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ANTIOXIDANT POTENTIAL AND PHYTOCHEMICAL CONTENT FROM PEELS OF THREE DIFFERENT CITRUS SPECIES: A MINI REVIEW

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Abstract: The *Rutaceae* family includes various species of citrus, with a significant percentage of citrus peel considered waste. These peels contain natural compounds, such as phenolic compounds and carotenoids, that have been found to enhance health. Consequently, multiple studies have been conducted to identify the beneficial phytochemicals present in citrus peels, including those from *Citrus limon*, *Citrus sinensis*, and *Citrus hystrix*. However, limited research has been carried out to compare these three citrus peels' antioxidant and phytochemical content. This study aims to review existing research on the antioxidant and phytochemical contents of the peels of these three citrus species. Information was gathered from electronic databases, including Science Direct, Scopus, and Web of Science, from 2016 to 2021. The results indicated that the peels of Citrus limon, Citrus sinensis, and Citrus hystrix exhibited different levels of antioxidant activity, such as the ability to scavenge free radicals and ferric-reducing antioxidant power. Additionally, the phytochemical contents, such as tannin, saponin, TPC, and TFC, showed varying amounts among the three citrus peels. These differences can be attributed to the bioactive compounds present in each peel, as well as the solvent and extraction method used. Despite the variations in antioxidant activity and phytochemical content, these findings suggest that citrus peels are beneficial and have the potential to serve as natural antioxidants. However, further research is necessary to fully realise the benefits of these peels.

Keywords: Antioxidant, *Citrus hystrix*, *Citrus limon*, *Citrus sinensis*, phytochemical.

Introduction

The genus Citrus belongs to the Rutaceae family and comprises over 40 different species. These species produce the world's most plentiful fruits, which are considered to contain significant beneficial phytochemicals. Some citrus examples that are well thought out as the primary industrialised citrus crops and are well-known for nutritional properties include lemons, oranges, mandarins, grapefruits, and limes (Satari & Karimi, 2018). In China, dried citrus peel is also known as 'Chenpi'. These peels are known to be a natural source of antioxidants and have been used as a traditional Chinese remedy to heal indigestion and inflammatory syndromes. According to Singh *et al*. (2020) citrus peels typically account for 40 to 50% of the total mass of citrus fruits produced and are widely regarded as a waste product. However, these peels contain phenolic compounds and carotenoids that are known and believed to be a significant source of naturally occurring health-enhancing compounds (Singh *et al*., 2020). Thus, several studies have been done to identify the beneficial phytochemicals found in the peels of citrus species and these include the peels of *Citrus limon*, *Citrus sinensis* and *Citrus hystrix*.

Citrus limon, also known as lemon, is a 3 to 5-meter-tall shrub or tree with evergreen leaves and edible yellow fruits from the family Rutaceae. The Citrus genus is regarded as one of the most important taxonomic subunits in the Rutaceae family. The fruit, in particular, the juice and essential oil extracted from *C. limon* serves as the primary raw material of the species. Although lemon fruit is very well known for having excellent nutritional value, precious biological activities are often overlooked in modern cosmetology and phytotherapy. Lemon juice, or *Citrus limon* fruit juice, was traditionally used as a common cure for scurvy until the recognition of vitamin C. Studies have now confirmed the traditional use of *Citrus limon*, which has been regarded as a natural remedy since ancient times (Klimek-szczykutowicz *et al*., 2020).

One of the major citrus cultivar classes is sweet orange, scientifically known as *Citrus sinensis*. In general, it accounts for approximately 70% of the overall yearly production of citrus species. While sweet orange is native to Asia, it is now widely distributed in the Pacific and warm regions of the globe. Sweet orange is a blooming evergreen tree. Typically, *Citrus sinensis* trees are around 9 to 10 metres tall, with large spines on the branches. It consists of two regions that are anatomically classified as the pericarp or the peel, the pulp also known as the endocarp with juice sac glands and the rind or skin. Due to the strong natural antioxidant that helps develop the immune system of the body, sweet orange is consumed as a great source of vitamin C throughout the world (Favela-Hernández *et al*., 2016).

