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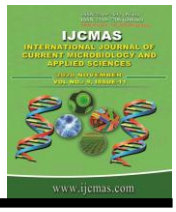

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Study on Different Extraction Methods and Antimicrobial Potential of Citrus Peel and Leaf

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A B S T R A C T

Citrus peels are a rich source of essential oils which can be extracted by using a hydro-distillation process. The extracts contain D-limonene (70-90%) are major component. The essential oil from blood orange results the great amount of antimicrobial properties which are used against the bacteria (*E. coli, B. aureus, S. typhimurim, L. moncytogene* and *E. faecalis*). Citrus peel contains a very good amount of flavonones including several polymethoxylated flavones. The numerous therapeutic properties have been reported in fruit like anticancer, antiviral, antitumor, anti-inflammatory activities. High amount of phyto-chemical and bio-active compounds like carotenoids, minerals, flavonoids and vitamins present in citrus fruit. The bitterness in juice of citrus is mainly due to the presence of naringenin. It also contains antioxidants, antimicrobial, insect repellent and some medicinal properties.

Introduction

Citrus is the world's most valuable group of fruits which are commercially produced and anywhere the environment is acceptable. It is commonly cultivated in many areas with sufficient tropical, subtropical and temperate climatic conditions. The Citrus genus belongs to the Citrineae subtribe, sub-family Aurantioideae of the family Rutaceae. The other economically important genera of this family are Poncirus and Fortunella. India, a natural home of many species of citrus harbours vast reservoir of diverse types and forms ranging from oranges, mandarins, pummelo, grapefruit, lime and lemon, wild and semi wild species and other related genera.

Citrus species are also used as a medicinal plant. Like *Citrus limon* is mainly used for its anti-cancer and anti-bacterial activity alkaloids in crude extracts of different sections (stem, leaves, juice, flower and peel) against clinically significant bacterial strains (Kawaii *et al.,* 2000). The limonoids were derived from *C. limon* or exhibit strong antifungal and antibacterial activity. Citrus fruit extracts are among the most commonly studied therapeutic agents (e.g. grape fruit, limon and orange) for food applications and have shown an important decrease in bacterial

growth (Corbo *et al.,* 2008). There are many Citrus sp. of these *C. paradise* (grape fruit), *C. limetta* (sweet lemon), *C. aurantium* (bitter orange), *C. jambhiri* (Rough lemon) and *C. limon* (lemon) (Tawfik *et al.,* 2010). Despite this vast cultivation, phylogeny and taxonomy of many citrus varieties remain uncertain which has been complicated by several factors such as hybridization, apomixis, polyploidy and bud mutations.

An antimicrobial is a substance which kills the microbes (i.e. fungi, protozoa, bacteria or viruses) and also inhibits their growth. Antibiotics are those substances which are formed by microorganisms and destroy or block other microorganisms from growing. Some Indian herbal oils have antimicrobial properties to investigate the properties for use as botanical protection in food industries. Lime exocarp comprises 7% of all the usual volatile essential oils, the main components of which are citral, fenchone, B-pinene and limonene. Major fraction of peel oil includes esquiterpenes, monoterpenes (76%) and oxygen containing compounds like alcohols, aldehydes and esters. Citrus contains unique forms of phenolic compounds such as flavonoids, which are considered to exhibit antiviral, anti-oxidant, anti-allergenic, anti-carcinogenic, anti-inflammatory, anti-microbial activity (Escobedo-Avellaneda *et al.,* 2014). Citrus flavonoids have a wide range of biological activity including anti-diabetic, anticancer, antibacterial, and antiviral antifungal activities (Burt, 2004; Ortuño *et al.,* 2006). Antimicrobial study was performed to detect antimicrobial property of the different extracts of leaves, peel in methanol and tertra cycline and streptomycin (1mg / ml) levels against *E.coli* were calculated using the agar plate diffusion process.

In their peels, the citrus contain essential oils which were extracted by the hydrodistillation process. Alpha-pinene, m-cymene, sabinene, 4-terpineol, d-limonene, linalool, B-myrcene and B-pinene were the key components of the oils extracted. Among the all the most important component was D-limonin (66-93%). The highest antimicrobial activity of bacteria was found (*S. typhimurium, B. cereus, E. coli, S. aureus* etc) with essential oil of the blood orange (*C.sinsesis*).

