins, and xylem. Until yet, lignans have been discovered in over 70 plant families, with over 100 neolignans and 200 classical lignans identified. Cinnamic acid, propenyl benzene, allyl benzene, and cinnamyl alcohol are the monomers that make up lignans. They have a basic scaffold of two or more phenylpropanoid units, and the monomers that make up lignans are cinnamic acid, propenyl benzene, allyl benzene, and cinnamyl alcohol. Lignans have a wide range of structural characteristics. Almost two thousand lignans have been identified thus far [53].



Enterodiol and enterolactone are two mammalian lignans [54].

1.1.2.5 Stilbenes:

Stilbenes are phenylpropanoids with a 1,2-diphenylethylene backbone that belong to a small category of phenylpropanoids. They are a group of secondary metabolites formed by substituting a phenyl on both carbon atoms of a cis- or trans-ethene double bond with a phenyl on both carbon atoms of the double bond in either the phenylpropanoid or polyketide pathways. One of the components of such a system could be naturally occurring nucleosides modified by stilbene derivatives [55]. Stilbenes, particularly trans-resveratrol and its glycoside, are good for your health because they're antioxidative, anticarcinogenic, and anticancer [56]. The majority of stilbenes and derivatives are found in specific plant families. Grape, pine, peanut, and sorghum are some examples of common stilbenes isolated from various plant families. Plant stilbenes have attracted a lot of attention in recent years due to their biological activity and potential pharmaceutical applications. Since resveratrol is thought to play a role in health benefits. It has been one of the most thoroughly researched natural products [57].



The structure of stilbenes in general [54].

1.2 Extraction of bioactive compounds:

Extraction is the process of separating desired components from plant materials by employing various solvents. Filtration is used to separate soluble plant material from insoluble plant

material during extraction. Two factors influence the recovery of bioactive components from plant material:

1) Extraction solvents

2) Extraction techniques

1.2.1 Extraction Solvents:

We used green solvents such as methanol, ethanol, and their aqueous mixes to extract bioactive components. Because of the following reasons, green solvents are preferred:

a) These supercritical solvents are often regarded as safe, biodegradable, nontoxic, and environmentally benign. For the nutraceutical industry, green solvents are excellent for the separation of bioactive components [58].

b) These solvents are polar in nature, meaning that while most of the plant's polar bioactive components are soluble, non-polar components are only moderately soluble.

1.2.2 Methods of Extraction:

Given the wide range of bioactive chemicals and the large number of plant species, a standard and integrated approach to identifying these compounds with human health benefits is required [59]. An integrated strategy was published in a study that showed the process of medicinal plant study, which began with name collection of commonly used herbs and finished with industrialisation [60].

Only after further separation, identification, and characterization of bioactive chemicals, as well as a suitable extraction procedure, is it viable to proceed. To understand the extraction selectivity from various natural sources, different extraction procedures should be performed in varied settings. Various procedures, many of which have remained virtually unchanged for hundreds of years, can be employed to extract bioactive chemicals. All of these techniques have the same goals: (a) extracting targeted bioactive compounds from complex plant samples, (b) increasing analytical method selectivity, (c) increasing bioassay sensitivity by

increasing the concentration of targeted compounds, (d) converting bioactive compounds into a more suitable form for detection and separation, and (e) providing a strong and repeatable signal.

1.2.2.1 Conventional extraction techniques:

Various traditional extraction procedures can be used to extract bioactive chemicals from plant sources. The majority of these methods rely on the extraction capacity of the various solvents in use, as well as the use of heat and/or mixing. The established conventional procedures for extracting bioactive chemicals from plants are: (1) Soxhlet extraction, (2) Maceration, and (3) Hydrodistillation [59].

1.2.2.1.1 Soxhlet:

Franz Ritter Von Soxhlet, a German chemist, was the first to propose the Soxhlet extractor (1879). It was created primarily for the extraction of lipids, although it is no longer confined to that. The Soxhlet extraction method has been widely utilized to extract important bioactive chemicals from a wide range of natural sources. It's used as a benchmark for comparing new extraction methods. In most cases, a thimble is used to hold a little amount of dry sample. After that, the thimble is placed in a distillation flask containing the solvent of interest. The thimble-holder solution is sucked by a siphon once it reaches an overflow level. The solution is siphoned back into the distillation flask. The extracted solutes are carried into the bulk liquid by this solution. The solute is retained in the distillation flask, and the solvent is returned to the plant's solid bed. Until the extraction is complete, the operation is repeated [62].

1.2.2.1.2 Maceration:

Maceration has long been utilized in the production of tonics at home. It quickly became a popular and cost-effective method of obtaining essential oils and bioactive chemicals. Maceration is usually done in numerous steps for small-scale extraction. To begin, plant materials are ground into minute particles to enhance surface area for optimal solvent mixing. Second, a suitable solvent known as menstruum is added to a closed vessel during the maceration phase. Finally, the liquid is strained out, but the marc, which is the solid residue

left over from the extraction process, is pressed to remove a considerable number of occluded solutions. Filtration is used to separate contaminants from the obtained strained and push out liquid. Occasional shaking in maceration aids extraction in two ways: (a) it increases diffusion, and (b) it removes concentrated solution from the sample surface, allowing additional solvent to reach the menstruum, resulting in a higher extraction yield [59].

1.2.2.1.3 Hydrodistillation:

Hydrodistillation is a method for extracting bioactive chemicals and essential oils from plants that has been used for centuries. It does not require the use of organic solvents and can be done prior to the dehydration of plant materials. Water distillation, water and steam distillation, and direct steam distillation are the three methods of hydrodistillation [63]. First, the plant materials are packed in a still compartment; then, sufficient water is added and the process is brought to a boil. Direct steam is injected into the plant sample as an alternative. The key influencing elements for free bioactive chemicals in plant tissue are hot water and steam. The vapor mixture of water and oil condenses due to indirect cooling with water. The condensed mixture goes from the condenser to a separator, which separates the oil and bioactive chemicals from the water automatically [64]. Hydrodistillation is made up of three physicochemical processes: diffusion, hydrolysis, and heat decomposition. Some volatile components may be lost at high extraction temperatures. Because of this limitation, it can only be used to extract thermally labile compounds.

1.2.2.2 Non-conventional extraction techniques:

Novel extraction approaches have been developed to address some of the drawbacks of traditional extraction methods [65]. The food sector is interested in helped new extraction techniques such as ultrasound-assisted [66, 67], pulsed electric field assisted [68], enzyme-assisted [69, 70], microwave-assisted [71], supercritical fluid [72], and pressurized liquid [73]. Many studies have found that combining innovative extraction approaches can be effective at extracting data quickly and efficiently [66, 74, 75]. Ultrasound-assisted extraction (UAE), enzyme-assisted extraction (EAE), and microwave-assisted extraction (MAE) are the most regularly described and widely used new extraction methods for bioactive chemical extraction.

1.2.2.2.1 Ultrasound-assisted extraction (UAE):

In recent decades, the uses of UAE technology as a clean, green, and cost-effective alternative to conventional techniques have been extensively researched [66, 75].

Ultrasound propagates through any media by causing cycles of expansions and compressions as a mechanical wave with a frequency ranging from 20 kHz to 100 MHz. When the acoustic power is high enough, it can induce the development, growth, and collapse/implosion of cavitation bubbles in a liquid media, which has been widely used in improving numerous food processes. The phenomena referred to above is known as 'acoustic cavitation,' and it is the driving force behind ultrasonic extraction enhancement. Cavitation-induced high shear forces result in increased mass transfer of target components through turbulent mixing and acoustic streaming [76, 59]. Micro-turbulence, severe agitation of the extractant, and inter-particle collisions of sample particles in the extractant can all arise from cavitation bubbles collapsing [77]. At the same time, the implosion of cavitation bubbles at liquid–solid interfaces could result in matrix particle breakup and surface erosion by microjets and high-power ultrasonic shockwaves [78, 66].

1.2.2.2.2 Enzyme assisted extraction (EAE):

Based on the inherent ability (specificity and regioselectivity) of enzymes, EAE, enzyme or enzyme combination preparations were frequently used to catalyze hydrolysis of components resistant to mass transfer like cell wall and/or binding to the target components like pectin in the material matrix. By degrading and disturbing the structural integrity of cell walls and membranes, particular enzymes such as pectinases, cellulases, and hemicellulases can improve target drug recovery during extraction [79, 80]. To effectively use enzymes for EAE, it is necessary to first understand their catalytic specificity and mechanism of action, as well as explore optimal conditions and which enzyme or enzyme combination is best for the raw materials. Before selecting a good enzyme or enzyme combination, it is also preferable to know the cell wall composition of the sample matrix [81].

1.2.2.2.3 Microwave-assisted extraction (MAE):

Microwave is an electromagnetic wave that is used for industrial and home heating at two frequencies (915 MHz and most commonly 2450 MHz). MAE is primarily caused by the heating impact of microwaves, which results in a greater extraction temperature and, as a result, a faster mass transfer rate [82]. Microwave can also produce direct heating/bulk heating inside the solvent body and sample matrix due to microwave penetration to a certain depth and contact with the polar components [59]. Direct and bulk heating cut down on the time it takes to heat up the solvent and samples, which is especially beneficial for large-scale extractors. The heating effect of microwave in a solvent-sample mixture is determined by their dielectric characteristics, which are influenced by temperature, microwave frequency, solvent composition, and even the samples in the solvent [83]. Furthermore, direct heating penetrates the matrix during MAE, increasing local temperature and pressure, which can push target components from the matrix into the solvent body. This can improve solvent penetration into the sample matrix and target component transfer from the matrix to the solvent [84, 59].

1.3 Essential oils:

Essential oils (EOs) are liquid combinations of volatile substances extracted from aromatic plants, most generally through steam distillation. They constitute what is termed the "essence" of a plant and typically have pleasantly perfumed odours. Aromatic plants and EOs have been employed for millennia for their beneficial properties, well recorded in ancient literature [85]. Recent scientific research has supported some of the claimed health characteristics, including antiseptic, antioxidant, and anti-inflammatory capabilities. [86,87]. Hundreds of chemicals (secondary metabolites) with low boiling points have been detected in Essential oils, and the chemical variability of their constituents affect EO oxidative stability.

1.3.1 Biological uses of essential oils:

Numerous studies are done in the field of essential oils and their role in treatment of a variety of infectious diseases. Plants medicinal properties are widely used as bioactive compounds [88]. Eessential oils were used for diagnoses of numerous types of disease all over the world

in old times. In current day and age, the role of essential oils is expanding as they are widely used by researchers in food industries, as well as the aesthetics and smell industries, to generate valuable aromas and with a variety of biological activities [89].

Comprehensive examinations of essential oils revealed that they fungicidal activity and phytochemicals. The essential oils do not exhibit properties, in vivo and in vitro studies are need of time to combat pests, and most of the oils have strong antioxidant effects [90]. Eos with high antioxidant potential help defend lipids and also act as hepatoprotective negotiators in mammals. Because oxygen is a toxic element causing change in metabolic functions while being in most reactive forms such as superoxide and free radicals, antioxidant substances are crucial for human health. [91]. Essential oils are best known for their antibiotic and other therapeutic properties, dynamic essential oil composition are exhibited resulting in a variety of activities based primarily on the chemo groups [92].

1.3.2 Sources and isolation of essential oils

These plants are distributed in different regions. Natives employ them to cure disorders making them important for research. Plant yields essential oil. Essential oils are commonly isolated from plant parts, including flowers, leaves and stems and barks [93].

Essential oils extracted from a variety of fragrant plants are accumulated in the various parts of plant [94].The essential oils were isolated plant components. Diffusion via plant tissues regulates the extraction rate. The extraction of essential oil is dependent on the stability of EO. For essential oil extraction, methods have been employed like steam distillation and hydro distillation, which are considered appropriate extraction technologies [95]. Other novel techniques were also investigated [96]. The essential oil extracted by steam is extensively employed in pharmaceutical activities but the essential oil used in the fragrance or perfume industry is extracted using supercritical carbon dioxide [97].

1.4 Citrus Fruits:

Citrus fruits, wildly cultivated fruits, contain valuable bioactives. [98, 99]. They are members of the Rutaceae family and has large amount of species (40 different species), distributed in different regions of the world. Many unique citrus cultivars have been developed. The main citrus crops are oranges, grapefruits, lemons, mandarins, and limes, which have been highly nutritive value [99]. According to the 2016 statistical report, the global total citrus production

was 124.24 million tons. Asian countries are among the ten largest citrus-producing countries [100]. Citrus fruits are eaten fresh or in juice all over the world.

1.4.1 Structure of citrus fruit

The fruit structure consists of a peel, thin tissue (flavedo) containing various oil sacks loaded with scented essential oil of tremendous economic interest [101]. The figure represents a schematic description of the structural makeup of citrus. The flavedo is followed by layer of parenchyma cells called albedo.. The fruit's pulp has segments (locules) separated by a epidermal membrane and carrying vesicles and seeds [102]. The core of the fruit has tissue analogous to albedo. The inner and segment layers are termed together as the "rag." [103].

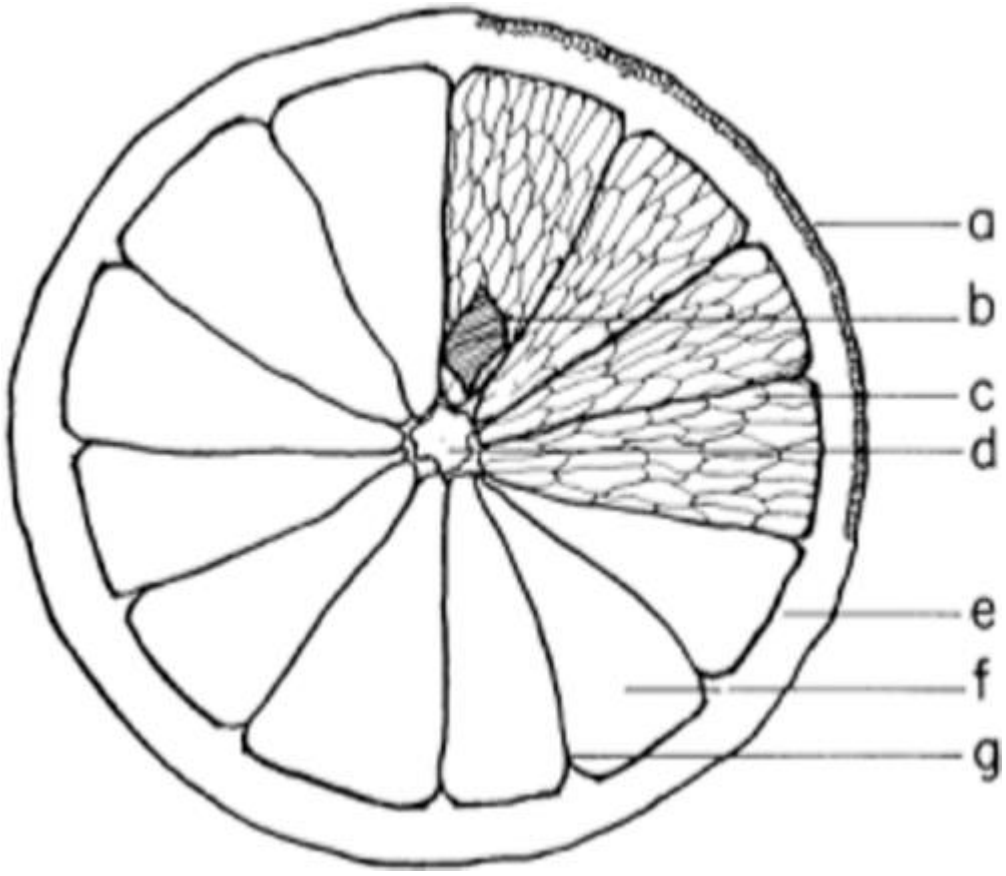


Figure: Schematic view of the cross section of an orange: (a) oil sacs in flavedo; (b) seed; (c) juice sacs; (d) center core; (e) albedo; (f) segment; (g) segment membrane [104].

Nutritional value of citrus fruit:

Source of vitamin C, citrus fruit like most other whole foods contain essential nutrients and a variety of phytoconstituents. Furthermore, citrus has no fat and cholesterol because this is plant food. Fresh citrus has a suitable average energy value which removes concerns about putting on weight. A medium orange, for example, provides 70-80 kilocalories. while a tablespoon of lemon juice only has 5 kilocalories [105].

1.4.2 Bioactive Compounds/phytochemicals in citrus fruits:

Plant-derived bioactives have physiological effects of protection against various disorders. The quantity of identified phytochemicals is expanding with current understanding of their role [106]. Several classes of phytochemicals and their anticarcinogenic actions may include antioxidant capacity and impacts on cell differentiation that detoxify carcinogens, and altered colonic milieu. The only approach to have a wide variety of phytochemicals daily is to consume plant-based foods like citrus as part of a balanced diet.

The combination of essential organic acids, polysaccharides, and proteins is the principal cause of the citrus fruits' popularity. Fruit high in vitamins, phenolic compounds and phytochemicals such as limonoids have significant health advantages. Flavonoids especially (PMFs) present in citrus fruits are antioxidant [107] and anti-cancer [108], resulting in considerable amount of recent interest.

Citrus carotenoids and hydroxycinnamic acid have been isolated. There is substantial proof that fruit exhibits antioxidant capabilities and no cholesterol could be associated with bone and immune system function. [109, 110].

1.4.3 Classification:

Citrus species are in thousands because of hybridization and therefore, the characterization of citrus is complex because of varieties. Citrus can be grouped into five broad types that are economically valuable.

1.4.3.1 Sweet oranges (*Citrus sinensis* Osbeck):

Sweet oranges are grown worldwide and produce the freshest juice. It has spherical form, orange color, tight skin with a juicy pulp. It's easy to and utilized as a fresh ingredient in salads. Oranges can be of four types: common one, navel-like, acid-free oranges, and blood

oranges. Valencia, Mausami and blood orange are some of the most popular sweet orange varieties [111].

1.4.3.2 Mandarin oranges (Citrus reticulata Blanco):

China is the largest producer of Mandarin. Tangerine is often used interchangeably with the term mandarin, tangerine usually refers to species that produce rich orange-colored fruits. Mandarin has a spherical form and a sweet flavor, as well as a loose, orangey tint. Its components can be separated. It is generally used for eating by hand, in juice, and to a lesser amount for processing. Satsuma group, Mediterranean mandarin, Tangerine or Clementine group, and other mandarin are the four classes. The Dancy, Ponkan, Mikan, and Temple of Mandarin families are some of the most important commercial cultivars [112].

1.4.3.3 Grapefruits (*Citrus paradisi* Macfadyen*):*

Grapefruit is thought to be a cross between a pummelo and a sweet orange. It has a thick and spongey rind and is juicy, sweet, medium to large in size. It has a small number of white-fleshed, red-fleshed, and pink-fleshed varieties. Because of their rejuvenating flavor and slight sourness, the cultivars are prized as breakfast fruits, salads, and in juicy form. Popular grapefruit varieties include the Marsh, Ruby Red, and Foster [113].

1.4.3.4 Lemons (Citrus limonBurmann):

Lemon is an important fruit that is fresh in the group, albeit it is not as widely consumed as mandarins and oranges. They have a high acid content in general, however acid-free cultivars do exist. It is mostly used for beverages and fresh juice, as well as for cooking, particularly in the production of lemon pies sweets, jams, and marmalades, and for medicinal uses due to its high vitamin content. The fruit is elliptical in shape having distinct necks and nipples. Yellow skin of lemon contains the well-known oil glands. This fruit's flesh is a pale yellow color and has a sour taste. There are three major groupings of lemons: Femminello, Verna, and Sicilian [114].

1.4.3.5 Lemons (Citrus aurantifolia Swingle):

Lime is commonly used in lime-soda and beverages, and in other alcoholic beverages. They can also be used for pickling; for culinary uses, such as flavorings in jellies, jams, and marmalades; for medical applications and as a source of lime oil. It has a greenish-yellow tint and thin skin. The juice has a high acidity level. Tahiti and Key (Mexican) limes are the two most economically important acid lime cultivars [115].