Citrus hystrix, also widely referred to as kaffir lime, is commonly cultivated in Thailand, and the natives call it Makrut. Even though it is extensively cultivated in Thailand, kaffir lime is also broadly grown and distributed in many other countries, including Southeast Asia. The leaves of *Citrus hystrix* have a dark green colour and a shiny sheen. For different flavouring purposes, such as seasoning or preparing savoury curry pastes, the aromatic leaves and peels are typically used as spices. It was also used as a common medicine in Thailand to protect healthy gums and teeth. Furthermore, it has been used as a remedy for scurvy. According to Anuchapreeda *et al*. (2020), *Citrus hystrix* peel extracts contain a variety of chemical components, the most important of which are β-pipene, limonene, sabinene and citronellal. Several studies have also documented the various biological activities of kaffir lime peel and leaf as a natural source of antioxidants and phenolic compounds (Anuchapreeda *et al*., 2020).

Antioxidant refers to any substance that will significantly delay or prevent oxidation of an oxidisable substrate such as proteins, lipids, carbohydrates, and DNA (Rubio *et al*., 2016). The major role of antioxidants is to function as a defence mechanism by removing the free radicals that are known to have destructive effects. It also can prevent oxidative stress as well as repair the molecules that are damaged; therefore, it is also known as a reactive oxygen species (ROS) scavenger (Ruwali *et al*., 2019). Free radicals may exist internally in cells and tissues through inflammation or disease. It can also be generated externally from sources such as irradiation, drugs, foods, pollution or because of decreased protective capacity. Hence, if there is an increase in the production of free radicals, it may induce oxidative damage in our bodies.

Antioxidants are bioactive substances that can be obtained from plant-based foods. Other plant-derived bioactive substances include flavonoids, alkaloids, carotenoids, tannins, and phenolic compounds. In order to uncover these medically active substances found in plants, phytochemical screening or analysis is necessary. The term phytochemical refers to a range of therapeutic plant-derived compounds. Some of the well-known examples of plant-derived compounds with therapeutic activities also have anticarcinogenic, antimutagenic, anti-inflammatory and antioxidative properties (Frank *et al*., 2020). Akhtar *et al*. (2018) pointed out that because of the vast and diverse assortment of organic compounds that can produce a definite physiological action in the human body, medicinal plants have been used for thousands of years to heal human diseases. Alkaloids, flavonoids, saponins, tannins, terpenoids, and phenolic compounds are the most significant compounds that are tested because of their therapeutic performance and low toxicity (Akhtar *et al*., 2018).

According to Walia *et al*. (2019), the classification of the phytochemical components exhibited in medicinal plants, qualitative and quantitative analyses are essential. Phytochemical screening may be divided into two main categories, which are qualitative and quantitative analyses of phytochemicals (Walia *et al*., 2019). There are various types of qualitative analyses of phytochemicals that researchers have done. These types of analyses include tests for carbohydrates, tannins, saponins, alkaloids, flavonoids, glycosides, phenols, and quinones. Different methods were used for the quantitative analysis of phytochemicals, and these include total phenolic content, total flavonoid content, gas chromatography-mass spectrometry (GCMS), and high-performance liquid chromatography (HPLC). The presence of specific bioactive constituents is attributed to the therapeutic benefit of plants. Bioactive compounds are classified as food components which affect the cellular or physiological activities of either humans or animals that consume these compounds.

Methodology

The objective of this study is to review the antioxidant and phytochemical contents of peels of three different citrus species. The development of research topics was critical for determining the direction of the review and the method for identifying and selecting relevant articles. Hence, the research questions for this review are: (1) What is the level of antioxidant activity exhibited from peels of *Citrus limon, Citrus sinensis* and *Citrus hystrix*? (2) What are the levels of total phenolic content and total flavonoid content present in peels of *Citrus limon, Citrus sinensis* and *Citrus hystrix*? Based on these research questions, three databases were used in this study: SCOPUS (life and health sciences), Science Direct (science and medicine), and Web of Science (WoS) from the year 2016 to 2021. Three databases were believed to be sufficient to rule out bias and would be able to access all the relevant papers within the area of interest.

Results and Discussion

Antioxidant Potential from Peels of Citrus Species

Two types of antioxidants are endogenous antioxidants known to be generated by the body and exogenous antioxidants, which can be obtained from outside of the body (Irshad & Chaudhuri, 2002). There are many examples of exogenous antioxidants such as vitamin C, vitamin E, carotenoids and polyphenols, that can be obtained easily by consuming plant-based food. Citrus fruits are rich sources of phytochemicals that are useful, such as vitamins A, C and E, minerals, flavonoids, coumarins, limonoids, carotenoids, and pectins (Zou *et al*., 2016). Thus, this paper focused on reviewing the antioxidant potential exhibited by *Citrus limon, Citrus sinensis* and *Citrus hystrix* peels in terms of the ability to scavenge free radicals by using common methods such as 2,2-diphenyl-1-(2,4,6-tri nitrophenyl) hydrazyl (DPPH) radical scavenging activity, ferric reducing power and 2,2′-Azinobis-(3-Ethylbenzthiazolin-6- Sulfonic Acid) (ABTS) radical reduction activity.