Antimicrobial impact of aqueous juice and peel extract from dried and fresh citrus and sweet lemon against 6 gram +ve and 8 gram -ve bacteria or yeast isolates like *Entenococcus faecalis*.

Antimicrobial effect of aqueous extract of juice and peel from dried and fresh citrus and sweet lemon against 8 gram –ve and 6 gram +ve bacteria and yeast isolates including *Entenococcus faecalis, Enterobacteraeroge, Staphylococcus pneumoniae, Staphylococcus pyogenes, Staphylococcus agalactiae, S. aureus, Pseudomonas aeruginosa* etc. Antimicrobials and antibiotics from plant sources have been shown to function more effectively with less side-effects and additional beneficial effects (Akhilesh *et al.,* 2012). Commonly bacteria have a genetic capacity to spread and develop tolerance to drugs used as therapeutic agents (Nascimento *et al.,* 2000). In therapeutic therapies use of plant extracts and phytochemicals with both known antimicrobial properties may play a major role (Prabuseenivasan *et al.,* 2006).

Plant parts used

Peel: Although the peel of citrus is bitter in taste if it is eaten as a raw but it also play a major role in making of marmalade or other soups. Peel is the protective outer covering or outer skin of the fruit. Botanically it is also called as exocarp. Fruit type like Hesperidium contains very thick type peel. In citrus species the inner and outer part peeled off together

and after combining both the parts it is called as peel. The layer which is the juicy part of the fruit is called endocarp. In some of the fruits like apple, pear the peel can be eaten with fruit while fruits like grapefruit, pomegranate and banana the peel should have to be removed before eating of the fruit. In fruit about 25-30% area covered by the peel and it is wasted by almost everyone. The citrus peel byproduct contains high amount of antioxidants and antimicrobial compounds these can be used as an antioxidant agents or phytochemicals. The wasted peel of citrus contains wide range of biological activities like antiviral, antibacterial, antidiabetic, anticancer, antifungal etc. The citrus peel generally contains two types of layers, the outermost layer of the peel is called zest and it is made up from citral (5%) and limonene (90%). By proper management of the peel we can decrease the level of solid waste and also helps in adding value for this after cutting the outer layer of the peel which is called as zest. Now-a-days it is used as a flavoring agent in biscuits, pies, candies, cakes or chocolates.

Citrus peel is very good source of nutrients as it contains phytochemicals and these are Y-sitosterol, B-sitosterol, glycosides and volatile oils. Certain flavones, phenolic compounds and ascorbic acid contains special activities. The peel is used against digestion, peptic, ulcer, scurvy, eye infections, respiratory disorders, gums, piles, skin care, weight loss etc (Ali *et al.,* 2017).

John *et al.,* (2017) was conducting an experiment for evaluating the antioxidant and antimicrobial properties from the peel of citrus lemon. They collected the fresh lemons from the Chennai local market and removed the peel of lemon after washing it and kept it for 4-5 days at room temperature. After that they pulverized the dried peel using an electric blender and used it for methanol and acetone methods of extraction.

Leaf: Citrus leaves are generally compound which varies from trifoliate (poncirus trifoliate) to palmately compound (e.g. *Casimiora* spp.) to pinnately compound (e.g. *Citropsis gilletaiana*). During the time of full sun, the leaves of citrus spp. become strongly conduplicate while in case of shady conditions the leaves are more or very less conduplicate.

The leaf of the citrus is a rich source of the antimicrobial properties which show susceptibility against certain bacteria like *Escherichia coli, Salmonella typhi* and *Staphylococcus aureus*. The leaves of the citrus also contain essential oils. Saeb *et al.,*(2016) were collected fresh leaves of citrus and used for the extraction of antimicrobial activities against the bacteria (*Escherichia coli, S. aureus, Bacillus subtilis, Salmonella typhi*). They were used the shade drying method for drying of leaves at room temperature for 72 hours and used the powdered form of leaves with 80% methanol. They were used three different species of citrus (*C. grandis, C. reticulata* and *C.limon*).