1.4.4 Citrus peels as a source of bioactive compounds:

Processing of citrus fruit produce peels which are usually regarded as agricultural wastes [116]. Citrus peel is a source of environmental due to fermentation and decomposition processes. [117]. Citrus peel is a valuable byproduct of the citrus market and can be employed in pharmacology, and nutraceuticals [118]. Citrus peel industrial waste are easily accessible and a source of renewable biomass [119]. It naturally contains flavonoids which are promising [120]. Furthermore, citrus peel is an easily accessible, affordable, and cost-effective plant-based resource for managing lifestyle-related illnesses. It is also a rich source of dietary fibre and nutrients. Consequently, the citrus peel can be employed in food products as a functional component and an alternative for chemical preservatives.

Bioactivites and minerals contained in citrus peels have the potential to be explored for their health-promoting capabilities in diets [121]. It can be used as a medication or as a food additive. It amounts to huge amount of the overall weight of the fruit. Soy sauce addition to citrus peels has shown to enhanced nutritional and bioactive characteristics [122]. Carotenoids, ascorbic acid, and phenolic metabolites are identified in the Citrus peel. Phenolic chemicals have antibacterial, antioxidant, anticancer, antimutagenic, antiallergic and anti-inflammatory effects.[123, 124, 125, 126, 127]. The extraction of phenolic compounds from citrus peel is a viable and innovative technique of marketing citrus by-products. [123, 128].

1.4.5 Citrus peels antioxidative potential:

Bioactive chemicals found in citrus peels have a solid antioxidative capacity against free radicals. The citrus peel contains a high concentration of natural antioxidants [129]. The

antioxidant potential of citrus peel is due to the enormous amount of phenolics [130]. Citrus peel includes phenolic contents and flavonoids (hesperidin, nobiletin, tangeritin and narirutin) that help stabilize free radicals by transferring protons or electrons [131]. Orange peel flavonoids have antioxidant potential corresponding to hydroxyl instead of radical elimination mechanism [132].

1.4.6 Essential oils in citrus peels:

Aside from phenolic chemicals, citrus peel is high in essential oils, blends of volatile substances [133]. Citrus peel essential oils are cheaper and ecologically friendly to chemical additives [134]. Essential oil is a saturated hydrophobic material found in citrus peel oil cells. It accounts for nearly 0.5–5% of the fresh weight of citrus peel and is comprised of volatile aromatic components. The most citrus peel contains bioactives. Citrus fruits with thick peels have a greater concentration of essential oils than citrus species with thinner rinds. In the peels of citrus varieties, the predominant essential oils were non-terpenoid ester derivatives. Mono- and sesquiterpene hydrocarbons, on the other hand, were prevalent in citrus peels [135]. Limonene a colourless aliphatic compound found in essential oils of many citrus species. The predominant essential oil components found in orange peels were monoterpene hydrocarbo. Other essential oil components found were beta-humulene, and oxygenated sesquiterpenes. Lemon peels comprised fatty alcohol esters, sesquiterpenes, and oxygenated monoterpenes. The level of essential oil was also reported to decrease during the ripening process [137].

1.5 Aims and objectives:

The main aims and objectives of the present work are as follows:

1- Evaluation of anti-oxidant potential of citrus fruit waste obtained as an agro-industrial waste.

2- Extraction of functional components (phenolic components, carotenoids and ascorbic acid).

3- Isolation of essential oil from citrus fruit peels using clevenger apparatus.

4- Profiling of functional components in citrus fruit waste extracts/fractions using state-of-the-art spectroscopic and chromatographic tools such as UV/Vis, FTIR, GC-MS, HPLC, LC-MS/MS, where applicable.

CHAPTER 2

REVIEW LITERATURE

Gorinstein et al., (2001) evaluated the antioxidant potential of various citrus fruits. Lemons, oranges, and grapefruits were tested for total polyphenols, dietary fibre, ascorbic acid, essential phenolics, , and a few trace elements, and their total radical-trapping antioxidative capability (TRAP) was also examined. In the fruits with peels investigated, there were no considerable variations in dietary fibre content in total including soluble and insoluble ones. Fruit peels had significantly higher levels of dietary fibre in total including soluble and insoluble ones than peeled fruits. Total polyphenols in peeled grapefruits, lemons, oranges, are 13510.1, 16410.3, 15410.2, mg/100 g, respectively, while in their peels, the values are 19010.6, 17910.5, and 15510.3 mg/100 g, respectively. Total polyphenol content was substantially higher in peeled lemon as compared to peeled oranges and grapefruits. Total polyphenol content was substantially higher in the peels than in the peeled fruits. Vital phenolic content and ascorbic acid content yielded the same results according to research. The levels of iron were significantly greater in lemons and their peels than in oranges and grapefruits and their peels, respectively. In addition, in lemons and their peels TRAP was considerably greater than in oranges and grapefruits and their peels, respectively. The TRAP was substantially higher in peels than in peeled fruits in all three fruits (P 0.05). Finally, among the citrus fruits tested, lemons have the highest antioxidant capacity and are preferred for dietary protection of cardiovascular and other disorders. Peels of *citrus* species are high in dietary fibers and phenolic chemicals, making them ideal for industrial processing [138].

Bast et al., (2002) studied antioxidants being widely employed in an attempt to achieve and maintain good health. Antioxidants are commonly found in large doses in nutraceuticals and food supplements. Antioxidant intake in excess can have negative consequences, which is not often recognized. To illustrate broad considerations on antioxidant toxicity, three antioxidants are used: vitamin E, -carotene, and lipoic acid. The following suggestions for assessing antioxidant toxicity are made based on the examples: I Traditional safety elements should be avoided. (ii) More research into the mechanisms of antioxidant activity and toxicity is needed. (iii) Bio-kinetic/bio-efficacy modeling could aid in dosage optimization.
(iv) When antioxidant supplementation becomes a medicine, more precise risk/benefit analyses are required [139].

Silalahi et al., (2002) conducted experimental and epidemiological research stating that persons have a lower risk of degenerative illnesses who eat a lot of fruits and vegetables,. The antioxidant potential of bioactive components present in fruits and vegetables, and other physical and biochemical features of bioactive compounds are thought to be basically responsible for this protective impact. The bioactive components found in citrus fruits include -carotene, dietary fibre, flavonoids etc. Citrus fruit consumption may lower the risk of degenerative diseases [140].

Ahmad et al., (2006) studied cold pressing extraction of essential oils from the peels of Mousami, Grapefruit, Malta and Eureka lemon. Malta peel produced the highest oil yield 0f 1.21 percent, followed by Eureka lemon having 1.12 percent, Mousami and Grapefruit having 0.98 percent each. the composition of the oils extracted was determined. 61.08 percent of limonene, 0.11 percent of –thujene, 0.84 percent of –pinene, 0.32 percent of camphene, 4.18 percent of citronellol, 7.74 percent of citral, 5.62 percent capraldehyde, 2.10 percent of caprinaldehyde, 7.63 percent of borneol), 2.06 percent of terpinolene, 1.28 percent of linalool and 0.22 percent of citran were found in Malta. 76.28 percent of limonene, 1.26 percent of -pinene, 1.56 percent of -terpinolene, 1.74 percent of citral 0.35 percent of capraldehyde, 1.26 percent of 2-hexene-1-ol, 0.35 percent of decanol and 0.35 percent of linalool were found to be the primary components in Mousami. The chemical makeup of essential oils from these species differed greatly, possibly due to genetic differences [141].

Chodak et al., (2007) investigated the antioxidant activity of various fruit seeds and peels. Antioxidant activity, total polyphenol content and tannin levels were evaluated. The findings revealed significant differences in the studied parameters among the materials. Citrus fruit peels imported to Poland have a higher content of polyphenol contents as well as stronger ability to scavenge free radicals than the seeds. The highest antioxidant activity had been found in the peels of apples and grapes. The seeds of apples and oranges had considerable antioxidant potential, Tannins are important antioxidants found in grape, apple, and gooseberry fruits. In the peels and seeds of numerous fruits, which are waste products in the fruit and vegetable business, antioxidant potential may be found [142].

Abeysinghe et al., (2007) investigated the concentrations of phytochemicals as well as total antioxidant capabilities of citrus fruit edible tissues including juice sacs, segment, segment membrane of four species. Flavanones (hesperidin and naringin) were identified by HPLC. In

Citrus species, hesperidin accounted for 18.5–38.5 percent of total phenolic contents. Naringin was only found in *Citrus changshanensis*. In this species' SM, it accounted for 53.7 percent of total phenolics. In SM of all tested species, the concentration of phenolic compounds and TAC were considerably higher than in juice sacs and Segment. The segment membrane of citrus *changshanensis* had the highest total phenolic contents. The results indicated that the segment membrane of citrus fruit had a high amount of bioactive components and antioxidant capacity; therefore, eating citrus fruit with all edible tissues is advisable rather than just the juice or juice sacs [143].

Kamal et al., (2011) investigated applications of essential oils of *citrus* peels as they are having a wide range of medical and culinary applications. Essential oils were isolated from peels of three Citrus species, and their yield and chemical composition were determined in the research. These differently treated peels of citrus species had essential oil concentration of 0.30-0.50, 0.24-1.07, and 0.20- 0.40 g/100 g, respectively using hydro-distillation technique. The smallest amount of oil was found in fresh peel samples, whereas, the largest amount of oil was found in oven-dried peel samples. In the essential oils of peels of citrus species using GC and GC/MS, a total of 16-27, 17-24, and 18-40 bioactive components were detected, respectively. The most common chemical ingredient was limonene. The production and concentration of major bioactives in the studied essential oils varied, according to the procedures used for drying and species tested [144].

Espina et al., (2011) examined essential oils from citrus species of Spain's composition and their antimicrobial activity against pathogenic and spoiling microorganisms including their possible synergistic harmful effects when mild heat was used in combination. Using gas chromatography-mass spectrometry analysis, 65 chemicals were isolated as primary volatile components. Limonene (59-85 percent) was found to be the main costituents in essential oils of three different species. The disc agar diffusion experiment revealed that essential oil of mandarin caused maximum growth inhibition, while essential oils of orange and lemon were eliminated. Essential oil of mandarin was shown to have the largest spectrum of activity when the bacteriostatic and bactericide effects were assessed. Three essential oils low concentration in combination with a treatment of mild heat had interactive harmful effects, inactivating of bacterial cells 5 log cycles and demonstrating effective combined food preservation treatments potential [145].

Goulas et al., (2012) studied the antioxidant potential of the major *Citrus* varieties growing in Cyprus. The antioxidant potency and was found highest in the 'Valencia' fruit, followed by the 'Mandora' fruit and the other three varieties of grapefruit. Studies found flavonoids derivatives and a furocoumarin among the fruits studied by HPLC. Analysis by LC–MS revealed the presence of flavonoids and the research overall supported the supremacy of the orange fruit named Valencia as a prime source of bioactive compounds. Furthermore, the study described the bioactives profile of the citrus fruits grown in Cyprus [146].

G. Oboh and Ademosun (2012) evaluated the antioxidants and determined the bound and free phenolics distribution in various citrus peels of Nigerian species. In 80% acetone, free phenolics were extracted whereas phenolics bounded were extracted using ethyl acetate from the alkaline and acid hydrolyzed leftover. Bound phenolic extracts had considerably higher DPPH* scavenging capacity than free phenolic extracts except in orange peels, whereas bound phenolic extracts had lesser DPPH radical scavenging ability than free phenolic extracts. It had significantly higher DPPH radical scavenging ability. Ability to scavenge DPPH*, ferric reducing antioxidant properties and ABTS* scavenging ability were found in orange peels bound phenolics, while the lowest ABTS* scavenging ability were found from shad bound phenolic contents. Bound phenolics in grapefruit peel had the highest OH* scavenging ability. The shad peel bound phenolics had the minimum. The phenolics chelated Iron (ii) and prevented formation of malondialdehyde in the pancreas of rat in dose dependent way. The high antioxidant potential of extracts from orange peels phenolics could be used in the nutraceuticals formation and food preservatives because of their additive and/or synergistic action, which is considered to have contributed in the observed medicinal properties of the *citrus* peels [147].

Pereira et al., (2013) determined the physicochemical composition and potential of antioxidants in three native species, namely pindo palm with the aim of cultivation encouragement. According to the ABTS* method, the fruit having highest overall concentration of phenolic component was the mandacaru-de-tres-quinas. The araticu-do-mato did not differ statistically from the mandacaru-de-tres-quinas fruit although having the highest vitamin C content. On the other hand, it was found almost equal to the pindo palm fruit. The total soluble solids and total titratable acidity ratio of the araticu-do-mato (41.92) was also maximum which indicate that it was good for processing and in-nature intake [148].

Al-Juhaimi et al., (2014) measured phenolic chemicals, ascorbic acid concentration, and free radical scavenging capabilities in the peel and pulp of Eureka lemon fruits, Orlando orange and mandarin. Total phenolic contents; total ascorbic acid content; capabilities of DPPH radical scavenging were found in three different species, mandarin, lemon pulp and orange. Orange, lemon peels and mandarin had the values of high total phenolic contents. According to the research, the citrus peels are rich in phytochemicals and have antioxidant components which could be used in the development of functional foods [149].

Zhang et al., (2015) assessed the total antioxidant capacity of various *citrus* species, and was evaluated using an on-line high performance liquid chromatography-free radical scavenging detection system. All citrus samples were evaluated in 5 minutes using this technology. Quantification limits, outstanding recovery rate, low detection and stability, precision and repeatability are the characteristics of an on-line HPLC-FRSD system. It was found better than conventional on-line High Performance Liquid Chromatography methods in the study of *Citrus* antioxidant capacity checked by using a guard column instead of an analytic column. This newly designed technology is faster and more resilient than the conventional scavenging methods. The methodology could be useful for determining antioxidant capabilities in *Citrus* species [150].

Diab et al., (2016) tested total polyphenol content, antioxidant activity using DPPH and total flavonoids. To investigate their mitogenic proliferative capabilities and cytotoxicity in human leukemia HL-60 cells and mice spleen cells, the CCK-8 assay was used. Chromosomal aberrations (CAs) assay was used for the investigation of activities related to genotoxicity/antigenotoxicity in spleen cells of mouse. Phenolic contents were found maximum in lemon peels which is followed by mandarin and also grapefruit. On the other hand, mandarin had the highest flavonoids concentration which is followed by peels of lemon and grapefruit. Peels of lemon had the highest antioxidant potential, as proved by the maximum DPPH radical scavenging. Moderate anti-leukemia efficacy against HL-60 cells was found in peels of mandarin. Peels of grapefruit and lemon remained unsuccessful. Peels of *Citrus* species also had immunostimulation related properties resulted in an increased proliferation of mice spleen cells (T-lymphocytes). Citrus extracts reduced cytotoxicity generated by cisplatin in mouse splenocytes over 24 hours, demonstrating non-cytotoxic and antigenotoxic properties. Conclusions: Citrus peel phytochemical elements may have biological effects such as anticancer, immunostimulation, and antigenotoxic potential [151].

Putnik et al., (2017) studied that citrus fruit is a highly used crop resulted in huge amounts of waste by-products containing bioactive compounds such as pectins, essential oils and antioxidants (water soluble and insoluble). Some of these wastes are now valorized using various methods. Effective, non-toxic, and low-cost extraction systems might significantly improve valorisation and result in both increased earnings and improved quality bioactive components. The most recent research on new and environmentally friendly ways for valorizing citrus by-products was reviewed. Ultrasonic, pulsed electric fields, microwaves, and high pressure are compared to both old and new methods of valorization in order to stress the benefits and scalability of these enabling technologies. The reported innovative technologies can often offer a "greener" valorisation procedure of extraction than the traditional technique due to the lower energy consumption and reduced use of toxic solvents [152].

Fu et al., (2017) studied flavonoids in Pericarpium of Citri Reticulatae were evaluated simultaneously using an ideal HPLC condition. It was found by changing compositions of mobile phase and wavelengths detection, under which the calibration curves of all six substances displayed good linearity results. The relative standard deviation was approximately less for the chemicals examined. The approach used was successfully employed to check changes in the six flavonoids in PCR over a three-year storage period at 25°C. To evaluate the sample quality and antioxidant capacity were measured. The concentration of hesperidin declined and antioxidant activity rose throughout the storage time, suggesting that flavonoids might be used as indices for the change in quality of citrus' PCR. The findings revealed that PCR quality was improved by storing it for extended periods of time [153].

Garibay et al., (2017) explored that essential oils of various *citrus* species have antimycotic activity extracted from the oral cavity. According to the Ames test, they are neither mutagenic nor cytotoxic. They studied antimutagenic and antioxidant potential they have. Antimutagenic qualities were tested. Antimutagenic properties were tested. Only C. latifolia was antimutagenic, whereas both were antimutagenic against. They were antioxidants in the presence of the ROS-producing chemical. The antioxidant activity in the DPPH assay ranged from 6–23% for C. sinensis and for C. latifolia 22–71 percent in the antioxidant potential evaluation. In a -carotene bleaching assay, both *citrus* species were antioxidants and were

able to reduce self cell-death activated with hydrogen peroxide. The levels of superoxide ion were reduced in the presence of both oils. It was found that the essential oils of *citrus* species showed antimutagenic and antioxidant activities against different types of mutagens [154].

Rafiq et al., (2018) studied citrus plants including *citrus* species fruits such as mandarin, lime, orange, lemon, grapefruit and sour orange belonging to the Rutaceae family and appear to be a promising source of various well-known beneficial nutrients for humans. Due to the high volume of peel produced by citrus processing, it's considered as a rich source of phenolics. *Citrus* fruit by-products could be used as a source of nutraceuticals which are usually dumped as wastage in the environment. Such by-product wastes are capable of providing considerable low-cost nutritional supplements in diet due to their convenient availability. The use of these bioactive-rich citrus by-products could provide a profitable and environmentally friendly foundation for new nutraceuticals development or the improvement of existing bioactives. The possible components present in citrus peels discarded as garbage were thoroughly reviewed [155].

Sir Elkhatim et al., (2018) studied wasted sections of citrus fruits' phenolics, vitamin C, and antioxidant potential. Ethanolic extracts were made from the complete citrus variety. Results showed that the citrus peels had more important phytochemicals and antioxidant activity than the inner wasting sections like pulp and seeds. The highest total phenolic contents was discovered in peels of grapefruit which is followed by peels of lemon and orange peels. The peels of the other citrus fruits utilized when compared, citrus peels of orange have the highest levels of flavonoids and vitamin C. Citrus waste, especially peels, have a high antioxidant capacity and other activities indicating that they may provide health and nutritional benefits when used as a natural antioxidant in the food business [156].

Damian et al., (2018) studied that food waste is generated across the food supply chain. Cradle to cradle and circular economy like concepts of industrial ecology are increasingly being seen as basic principles for ecological innovation, with aim of of achieving a "zero waste economy" in which trash is utilized as a raw material for applications of product. The vast waste amount generated by food sector not only represents a significant loss of valuable commodities, but it also poses substantial management challenges, both economically and environmentally. Environmental law has aided in the implementation of sustainable waste management practices across the European Union. In light of the issues facing the food

business, efforts are being made to optimize processing technology in order to reduce waste. Citrus peels and seeds residues of the citrus juice extraction could be used as natural antioxidants as a means to reutilize them. Several citrus fruits extracts of peel and seed were evaluated for antioxidant potential. Seeds had more antioxidant activity than peels in general. The phenolics and antioxidative capacity of the extracts did not have an obvious link [157].

Teneva et al., (2018) studied chemical makeup of citrus aurantium L zest essential oil, it's antioxidant activity and antibacterial capability. The chromatography analysis was used to identify the chemical components. the evaluation of antioxidant activity was done. The primary compounds found in the essential oil were limonene, -myrcene, and -pinene, according to the findings. In terms of DPPH radical scavenging activity, the limonene outperformed zest essential oil. For essential oil's antibacterial activity, the disc-diffusion method was employed against pathogenic pathogens. Gram-positive bacteria were more susceptible to the oil, whereas Gram-negative bacteria were less responsive. As a biopreservative agent, the essential oil when used yielded good results [158].