Free Radical Scavenging Activity

Free radicals are more commonly classified as reactive nitrogen species (RNS) or reactive oxygen species (ROS) in the diverse fields of biology and medicine (Poprac *et al*., 2017). Molecular particles or molecules containing one or more unpaired electrons are known as free radicals. Their existence normally makes them extremely reactive. It is very crucial in sustaining the balance between the beneficial and harmful effects of ROS in healthy organisms and this can be accomplished by a set of mechanisms known as redox regulation. Generally, antioxidants can counteract the harmful effects of free radicals and it is thought that plant-based foods are rich in antioxidants. Those antioxidants can scavenge free radicals by interrupting the radical chain reactions or stop the reactive oxidants from being generated (Lalhminghlui & Jagetia, 2018). DPPH radical scavenging assay is considered one of the methods that are widely used to measure the antioxidant activity of plant extracts due to the relatively short time required for the analysis. In the DPPH assay, violet colour DPPH solution is reduced to a yellow-coloured product, diphenyl picryl hydrazine, by the addition of the extract in a concentration-dependent manner.

According to Papoutsis *et al*. (2016) the extraction process was performed on peels of *Citrus limon* by dividing the samples into seven different extracts which were then used to evaluate the antioxidant properties and the results were compared between the solvents used. These extracts include absolute methanol, ethanol, acetone, 50% methanol, 50% ethanol, 50% acetone and water extracts. The results obtained using 50% acetone showed the greatest antioxidative properties (0.15 mg TE/g dw), followed by absolute methanol, while the lowest antioxidative properties were seen in the water and absolute acetone extracts (0.03 and 0.02 mg TE/g dw, respectively) (Papoutsis *et al*., 2016). Another study done by Xi *et al*. (2017) proved the ability of the peels from *Citrus limon* to scavenge the free radicals using 80% methanol and the number of antioxidants exhibited from different parts of *Citrus limon* fruits, including peels, pulp, juice, seed, and the whole fruits were compared. The results showed that the *Citrus limon* peels yield the highest antioxidant activity which is 8.20% (8.20 ± 0.33) compared to any other part of *Citrus limon* in 80% methanol (Xi *et al*., 2017). These results suggest that the peels of *Citrus limon* contain a significant number of potential antioxidants.

In the study investigating the antioxidant properties in *Citrus sinensis* peel extracts, which were done using several different extracts, Liew *et al*. (2018) reported that antioxidants in considerable amounts were exhibited in 50, 70, and 100% methanol, ethanol as well as acetone extracts. The peels of *Citrus sinensis* were prepared by drying the samples and extracted by using methanol, ethanol and acetone with several different concentrations and the result showed that the greatest amount of antioxidant found was in 70% acetone, which was 18.20 ± 1.62 (mg TE/g) (Liew *et al*., 2018). In the same vein, a study conducted by Ghazian *et al*. (2016) on essential oils from *Citrus sinensis* peels reported that the essential oil from the peels yielded strong antioxidative properties with 3.68 (mg TE/g dw) (Ghazian *et al*., 2016). It was suggested that these potent antioxidative properties yielded by the essential oils from the peels of *Citrus sinensis* were because of their flavonoid and ascorbic acid.

In a study which set out to determine the antioxidant potentials of *Citrus hystrix* peels conducted by Wungsintaweekul *et al*. (2010), the plant extracts and volatile oils were tested according to the radical scavenging properties for their antioxidant activity. The extraction process was done using methanol as the extraction solvent. When tested for antioxidant activity against DPPH radicals, peel extract and oil of *Citrus hystrix* were shown to result in half-maximal inhibitory concentration (IC50) of 66.3 µg/mL and > 250 µg/mL respectively (Wungsintaweekul *et al*., 2010). This result was supported by Wijaya *et al*. (2017), who found that there was a presence of antioxidant activity in the peels of *Citrus hystrix*. In this research, ethanol was used as the extraction solvent and the crude extracts were further fractionated into several extracts including hexane, ethyl acetate, and n-butanol. The crude extract needs about 0.09 mg of the crude extract to inhibit DPPH neutralisation by 50% and it showed the lowest IC50 (Wijaya *et al*., 2017). Thus, the lower the IC50 value, the stronger the ability of the sample to quench DPPH. The summary findings of radical scavenging activity by DPPH in peels of three different citrus species are shown in Table 1.