Ethyl alcohol extract

Ethanol is the one of the important organic compound. It is called by different names (like ethyl alcohol, drinking alcohol, simple alcohol, grain alcohol and spirit) having chemical formula (C_2H_6O). It is flammable, volatile colorless liquid having a characteristic odor. Ethanol is used in wide range of industries. It is used in making antiseptic, hand sanitizer liquid used against the bacteria. Ethanol plays a major role for extracting the antimicrobial potential from the different parts of the citrus species. Various scientists used ethanol method of extraction with the parts of citrus like peel, seed and leaf. EL-Desoukey (2018) conducted an experiment for evaluating the antimicrobial effect of orange. They were used 10 ml of

ethanol as a solvent with 5g of dried plant peel for the extraction. After the extraction it is used against various bacterial strains. Different scientists used different concentration of ethanol. Yashaswini and Arvind (2018) were used 4g of orange peel with 20ml of ethanol for the extraction of antimicrobial properties of orange (*Citrus reticulata*). Pandey Amit *et al.,* (2011) were used 70% ethanol as a solvent for the extraction of antimicrobial properties from the peel of citrus limon. Ahmad and Ahmad, (2016) were conducting an experiment for evaluating the antibacterial activity of citrus limon. They were used 250 ml of ethanol (95% v/v) solvent with 25g dried powdered leaves.

Extract of methanol

Methanol also called by other name methyl alcohol having the chemical formula CH_3OH. It is a volatile, flammable, colorless, light liquid with odor similar to ethanol. Methanol acts as a major solvent for the extraction of antimicrobial, antibacterial or antifungal properties from the citrus species. Dhanavade *et al.,* (2011) were conducting an experiment for evaluating the antimicrobial activity of lemon (*Citrus lemon L.*) from the peel. They used the methanol solvent for the method of extraction. Saeb *et al.,* (2016) were used 80% methanol as a solvent agent with the shade dried leaf powder of citrus limon, *Citrus reticulata* and *Citrus grandis* for evaluating the antibacterial activities against some of the pathogenic bacteria. Yashaswini and arvind, (2018) were used the 20 ml of methanol solvent with 4 g of orange peel powder used for the extraction method of antimicrobial properties. Abd *et al.,* (2016) were conducting an experiment for finding the antioxidant properties from the peel of orange and lemon. They used the 80ml of methanol (80%) with 10g dried peel powder and after kept at room temperature the extract were centrifuged at 5000 rpm for 30 min at room temperature. They were filtered by using Whatman no. 4 filter paper.

Aqueous extract

It is one of the easy and important methods of extraction for evaluating the antimicrobial properties from the different parts of the citrus species. Different scientists use this aqueous method of extraction with different quantity of the aqueous. EL-Desoukey (2018) were used this method. They were added the dry peel of citrus into the boiled distilled water and kept at 4°C. Amengialue *et al.,* (2016) were used the 90ml of distilled water in 10g powder of peel. After that they were cover it with aluminium foil and filter the product before use. Ahmad and Ahmad *et al.,* (2016) were used this method of extraction for evaluating the antibacterial activity of citrus species. They used 25g of dried powdered with hot distilled water and boiled the matter for 30 min. This aqueous method plays a major role in evaluating the antibacterial and antimicrobial potential of citrus limon.

Extract of acetone

Acetone is called propanone, it is an organic compound having chemical formula $(CH_3)_2CO$. It is highly volatile, flammable or colorless liquid having a pungent odor. It has various benefits many scientists used it for the antimicrobial properties extraction method. Dhanavade *et al.,* (2011) were used acetone at their experiment for evaluating the antimicrobial activity of citrus lemon peel and check their impact on different bacterial pathogens. Ahmad and Ahmad, (2016) were used acetone solvent at 95% (v/v) concentration with the dried powder of leaves. They used 250 ml of solvent acetone with 25g powder. Yashaswini and Arvind, (2018) used the 4g of dry orange powdered from peel and after that stored at 4°C and treat with 20 ml of acetone extract. They incubate the mixture at