Ferreira et al., (2018) studied that *Citrus reticulata* Blanco used in industry by-products producing negative influence on the environment. Extraction efficiency of water and 70% ethanol was compared for extracting phenolic contents from peels of *Citrus reticulata*. A simple solid phase extraction procedure was applied to get enhanced phenolic contents extraction. The extraction efficiency of the two solvents utilized did not differ significantly. Hesperidin, tangeritin, rutin, and naringin were the predominant constituents constituting 86 percent of the phenolics extracted. The extracts were enriched in phenolics and antioxidant activity 4-5 folds owing to solid phase extraction. The extracts' anti-proliferative action was shown to be dose-dependent, as well as cell line-dependent. The findings suggested that *Citrus reticulata* peels constitute a low-cost, high-volume antioxidant source and potentially bioactive phenolics [159].

Chavan et al., (2018) evaluated citrus fruits and *citrus* essential oils. A substantial number of by-products are thrown as waste in a farm field or a disposal hole. The research focused on the technologies and procedures for utilizing citrus wastes, such as feed for essential oil extraction, bio-oil production, charcoal and hydrogen syngas, and so on. *Citrus* pulp is a highly effective adsorption material. The next generation potential power is the PEM Fuel Cell; it was developed by preparing activated carbon from pulp of citrus. The study

investigated thorough use of these value added byproducts for making logical use of this worthy resource while protecting the environment also [160].

Peng et al., (2018) studied that soy sauce includes several bioactives having powerful antioxidative capabilities. *Citrus* peels contain huge varieties of flavonoid contents and have effective antioxidants. The influence of citrus peels on the variations of koji's enzymatic activities, the changes in antioxidant capabilities of soy sauce was examined during fermentation of moromi. Findings suggested that in koji production using citrus peels, helps in increasing -glycosidase activity while having no influence in the koji on the activity of other enzymes. During moromi fermentation, total flavonoid content, total phenolic content, polymethoxylated flavones, contents of aglycone-form isoflavones, and organic acid, their antioxidant potential capabilities were found to be higher in soy sauce added with *Citrus* peel than in soy sauce added without *Citrus* peel, indicating antioxidant activities strong positive correlation. Using *Citrus* peel in koji production is a simple and effective way to improve bioactive and functional characteristics of soybean products was demonstrated while reducing waste [161].

Aboudaou et al., (2019) compared the extraction of essential oil from orange peel by using GC–MS involving three different methods in terms of effectiveness. The microstructure and behavior of epithelial cells in the orange peel bark was evaluated. The standard hydro-distiillation extraction method resulted in more changes in cellular structure than the solvent-free microwave assisted extraction method, according to the findings. When solvent-free microwave assisted and hydrodistillation were compared, it was found that solvent-free microwave assisted extraction had advantages such as higher efficiency and faster rates.The antioxidant potential of essential oil was tested in-vitro using the DPPH assay, which showed that it has a greater radical scavenging activity (more than 80%). The essential oil was added to liquid whole egg at three different concentrations (0.1, 0.3, and 0.5 percent, v/v) to evaluate its effect on oxidative stability, as well as on color and odor under circumstances of simulated cold commercial retail. It was revealed by the thiobarbituric acid reactive chemicals analysis that adding essential oil reduced lipid oxidation sufficiently. The findings supported the usage of orange peel essential oil as an important food additive [162].

Calderon et al., (2019) explored that byproducts like peel of citrus fruits have potent uses due to certain bioactives. The absence of effective procedures, as well as the high amounts of

pollution caused by traditional bioactive chemical extraction causes the use of green solvents increased importance of green chemistry. The study investigated the use of ultrasound for extraction of bioactive components from citrus peel. A physicochemical analysis was used to evaluate the antioxidant capability, phenolics, ascorbic acid content, carotenoids, and HPLC phenolic profile in extracts of orange peel. The results showed that a time of thirty minutes, fifty percent ethanol in water and a power of 400 watts were the optimum conditions for UAE of bioactive components of citrus peel. TC content of 0.63mg ß-carotene/100g and phenolic concentration of 105.96mg GAE/100g were obtained under optimum conditions. Hesperidin was the most common phenolic component found in all extracts of citrus peels, with a maximum concentration of 112.040±08mg/100g [163].

Esfahlan et al., (2019) studied walnut being an all known member of the family named Juglandaceae as an important tree nut. Walnut fruit's kernel is responsible for its nutraceutical value. The study of the phenolics and antioxidant potential of different parts of walnut was done. Extracting and quantifying phenolics from each portion of the walnut tree and fruit using different solvent systems was analyzed and compared. The antioxidant capabilities of phenolic extracts obtained from various portions of the walnut tree and fruit were described. Antioxidant and antiradical activity of the extracted materials were evaluated as well [164].

Dong et al., (2019) studied that lemons are considered important for their economic, health and nutritional advantages. The antioxidant capacity, phenolic components, and maturity degree of *Citrus limon* Burm. f.] fruits collected at various times of the year were studied. In terms of weight, soluble solid components, and total sugar, it was observed that fruits harvested in November outperformed others in soluble solid components. The main phenolics found in lemon fruits were ferulic acid, eriocitrin, -coumaric acid and hesperidin. April fruits' pulp had the highest total phenolis about 3.49 GAE mg/g of fruit weight. Total flavonoid content was 1.46 RE mg/g of fruit weight. Sum of individual phenolic acids content 269.36 g/g of fruit weight), and antioxidant potency composite scored 100.00%. August saw the highest individual flavonoids (3098.42 g/g of fruit weight. The maximum total flavonoids (6.35 mg/g RE of fruit weight) and APC of 95.14 percent were found in fruits grown in April. The highest total phenolics (7.96 GAE mg/g of fruit weight) and sum of individual flavonoids about 9003.91 g/g of fruit weight were found in fruits grown in August. However, the highest individual phenolic acids about 769.28 g/g of fruit weight were found in fruits grown in June. The soluble solid content of pulps of lemon fruit was found to be inversely linked with their

total phenolic contents and antioxidant potency. With the increase in soluble solid contents of peels, total phenolics fell as well. Most importantly, the findings revealed that phenolic contents and antioxidant potential of fruits of lemon differed greatly depending on their maturity degrees and harvest time, which is critical information for making better use of different lemon fruit resources [165].

Mejia et al., (2019) studied that a method of solid liquid extraction for extraction and evaluation of polyphenols from peels of orange could be used on ethanolic aqueous solution. Chemical profiling was also done using liquid chromatography-mass spectrmetry/mass spectrometry. To investigate the effect of extraction factors on recovery, an experimental factorial design was used. Response surface analysis and Principal Component Analysis were used to analyze the data using multifactorial ANOVA. All extracts of peels contained trace levels of antioxidants. All samples contained flavonoid, while rutin flavonol was found in concentrations ranging from 3.3 to 4.7 mg•g1. The flavanone hesperidin (280–673 mg•g1) was the most important polyphenol found in the extracts from all tested citrus species. Furthermore, total polyphenols and flavonoids, total antioxidant capacity, and DPPH free radical scavenging were evaluated in extracts of peels. The findings revealed that citrus peel by-products waste could be reutalized as a vital source of polyphenols and could be an important value-added bioactives source [166].

Azman et al., (2019) explored the peel of a citrus fruit as a useful food. It's chock-full of antioxidants. They studied the antioxidant capabilities of some peels of *citrus* species. Citrus peels were examined for antioxidant capabilities in citrus peels. DPPH radical scavenging and FRAP were also measured. The antioxidant profile of frozen citrus peels was significantly higher than that peels of fresh fruits. When compared to fresh peels of citrus, the peels frozen gave worth noticing antioxidant potential, as seen by their higher ferric ion reducing power. Furthermore, orange peel that were frozen has higher antioxidant potential, as obseverd by decreased EC_{50} values ranging from 0.813 0.1 to 3.16 mg mL-1. The ferric ion reducing power value and total phenolics and total flavonoid content had a relatively strong connection. Peels of frozen citrus are beneficial sources of antioxidant, according to the study [167].

Anticona et al., (2020) studied that citrus species are widely cultivated and consumed all over the world. Eighteen percent of all citrus species cultivate in processes of industry, resulting in

a huge amount of garbage. There is a growing desire to reduce the damaging effect of citrus waste on enviroment and to manage responsibly. As a result, research into the utilization of non-traditional ways for extraction of value-added chemicals including polyphenols, essential oils, carotenoids, and pectins from this type of waste has grown more substantial in recent years. The effectiveness of different techniques was evaluated. A wide range of information regarding the most common novel ways for extracting value-added bioactive chemicals from citrus waste was considered, as well as the most important influencing elements for each technology were examined [168].

Bora et al., (2020) studied that citrus is a Rutaceae genus that comprises major crops such as oranges, pummelos, lemons, grapefruits, limes, and other citrus varieties. Essential oils of citrus fruits are made up of biologically active chemicals. The phytochemicals offer a variety of health-promoting qualities. All of which have enormous potential for food applications. Essential oil of citrus extraction and detection and purification technologies and possible allergic reactions associated with the use of essential oil of citrus in food applications were briefly discussed. All of which should be addressed in future researches. Environmental friendly, low-cost, and "green" extraction methods should be employed to ensure citrus essential oils being cost-effective and environmentally friendly products [169].

Singh et al., (2020) studied that peels of citrus species account for 40–50 percent of the overall fruit mass but still widely considered as waste. It is a substantial source of naturally occurring health-promoting bioactive components make up the majority of phenolics in citrus peel especially nobiletin and tangeretin. It is also important to note that citrus peels have higher concentrations of phytochemicals than the edible portion of the citrus fruits. Phenolics in citrus peels act as antioxidants which protect cells from free radical damage and lower the risk of many diseases by either donating protons or electrons. Citrus peels contain more polyphenols and their antioxidant potency is higher than that of other edible fruit parts. Therefore, citrus peels can be utilized as a source of useful phytochemicals and preservatives in the production of food products that are safe and also advantageous to one's health [170].

Safranko et al., (2021) using new and green extraction procedures, examined the efficient usage and valorization of peel of mandarin (*Citrus unshiu* Marc. variety Kuno). The extraction and analysis of volatile chemicals using supercritical CO2 extraction under different operating pressure was the initial stage of the research. GC-MS analysis was used for the extraction of the volatile components in the extracted extracts. Limonene was found to

be the primary volatile component (13.16 percent at 100 bar). After super critical carbon dioxide treatment, the bioactive peel of orange was treated to subcritical water extraction in a dynamic temperature range (130–220 degrees) with different solvent-solid ratios of 10 to 30 mL/g over time periods ranging from five to fifteen minutes to get flavonoids. HPLC was used to qualify and quantify present flavonoids. Hesperidin with the value of 0.16 to 15.07 mg/g was found to be the most prominent flavanon in peel of mandarin, along with other polyphenolics that could be produced as a result of thermal degradation. The presence of 5-hydroxymethylfurfural and chlorogenic acid was found at increased temperatures. To measure antiradical activity and total phenolics in the extracts spectrophotometric methods were utilized, while response surface approach was used to make the process suitable [171].

Czech et al., (2021) explored citrus fruits being used in the food sector while valuable citrus peel is exploited creating consumer worries about health. Sugar, dietary fiber, lead, redox chemicals, and cadmium contents in citrus fruits were compared for orange, mandarin, lemon, pomelo, key lime, and yellow, green and red grapefruit. The pulp has less fibre, phenolics and tannins as compared to the peels of all fruits. There was much lower sugar content in whole citrus fruits, whereas dietary fibre and phenolics, particularly ferulic acid, were substantially higher. Ascorbic acid were greater in grapefruits. Due to their higher quantity of ascorbic acid, phenolics and tannins, lemons, mandarins and limes had an antioxidant potential greater than their pulp. While the heavy metals like lead and cadmium in citrus fruits were higher in peels than in the pulps but still considerably below the daily limit [172].

Lu et al., (2021) studied that the bakery uses lemon flavedo which is the outer green layer of peel of lemon for its pleasant smell and attracting color. There are currently no data on specific flavedo bioactive chemicals but a lot of data on lemon peel. The effect of extracts of flavedo was determined and comparison of three extraction processes on two dried samples was made. Treated samples were compared. The primary components of total phenolic contents in OD and EOR samples were synapic acid and esculetin. As a result, lemon flavedo could be considered as a source for development of functional foods [173].

CHAPTER 3

MATERIAL AND METHODS

The research work presented in this thesis was carried out in the laboratories of the Institute of Chemistry, Institute of Food Sciences and Nutrition, Department of Pharmacy, University of Sargodha and University of Veterinary and Animal Sciences, Lahore.

3.1 Plan of Work:

The current work was planned in order to extract and characterize selected bioactives and essential oil from peels of citrus fruit and their antioxidant potential investigation.

3.2 Analytical Instruments Used:

Following instruments were used throughout the research work:

1. Atomic absorption spectrophotometer (Perken Elmer, A. Analyst 300)
2. UV visible spectrophotometer (U-2001, Hitachi Instruments Inc. Tokyo, Japan)
3. Commercial blender (TSK-949 Westpoint, France)
4. Orbital shaker (Gallenkamp, UK)
5. Water Bath Memmert
6. Analytical Balance (AUY-220 Shimadzu)
7. Rotary vacuum evaporator (EYELA, N-N Series, Rikakikai Co. Ltd. Tokyo, Japan)
8. HPLC (Varian, USA)
9. Oven (Haier)
10. Pipetmean Set (Gilson ,Switzerland)
11. Refigerator (orient)
12. Autoclave (Keller Technology corp.)
13. GC-MS (GC 17-a Schimadzu)
14. Clavenger Apparatus (Locally Designed Apparatus)
15. Miilpore Filter (pore size 45 micrometer)
16. Syringe (10 ml)

3.3 Chemicals and Reagents:

Analytical grade Chemicals of Sigma-Aldrich Chemicals Corporation, Germany was used in research work.

1. Ethanol
2. Methanol
3. Distilled water
4. Aluminum chloride
5. Gallic acid
6. Butylated hydroxyl tolein (BHT)
7. Catechin
8. Ferric chloride
9. Folin-Ciocalteu reagent
10. Nitrium hydrogen phosphate
11. Potassium ferricynide
12. Sodium carbonate
13. Sodium dihydrogen phosphate
14. Sodium hydroxide Trichloroacetic acid (TCA)
15. 1,1-diphenyl-2-picrylhydrazyl (DPPH)
16. Sodium Sulphate
17. Terpinene
18. Citranellal
19. Eugenol
20. Dodecanol
21. Limonene
22. Linalol
23. Linalol acetate
24. Myrcene
25. Nerol
26. Octanol
27. Nerol Oxide
28. Triton X-100
29. Flumequin

30. Amoxycillin

3.4 Apparatus used:

1. Aluminium Foil
2. Beakers
3. Condenser
4. Conical Flasks
5. Funnels
6. Filter Papers
7. Measuring Cylinders
8. Reagent Bottles
9. Round Bottom Flask
10. Micro Pipettes
11. Test Tubes
12. Test Tube Stands
13. Stirrers
14. Volumetric Flasks

3.5 Sample Collection:

Samples of selected *Citrus* species Fruiter (*Citrus reticulata* Blanco 'Merisol', Kinnow (Citrus *reticulata*), Mausambi (*Citrus sinensis* 'Valencia') were acquired from local fruit market and agricultural farms. The *citrus* species were confirmed and authenticated by a taxonomist at department of Botany, University of Sargodha, Sargodha, Pakistan.

3.5.1 Drying and Grinding of Sample:

Three different *citrus species* samples were washed with distilled water to remove the associated debris, peeled off and then shade-dried for about 1 month. The material was crushed into smaller pieces after complete shade drying and then milled, using a grinder mill, into fine particles. The powder thus formed was then placed at room temperature (25°C) in polythene bags.

3.6 Preparation of Extracts:

To make the extracts, the 20g crushed material was placed in conical flasks with different green solvents (200ml absolute ethanol, 80 percent ethanol, 50 percent ethanol, and 200ml distilled hot water) and shaken for 6-8 hours at 300 rpm on orbital shaker. The extracts were separated from the leftovers after 6-8 hours of shaking using Whatsman filter paper No. 1. After filtration, the appropriate solvents were added to the residues (remains) and the whole process was repeated three times, with all of the filtrate collected in 1000ml conical flasks. The surplus solvent from the extracts was evaporated using a rotary evaporator under decreased pressure after filtration. We were able to obtain crude concentrated extracts. To make semi-liquid extracts, the extracts were concentrated in a water bath. The semi-liquid extracts were then placed in an eppendorf tube and stored at -2°C for future experimentation.

3.7 Isolation of Essential Oil:

Using clavenger apparatus, the dried materials were exposed for three to four hours to the Steam Hydro-Distillation process. It has a round bottom flask of thousand mL. In this, the samples and distilled water were added until quarter of the flask was left empty. The water supply to the clavenger apparatus's condenser was maintained. The temperature was nearly 100 degrees. The distillates of the essential oil were dried by the addition of anhydrous Sodium sulphate. After being filtered via millipores these sample oils were stored at -4 degrees centigrades.

3.8 Estimation of Antioxidant Potential of Extracted Crude Content:

The antioxidant potential of citrus extracts was evaluated using the following in-vitro assays:

3.8.1 Total Phenolic Contents (TPC) Determination:

Extracts' total phenolic content was measured using the Folin-Ciocalteu reagent, as reported by Hussain et al. (2012) [174]. In this process, 0.5mL (0.05g/5mL) of each extract solution was obtained in separate test tubes, and then 0.5mL of Folin-Ciocalteu reagent (1:1) was added to each test tube solution, which was then diluted with 7.5mL deionized water. After that, all of the mixes were allowed to sit at standard temperature for 10 minutes. 1.5mL of 20% sodium carbonate (w/v) was added after 10 minutes of incubation at 25°C. After 20 minutes of incubation at 40°C in a water bath, the mixture was cooled in an ice bath, and the absorbance was measured at 755nm with a spectrophotometer (Hitachi U-2001, model 121-

0032). Standards for gallic acid (100-1300ppm) were dealt in the same way. Using a gallic acid calibration curve, the total phenolic content was estimated and reported in Gallic Acid Equivalents (GAE) mg/100 gram of dry weight.

3.8.2 Total Flavonoids Contents (TFC) Determination:

Hussain et al. (2012) [174] described a technique for determining the total flavonoids content of extracts. Briefly, the extracts were reconstituted in water individually, then 1mL of aqueous extract (containing 0.01g/mL of DW) was placed in a 10mL volumetric flask, and the total volume of each flask was increased to 5mL by adding distilled water. After that, each flask received 0.3mL of 5% $NaNO_2$ and was maintained at room temperature for 5 minutes. 0.6 mL 10% $AlCl_3$ was added after five minutes, and the mixtures were maintained at 25 degrees centigrade for another five minutes. 2mL of 1M sodium hydroxide was added after 5 minutes, followed by distilled water to bring the total volume to 10mL. The mixtures were properly mixed, and by using a spectrophotometer at 510nm, the absorbance of the mixtures and the standard solution was measured. The total flavonoids content was estimated using the catechin (10-130ppm) calibration curve and reported as catechin equivalents (CE) milligrams per 100 gram.

3.8.3 DPPH Radical Scavenging Assay:

The spectrophotometric 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical test of citrus extract was performed according to the methodology reported by Zhuang et al. (2012) [175]. The samples were mixed with 1 mL of 90 M DPPH solution to form a total volume of 4 mL with 95 percent methanol at varied concentrations (0.5 to 15.5 g/mL). The absorbance of the resultant solutions and blank were measured at 515nm after incubation for 1 hour at room temperature. As a positive control, butylated hydroxy toluene (BHT) was used. The % inhibition of free radicals by DPPH was computed as follows:

$$I\ (\%) = 100 \times A_{blank} - A_{sample} / A_{blank}$$

Where A_{blank} is the absorbance of the control reaction mixture excluding tested compound (sample) and A_{sample} is the absorbance of tested compound. Extract concentration providing 50% inhibition was calculated from the plot of inhibition percentage against concentration.

3.9 HPLC of Citrus Extracts:

Citrus extracts were analyzed using HPLC with a UV-visible detector to determine individual phenolics and flavonoids. The retention durations of phenolic contents and flavonoids were compared to those of reference standards for identifying them (Sigma Chemicals Co., St Louis, MO, USA). The standard calibration curves were used for determining them quantitatively.