ABTS Radical Scavenging Activity

2,2′-Azinobis-(3-ethylbenzthiazolin-6-sulfonic acid) (ABTS) is a compound that is extremely soluble in water and is considered chemically stable. It serves as a peroxidase substrate. When it is exposed to oxidation either by hydrogen peroxide or ferryl myoglobin, it will create a metastable cation (Kumar *et al*., 2017). The ABTS assay is one of the most sensitive methods

Table 1: DPPH free radical scavenging assay in peels of three different citrus species

Citrus Species	Extract	Antioxidant Activity	Reference
Citrus limon	Methanol, ethanol, acetone (100% and 50%) and water	(mg TE/g dw) Absolute methanol- 0.13 Absolute ethanol- 0.05 Absolute acetone- 0.02 50% methanol- 0.11 50% ethanol- 0.09 50% acetone- 0.15 Water- 0.03	Papoutsis *et al*. (2016)
Citrus limon	80% methanol	(TE)/g FW Peels- 8.20	Xi *et al*. (2017)
Citrus sinensis	Methanol, ethanol, acetone (50%, 70% and 100%) and water	(mg TE/g dw) Absolute methanol- 13.96 Absolute ethanol- 11.61 Absolute acetone- NA 70% methanol- 16.69 70% ethanol- 16.52 70% acetone- 18.20 50% methanol- 15.98 50% ethanol- 15.96 50% acetone- 16.87 Water- 8.35	Liew *et al*. (2018)
Citrus sinensis	Essential oil	(mg TE/g dw) 3.68	Ghazian *et al*. (2016)
Citrus hystrix	Methanol	IC_{50} (µg/ ml) Peel extract- 66.3 Peel oil- >250	Wungsintaweekul *et al*. (2010)
Citrus hystrix	Ethanol, hexane, ethyl acetate, and n-butanol	IC_{50} (mg/ ml) Ethanol- 0.09 Hexane- 53.84 Ethyl acetate- 1.54 N-butanol- 0.44	Wijaya *et al*. (2017)

of detecting antioxidant activity since antioxidant reactions require faster kinetic reactions. This technique was originally documented by Miller and colleagues in 1997 and is determined by the ability of an antioxidant to stabilise the coloured ABTS cation radical that was formed by ABTS oxidation. DPPH and ABTS are two of the most commonly utilized techniques for assessing the antioxidative properties of plant extracts, foods, and specific compounds. Their stability, cost-effectiveness, and manageable protocols are the driving forces behind their widespread use in determining antioxidant activity. Multiple studies have established a direct association between flavonoid concentration and DPPH radical or ABTS radical scavenging activity. This indicates that species with higher total flavonoid content possess the most potent antioxidant activity (Hernández-Rodríguez *et al*., 2018).

In an analysis which was set out to investigate the antioxidant properties in peels of lemon fruit, Papoutsis *et al*. (2016) found that peels that were extracted using absolute methanol and 50% methanol exhibited the highest antioxidant activity. In this study, seven extraction solvents were used as a comparison to one another to find out which solvents can yield the greatest number of antioxidants present in the respective peels. These extraction solvents include water, absolute methanol, ethanol, acetone, 50% methanol, 50% ethanol and 50% acetone. Upon testing of the antioxidant activity, all the peels that were extracted exhibited the presence of antioxidants. However, the highest antioxidant was presented in absolute methanol and 50% methanol with 0.46 TE/g dw and 0.43 mg TE/g dw, respectively (Papoutsis *et al*., 2016). This view is supported by Mcharek and Hanchi (2017), who found that the lemon peels extracted using methanol as the extraction solvent exhibited antioxidant activity (Mcharek & Hanchi, 2017). Furthermore, Xi *et al*. (2017) also stated in their study that after investigating the antioxidant activity on different parts of the lemon fruit, they found that peels of *Citrus limon* showed the highest activity of antioxidants ranging from 8.65 ± 0.09 to 14.40 ± 0.96 µM TE/g fw when compared to other parts of the lemon fruit including the pulp and juice (Xi *et al*., 2017).