130 rpm for 36 hours at 30°C for the successful extraction of the antimicrobial properties of the (*Citrus reticulata* var. kinnow) and were used it against the pathogenic like *Staphylococcus aureus, Klebsiella pneumoniae, Escherichia coli* and *Pseudomonas aeruginosa.*

Antimicrobial properties of citrus

Abd*et al.,* (2016) had isolated various antimicrobial and chemical properties of lemon and orange peel. They were used the extract of ethanolic and methanolic of the dried peel (air oven drying methods or microwave). Ahmad and Ahmad, (2016) had isolated the antibacterial activity of *Citrus limon* and *Morus nigra*. They used ethyl acetate and acetone extract from *Citrus limon* and *Morus nigra.* Agar well diffusion method for bacterial pathogens (*Bacillus* and *Staphylococcus aureus*) had been used to evaluate anti-bacterial behaviour. Akinnibosun *et al.,* (2015) were demonstrated the antimicrobial potential from the leaf extract of the *Citrus aurantifolia, Bryophyllum pinnatum L.* The agar well diffusion method is used to test the various organisms like *Staphylococcus aureus, Penicillium notatum, Escherichia coli, Pseudomonas aeruginosa, Klebsiella pneumonia, Mucormucedo, Aspergillus niger* and *Candida albicans*. The result shows that the *B. pinnatum* was less effective compared to the extract of *C. aurantifolia* against the organisms. Aladekoyi *et al.,* (2016) was worked on the antimicrobial properties which were extracted from the seeds of three *Citrus* sp. (*Citrus aurantifolia, Citrus limon,* and *Citrus aurantium*). By using the method of agar well diffusion against different bacterial pathogens, they had isolated the antimicrobial properties. They also said that the oils which were extracted from citrus seeds are used in perfumery industries, confectionary and toiletry. Oils which were extracted from the seeds of the bitter lemon show very high inhibition against *Klebsiella*, against proteins and against the E. coli. Ali *et al.,* (2017) had evaluated the activity of antimicrobial of methanolic extract from Citrus limon peel. The methanolic extract from the peel was separated from fruits, shade dried and powdered using methanol. These extracts are using against the one fungal strain (*Candida albicans*) two bacterial strains among and other gram +ve (*S.aureus*) and gram -ve (*Escherichia coli*) using agar well diffusion method. It results that the *Citrus limon* peel contains high amount of phytochemicals. Amengialue *et al.,* (2016) has responded for the phytochemical and antimicrobial properties of citrus lemon. These antimicrobial properties were evaluated against some fungi and bacterial pathogens like *Bacillus subtillis, Staphylococcus aureus, Fusarium oxysporum, Pseudomonas, Aspergillus niger.* The isolation done from the seed and peels by using ethanol and methanol extraction methods.

Citrus is a rich source of essential oils which consist of D-dimonene. Bozkurt *et al.,* (2017) was extracted the essential oils from different species of citrus fruit peels like lemon (*C. limon*), orange (*C. sinensis*), mandarin (*C. reticulate*), grapefruit (*C. paradise*). These essential oils are used to assess the antimicrobial activity of a variety of bacteria and to know the various elements of essential oils by GC-MS. Citrus fruit peel contains flavonones and polymethoxylated flavones in good amount which are also present in other plants. Dhanavade *et al.,* (2011) studied the antimicrobial function of the lemon. They also tested MLC antimicrobial activity with various solvents against micro-organisms such as *Pseudomonas aeruginosa*. El-Desoukey, (2018) were studied about the antimicrobial effect of *Citrus sinensis* (orange) peel. These antimicrobial activities were tested against the pathogens isolated from poultry or animals (*Pseudomonas aerogenes, Bacillus cereus, Escherichia coli,*

Staphylococcus aureus and *Candida albican*). The method used for isolation was Agar well diffusion method. The results observed that the peel of *Citrus sinensis* (orange) contains Tannins, alkaloids, saponins, flavonoids. Their antibacterial effect act against the Gram +ve and Gram -ve bacteria. Ewansiha, (2020) in his experiment found antibacterial activity of Citrus Limon juice against the pathogenic organisms. He used column chromatography and cold pressing extraction method to obtain the crude juice extracts. Over *Streptococcus Pyogenes, Klebsiella pneumoniae* and *Salmonella enterica* he used other processes such as Agar well diffusion and tube dilution. The peel of citrus waste from various industries is a good source of polyphenols. Hegde *et al.,* (2015) were isolates the different polyphenols from the orange fruit by using different drying technique.