3.10 GC-MS Analysis of Citrus Essential Oil (CEO):

To determine the chemical composition of citrus essential oils, GC-MS analysis was used. Hewlett Packard 5890 Gas Chromatograph JOEL model JMS-A 5050 H mass spectrometer (JOEL, Japan) (JOEL, Japan). Helium as carrier gas, split ratio 1:100, electrical energy 70 eV, ionization current 200 A, ionization temperature 250°C, column temperature rising to 230°C at a rate of 6°C/min

3.10.1 Identification:

The chemical ingredients were identified using their retention time and a known spectrum from the National Institute of Standards and Technology (NIST) library (NIST147.LIB) [176].

3.11 Estimation of Antioxidant potential of Citrus Peel Essential Oil:

The antioxidant capabilities of citrus peel essential oil were assessed using the following in-vitro assays:

3.11.1 Total Phenolic Contents (TPC), Total Flavonoid Contents (TFC) and DPPH* radical scavenging activity:

The total phenolic contents (TPC), total flavonoid contents (TFC) and DPPH* radical scavenging activities of the investigated oils were determined after the antioxidant capacity of the oils was determined. Using the Folin Ciocalteu Reagent (FCR) method with slight modifications, the amount of TPC in the citrus peel essential oils was determined colorimetrically. Briefly, the oil (10 mg/1 mL methanol) was combined with 0.5 mililitres of

Folin Ciocalteu Reagent and deionized water in a test tube (7.5 mililitres). After allowing the mixture to cool to room temperature (ambient temperature), 1.5 mililitres of sodium carbonate solution (twenty percent w/v) was added. This combination was heated to 40°C in a water bath for twenty five minutes before cooling in an ice bath. The absorbance (A) of the colorful complex reaction mixture generated was measured at 755 nm by using a ultra-violet/visible spectrophotometer, (V-630, JASCO International Co. Ltd., Hachioji, Tokyo, Japan). The levels of TP was assessed using a Gallic Acid (GA) standard calibration curve (R2 = 0.9982) that was created by running a series of standard solutions made in the concentration range of 0.25-6.50 mg LG1. GAE mg/100 g of essential oil was used to represent the results. The total flavonoids (TF) content was estimated using the catechin (10-130ppm) calibration curve and reported as catechin equivalents (CE) milligrams per 100 gram.

The capacity to scavenge stable free radicals (DPPH*) of citrus peel essential oils and the main component were measured to determine their antioxidant potential. This test was carried out, with minor modifications, according to the procedure reported in a prior study. Methanolic (0.5-10 g/mL methanol) solutions of the test citrus peel essential oils were combined with 1 mL of newly made 90 M DPPH solution at dynamic concentrations. By adding 95 percent methyl hydroxide, the volume was increased to 4 mL. The reaction mixture was placed at room temperature for thirty minutes in the dark after shaking. A ultra-violet/visible spectrophotometer was used to measure the absorbance of the incubated mixture and the control at 515 nm (V-630, JASCO International Co. Ltd., Hachioji, Tokyo, Japan). In parallel measurements, butylated Hydroxyl Anisole (BHA) considered a food-grade synthetic antioxidant was employed as a positive control. The DPPHE scavenging power and corresponding values of the essential oils were calculated using the equation already reported [177, 178].

3.12 Statistical Analysis:

In triplicate, each parameter was examined. To calculate means and standard deviations, conventional statistical methods were employed. Statistical analysis (ANOVA) was applied to the data to determine significant variations ($p < 0.05$).

CHAPTER 4

RESULTS AND DISCUSSIONS

The purpose of this work was to extract and analyse selected bioactives and essential oils from citrus fruit peels, as well as to assess their antioxidant capacity. To make the extraction procedure more environmentally friendly, green solvents were used. In-vitro antioxidant tests were used to determine the antioxidant capability of both the extracts and essential oils, which comprised total phenolic contents, total flavonoid contents, DPPH radical scavenging capacity, and specific phenolic acid and flavonoid compositions. Fruit peels from various citrus species (Kinnow, Fruiter, and Mausami) were analyzed using green solvents (ethanol and water), and the essential oils from the fruit peels were obtained using steam hydro-distillation.

4.1 Yield of Extracts:

The solid/liquid extraction approach is the most successful for extracting bioactive components, although numerous factors such as time, sample composition, temperature, and solvent polarity influenced the yield. Pure solvents (ethanol, water) and their aqueous mixes were used to extract bioactive components from citrus species, due to the varying polarity of plant phenolics. With diverse solvent systems, such as absolute ethanol, aqueous ethanol (80:20 ethanol: water; 50:50 ethanol: water), and 100 percent hot distilled water, the yield (g/100g) of extractable components varied. The antioxidant component yield of kinnow extracts in various solvent systems ranged from 49.81 to 16.87 percent (g/100g). The most effective extracting solvent was determined to be aqueous ethanol (80:20), which yielded the highest yield of extract from citrus species. For kinnow (49.81 percent), the highest yield was obtained with 80 percent aqueous ethanol (21.60 percent), while the lowest yield was obtained with hot water (16.87 percent). Mausami had a yield range of 30.23-39.35 percent, fruiter had a production range of 16.87-41.21 percent, and kinnow had a yield range of 40.12-49.87 percent. The extraction efficacy of the various solvents used in the current investigation was determined to be: 80 percent ethanol > 50 percent ethanol > absolute ethanol > hot water, based on the data. While sample yields ranged as follows: kinnow > mausami > fruiter. The largest yield of kinnow was recovered with 80 percent ethanol, indicating that 80 percent ethanol is more effective at recovering antioxidant components from citrus species. The effects of different solvents and sample types on percent extraction yield were found to be

significant ($P < 0.05$). As a result, it's safe to suppose that the extract yield varies significantly ($P < 0.05$) depending on the solvent system and citrus species employed.

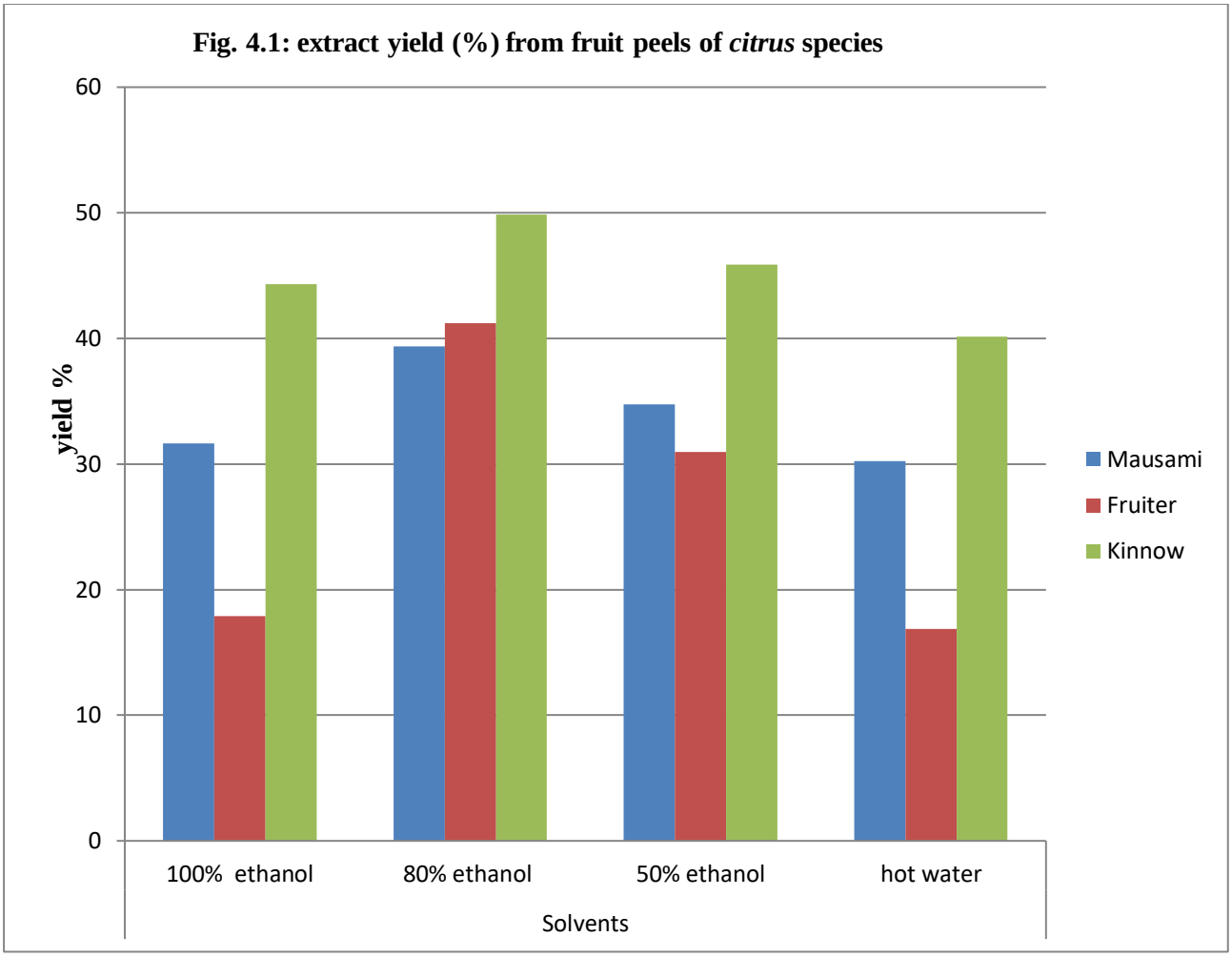
The current finding, which shows a larger extract yield with ethanol than with water, agrees well with Kim's percent yield of citrus species peels [179]. Kumar et al., [180] also showed a higher ethanol extraction yield, however this percent yield was lower than the current study's. Sultana et al. [181] found higher extraction yields with aqueous organic solvents than with absolute solvents, and their results are equivalent to the current yields. In this study, the yield of water extraction was much lower than that of ethanol extraction, which is consistent with the findings of Liew et al., [182]. Geographical differences, environmental circumstances, extraction processes, and the nature of the solvent and extractable components could all contribute to modest differences in % yield numbers. In comparison to water, ethanol was found to be a more effective solvent for extracting antioxidant chemicals in this investigation.

Table 4.1: Extract yield (g/100g) from fruit peels of different *Citrus* species:

Citrus species	Percent yield (g/100g)			
	100% ethanol	80% ethanol	50% ethanol	100% hot water
Mausami	31.63±0.58	39.35±0.13	34.76±0.67	30.23±0.32
Fruiter	17.87±0.76	41.21±0.34	30.98±0.56	16.87±0.45
Kinnow	44.34±0.12	49.87±0.15	45.87±0.23	40.12±0.57

Values are means ±SD (n=3) of three separate experiments.

Fig. 4.1: extract yield (%) from fruit peels of *citrus* species



4.2 Total Phenolic Contents:

Plant polyphenols are a wide collection of secondary metabolites with potent antioxidant properties that aid in the prevention of chronic diseases such as type 2 diabetes, heart disease, and cancer. Citrus fruits are high in phenolic compounds, making it one of the most significant horticultural crops on the planet. Citrus fruits such as lime, grapefruits, sweet orange, lemon, and tangerine have been studied extensively for their phenolic components and antioxidant activity [183].

The total phenolic contents of various citrus species were determined using Velioglu et al method with certain modifications (1998). In Folin-Ciocalteu reagent (FCR) several citrus juice samples (100 L) were combined [184].

Total phenolic concentrations in the studied extracts, estimated as gallic acid equivalent (GAE), ranged from 6.40 to 156.40 (mg/100g) of dry weight in different solvent systems (DW). For kinnow, 80 percent ethanol 156.40 GAE (mg/100g) recovered the highest phenolic content, whereas hot water 6.40 (mg/100g) recovered the lowest. The TPC values for DW extract of different species were 106.40-138.95 for mausami, 6.40-36.63 for fruiter, and 125-156.40 for kinnow (GAE mg/g). The phenolic content of hot water was found to be the lowest overall. Total phenolic components extracted from citrus species in various solvents differed significantly ($P < 0.05$) between extracts.The difference in the total phenolic contents of the tested extracts might have been due to the extraction potential of different solvents to dissolve antioxidant compounds. So, the order of different solvents to extract total phenolic compounds as: 80% ethanol > 50% ethanol > absolute ethanol > hot water. The effect of the ethanol concentration used in the extraction is in accordance with Li et al., [185] which say that the recovery of total phenolics increased with increase in the ethanol concentration, until the concentration reached 85%; after which, the recovery reduced with the increase of ethanol concentration. Liew et al., [182] reported that the other extracts exhibited relatively high TPC too except for 100% ethanol extraction and water extraction which showed significantly lower TPC than the other extracts and the results are comparable to the present study.

TPC for several citrus species was discovered to be in the following order: kinnow > Mausami > fruiter, with considerable ($P > 0.05$) variance amongst citrus species. The current findings are consistent with the TPC values found by Canen et al., [186] for citrus reticulata peel (42.64) GAE mg/g) and Citrus sinensis peel (42.68 GAE mg/g) of DW. According to Fejzi, A., and Avar, S. [187], grapefruit has the highest total phenolic content, followed by

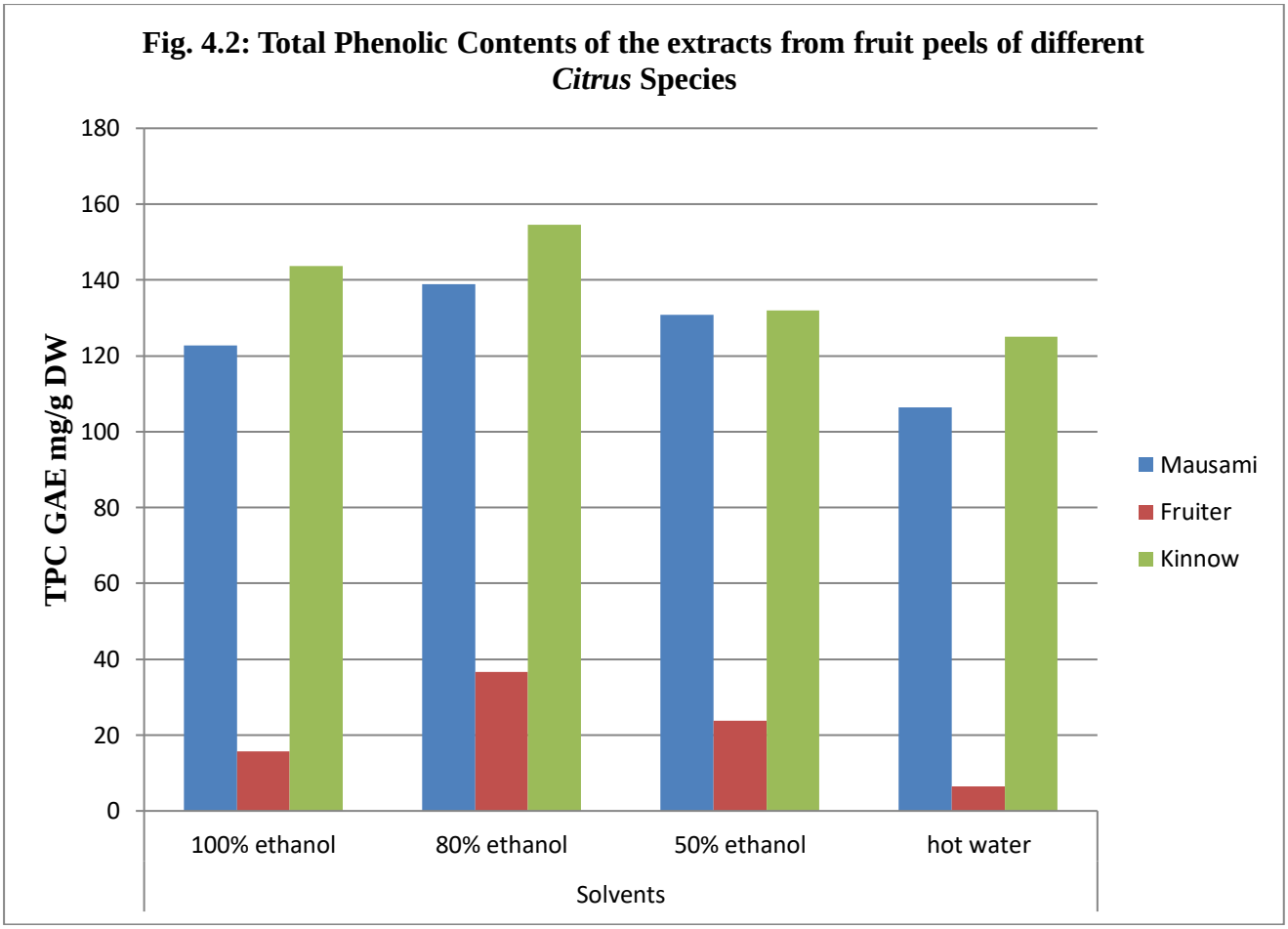
mandarine. Lemon, orange, and mayor lemon peel from Yeb Ben. Asjad et al., [188], reported TPC values that are similar to ours.

Table 4.2: Total phenolic contents (GAE mg/g (DW) of the extracts from fruit peels of different *Citrus* species

Citrus species	TPC (GAE mg/g DW)			
	100% ethanol	80% ethanol	50% ethanol	100% hot water
Mausami	122.67±0.54	138.95±0.23	130.81±0.52	106.40±0.63
Fruiter	15.70±0.12	36.63±0.85	23.84±0.49	6.40±0.72
Kinnow	143.60±0.84	156.40±0.39	131.98±0.59	125.00±0.70

Values are means ±SD (n=3) of three separate experiments.

Fig. 4.2: Total Phenolic Contents of the extracts from fruit peels of different *Citrus* Species



4.3 Total Flavonoid Contents:

Flavonoids are one of the primary classes of phenolic compounds, and they play a key role in the flavor and color of many fruits and vegetables, as well as goods derived from them like wine and tea. The biological effects of flavonoids have sparked a lot of curiosity, especially since there's evidence that eating a diet rich in fruits and vegetables can help prevent heart disease and cancer. Flavonoids can serve as antioxidants through a variety of mechanisms. Free radical scavenging, in which the polyphenol can break the free radical chain reaction, is likely to be the most important. [41].