In a study conducted by Guo *et al*. (2020), it was shown that the peels of sweet oranges can scavenge free radicals. The peels were extracted using 95% ethanol and were further fractionated into three more fractions petroleum ether extract, ethyl acetate extract and water extract. The result showed that ethyl acetate extract was the most potent (IC50 25.81 µg/mL), which was significantly different from the other three extracts (Guo *et al*., 2020). Similarly, Siahpoosh and Javedani (2016) assert that *Citrus sinensis* peels were also able to scavenge free radicals. The study comparing the antioxidant activity in peels from two different species of citrus used absolute methanol as the extraction solvent and showed that although the values of antioxidant activity were different between those two species, sweet orange peels exhibited higher antioxidant activity which ranged from IC50 of 64.31 µg/mL to IC50 of 52.89 µg/mL compared to the other species (Siahpoosh & Javedani, 2016). These studies also supported Pereira *et al*., which found that there was the presence of antioxidant activity in the peel of *Citrus sinensis*.

Although the studies exploring antioxidant activity using ABTS assay on the peels of *Citrus hystrix* were quite limited in recent years, Pattarachotanant and Tencomnao found that there was evidence of antioxidant properties present in the peels of kaffir lime. In this research that investigates the antioxidant activity in kaffir lime peels and leaves, 70% ethanol was used as the extraction solvent and was extracted by the maceration method. The results showed that in *Citrus hystrix* peels, the percentage of radical scavenging activity was higher compared to the percentage in leaves with 90.38 ± 0.11% and 65.18 ± 0.33%, respectively (Pattarachotanant & Tencomnao, 2020). These results reflect those of Saeio *et al*. (2011) who also found that there was a presence of antioxidant activity in kaffir lime peels when it was examined and showed to have the ability to scavenge free radicals (Saeio *et al*., 2011). Due to the lack of studies regarding kaffir lime peels in determining the ability to scavenge free radicals using the ABTS assay, further studies are still needed. The summary findings of ABTS radical scavenging activity in peels of three different citrus species are shown in Table 2.

Ferric Reducing Antioxidant Power

The mechanism of ferric reducing antioxidant power (FRAP) is based on electron transfer instead of hydrogen atom transfer. It is based on the pH's ability to reduce ferric ions (Fe^{3+}) to ferrous ions (Fe^{2+}). The reaction was conducted at an acidic pH of 3.6 which will then increase the redox potential to maintain the iron solubility. In test samples, it measures the total antioxidant activity according to the reduction of Fe^{3+} to Fe^{2+} in the presence of 2,4,6-try pyridyl-s-triazine as the reaction signal or indicator, and this is associated with a colour shift. This assay uses a method that is quite easy, rapid and low-cost and directly measures the total antioxidant activity of electron donation (Benzie & Devaki, 2018).

According to Mcharek and Hanchi (2017), their evaluation of the antioxidant activities of the peels of *Citrus limon* showed that the peels were able to reduce ferric ions into ferrous ions. The results varied according to mature and

Table 2: ABTS radical scavenging activity in peels of three different citrus species

Citrus Species	Extract	Antioxidant Activity	Reference
Citrus limon	Methanol, ethanol, acetone (100% and 50%) and water	(mg TE/g dw) Absolute methanol- 0.46 Absolute ethanol- 0.15 Absolute acetone- 0.03 50% methanol- 0.43 50% ethanol- 0.27 50% acetone- 0.05 Water- 0.13	Papoutsis *et al*. (2016)
Citrus limon	80% methanol	(µM TE/g fw) Peels- 8.65–14.40	Xi *et al*. (2017)
Citrus limon	Methanol	(µM TE/g extract) Mature- 32.28 Immature- 87.21	Mcharek & Hanchi (2017)
Citrus sinensis	95% ethanol, petroleum ether extract (PEE), ethyl acetate extract (EtO AcE) and water extract	(IC_{50} µg/ml) 95% ethanol- 131.88 PEE- 115.74 EtO AcE- 25.81 Water- 115.86	Guo *et al*. (2020)
Citrus sinensis	Methanol	(IC_{50} µg/ml) 2 min- 64.31 4 min- 56.82 6 min- 52.89	Siahpoosh & Javedani (2016)
Citrus sinensis	Beer brew	(IC_{50} µM/L) 1736.9 ± 58.8	Pereira *et al*. (2020)
Citrus hystrix	70% ethanol	(% of 1mg/mL extract) 90.38 ± 0.11	Pattarachotanant & Tencomnao (2020)
Citrus hystrix	Hydrodistillation	(mM TEAC/mL) 0.2	Saeio *et al*. (2011)