Hindi and chabuck, (2013) were isolate the antimicrobial properties of citrus from their aqueous extract like from peel or juice against 8 Gram -ve bacteria and 6 Gram +ve bacteria. For this, they used agar well diffusion method. John *et al.,* (2017) was evaluated the antioxidant and antimicrobial efficiency of citrus lemon (*C. limon* L.) from the peel. The method used for determining the antimicrobial potential was DPPH assay, FRAP and Phospho molybdenum assay and these were tested against four bacterial strains. Kaur, (2014) has responds to determine phenolics from Kinnow peel and also for antimicrobial properties. The effects of various solvents like (Methanol, Ethanol, Ethyl acetate, Isopropyl alcohol, Dichloro methane) for the extraction of phenolics were studied. The extraction for this drop done by two methods i.e. Soxhlet and conventional extraction method. She also said that the Hesperidium which is dominant in flavonone was isolated and investigated for antimicrobial properties. Kumari *et al.,* (2016) was performed an experiment to determine the antimicrobial properties from leaf (young and mature), peel in methanol and tetracycline and streptomycin (1mg / ml) levels against E.coli were calculated using the agar plate diffusion process. Yashaswini and Arvind, (2018) was determined the antimicrobial properties and phenolic content of the orange (Kinnow) against the pathogenic bacteria *Pseudomonas aeruginosa, Escherichia coli, Klebsiella pneumoniae* and *Staphylococcus*. For evaluating the antimicrobial potential Agar well diffusion method was used. Pandey *et al.,* (2011) has demonstrated the phytochemical and antimicrobial activity from the seed and peels of Citrus limon. The analysis was done by using the agar well diffusion method. And these properties were tested against the fungal and bacterial pathogens (*S. aureus, P. aeruginosa, E. coli*).

Halima and Allem, (2016) worked together on evaluating the antimicrobial property of algerian lemon (Citrus limon L.). They were isolated these properties from the peel and citrus limon seeds and also use disk diffusion method against six pathogenic bacteria and fungal strain. The result of this experiment teaches us that both peel and seeds of Citrus contain quercentin, a flavonoid which is a very good antimicrobial property. Saeb *et al.,* (2016) was studied to determine the antimicrobial properties present in the three species of the citrus against the pathogenic bacteria. The three species of citrus were *Citrus grandis, C. limon, C. reticulata*. They worked on the antimicrobial effects from leaf extract and other essential oils of the citrus species. They were using the three citrus species against *S.aureus, Bacillus subtilis, Escherichia coli* and *Salmonella typhimurium* by using E- test and agar disc diffusion method. Safdar *et al.,* (2017) conducted a maceration and ultrasound extraction test to extract polyphenols from Kinnow peel (*Citrus reticulata*). The antimicrobial properties of various polyphenols were extracted by using

ferric reducing antioxidant power (FRAP) and these properties were used against the different strains of *Staphylococcus aurens, Bacillus cereus* and *salmonella typhimurium*. Shetty *et al.,* (2016) worked for evaluating the in-vitro antimicrobial potential from the extract of *Citrus sinensis* peel. The method used for checking the antimicrobial activity was agar well diffusion method and their activity checked against *Streptococcus mutans* and *Lactobacillus acidophilus.*

In conclusion the citrus peel, leaf and seed which are wasted by the peoples and industries are rich source of the antimicrobial properties and having numerous therapeutic properties like anticancer, antiviral, anti-inflammatory activities. High amount of phyto-chemical and bio-active compounds like carotenoids, minerals, flavonoids and vitamins present in citrus fruit. Various methods of extraction were used like Hydro-distillation method, GC/MS analysis, Agar well diffusion method, Ethyl alcohol extract, Methanol extract, Aqueous extract, Acetone extract etc.

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