The catechin equivalent (mg/100g) of dry weight content of total flavonoids in the studied extracts was calculated (DW). The concentration of total flavonoids in citrus extracts in various solvents ranged from 43.76 to 131.76 CE mg/100g dry weight. For mausami, the highest flavonoids content was produced with 80 percent ethanol (131.76 CE mg/100g), whereas the lowest value was obtained with hot water for kinnow (43.76 CE mg/100g). The varying extraction ability of solvents to dissolve antioxidant molecules accounts for the variance in total flavonoids content. The amount of total flavonoids extracted from citrus peels differed considerably (p 0.05) among extracts. The following was the order of total flavonoid content in various solvents: Absolute ethanol > 50 percent ethanol > hot water > 80 percent ethanol

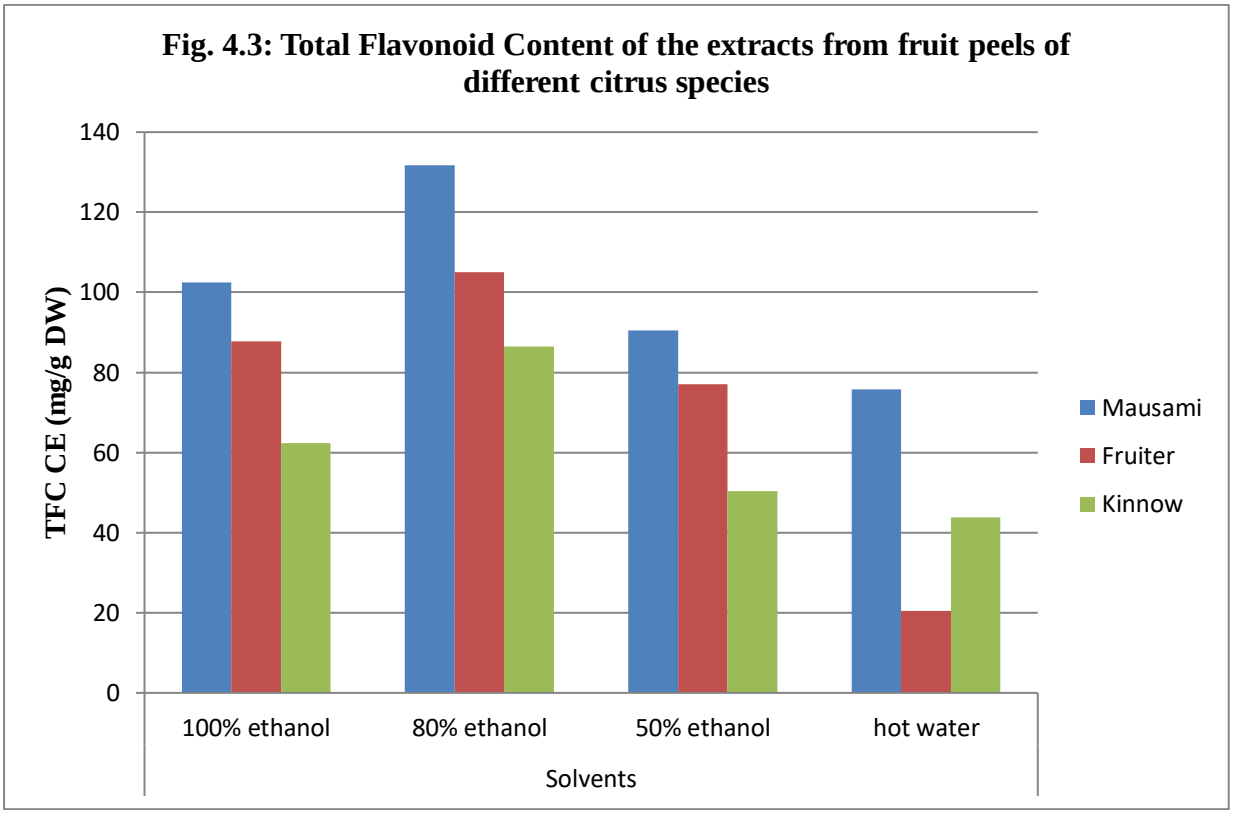
According to the current investigation, the order of flavonoid content in different citrus species peels is mausami > kinnow > fruiter, which is consistent with the results given by Ghasemi et al., (2009) [189]. TFC values for ethanolic extracts of citrus species peels were significantly higher than those reported by Chen et al., (2020) [190] for various citrus species fruits.

Table 4.3: Total flavonoid contents (CE mg/g DW) of the extracts from fruit peels of different *citrus* species.

Citrus species	TFC (CE mg/g DW)			
	100% ethanol	80% ethanol	50% ethanol	100% hot water
Mausami	102.43±0.33	131.76±0.26	90.43±0.59	75.76±0.69
Fruiter	87.76±0.18	105.09±0.80	77.09±0.78	70.43±0.70
Kinnow	62.43±0.81	86.43±0.40	50.43±0.76	43.76±0.71

Values are means ±SD (n=3) of three separate experiments.

Fig. 4.3: Total Flavonoid Content of the extracts from fruit peels of different citrus species



4.4 DPPH radical scavenging activity:

DPPH is a stable, nitrogen-centered free radical that is used to estimate plant material's antioxidant capacity. The DPPH scavenging assay, which yields maximal absorption in the wavelength region of 515-528nm, is extensively used to assess antioxidant scavenging activity. The antioxidant capacity of plant material affects the color of DPPH solution. The color of DPPH changes to yellow after it receives a proton from a hydrogen donor, primarily phenolics from plants. As the concentration of phenolic compounds rises, so does the DPPH radical scavenging activity.

The DPPH free radical scavenging activity of citrus peel extracts ranged from 49.43 to 72.35 percent. The greatest free radical scavenging activity for mausami was found in an aqueous ethanol (80%) extract (84.86%). Hot water, on the other hand, demonstrated the least amount of free radical scavenging action for fruiter (43.44 percent). Values for mausami, fruiter, and kinnow peels were 78.70-84.86, 43.44-72.74, and 73.75-82.84 % respectively. The current findings showed that free radical scavenging activity was significantly higher (p 0.05). Citrus species extracts scavenged free radicals in the following order: 80% ethanol > 50% ethanol > absolute ethanol > hot water. The standard for this study was butylated hydroxy toluene (BHT). The ability of the studied citrus species extracts to scavenge free radicals is directly related to the presence of phenolic components. Liew et al., (2018) [182] reported similar findings in their investigation.

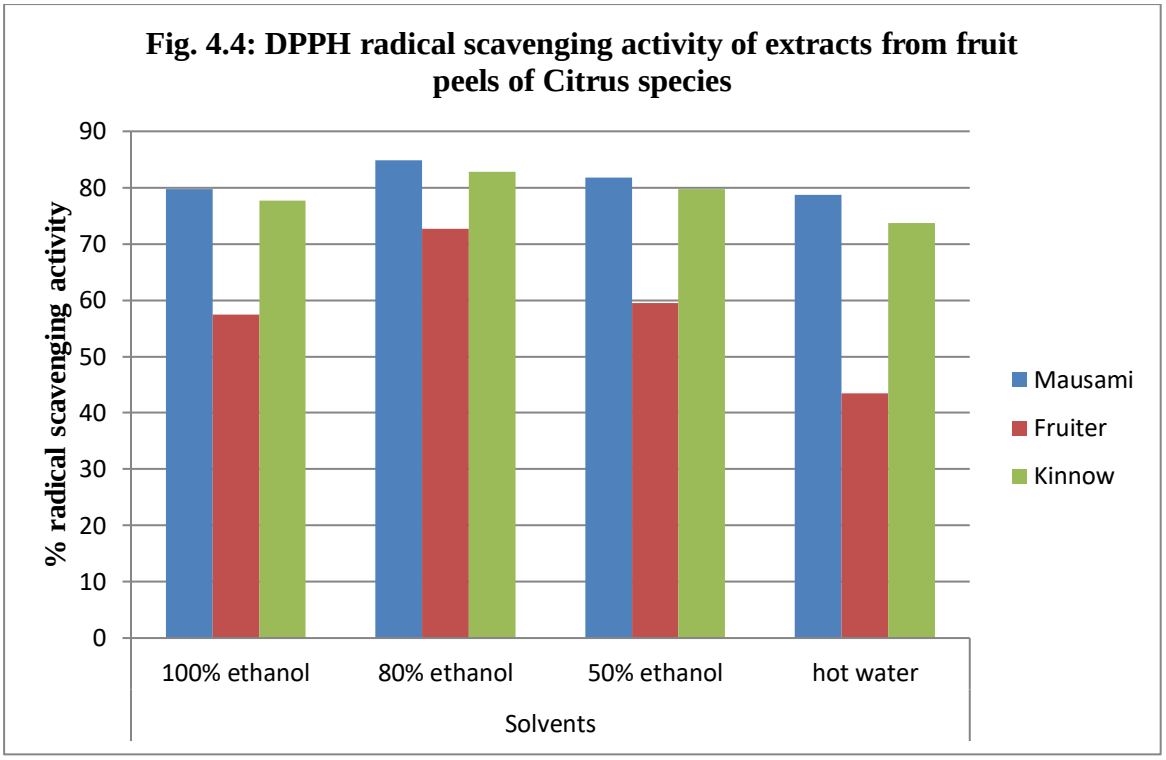
The ability to scavenge free radicals in citrus species peels is ranked as follows: mausami > kinnow > fruiter. The strongest radical scavenging activity was seen in Mausami, with substantial (P > 0.05) differences between species.

Table 4.4: DPPH radical scavenging activity of the extracts from fruit peels of different *citrus* species.

Citrus species	DPPH radical scavenging activity (%)			
	100% ethanol	80% ethanol	50% ethanol	100% hot water
Mausami	79.71±0.31	84.86±0.27	81.73±0.56	78.70±0.62
Fruiter	57.48±0.20	72.74±0.81	59.51±0.71	43.44±0.35
Kinnow	77.69±0.18	82.84±0.47	79.71±0.73	73.75±0.56

Values are means ±SD (n=3) of three separate experiments.

Fig. 4.4: DPPH radical scavenging activity of extracts from fruit peels of Citrus species



4.5 Phenolic and Flavonoid profile:

Using HPLC with a UV-visible detector, the contents of specific phenolics and flavonoids in citrus species peels were determined. The signals were obtained at a wavelength of 280nm. By comparing the retention time of the extracts to the retention time of standards, the components present in the extracts were determined. Galic acid, chlorogenic acid, caffeic acid, synapic acid, and benzoic acid were used as standards for phenolic acid analysis of extracts. Flavonoids profile requirements included myricetin, quercetin, and kaempferol..

Table 4.5: Individual phenolic acids (mg/g of extract) profile of extract from fruit peels of different *citrus* species.

Extracts	*Citrus* species	Phenolic Acids mg/g)					Total amount of phenolic acids mg/g)
		Galic acid	Chlorogenic acid	Caffeic acid	Synapic acid	Benzoic acid	
100% ethanol	Mausami	0.170	Traces	2.080	-	-	2.25
	Fruiter	-	0.168	-	3.331	-	3.499
	Kinnow	-	-	0.212	19.939	-	20.151
80% ethanol	Mausami	0.306	0.105	0.648	11.169	-	12.228
	Fruiter	0.195	0.956	0.234	10.774	-	12.159
	Kinnow	0.201	0.054	0.272	24.967	-	25.494
50% ethanol	Mausami	0.386	-	0.579	5.953	-	6.918
	Fruiter	-	1.689	0.220	8.271	-	10.18
	Kinnow	-	-	0.318	10.890	-	11.208
100% hot water	Mausami	0.163	-	0.569	4.611	-	6.343
	Fruiter	-	-	0.175	2.153	0.005	2.333
	Kinnow	-	-	0.265	3.652	-	3.917

Table 4.6: Individual flavonoids (mg/g of extract) profile of extract from fruit peels of different *citrus* species.

Extracts	*Citrus* species	Flavonoids (mg/g)			Total amount of phenolic acids mg/g)
		Myricetin	Quercetin	Kaempferol	
100% ethanol	Mausami	5.875	-	-	5.875
	Fruiter	0.546	-	-	0.546
	Kinnow	0.418	-	0.203	0.621
80% ethanol	Mausami	7.396	0.523	0.070	7.989
	Fruiter	0.636	-	0.089	0.725
	Kinnow	0.558	-	0.412	0.97
50% ethanol	Mausami	4.143	-	0.063	4.206
	Fruiter	0.740	-	-	0.740
	Kinnow	-	-	0.303	0.303
100% hot water	Mausami	-	0.902	-	0.902
	Fruiter	-	-	-	-
	Kinnow	-	-	-	-

4.7 Essential oils yield:

The essential oil was extracted from citrus peels that had been air dried (Citrus sinensis, Citrus reticulate Blanco ‘merisol', Citrus reticulate) using a Clevenger device. The yield of citrus peel essential oils is shown in Table 4.7.

Mausami (Citrus sinensis) peels yielded the most oil of the Citrus species examined (1.76 percent). The actual oil output of fruiter (C. reticulate Blanco ‘Merisol') and kinnow (C. reticulata) peels was (1.07 percent) (0.86 percent). The oil output of kinnow peels was lower than that of musammi and fruiter peels. These findings are consistent with those of Anwar et al. (2008) [191]. These findings are also consistent with Palazzolo et al., (2013) [193], who found that EO concentration varied by citrus species and was found to be in the range of 0.5–5.0 percent (w/v) in several citrus species. Total oil outputs from sweet orange, mandarin, and eureka lemon were 0.80, 0.80, and 0.90 percent, respectively, according to Weiss (1997) [192]. The oil output of Mousami, Kinnow, Fewtrell, and Grape fruit was 0.98, 0.32, 0.22, and 0.73 percent, respectively, according to Ahmad et al. (2006) [141]. This difference in the % production of peel oil could be attributable to seasonal variations or the timing of citrus fruit harvest.

Due to the increased output of essential oils from C. sinensis peels, this species could be investigated as a possible source for essential oil extraction for use in medicines, the food sector, and cosmetics.

Table 4.7: Yield of fruit peels essential oil from Citrus Species

Common Name	**Specie Name**	**% yield**
Mausami	*Citrus sinensis*	1.76 ± 0.05
Fruiter	*Citrus reticulata* Blanco	1.07 ± 0.12
Kinnow	*Citrus reticulate*	0.86 ± 0.06

Results are mean ±S.D of three peel samples from citrus species analyzed in triplicate.

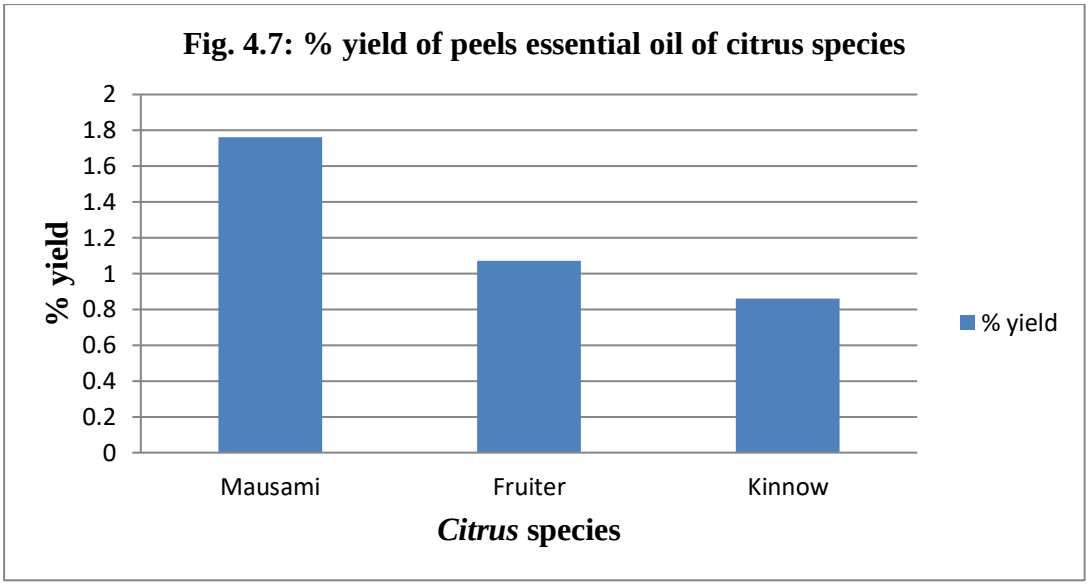


Table 4.8: Total phenolic contents (GAE mg/mL) of the essential oils from fruit peels of different *Citrus* species

Citrus species	TPC (GAE mg/mL)
Mausami	23.89±0.15
Fruiter	8.03±0.23
Kinnow	11.89±0.43

Results are mean ±S.D of three peel samples from citrus species analyzed in triplicate.

Table 4.9: Total flavonoid contents (CE mg/mL) of the essential oils from fruit peels of different *citrus* species.

Citrus species	TFC (CE mg/mL)
Mausami	30.81±0.18
Fruiter	16.86±0.27
Kinnow	47.09±0.41

Results are mean ±S.D of three peel samples from citrus species analyzed in triplicate.

4.10 DPPH radical scavenging activity:

The essential oil in citrus peel is a natural antioxidant source that aids in the prevention of oxidative stress and illness. In the food processing sector, citrus peel essential oil is an excellent substitute for artificial antioxidants. The DPPH assay was used to assess the antioxidant activity of essential oils isolated from the peels of fourteen Citrus species grown in China [194].

Table 4.10 illustrates the DPPH* radical scavenging values for essential oils from several citrus species peels in terms of percentage. values range from 47.09 to 78.70 percent. Mausami (citrus sinensis) received the highest score, which is comparable to Singh et al., (2021) [194]. Citrus peel Essential Oil of C. sinensis displayed high DPPH radical scavenging activity with IC_{50} values of 9.45 l/ml, according to Singh et al., (2021) [194], showing its significant antioxidant

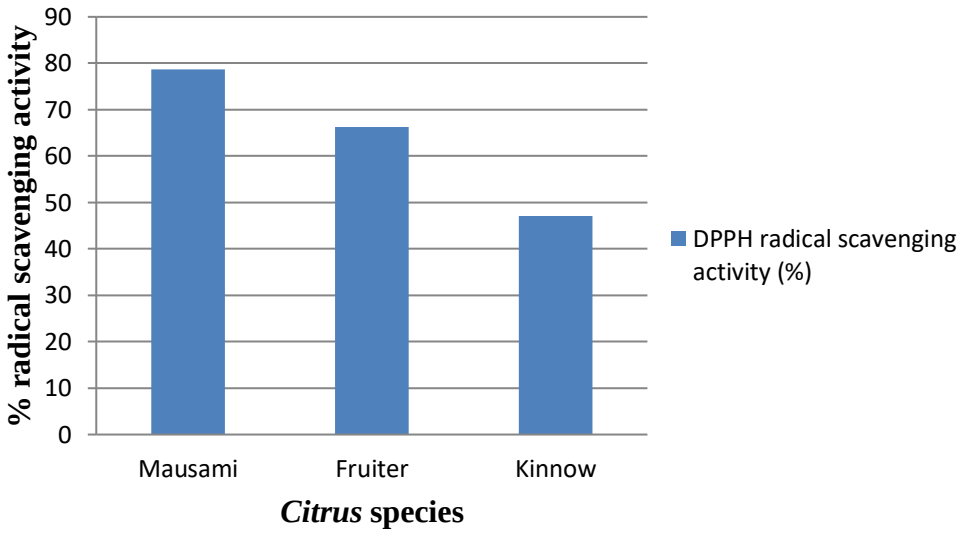
efficiency.

Table 4.10: DPPH radical scavenging activity of the essential oil from fruit peels of different *citrus* species.

Citrus species	DPPH radical scavenging activity (%)
Mausami	78.70±0.11
Fruiter	66.27±0.47
Kinnow	47.09±0.40

Results are mean ±S.D of three peel samples from citrus species analyzed in triplicate.

Fig. 4.10 DPPH radical scavenging activity (%) of peels essential oil of *Citrus* species

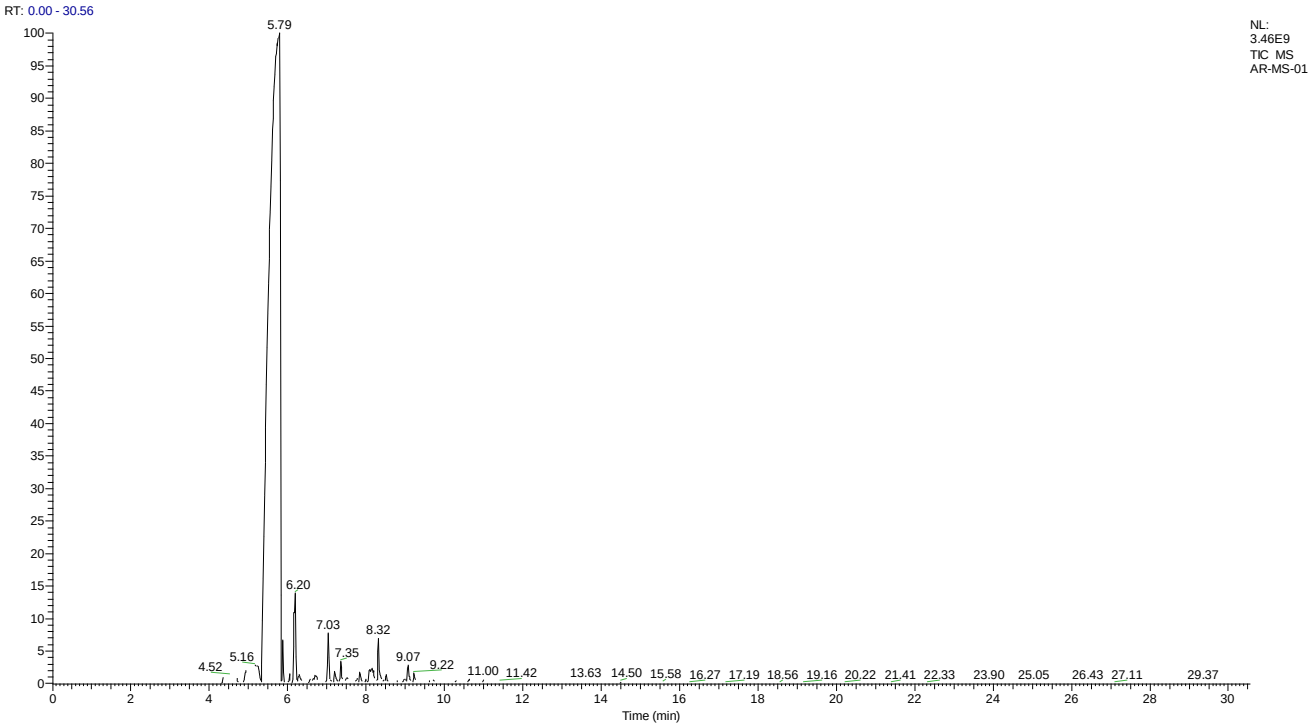


4.11 Chemical Composition (%) of Citrus peel Essential oils:

GC-MS was used to determine the components of the citrus peel essential oils. Essential oils of *Citrus* species were found to contain the following components. The main component discovered in these citrus species was limonene. Essential oil components' chemical structure found in the citrus peel is depicted. The chemical composition and pattern of citrus peel essential oils in citrus species varies depending on genotype, origin, environmental conditions, and essential oil extraction and analysis procedures [194].