immature peels which were 70 mg TE/g and 290 mg TE/g, respectively. The methanol extraction of these peels was believed to exhibit antioxidant activity and was also significantly correlated with the total phenolic contents. This shows that the reduced ability of the extract was largely attributed to its phenolic compounds (Mcharek & Hanchi, 2017). Similarly, Azman *et al*. (2019) assert that when examining the antioxidant potentials in frozen and fresh *Citrus limon* peels, they found that although there were slightly different amounts of antioxidants yielded by these frozen and fresh peels, the differences for both values of the fresh and frozen citrus peels ranged from 0.38 mM Fe^{2+}/g fw to 0.52 mM Fe^{2+}/g fw (Azman *et al*., 2019). These are the proofs of the presence of antioxidants that can be found in the peels of *Citrus limon*.

Farahmandfar *et al*. (2020) examined the antioxidant properties in the peel of *Citrus sinensis* by using FRAP assay as the test method and found that there was a reduction from ferric ions to intensely blue-coloured ferrous ions. The extraction of essential oils from the peels went through the hydro distillation process. In the research, they claimed that with increasing concentrations of the essential oils from the peels, the ability to reduce the ferric ions to ferrous ions of those compounds was also increased (Farahmandfar *et al*., 2020). Likewise, Guo *et* *al*. (2020) also discuss the antioxidant properties in *Citrus sinensis* peels. However, in this paper, they studied the activity of antioxidants by using a different extraction solvent, which was 95% ethanol and was further fractionated into three different fractions of petroleum ether extract (PEE), ethyl acetate extract (EtO AcE) and water extract. The antioxidant values exhibited by these extracts and sub-extracts differed from each other with the ethyl acetate extract (EtO AcE) that yielding the greatest effects of 81.16 ± 3.05 (mg/g dw) (Guo *et al*., 2020).

The study carried out by Lubinska-Szczygel *et al*. (2018) proved that the peels of *Citrus hystrix* exhibited antioxidant activity. The research was conducted by measuring the ability of *Citrus hystrix* to reduce ferric ions to ferrous ions. The FRAP value resulting from this study was 48.65 ± 4.6 mM TE/g DW (Lubinska-Szczygeł *et al*., 2018). This is in line with Saeio *et al*. (2011) and Lim and Loh (2016) where similar results were shown by both studies while investigating the antioxidant properties of kaffir lime. Both studies claimed that the peels of *Citrus hystrix* were able to reduce ferric ions to ferrous ions. By donating hydrogens to the ferric tripyridyl triazine (Fe3+ TPTZ) complex, the antioxidant compounds present in citrus peels serve as a reducing agent and thus disrupt the radical chain reaction (Saeio *et al*., 2011; Lim & Loh, 2016). Therefore, the higher the FRAP value, the greater the activity of antioxidants in the peels. The summary findings of ferric reducing antioxidant power in peels of three different citrus species are shown in Table 3.

Phytochemical Content from Peels of Citrus Species

The term phytochemical means bioactive nutrient plant chemicals that exist in plant-based foods such as vegetables, fruits and grains that could offer useful health benefits to its consumers. Phytochemical analysis was performed to measure the phytochemical present in a plant. Phytochemical analysis which also can be referred to as phytochemical screening, is generally understood to mean the extraction, screening and detection of the substances present in medicinally active plants. Classic examples of bioactive substances which can be obtained from plants include flavonoids, carotenoids, alkaloids, tannins, phenolic compounds, and antioxidants.

Qualitative Phytochemical Analysis

In an investigation into the phytochemical analysis of the peel extract from *Citrus limon*, Duru and Enyoh (2020) found that numerous types of phytochemicals were identified qualitatively. This paper aims to compare the phytochemicals present in the peels of lemon and lemongrass essential oils and the peels of *Citrus limon* were reported to show more positive results compared to the essential oils from lemongrass. These include proanthocyanin, lunamarin, quinine, phenol, flavanones, and tannin. The only negative result when tested was spartan (Duru & Enyoh, 2020). Another study by Okigbo and Okoli (2020) measured the phytochemical compositions in plant extracts which include lemon peels, and also reported that there was quite solid evidence of phytochemical properties in peels of *Citrus limon*. Methanol and chloroform were used as the extraction solvents in this research and both extracts, the peels of *Citrus limon* appeared to possess flavonoids and alkaloids while there were also positive results when tested with hydrogen cyanide, tannin, saponin and sterol (Okigbo & Okoli, 2020).