Table 4.11: Chemical Composition (%) of essential oil from fruit peels of *citrus reticulata*

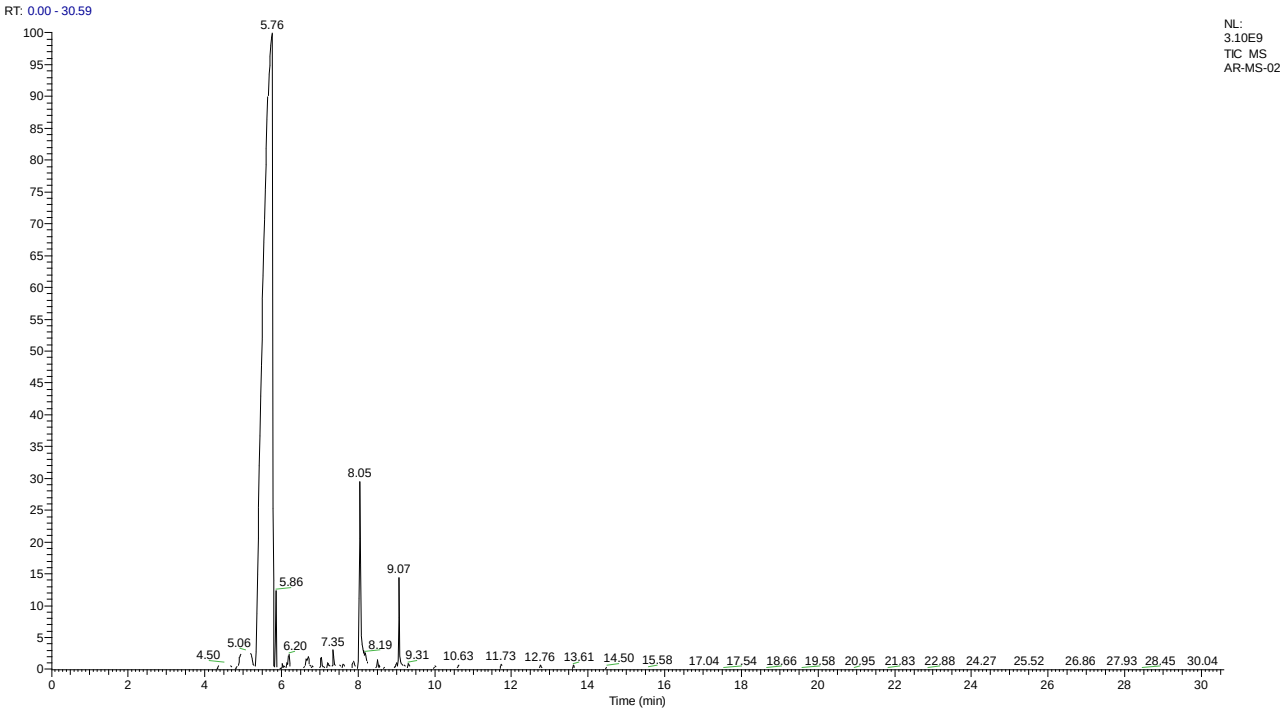
Sr.no	**Constituents**	**Retention. time**	**Content (%)**
1	Beta-sinensal	4.52	1.65
2	Alpha-copaene	5.16	1.49
3	Limonene	5.79	50.42
4	n-Hexadecanoic acid	6.20	5.65
5	Alpha-sinensal	7.03	3.14
6	Myrecene	7.35	3.03
7	Trans-carveol	8.32	3.09
8	Delta-cardinene	9.07	2.53
9	B-copaene	9.22	1.30
10	Others	-	27.7



There was a higher percentage of limonene, palmitic acid, sinensal, and myrecene in citrus reticulata, and our findings mirror those of Singh et al., (2021) [194].

Table 4.12: Chemical Composition (%) of essential oil from fruit peels of *citrus reticulata* Blanco (Fruiter)

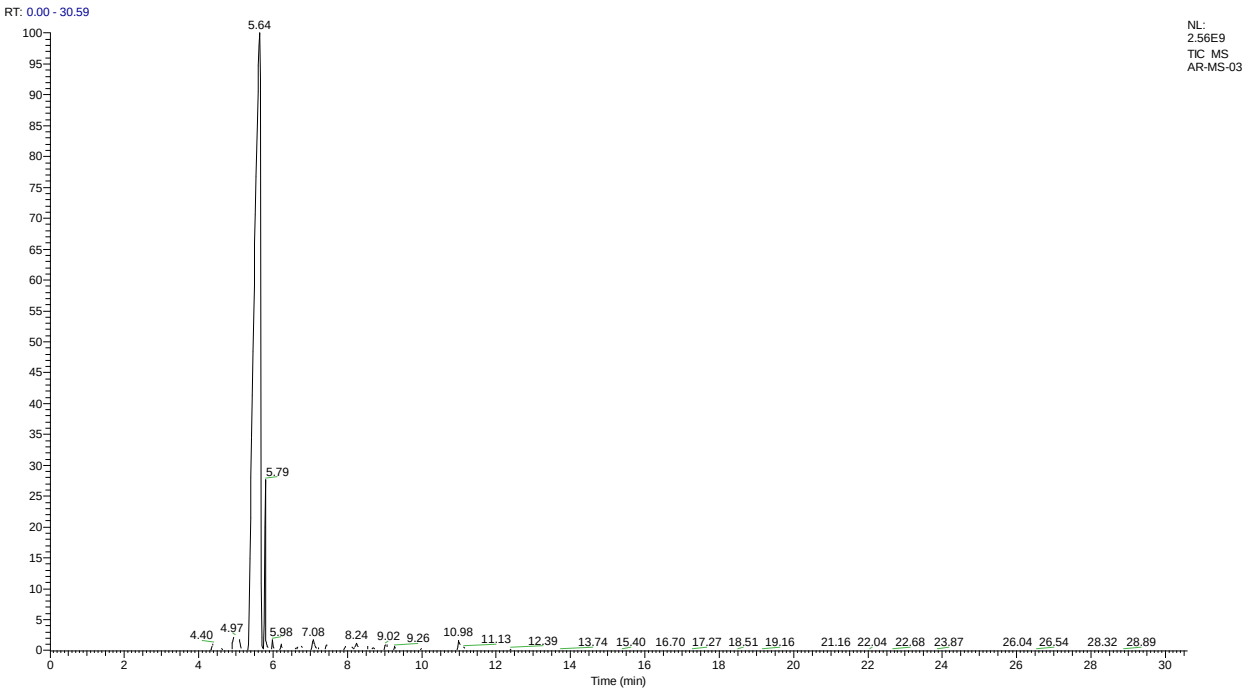
Sr.no	Constituents	Retention. time	Content (%)
1	Neryl Acetate	4.50	1.1
2	Nerol	5.06	2.3
3	Limonene	5.76	46.7
4	Geranyl Acetate	5.86	3.9
5	Citronellal	6.20	1.1
6	Geraniol	7.35	3.5
7	Geranial	8.05	19
8	Beta-caryophyllene	8.19	2.6
9	Neral	9.07	14.5
10	Others	-	5.3



There was a higher amount of limonene, geraniol, neral, and geranyl actate in citrus reticulata Blanco ‘merisol,' and our findings mirror those published by singh et al., (2021) [194].

Table 4.13: Chemical Composition (%) of essential oil from fruit peels of *citrus sinensis* (Mausami)

Sr.no	Constituents	Retention. time	Content (%)
1	Octanal	4.40	2.1
2	Alpha-terpenol	4.97	2.2
3	Limonene	5.64	73.6
4	Linalool	5.79	8.9
5	Neral	5.98	0.8
6	6-Methyl-5-hepten-2-one	7.08	1.9
7	Trans-citral	8.24	1.5
8	Terpene-4-ol	9.02	1
9	Decanal	10.98	0.8
10	Others	-	7.2



In *citrus sinensis*, there was greater percentage of limonene, linalool, alpha terpeneol and octanal and our findings match with the one reported by singh et al., (2021) [194].

SUMMARY

Fresh and processed foods both have a shorter shelf life due to oxidative processes. The majority of foods contain chemicals that are quickly oxidized. Free radicals (atoms or molecules with an unpaired electron in their atomic orbital) are extremely reactive and unstable molecules that degrade the overall quality of food and cause a variety of unfavorable changes in the nutritional composition of foods. An antioxidant is a chemical that slows down the oxidation process or neutralizes free radicals at lower concentrations than the substrate, decreasing the harmful effects. Antioxidants derived from natural resources such as plants, which are pharmacologically effective, are currently generating a lot of interest around the world. Phytochemicals with antioxidant capacity that are naturally present in food are of great interest because of their positive impact on human health by providing protection from oxidative degradation.

The presence of essential organic acids, sugars, and amino acids is the primary reason for citrus fruits' popularity. Fruit is high in vitamins (C, A, and B), minerals, fiber, phenolic compounds, and phytochemicals like carotenoids and limonoids, all of which are beneficial to one's health. Thousands of tons of peels produced during citrus juice processing are typically considered agro-industrial waste, and one-third of all citrus fruits are processed. Citrus peel is a readily available, inexpensive, and cost-effective plant-based source for ailments linked to a sedentary lifestyle. It also contains a lot of nutritional fiber and minerals. As a result, orange peel can be utilized in food as a functional component with possible health benefits and/or as a chemical preservative replacement. Citrus peel bioactive components have a great antioxidative potential against free radicals. Citrus peel is high in antioxidants that occur naturally. Citrus peel has a high antioxidant potential due to the presence of phenolic acids, flavonoids, and ascorbic acid. Citrus peel includes phenolics and flavonoids (hesperidin, narirutin, nobiletin, and tangeritin) that help stabilize free radicals by contributing protons or electrons.

Citrus peel also contains other types of antioxidants, such as essential oils, which are complex combinations of volatile chemicals (such as aldehydes, esters, alcohols, acids and ketones). Essential oils extracted from citrus peels are a cost-effective and environmentally friendly alternative to artificial food preservatives (sodium nitrate or benzoate).

Fruiter (Citrus reticulata Balnco ‘Merisol'), Kinnow (Citrus reticulata), and Mausami (Citrus sinensis) peels were evaluated for antioxidant activity of both bioactive components and essential oils in this study. The peel samples were extracted using four different solvent systems: 100% ethanol, 80 percent ethanol, 50 percent ethanol, and hot distilled water. The clavenger device was used to isolate essential oil using the steam hydro-distillation procedure.

Different assays, such as total phenolic content, total flavonoid content, DPPH radical scavenging activity, and phenolic aci and flavonoid profile, were utilized to assess the antioxidant capability of the peel extracts and essential oils extracted.

The dry matter yields of the citrus extracts ranged from 16.87 to 49.87 percent (g/100g). Ethanol at 80% yielded the highest extraction yield. Total phenolics and total flavonoids were present in significant amounts in all extracts, ranging from 6.40 to 56.40 GAE mg/g DW and 43.76 to 131.76 CE mg/g DW, respectively. The peels had a high rate of DPPH radical scavenging activity, ranging from 43.44 to 84.86 percent. 80 percent ethanol yielded the highest levels of total flavonoids, phenoilcs, and DPPH radical scavenging activity. The highest overall phenolic concentration was found in kinnow peel extracts. Total flavonid content and DPPH radical scavenging activity were highest in Mausami peels. All of the peel samples contained a significant amount of phenolic acids, with Synapic acid being the most prevalent. Synapic acid and caffeic acid were found to be the primary phenolic acids in the peels, with contributions of 0.175-2.080 mg/g and 2.153-24.967 mg/g of extract, respectively, according to HPLC analysis. Individual phenolic acid concentrations in the peels ranged from 2.25-25.494 mg/g of extract. Kinnow with 80 percent ethanol had the most total phenolic acids, whereas Mausami with 100 percent ethanol had the least. All of the peel samples had a significant amount of flavonoids, with myricetin being the most common flavonoid. According to HPLC analysis, the primary flavonoids in the peels were myricetin and kaempferol, which contributed 0.418-7.36 mg/g and 0.063-0.412 mg/g of extract, respectively. The mausami with 80 percent ethanol had the most total flavonoids, whereas the kinnow with 50 percent ethanol had the least. Overall, the results demonstrated that extracts from kinnow peel have stronger antioxidant activity and include more phenolic acids. Mausami has a higher flavonoid content. In terms of extraction efficacy, it was discovered that extracting antioxidants, phenolics, and flavonoids from citrus peels with 80 percent ethanol was more effective.

Mausami (Citrus sinensis) peels yielded the most oil of the Citrus species examined (1.76 percent). Fruiter (C. reticulate Blanco ‘Merisol') peels had a 1.07 percent oil yield, whereas kinnow (C. reticulata) peels had a 0.86 percent oil yield. The essential oils were chemically analyzed using a Gas Chromatographic System (Schimadzu) coupled to Mass Spectrometry. Limonene was discovered to be the most abundant component in all three citrus species. Total phenolic content, total flavonoid content, and DPPH radical scavenging activity are all higher in Citrus sinensis. As a result, it serves as an important antioxidant. The current findings suggest the use of citrus peels as a possible natural antioxidant source for the functional food and nutraceutical industries.

REFERENCES

1. Srinivasan, D., Nathan, S., Suresh, T., & Perumalsamy, P. L. (2001). Antimicrobial activity of certain Indian medicinal plants used in folkloric medicine. *Journal of ethnopharmacology*, *74*(3), 217-220.
2. Rahmatullah, M., Das, A. K., Mollik, M. A. H., Jahan, R., Khan, M., Rahman, T., & Chowdhury, M. H. (2009). An ethnomedicinal survey of Dhamrai sub-district in Dhaka District, Bangladesh. *American Eurasian Journal of Sustainable Agriculture*, *3*(4), 881-888.
3. Khanal, S., Adhikari, A., Tiwari, A., Singh, N. B., & Subedi, R. (2019). Efficiency of botanical extract against maize weevil Sitophilus zeamais (Motschulsky, 1855)(Coleoptera: Curculionidae). *World News of Natural Sciences*, *24*, 1-8.
4. Brielmann, H. L., Setzer, W. N., Kaufman, P. B., Kirakosyan, A., & Cseke, L. J. (2006). Phytochemicals: The chemical components of plants. *Natural products from plants*, *2*, 1-49.
5. Chukwuebuka, E., & Chinenye, I. J. (2015). Biological functions and anti-nutritional effects of phytochemicals in living system. *J Pharm Biol Sci*, *10*(2), 10-19.
6. Webb, D. (2013). Phytochemicals' role in good health. *Today's Dietitian*, *15*(9), 70.
7. Breslin, A. (2017). The chemical composition of green plants. *Sciencing*, *Leaf Group Ltd*, *76*.
8. Casadey, R., Challier, C., Senz, A., & Criado, S. (2019). Antioxidant ability of tyrosol and derivative-compounds in the presence of O2 (1Δg)-species. Studies of synergistic antioxidant effect with commercial antioxidants. *Food chemistry*, *285*, 275-281.
9. Papas, A. M. (2019). Diet and antioxidant status. *Antioxidant status, diet, nutrition, and health*, 89-106.
10. Chanda, S. V., & Nagani, K. V. (2010). Antioxidant capacity of Manilkara zapota L. leaves extracts evaluated by four in vitro methods. *Nature and science*, *8*(10), 260-266.
11. Adámez, J. D., Samino, E. G., Sánchez, E. V., & González-Gómez, D. (2012). In vitro estimation of the antibacterial activity and antioxidant capacity of aqueous extracts from grape-seeds (Vitis vinifera L.). *Food Control*, *24*(1-2), 136-141.

12. Botterweck, A. A., Schouten, L. J., Volovics, A., Dorant, E., & van den Brandt, P. A. (2000). Trends in incidence of adenocarcinoma of the oesophagus and gastric cardia in ten European countries. *International journal of epidemiology*, *29*(4), 645-654.
13. Scalbert, A., & Williamson, G. (2000). Dietary intake and bioavailability of polyphenols. *The Journal of nutrition*, *130*(8), 2073S-2085S.
14. Kris-Etherton, P. M., Hecker, K. D., Bonanome, A., Coval, S. M., Binkoski, A. E., Hilpert, K. F., ... & Etherton, T. D. (2002). Bioactive compounds in foods: their role in the prevention of cardiovascular disease and cancer. *The American journal of medicine*, *113*(9), 71-88.
15. Cieślik, E., Gręda, A., & Adamus, W. (2006). Contents of polyphenols in fruit and vegetables. *Food chemistry*, *94*(1), 135-142
16. Ahmad, I., & Aqil, F. (2007). In vitro efficacy of bioactive extracts of 15 medicinal plants against ESβL-producing multidrug-resistant enteric bacteria. *Microbiological Research*, *162*(3), 264-275.
17. Kasote, D. M., Katyare, S. S., Hegde, M. V., & Bae, H. (2015). Significance of antioxidant potential of plants and its relevance to therapeutic applications. *International journal of biological sciences*, *11*(8), 982.
18. Seigler, D. S. (1998). *Plant secondary metabolism*. Springer Science & Business Media.
19. Crozier, A., Clifford, M. N., & Ashihara, H. (Eds.). (2008). *Plant secondary metabolites: occurrence, structure and role in the human diet*. John Wiley & Sons.
20. Ornston, L. N., & Yeh, W. K. (1979). Origins of metabolic diversity: evolutionary divergence by sequence repetition. *Proceedings of the National Academy of Sciences*, *76*(8), 3996-4000.
21. Lattanzio, V. (2013). Phenolic Compounds: Introduction 50. *Nat. Prod*, 1543-1580.
22. Lehfeldt, C., Shirley, A. M., Meyer, K., Ruegger, M. O., Cusumano, J. C., Viitanen, P. V., ... & Chapple, C. (2000). Cloning of the SNG1 gene of Arabidopsis reveals a role for a serine carboxypeptidase-like protein as an acyltransferase in secondary metabolism. *The Plant Cell*, *12*(8), 1295-1306.
23. Manach, C., Scalbert, A., Morand, C., Rémésy, C., & Jiménez, L. (2004). Polyphenols: food sources and bioavailability. *The American journal of clinical nutrition*, *79*(5), 727-747.