In an analysis of phytochemicals in the peels from *Citrus sinensis*, Liew *et al*. (2018) discovered that there were a few types of phytochemicals found in the peel extracts. This paper classified these phytochemicals into phenolic acids, organic acids and flavonoids and was further divided into several subgroups for each property. The phytochemicals present include gallic acid, protocatechuic acid, 4-hydroxybenzoic acid, caffeic acid, ferulic acid, lactic acid, citric acid, L-maleic acid, kojic acid, ascorbic acid, catechin, epigallocatechin, vitexin, rutin, luteolin and apigenin (Liew *et al*., 2018). A study performed by Ghazian

Table 3: Ferric reducing antioxidant power in peels of three different citrus species

Citrus species	Extracts	Result	Reference
Citrus limon	Methanol	(mg TE/g extract) Mature- 58.06 Immature- 297.11	Mcharek & Hanchi (2017)
Citrus limon	70% ethanol	(mM Fe^{2+}/g fw) Frozen peel- 0.51 Fresh peel- 0.38	Azman *et al*. (2019)
Citrus sinensis	95% ethanol, petroleum ether extract (PEE), ethyl acetate extract (EtO AcE) and water extract	(VCEAC mg/g) 95% ethanol- 8.92 PEE- 15.36 EtO AcE- 81.16 Water- 5.23	Guo *et al*. (2020)
Citrus sinensis	Hydrodistillation	Inhibition (%) 0.52	Farahmandfar *et al*. (2020)
Citrus hystrix	Essential oil	(mM EC/mL) 0.2	Saeio *et al*. (2011)
Citrus hystrix	Ethanol, hexane, ethyl acetate, and n-butanol	(mM TE/g dw) 48.65 ± 4.6	Lubinska-Szczygeł *et al*. (2018)
Citrus hystrix	Methanol	(mM Fe^{2+}/g dw) 369.48 ± 20.15	Lim & Loh (2016)

et al. (2016) also showed the presence of phytochemical properties when investigated (Ghazian *et al*., 2016).

A recent study conducted by Pattarachotanant and Tencomnao (2020) that compared the phytochemical constituents in the leaves and peels of *Citrus hystrix* found that the peels consist of more phytochemical compounds. Even though there was not much research that explored the qualitative phytochemical constituents of these peels, the study reported that in the peels of *Citrus hystrix*, the phytochemical compounds that were found were sitosterol, citronellal, α-terpinol, citronellol, methyl 6-oxo heptanoate, α-copaene, caryophyllene, β-cubebene, cadinene and phytol (Pattarachotanant & Tencomnao, 2020). These results showed that the peels of *Citrus hystrix* exhibited some number of phytochemical constituents when tested qualitatively. The summary findings of the qualitative phytochemical analysis in peels of three different citrus species are shown in Table 4.

Quantitative Phytochemical Analysis

Total phenolic content (TPC), total flavonoid content (TFC), total saponins and total tannin contents (TTC) are a few examples of assays that were used to determine the quantity of the bioactive compound present in a plant. Xi *et al*. (2017) studied the phenolic profile characterisation and antioxidant capacity of different fruit parts from *Citrus limon* found that between all the different fruit parts of lemons, the TPC varied noticeably. In this study, 80% methanol was used as the extraction solvent for all the fruit parts. The highest TPC was exhibited by the peels of lemon, which resulted from 3.17 to 4.63 mg/g GAE fw and the lowest were found in the juice. For TFC, the study proved that the peels of *Citrus limon* exhibited quite an amount of TFC, ranging from 5.12 to 8.30 mg/g fw. Although the study found that the seeds yielded the greatest level of TFC, the peels are still considered to have a significant amount of TFC present (Xi *et al*., 2017). The study was supported by Papoutsis *et al*. (2016)

Table 4: Qualitative phytochemical analysis in peels of three different citrus species

Citrus Species	Extracts	Phytochemicals	Reference
Citrus limon	Hydrodistillation	Proanthocyanin, lunamarin, quinine, ribalinidine, epihedrine, anthocyanin, flavan-3-ol, sapogenin, phenol, flavanones, naringenin, steroids, epicatechin, kaempferol, phytate, flavone, oxalate, catechin, resveratrol and tannin.	Duru & Enyoh (2020)
Citrus limon	Ethanol and chloroform	Flavonoid, alkaloid, hydrogen cyanide, tannin, saponin and sterol.	Okigbo & Okoli (2020)
Citrus sinensis	Methanol, ethanol, acetone (50%, 70% and 100%)	Phenolic acids- gallic acid, protocatechuic acid, 4-hydroxybenzoic acid, caffeic acid, and ferulic acid. Organic acids- lactic acid, citric acid, L-maleic acid, kojic acid, and ascorbic acid. Flavonoids- catechin, epigallocatechin, vitexin, rutin, luteolin, and apigenin.	Liew *et al*. (2018)
Citrus sinensis	Hydrodistillation	Limonene, carvone, trans-carveol, etc.	Ghazian *et al*. (2016)
Citrus hystrix	70% ethanol	Sitosterol, citronellal, α-terpinol, citronellol, methyl 6- oxoheptanoate, α-copaene, caryophyllene, β-cubebene, cadinene, phytol.	Pattarachotanant & Tencomnao (2020)

who reported that peels of lemon also exhibited different phytochemical values when using methanol, ethanol, and acetone as the extraction solvents. Absolute methanol and 50% acetone resulted in the highest extraction yields of TPC which were 13.24 mg GAE/g dw and 12.37 mg GAE/g dw, respectively. Meanwhile, the greatest TFC were found in 100% methanol extracts which were 5.03 mg CE/g dw (Papoutsis *et al*., 2016).

A study investigated by Liew *et al*. (2018) on the phytochemical properties of *Citrus sinensis* peels revealed that the peels showed different values of phytochemicals. The study was conducted using methanol, ethanol, and acetone extracts at 100%, 70%, and 50% as the concentration for all extracts. All the extraction solvents showed various values of TPC and TFC. For TPC, the greatest level was yielded using 70% acetone extracts which were 38.24 ± 3.44 mg GAE/g and the lowest was demonstrated by 100% ethanolic extracts. However, for TFC, the highest level was 5.51 ± 0.43 mg CE/g which was shown in 50% acetone extracts (Liew *et al*., 2018). Likewise, the TPC and TFC assay was performed by Guo *et al*. and the results came out to be quite similar, thus proving that the peels of *Citrus sinensis* exhibit different values of phytochemicals (Guo *et al*., 2020). Although the levels of TPC and TFC might vary in each of the papers, there was still evidence of phytochemicals reported in *Citrus sinensis* peels.

According to a study performed by Wijaya *et al*. (2017) the phytochemical constituents in the peels of kaffir lime or *Citrus hystrix* exhibited certain levels of TPC and TFC through TPC and TFC assays. The analysis of those phytochemicals was examined using ethanol as the crude extract and was further fractionated into three different extracts which were hexane, ethyl acetate and n-butanol. For both the TPC and TFC assays, the crude extracts which were ethanol, exhibited the highest level at 0.318 mg GAE/mg and 0.206 mg RE/mg, respectively (Wijaya *et al*., 2017). This research was complemented by Lim and Loh (2016) who claimed that the peels of *Citrus hystrix* exhibited phytochemicals when assessed using TPC and TFC assays (Lim & Loh, 2016). The summary findings of the quantitative phytochemical analysis in peels of three different citrus species are shown in Table 5.

Conclusion

This comprehensive review evaluates and contrasts the antioxidant activity and phytochemical content of peels from three distinct citrus species: *C. limon*, *C. sinensis*, and *C. hystrix*. Through the use of various methods such as DPPH free radical scavenging assay, ABTS radical scavenging assay, FRAP assay, and qualitative and quantitative phytochemical analysis, the researchers discovered that each species' peel has varying values of antioxidant activity and phytochemical content. The differences are due to the distinct bioactive compounds present in each species' peel, as well as the extraction method and solvent used. Despite the variations in antioxidant activity and phytochemical content, the peels of all three citrus species have valuable antioxidant properties and phytochemical constituents, making them a promising natural antioxidant source. However, further research is necessary to fully exploit the benefits of these peels in the future.

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