24. Aguilera, Y., Martin-Cabrejas, M. A., & de Mejia, E. G. (2016). Phenolic compounds in fruits and beverages consumed as part of the mediterranean diet: their role in prevention of chronic diseases. *Phytochemistry reviews*, *15*(3), 405-423.
25. Udvardi, M., Parniske, M., Spaink, H., Saalbach, G., Webb, J., & Chiurazzi, M. (2005). *Lotus japonicus handbook* (pp. 1-384). A. J. Márquez, & J. Stougaard (Eds.). Dordrecht, The Netherlands:: Springer.
26. Naczk, M., & Shahidi, F. (2004). Extraction and analysis of phenolics in food. *Journal of chromatography A*, *1054*(1-2), 95-111.
27. González, C. S. M. (2002). Compuestos polifenólicos: Estructura y clasificación. Presencia en alimentos y consumo. Biodisponibilidad y metabolismo. *Alimentaria: Revista de tecnología e higiene de los alimentos*, (329), 19-28.
28. Gülçin, İ. (2006). Antioxidant activity of caffeic acid (3, 4-dihydroxycinnamic acid). *Toxicology*, *217*(2-3), 213-220.
29. Clifford, M. N., & Scalbert, A. (2000). Ellagitannins, occurrence in food, bioavailability and cancer prevention. *J Food Sci Agric*, *80*, 1118-25.
30. Gülçin, I., Bursal, E., Şehitoğlu, M. H., Bilsel, M., & Gören, A. C. (2010). Polyphenol contents and antioxidant activity of lyophilized aqueous extract of propolis from Erzurum, Turkey. *Food and Chemical Toxicology*, *48*(8-9), 2227-2238.
31. Coban, T. A., Beydemir, Ş., Gülçin, İ., & Ekinci, D. (2007). Morphine inhibits erythrocyte carbonic anhydrase in vitro and in vivo. *Biological and pharmaceutical bulletin*, *30*(12), 2257-2261.
32. Beyza Öztürk Sarıkaya, S., Gülçin, İ., & Supuran, C. T. (2010). Carbonic anhydrase inhibitors: Inhibition of human erythrocyte isozymes I and II with a series of phenolic acids. *Chemical biology & drug design*, *75*(5), 515-520.
33. Topal, F. (2011). In vitro inhibition of a-carbonic anhydrase isozymes by some phenolic compounds.
34. Innocenti, A., Gülçin, I., Scozzafava, A., & Supuran, C. T. (2010). Carbonic anhydrase inhibitors. Antioxidant polyphenols effectively inhibit mammalian isoforms I–XV. *Bioorganic & medicinal chemistry letters*, *20*(17), 5050-5053.
35. Şentürk, M., Gülçin, İ., Beydemir, Ş., Küfrevioğlu, Ö. İ., & Supuran, C. T. (2011). In vitro inhibition of human carbonic anhydrase I and II isozymes with natural phenolic compounds. *Chemical biology & drug design*, *77*(6), 494-499.
36. Robbins, R. J. (2003). Phenolic acids in foods: an overview of analytical methodology. *Journal of agricultural and food chemistry*, *51*(10), 2866-2887.

37. Ross, K. A., Beta, T., & Arntfield, S. D. (2009). A comparative study on the phenolic acids identified and quantified in dry beans using HPLC as affected by different extraction and hydrolysis methods. *Food Chemistry*, *113*(1), 336-344.
38. Topal, M., & GÜLÇİN, İ. (2014). Rosmarinic acid: a potent carbonic anhydrase isoenzymes inhibitor. *Turkish journal of chemistry*, *38*(5), 894-902.
39. Gülçin, İ., Scozzafava, A., Supuran, C. T., Koksal, Z., Turkan, F., Çetinkaya, S., ... & Alwasel, S. H. (2016). Rosmarinic acid inhibits some metabolic enzymes including glutathione S-transferase, lactoperoxidase, acetylcholinesterase, butyrylcholinesterase and carbonic anhydrase isoenzymes. *Journal of enzyme inhibition and medicinal chemistry*, *31*(6), 1698-1702.
40. Chen, J. H., & Ho, C. T. (1997). Antioxidant activities of caffeic acid and its related hydroxycinnamic acid compounds. *Journal of agricultural and food chemistry*, *45*(7), 2374-2378.
41. Croft, K. D. (1998). The chemistry and biological effects of flavonoids and phenolic acids a. *Annals of the New York Academy of Sciences*, *854*(1), 435-442.
42. White, P. J., & Xing, Y. (1997). *Antioxidants from cereals and legumes* (pp. 25-63). AOCS Press: Champaign, IL.
43. Macheix, J. J., Fleuriet, A., & Billot, J. (2018). *Fruit phenolics*. CRC press.
44. Bors, W. (1996). Flavonoids and polyphenols: chemistry and biology. *Handbook of antioxidants*, *409*.
45. Takahama, U. (1985). Inhibition of lipoxygenase-dependent lipid peroxidation by quercetin: mechanism of antioxidative function. *Phytochemistry*, *24*(7), 1443-1446.
46. Takahama, U. (1985). Inhibition of lipoxygenase-dependent lipid peroxidation by quercetin: mechanism of antioxidative function. *Phytochemistry*, *24*(7), 1443-1446.
47. Husain, S. R., Cillard, J., & Cillard, P. (1987). Hydroxyl radical scavenging activity of flavonoids. *Phytochemistry*, *26*(9), 2489-2491.
48. Takahama, U. (1984). Hydrogen peroxide-dependent oxidation of flavonols by intact spinach chloroplasts. *Plant physiology*, *74*(4), 852-855.
49. RAMANATHAN, L., & DAS, N. P. (1993). Effect of natural copper chelating compounds on the pro-oxidant activity of ascorbic acid in steam-cooked ground fish. *International journal of food science & technology*, *28*(3), 279-288.
50. Hu, C., & Ding, Y. (1996). Antioxidant effect of flavonoids in different oxidation systems. *Food Fermentation Industries*, *22*, 46-53.

51. Jovanovic, S. V., Steenken, S., Tosic, M., Marjanovic, B., & Simic, M. G. (1994). Flavonoids as antioxidants. *Journal of the American Chemical Society*, *116*(11), 4846-4851.
52. Lakhanpal, P., & Rai, D. K. (2007). Quercetin: a versatile flavonoid. *Internet Journal of Medical Update*, *2*(2), 22-37.
53. Markulin, L., Corbin, C., Renouard, S., Drouet, S., Gutierrez, L., Mateljak, I., ... & Lainé, E. (2019). Pinoresinol–lariciresinol reductases, key to the lignan synthesis in plants. *Planta*, *249*(6), 1695-1714
54. Gulcin, İ. (2020). Antioxidants and antioxidant methods: An updated overview. *Archives of toxicology*, *94*(3), 651-715.
55. Biais, B., Krisa, S., Cluzet, S., Da Costa, G., Waffo-Teguo, P., Mérillon, J. M., & Richard, T. (2017). Antioxidant and cytoprotective activities of grapevine stilbenes. *Journal of agricultural and food chemistry*, *65*(24), 4952-4960.
56. Krawczyk, H. (2019). The stilbene derivatives, nucleosides, and nucleosides modified by stilbene derivatives. *Bioorganic chemistry*, *90*, 103073.
57. Gülçin, İ. (2010). Antioxidant properties of resveratrol: A structure–activity insight. *Innovative food science & emerging technologies*, *11*(1), 210-218.
58. de Faria, E. L., Shabudin, S. V., Claúdio, A. F. M., Válega, M., Domingues, F. M., Freire, C. S., ... & Freire, M. G. (2017). Aqueous solutions of surface-active ionic liquids: remarkable alternative solvents to improve the solubility of triterpenic acids and their extraction from biomass. *ACS sustainable chemistry & engineering*, *5*(8), 7344-7351.
59. Azmir, J., Zaidul, I. S. M., Rahman, M. M., Sharif, K. M., Mohamed, A., Sahena, F., ... & Omar, A. K. M. (2013). Techniques for extraction of bioactive compounds from plant materials: A review. *Journal of food engineering*, *117*(4), 426-436.
60. Farnsworth, N. R., Akerele, O., Bingel, A. S., Soejarto, D. D., & Guo, Z. (1985). Medicinal plants in therapy. *Bulletin of the world health organization*, *63*(6), 965.
61. Smith, R.M., 2003. Before the injection—modern methods of sample preparation for separation techniques. Journal of Chromatography A 1000 (1–2), 3–27.
62. Soxhlet, F. V. (1879). Die gewichtsanalytische bestimmung des milchfettes. *Dingler's Polytechnisches Journal*, *232*, 461-465.
63. Vankar, P.S., 2004. Essential oils and fragrances from natural sources. Resonance 9 (4), 30–41.

64. Silva, L.V., Nelson, D.L., Drummond, M.F.B., Dufossé, L., Glória, M.B.A., 2005. Comparison of hydrodistillation methods for the deodorization of turmeric. Food Research International 38 (8–9), 1087–1096
65. Putnik, P., Lorenzo, J. M., Barba, F. J., Roohinejad, S., Režek Jambrak, A., Granato, D., ... & Bursać Kovačević, D. (2018). Novel food processing and extraction technologies of high-added value compounds from plant materials. *Foods*, *7*(7), 106.
66. Tiwari, B. K. (2015). Ultrasound: A clean, green extraction technology. *TrAC Trends in Analytical Chemistry*, *71*, 100-109
67. Bindes, M. M. M., Reis, M. H. M., Cardoso, V. L., & Boffito, D. C. (2019). Ultrasound-assisted extraction of bioactive compounds from green tea leaves and clarification with natural coagulants (chitosan and Moringa oleífera seeds). *Ultrasonics sonochemistry*, *51*, 111-119.
68. Redondo, D., Venturini, M. E., Luengo, E., Raso, J., & Arias, E. (2018). Pulsed electric fields as a green technology for the extraction of bioactive compounds from thinned peach by-products. *Innovative food science & emerging technologies*, *45*, 335-343.
69. Puri, M., Sharma, D., & Barrow, C. J. (2012). Enzyme-assisted extraction of bioactives from plants. *Trends in biotechnology*, *30*(1), 37-44.
70. Marathe, S. J., Jadhav, S. B., Bankar, S. B., Dubey, K. K., & Singhal, R. S. (2019). Improvements in the extraction of bioactive compounds by enzymes. *Current opinion in food science*, *25*, 62-72.
71. Rodsamran, P., & Sothornvit, R. (2019). Extraction of phenolic compounds from lime peel waste using ultrasonic-assisted and microwave-assisted extractions. *Food bioscience*, *28*, 66-73.
72. Pimentel-Moral, S., Borrás-Linares, I., Lozano-Sánchez, J., Arráez-Román, D., Martínez-Férez, A., & Segura-Carretero, A. (2019). Supercritical CO2 extraction of bioactive compounds from Hibiscus sabdariffa. *The Journal of Supercritical Fluids*, *147*, 213-221.
73. Pereira, D. T. V., Tarone, A. G., Cazarin, C. B. B., Barbero, G. F., & Martínez, J. (2019). Pressurized liquid extraction of bioactive compounds from grape marc. *Journal of Food Engineering*, *240*, 105-113.
74. Aires, A. (2017). Phenolics in foods: extraction, analysis and measurements. *Phenolic Compounds*, 61-88.

75. Chemat, F., Rombaut, N., Sicaire, A. G., Meullemiestre, A., Fabiano-Tixier, A. S., & Abert-Vian, M. (2017). Ultrasound assisted extraction of food and natural products. Mechanisms, techniques, combinations, protocols and applications. A review. *Ultrasonics sonochemistry*, *34*, 540-560.
76. Chemat, F., & Khan, M. K. (2011). Applications of ultrasound in food technology: processing, preservation and extraction. *Ultrasonics sonochemistry*, *18*(4), 813-835.
77. Putnik, P., Kresoja, Ž., Bosiljkov, T., Jambrak, A. R., Barba, F. J., Lorenzo, J. M., ... & Kovačević, D. B. (2019). Comparing the effects of thermal and non-thermal technologies on pomegranate juice quality: A review. *Food Chemistry*, *279*, 150-161.
78. Vilkhu, K., Mawson, R., Simons, L., & Bates, D. (2008). Applications and opportunities for ultrasound assisted extraction in the food industry—A review. *Innovative Food Science & Emerging Technologies*, *9*(2), 161-169.
79. de Moura, J. N., Campbell, K., Mahfuz, A., Jung, S., Glatz, C. E., & Johnson, L. (2008). Enzyme-assisted aqueous extraction of oil and protein from soybeans and cream de-emulsification. *Journal of the American Oil Chemists' Society*, *85*(10), 985-995.
80. Puri, M., Sharma, D., & Barrow, C. J. (2012). Enzyme-assisted extraction of bioactives from plants. *Trends in biotechnology*, *30*(1), 37-44.
81. Nadar, S. S., Rao, P., & Rathod, V. K. (2018). Enzyme assisted extraction of biomolecules as an approach to novel extraction technology: A review. *Food Research International*, *108*, 309-330.
82. Kaderides, K., Papaoikonomou, L., Serafim, M., & Goula, A. M. (2019). Microwave-assisted extraction of phenolics from pomegranate peels: Optimization, kinetics, and comparison with ultrasounds extraction. *Chemical Engineering and Processing-Process Intensification*, *137*, 1-11.
83. Singh, A., Nair, G. R., Liplap, P., Gariepy, Y., Orsat, V., & Raghavan, V. (2014). Effect of dielectric properties of a solvent-water mixture used in microwave-assisted extraction of antioxidants from potato peels. *Antioxidants*, *3*(1), 99-113.
84. Mandal, V., Mohan, Y., & Hemalatha, S. (2007). Microwave assisted extraction—an innovative and promising extraction tool for medicinal plant research. *Pharmacognosy reviews*, *1*(1), 7-18.
85. Valgimigli, L. (2012). Essential oils: an overview on origins, chemistry, properties and uses. *Essential oils as natural food additives*, 24.

86. Bakkali, F., Averbeck, S., Averbeck, D., & Idaomar, M. (2008). Biological effects of essential oils–a review. *Food and chemical toxicology*, *46*(2), 446-475.
87. Adorjan, B., & Buchbauer, G. (2010). Biological properties of essential oils: an updated review. *Flavour and Fragrance Journal*, *25*(6), 407-426.
88. Tepe, B., Daferera, D., Tepe, A. S., Polissiou, M., & Sokmen, A. (2007). Antioxidant activity of the essential oil and various extracts of Nepeta flavida Hub.-Mor. from Turkey. *Food chemistry*, *103*(4), 1358-1364.
89. Rios, J. L., & Recio, M. C. (2005). Medicinal plants and antimicrobial activity. *Journal of ethnopharmacology*, *100*(1-2), 80-84.
90. Dorman, H. D., & Deans, S. G. (2000). Antimicrobial agents from plants: antibacterial activity of plant volatile oils. *Journal of applied microbiology*, *88*(2), 308-316.
91. Pérez Gutierrez, R. O. S. A., Hernández Luna, H., & Hernández Garrido, S. (2006). Antioxidant activity of Tagetes erecta essential oil. *Journal of the Chilean Chemical Society*, *51*(2), 883-886.
92. Mimica-Dukić, N., Božin, B., Soković, M., Mihajlović, B., & Matavulj, M. (2003). Antimicrobial and antioxidant activities of three Mentha species essential oils. *Planta medica*, *69*(05), 413-419.
93. Başer, K. H. C., & Demirci, F. (1994). Essential oils. *Echinophora tenuifolia*.
94. Gilani, A. H., Khan, A. U., Jabeen, Q., Subhan, F., & Ghafar, R. (2005). Antispasmodic and blood pressure lowering effects of Valeriana wallichii are mediated through K+ channel activation. *Journal of ethnopharmacology*, *100*(3), 347-352.
95. Kulisic, T., Radonic, A., Katalinic, V., & Milos, M. (2004). Use of different methods for testing antioxidative activity of oregano essential oil. *Food chemistry*, *85*(4), 633-640.
96. Bousbia, N., Vian, M. A., Ferhat, M. A., Petitcolas, E., Meklati, B. Y., & Chemat, F. (2009). Comparison of two isolation methods for essential oil from rosemary leaves: Hydrodistillation and microwave hydrodiffusion and gravity. *Food chemistry*, *114*(1), 355-362.
97. Donelian, A., Carlson, L. H. C., Lopes, T. J., & Machado, R. A. F. (2009). Comparison of extraction of patchouli (Pogostemon cablin) essential oil with supercritical CO2 and by steam distillation. *The Journal of Supercritical Fluids*, *48*(1), 15-20.

98. Hou, H. S., Bonku, E. M., Zhai, R., Zeng, R., Hou, Y. L., Yang, Z. H., & Quan, C. (2019). Extraction of essential oil from Citrus reticulate Blanco peel and its antibacterial activity against Cutibacterium acnes (formerly Propionibacterium acnes). *Heliyon*, *5*(12), e02947.

99. Satari, B., & Karimi, K. (2018). Citrus processing wastes: Environmental impacts, recent advances, and future perspectives in total valorization. *Resources, Conservation and Recycling*, *129*, 153-167.

100. Fresh, F. C. F. (2017). Processed statistical bulletin 2016. *Food and Agriculture Organization: Rome, Italy*, 66.

101. Miller, E. V., Winston, J. R., & Schomer, H. A. (1940). Physiological studies of plastid pigments in rinds of maturing oranges. *J. Agric. Res*, *60*, 259-267.

102. Davis, W. B. (1932). Deposits of oil in the juice sacs of citrus fruits. *American Journal of Botany*, *19*(2), 101-105.

103. Matlack, M. B. (1931). The juice sac of the orange with some observations on the plastids of citrus. *Journal of the Washington Academy of Sciences*, *21*(17), 437-440.

104. Chavan, P., Singh, A. K., & Kaur, G. (2018). Recent progress in the utilization of industrial waste and by-products of citrus fruits: A review. *Journal of Food Process Engineering*, *41*(8), e12895.

105. Whitney, E. N., & Rolfes, S. R. (1999). Understanding Nutrition. Belmont, CA: West.

106. Steinmetz, K. A., & Potter, J. D. (1991). Vegetables, fruit, and cancer. II. Mechanisms. *Cancer Causes & Control*, *2*(6), 427-442.

107. Chen, X. M., Tait, A. R., & Kitts, D. D. (2017). Flavonoid composition of orange peel and its association with antioxidant and anti-inflammatory activities. *Food chemistry*, *218*, 15-21.

108. Ke, Z., Xu, X., Nie, C., & Zhou, Z. (2015). Citrus flavonoids and human cancers. *Journal of food and nutrition research*, *3*(5), 341-351.

109. Bermejo, A., & Cano, A. (2012). Analysis of nutritional constituents in twenty citrus cultivars from the Mediterranean area at different stages of ripening. *Food and Nutrition Sciences*, *3*(5), 639-650.

110. Codoñer-Franch, P., & Valls-Bellés, V. (2010). Citrus as functional foods. *Current Topics in Nutraceutical Research*, *8*(4).

111. Xu, Q., Chen, L. L., Ruan, X., Chen, D., Zhu, A., Chen, C., ... & Ruan, Y. (2013). The draft genome of sweet orange (Citrus sinensis). *Nature genetics*, *45*(1), 59-66.

112. Lota, M. L., de Rocca Serra, D., Tomi, F., & Casanova, J. (2000). Chemical variability of peel and leaf essential oils of mandarins from Citrus reticulata Blanco. *Biochemical Systematics and Ecology*, *28*(1), 61-78.

113. Aloui, H., Khwaldia, K., Sánchez-González, L., Muneret, L., Jeandel, C., Hamdi, M., & Desobry, S. (2014). Alginate coatings containing grapefruit essential oil or grapefruit seed extract for grapes preservation. *International Journal of Food Science & Technology*, *49*(4), 952-959.

114. Soni, S. L., & Randhawa, G. S. (1970). Changes in chemical constituents of rind of lemon (Citrus limon Burmann) during growth. *Indian Journal of Horticulture*, *27*(3/4), 106-116.

115. Chisholm, M. G., Wilson, M. A., & Gaskey, G. M. (2003). Characterization of aroma volatiles in key lime essential oils (Citrus aurantifolia Swingle). *Flavour and Fragrance Journal*, *18*(2), 106-115

116. Negro, V., Mancini, G., Ruggeri, B., & Fino, D. (2016). Citrus waste as feedstock for bio-based products recovery: Review on limonene case study and energy valorization. *Bioresource Technology*, *214*, 806-815.

117. Casquete, R., Castro, S. M., Martín, A., Ruiz-Moyano, S., Saraiva, J. A., Córdoba, M. G., & Teixeira, P. (2015). Evaluation of the effect of high pressure on total phenolic content, antioxidant and antimicrobial activity of citrus peels. *Innovative Food Science & Emerging Technologies*, *31*, 37-44.

118. Mahato, N., Sharma, K., Sinha, M., & Cho, M. H. (2018). Citrus waste derived nutra-/pharmaceuticals for health benefits: Current trends and future perspectives. *Journal of Functional Foods*, *40*, 307-316.

119. Chavan, P., Singh, A. K., & Kaur, G. (2018). Recent progress in the utilization of industrial waste and by-products of citrus fruits: A review. *Journal of Food Process Engineering*, *41*(8), e12895.

120. Cheigh, C. I., Chung, E. Y., & Chung, M. S. (2012). Enhanced extraction of flavanones hesperidin and narirutin from Citrus unshiu peel using subcritical water. *Journal of Food Engineering*, *110*(3), 472-477.

121. de Moraes Barros, H. R., de Castro Ferreira, T. A. P., & Genovese, M. I. (2012). Antioxidant capacity and mineral content of pulp and peel from commercial cultivars of citrus from Brazil. *Food chemistry*, *134*(4), 1892-1898.

122. Peng, M., Liu, J., Liu, Z., Fu, B., Hu, Y., Zhou, M., ... & Xu, N. (2018). Effect of citrus peel on phenolic compounds, organic acids and antioxidant activity of soy sauce. *LWT*, *90*, 627-635.

123. Ferreira, S. S., Silva, A. M., & Nunes, F. M. (2018). Citrus reticulata Blanco peels as a source of antioxidant and anti-proliferative phenolic compounds. *Industrial Crops and Products*, *111*, 141-148.

124. Nair, A., Kurup Sr, R., Nair, A. S., & Baby, S. (2018). Citrus peels prevent cancer. *Phytomedicine*, *50*, 231-237.

125. Shetty, S. B., Mahin-Syed-Ismail, P., Varghese, S., Thomas-George, B., Kandathil-Thajuraj, P., Baby, D., ... & Devang-Divakar, D. (2016). Antimicrobial effects of Citrus sinensis peel extracts against dental caries bacteria: An in vitro study. *Journal of clinical and experimental dentistry*, *8*(1), e71.

126. Singh, J. P., Kaur, A., Singh, N., Nim, L., Shevkani, K., Kaur, H., & Arora, D. S. (2016). In vitro antioxidant and antimicrobial properties of jambolan (Syzygium cumini) fruit polyphenols. *LWT-Food Science and Technology*, *65*, 1025-1030.

127. Sridharan, B., Mehra, Y., Ganesh, R. N., & Viswanathan, P. (2016). Regulation of urinary crystal inhibiting proteins and inflammatory genes by lemon peel extract and formulated citrus bioflavonoids on ethylene glycol induced urolithic rats. *Food and Chemical Toxicology*, *94*, 75-84.

128. Satari, B., & Karimi, K. (2018). Citrus processing wastes: Environmental impacts, recent advances, and future perspectives in total valorization. *Resources, Conservation and Recycling*, *129*, 153-167.

129. de Moraes Barros, H. R., de Castro Ferreira, T. A. P., & Genovese, M. I. (2012). Antioxidant capacity and mineral content of pulp and peel from commercial cultivars of citrus from Brazil. *Food chemistry*, *134*(4), 1892-1898.

130. Kurowska, E. M., & Manthey, J. A. (2004). Hypolipidemic effects and absorption of citrus polymethoxylated flavones in hamsters with diet-induced hypercholesterolemia. *Journal of agricultural and food chemistry*, *52*(10), 2879-2886.

131. Chen, X. M., Tait, A. R., & Kitts, D. D. (2017). Flavonoid composition of orange peel and its association with antioxidant and anti-inflammatory activities. *Food chemistry*, *218*, 15-21.

132. Kanaze, F. I., Termentzi, A., Gabrieli, C., Niopas, I., Georgarakis, M., & Kokkalou, E. (2009). The phytochemical analysis and antioxidant activity assessment of orange peel (Citrus sinensis) cultivated in Greece–Crete indicates a new commercial source of hesperidin. *Biomedical Chromatography*, *23*(3), 239-249.

133. Bustamante, J., van Stempvoort, S., García-Gallarreta, M., Houghton, J. A., Briers, H. K., Budarin, V. L., ... & Clark, J. H. (2016). Microwave assisted hydro-distillation of essential oils from wet citrus peel waste. *Journal of cleaner production*, *137*, 598-605.

134. Mahato, N., Sharma, K., Koteswararao, R., Sinha, M., Baral, E., & Cho, M. H. (2019). Citrus essential oils: Extraction, authentication and application in food preservation. *Critical reviews in food science and nutrition*, *59*(4), 611-625.

135. González-Mas, M. C., Rambla, J. L., López-Gresa, M. P., Blázquez, M. A., & Granell, A. (2019). Volatile compounds in citrus essential oils: A comprehensive review. *Frontiers in plant science*, *10*, 12.

136. Hosni, K., Zahed, N., Chrif, R., Abid, I., Medfei, W., Kallel, M., ... & Sebei, H. (2010). Composition of peel essential oils from four selected Tunisian Citrus species: Evidence for the genotypic influence. *Food Chemistry*, *123*(4), 1098-1104.

137. Di Rauso Simeone, G., Di Matteo, A., Rao, M. A., & Di Vaio, C. (2020). Variations of peel essential oils during fruit ripening in four lemon (Citrus limon (L.) Burm. F.) cultivars. *Journal of the Science of Food and Agriculture*, *100*(1), 193-200.

138. Gorinstein, S., Martín-Belloso, O., Park, Y. S., Haruenkit, R., Lojek, A., Ĉíž, M., ... & Trakhtenberg, S. (2001). Comparison of some biochemical characteristics of different citrus fruits. *Food chemistry*, *74*(3), 309-315.

139. Bast, A., & Haenen, G. R. (2002). The toxicity of antioxidants and their metabolites. *Environmental toxicology and pharmacology*, *11*(3-4), 251-258.

140. Silalahi, J. (2002). Anticancer and health protective properties of citrus fruit components. *Asia Pacific journal of clinical nutrition*, *11*(1), 79-84.

141. Ahmad, M. M., Iqbal, Z., Anjum, F. M., & Sultan, J. I. (2006). Genetic variability to essential oil composition in four citrus fruit species. *Pakistan Journal of Botany*, *38*(2), 319.

142. Duda-Chodak, A., & Tarko, T. (2007). Antioxidant properties of different fruit seeds and peels. *Acta Scientiarum Polonorum Technologia Alimentaria*, *6*(3), 29-36.

143. Abeysinghe, D. C., Li, X., Sun, C., Zhang, W., Zhou, C., & Chen, K. (2007). Bioactive compounds and antioxidant capacities in different edible tissues of citrus fruit of four species. *Food chemistry*, *104*(4), 1338-1344.

144. Kamal, G. M., Anwar, F., Hussain, A. I., Sarri, N., & Ashraf, M. Y. (2011). Yield and chemical composition of Citrus essential oils as affected by drying pretreatment of peels. *International Food Research Journal*, *18*(4), 1275.

145. Espina, L., Somolinos, M., Lorán, S., Conchello, P., García, D., & Pagán, R. (2011). Chemical composition of commercial citrus fruit essential oils and evaluation of their antimicrobial activity acting alone or in combined processes. *Food control*, *22*(6), 896-902.

146. Goulas, V., & Manganaris, G. A. (2012). Exploring the phytochemical content and the antioxidant potential of Citrus fruits grown in Cyprus. *Food Chemistry*, *131*(1), 39-47.

147. Oboh, G., Ademosun, O., & Lajide, L. (2012). Improvement of the nutritive value and antioxidant properties of citrus peels through Saccharomyces cerevisae solid substrate fermentation for utilization in livestock feed. *Livestock Res Rural Dev*, *24*(1), 1-10.

148. Pereira, M. C., Steffens, R. S., Jablonski, A., Hertz, P. F., Rios, A. D. O., Vizzotto, M., & Flôres, S. H. (2013). Characterization, bioactive compounds and antioxidant potential of three Brazilian fruits. *Journal of Food Composition and Analysis*, *29*(1), 19-24.

149. Al-Juhaimi, F. Y. (2014). Citrus fruits by-products as sources of bioactive compounds with antioxidant potential. *Pak. J. Bot*, *46*(4), 1459-1462.

150. Zhang, H., Xi, W., Yang, Y., Zhou, X., Liu, X., Yin, S., ... & Zhou, Z. (2015). An on-line HPLC-FRSD system for rapid evaluation of the total antioxidant capacity of Citrus fruits. *Food chemistry*, *172*, 622-629.

151. Diab, K. A. (2016). In vitro studies on phytochemical content, antioxidant, anticancer, immunomodulatory, and antigenotoxic activities of lemon, grapefruit, and mandarin citrus peels. *Asian Pacific Journal of Cancer Prevention*, *17*(7), 3559-3567.

152. Putnik, P., Bursać Kovačević, D., Režek Jambrak, A., Barba, F. J., Cravotto, G., Binello, A., ... & Shpigelman, A. (2017). Innovative “green” and novel strategies for the extraction of bioactive added value compounds from citrus wastes—A review. *Molecules*, *22*(5), 680.

153. Fu, M., Xu, Y., Chen, Y., Wu, J., Yu, Y., Zou, B., ... & Xiao, G. (2017). Evaluation of bioactive flavonoids and antioxidant activity in Pericarpium Citri Reticulatae (Citrus reticulata ‘Chachi’) during storage. *Food chemistry*, *230*, 649-656.

154. Toscano-Garibay, J. D., Arriaga-Alba, M., Sánchez-Navarrete, J., Mendoza-García, M., Flores-Estrada, J. J., Moreno-Eutimio, M. A., ... & Ruiz-Pérez, N. J. (2017). Antimutagenic and antioxidant activity of the essential oils of Citrus sinensis and Citrus latifolia. *Scientific reports*, *7*(1), 1-9.

155. Rafiq, S., Kaul, R., Sofi, S. A., Bashir, N., Nazir, F., & Nayik, G. A. (2018). Citrus peel as a source of functional ingredient: a review. *Journal of the Saudi Society of Agricultural Sciences*, *17*(4), 351-358.

156. Sir Elkhatim, K. A., Elagib, R. A., & Hassan, A. B. (2018). Content of phenolic compounds and vitamin C and antioxidant activity in wasted parts of Sudanese citrus fruits. *Food Science & Nutrition*, *6*(5), 1214-1219.

157. Damian, C. (2018). Antioxidant activity of citrus peel and seeds extracts. *International Multidisciplinary Scientific GeoConference: SGEM*, *18*(6.2), 19-26.

158. Kalogeropoulos, N., Konteles, S. J., Troullidou, E., Mourtzinos, I., & Karathanos, V. T. (2009). Chemical composition, antioxidant activity and antimicrobial properties of propolis extracts from Greece and Cyprus. *Food chemistry*, *116*(2), 452-461.

159. Ferreira, S. S., Silva, A. M., & Nunes, F. M. (2018). Citrus reticulata Blanco peels as a source of antioxidant and anti-proliferative phenolic compounds. *Industrial Crops and Products*, *111*, 141-148.

160. Chavan, P., Singh, A. K., & Kaur, G. (2018). Recent progress in the utilization of industrial waste and by-products of citrus fruits: A review. *Journal of Food Process Engineering*, *41*(8), e12895.

161. Peng, M., Liu, J., Liu, Z., Fu, B., Hu, Y., Zhou, M., ... & Xu, N. (2018). Effect of citrus peel on phenolic compounds, organic acids and antioxidant activity of soy sauce. *LWT*, *90*, 627-635.

162. Aboudaou, M., Ferhat, M. A., Hazzit, M., Ariño, A., & Djenane, D. (2019). Solvent free-microwave green extraction of essential oil from orange peel (Citrus sinensis L.): effects on shelf life of flavored liquid whole eggs during storage under commercial retail conditions. *Journal of Food Measurement and Characterization*, *13*(4), 3162-3172.

163. Montero-Calderon, A., Cortes, C., Zulueta, A., Frigola, A., & Esteve, M. J. (2019). Green solvents and Ultrasound-Assisted Extraction of bioactive orange (Citrus sinensis) peel compounds. *Scientific reports*, *9*(1), 1-8.

164. Jahanban-Esfahlan, A., Ostadrahimi, A., Tabibiazar, M., & Amarowicz, R. (2019). A comparative review on the extraction, antioxidant content and antioxidant potential of different parts of walnut (Juglans regia L.) fruit and tree. *Molecules*, *24*(11), 2133.

165. Dong, X., Hu, Y., Li, Y., & Zhou, Z. (2019). The maturity degree, phenolic compounds and antioxidant activity of Eureka lemon [Citrus limon (L.) Burm. f.]: A negative correlation between total phenolic content, antioxidant capacity and soluble solid content. *Scientia Horticulturae*, *243*, 281-289.

166. Gómez-Mejía, E., Rosales-Conrado, N., León-González, M. E., & Madrid, Y. (2019). Citrus peels waste as a source of value-added compounds: Extraction and quantification of bioactive polyphenols. *Food chemistry*, *295*, 289-299.

167. Azman, N. F. I. N., Azlan, A., Khoo, H. E., & Razman, M. R. (2019). Antioxidant properties of fresh and frozen peels of citrus species. *Current Research in Nutrition and Food Science Journal*, *7*(2), 331-339.

168. Anticona, M., Blesa, J., Frigola, A., & Esteve, M. J. (2020). High biological value compounds extraction from citrus waste with non-conventional methods. *Foods*, *9*(6), 811.

169. Bora, H., Kamle, M., Mahato, D. K., Tiwari, P., & Kumar, P. (2020). Citrus essential oils (CEOs) and their applications in food: An overview. *Plants*, *9*(3), 357.

170. Singh, B., Singh, J. P., Kaur, A., & Singh, N. (2020). Phenolic composition, antioxidant potential and health benefits of citrus peel. *Food Research International*, *132*, 109114.

171. Šafranko, S., Ćorković, I., Jerković, I., Jakovljević, M., Aladić, K., Šubarić, D., & Jokić, S. (2021). Green Extraction Techniques for Obtaining Bioactive Compounds from Mandarin Peel (Citrus unshiu var. Kuno): Phytochemical Analysis and Process Optimization. *Foods*, *10*(5), 1043.

172. Czech, A., Malik, A., Sosnowska, B., & Domaradzki, P. (2021). Bioactive substances, heavy metals, and antioxidant activity in whole fruit, peel, and pulp of citrus fruits. *International Journal of Food Science*, *2021*.

173. Lu, S. Y., Chu, Y. L., Sridhar, K., & Tsai, P. J. (2021). Effect of ultrasound, high-pressure processing, and enzymatic hydrolysis on carbohydrate hydrolyzing enzymes and antioxidant activity of lemon (Citrus limon) flavedo. *LWT*, *138*, 110511.

174. Hussain, A. I., Chatha, S. A., Noor, S., Khan, Z. A., Arshad, M. U., Rathore, H. A., & Sattar, M. Z. (2012). Effect of extraction techniques and solvent systems on the extraction of antioxidant components from peanut (Arachis hypogaea L.) hulls. *Food Analytical Methods*, *5*(4), 890-896.

175. Zhuang, Y., Chen, L., Sun, L., & Cao, J. (2012). Bioactive characteristics and antioxidant activities of nine peppers. *Journal of functional foods*, *4*(1), 331-338.

176. Javed, S., Javaid, A., Nawaz, S., Saeed, M. K., Mahmood, Z., Siddiqui, S. Z., & Ahmad, R. (2014). Phytochemistry, GC-MS analysis, antioxidant and antimicrobial potential of essential oil from five citrus species. *Journal of Agricultural Science*, *6*(3), 201.

177. Hussain, A. I., Anwar, F., Sherazi, S. T. H., & Przybylski, R. (2008). Chemical composition, antioxidant and antimicrobial activities of basil (Ocimum basilicum) essential oils depends on seasonal variations. *Food chemistry*, *108*(3), 986-995.

178. Hussain, A.I., F. Anwar, P.S. Nigam, M. Ashraf and A.H. Gilani, 2010. Seasonal variation in content, chemical composition and antimicrobial and cytotoxic activities of essential oils from four Mentha species. J. Sci. Food Agric., 90: 1827-1836.

179. Kim, J. S. (2013). Preliminary evaluation for comparative antioxidant activity in the water and ethanol extracts of dried citrus fruit (Citrus unshiu) peel using chemical and biochemical in vitro assays.

180. Kumar, K. A., Narayani, M., Subanthini, A., & Jayakumar, M. (2011). Antimicrobial activity and phytochemical analysis of citrus fruit peels-utilization of fruit waste.

181. Sultana, B., Anwar, F., & Ashraf, M. (2009). Effect of extraction solvent/technique on the antioxidant activity of selected medicinal plant extracts. *Molecules*, *14*(6), 2167-2180.

182. Liew, S. S., Ho, W. Y., Yeap, S. K., & Sharifudin, S. A. B. (2018). Phytochemical composition and in vitro antioxidant activities of Citrus sinensis peel extracts. *PeerJ*, *6*, e5331.

183. Zhang, Y., Sun, Y., Xi, W., Shen, Y., Qiao, L., Zhong, L., ... & Zhou, Z. (2014). Phenolic compositions and antioxidant capacities of Chinese wild mandarin (Citrus reticulata Blanco) fruits. *Food chemistry*, *145*, 674-680.

184. Ghafar, M. F., Prasad, K. N., Weng, K. K., & Ismail, A. (2010). Flavonoid, hesperidine, total phenolic contents and antioxidant activities from Citrus species. *African Journal of Biotechnology*, *9*(3).

185. Li, B. B., Smith, B., & Hossain, M. M. (2006). Extraction of phenolics from citrus peels: I. Solvent extraction method. *Separation and Purification Technology*, *48*(2), 182-188.

186. Canan, I., GÜNDOĞDU, M., Seday, U., Oluk, C. A., KARAŞAHİN, Z., EROĞLU, E. Ç., ... & ÜNLÜ, M. (2016). Determination of antioxidant, total phenolic, total carotenoid, lycopene, ascorbic acid, and sugar contents of Citrus species and mandarin hybrids. *Turkish Journal of Agriculture and Forestry*, *40*(6), 894-899.

187. Fejzić, A., & Ćavar, S. (2014). Phenolic compounds and antioxidant activity of some citruses. *Bulletin of the Chemists and Technologists of Bosnia and Herzegovina*, *42*, 1-4.

188. Asjad, H. M. M., Akhtar, M. S., Bashir, S., Din, B., Gulzar, F., Khalid, R., & Asad, M. (2013). Phenol, flavonoid contents and antioxidant activity of six common citrus plants in Pakistan. *Journal of Pharmaceutical and Cosmetic Sciences*, *1*(1), 1-5.

189. Ghasemi, K., Ghasemi, Y., & Ebrahimzadeh, M. A. (2009). Antioxidant activity, phenol and flavonoid contents of 13 citrus species peels and tissues. *Pak J Pharm Sci*, *22*(3), 277-281.

190. Chen, Q., Wang, D., Tan, C., Hu, Y., Sundararajan, B., & Zhou, Z. (2020). Profiling of flavonoid and antioxidant activity of fruit tissues from 27 Chinese local citrus cultivars. *Plants*, *9*(2), 196.

191. Anwar, F., Naseer, R., Bhanger, M. I., Ashraf, S., Talpur, F. N., & Aladedunye, F. A. (2008). Physico-chemical characteristics of citrus seeds and seed oils from Pakistan. *Journal of the American Oil Chemists' Society*, *85*(4), 321-330.

192. Weiss, E. A. (1997). *Essential oil crops*. Cab International.

193. Palazzolo, E., Laudicina, V. A., & Germanà, M. A. (2013). Current and potential use of citrus essential oils. *Current Organic Chemistry*, *17*(24), 3042-3049.

194. Singh, B., Singh, J. P., Kaur, A., & Yadav, M. P. (2021). Insights into the chemical composition and bioactivities of citrus peel essential oils. *Food Research International*, 110231.