International Journal of Pharmaceutical Research and Applications
Volume 7, Issue 1 Jan-Feb 2022, pp: 773-779 www.ijprajournal.com ISSN: 2249-7781

Orange (Citrus Aurantium) Benefits and Extraction of Essential Oil from Orange Peel

[1]Yogeshwaran S, [1]Aravindhan P, [1]Siva R, [1]Gunapriyanga P, [1]Sangeetha V, [2]Jeevanandham S, [3]Gokul Manikandan P

[1]*Student, PPG College of Pharmacy, Coimbatore, Tamilnadu*
[2]*Principal, PPG college of Pharmacy, Coimbatore, Tamilnadu*
[3]*Assistant professor, PPG college of pharmacy, Coimbatore, Tamilnadu*
Corresponding Author: Yogeshwaran S

Submitted: 25-01-2022 Accepted: 08-02-2022

ABSTRACT: Orange is the one of the most famous fruit in the world. Orange (Citrus aurantium) is well known for its nutritional and medicinal properties throughout the world. whole Orange plant which consist of lot of medicational properties which including anti-bacterial, anti-fungal, anti- diabetic, cardio- protective, anti-cancer, anti-arthritic, anti-inflammatory, anti-oxidant, anti-tubercular, anti-asthmatic and anti-hypertensive, activities present. Orange peels are throw away as wastes after consumption of edible parts of orange fruits. To get the highest quality and quantity of orange peel oil, it is necessary to know the suitable methods for drying and the appropriate form of the peel, whether in the form of pieces, grated or powder. Essential oil are highly concentrated substance, which are extracted from various parts of orange plant such as fruit peels, flowers, leaves, stems, roots and seeds. These oil are often used for their flavour and their therapeutic properties, in wide selection products such as foods, pharmaceuticals, medicines, perfume industry and cosmetics.

KEYWORDS: Citrus aurantium, Orange, Essential oil, Extraction.

I. INTRODUCTION

The world health organization (WHO) estimates that about 80% of the population still depends upon herbal medicines for the treatment of various diseases due to easy availability, economic reasons and less side effects. Herbal remedies have formed the basis of traditional systems of medicine for ages and have formed the foundation of modern pharmacology. Herbal medicines have long history of popularity, better patient tolerance as well as acceptance. Availability of medicinal plants is not a problem especially in developing countries like India, which is having rich agro climatic, cultural and ethnic biodiversity. In worldwide trades citrus fruits generate about 105 billion dollars per year all over the world. Orange fruit is cultivated in more than 130 countries including India, UK, France, Germany, Holland, Brazil, China, USA and Spain. Oranges are generally available from winter through summer with seasonal variations depending on the variety. [1]

Essential oil is obtained from plant material which is held within certain part of the plant or specific part of the plant cells; it may be from leaves, seeds, peels or stalks, depending on the species. The methods used for obtaining essential oil include Steam-distillation, Solvent extraction, Supercritical fluid extraction, Cold pressing, Microwave extraction. Citrus fruits belong to six genera (Fortunella, Eremocitrus, Clymendia, Poncirus, Microcitrus, and Citrus) which are native to the tropical and sub-tropical regions of Asia, but the major commercial fruits belong to genus Citrus. The genus citrus includes several important fruits such as oranges, mandarins, lime, lemons and grape fruits. The essential oil is present in the fruit's peel in great quantities. The citrus essential oil is a mixture of volatile compounds and mainly consists of monoterpene hydrocarbon. [2]

In many food products, such as marmalades, oils, ice creams, alcoholic and non-alcoholic beverages, gelatins, soft drinks, sweets, candies and cakes, they are applied as aroma flavour. Citrus fruit contain a wide range of bioactive compounds such as phenolics which possess health benefits. Bitter orange is used principally as a Citrus rootstock in Tunisia. Because of its sour and bitter taste, it is not considered an edible fruit. Its juice, characterized by a sour taste, is used in salads to replace lemon juice and the peel is used in jam production. [8]

Bitter (sour) orange refers to Citrus aurantium L. and is an interspecifc hybrid between

International Journal of Pharmaceutical Research and Applications
Volume 7, Issue 1 Jan-Feb 2022, pp: 773-779 www.ijprajournal.com ISSN: 2249-7781

Citrus reticulata and Citrus maxima. This plant belongs to the cultivated sour citrus fruits with a sugar/acid ratio of less than 1 and is native of southeastern Asia. Overall production of bitter orange is less than sweet citrus fruits like orange, pomelo and mandarin, however they are well-known for their essential oil (EO) which frequently used in flavoring, cosmetics, and perfume as well as their applications in culinary materials like fruit juice vinegar .[9]

II. CITRUS AURANTUM

Citrus aurantium L. (Rutaceae), commonly known as bitter orange, is usually utilized as a flavoring and acidifying agent for food. Besides the essential oil and its components the fruits of C. aurantium are sources of flavonoid-type compounds with diverse biological effects. Due to the abundance of health-giving secondary metabolites, C. aurantium is also used for the treatment of several ailments such as anti bacterial, anxiety, lung and prostate cancers, and gastrointestinal disorders and obesity. C. aurantium has found an important place as a preferable agent to replace ephedra, as it contains p-synephrine, a phenyl ethanolamine type alkaloid, which is chemically similar to adrenergic agents, as appetite suppressants. Recently, several scientific studies investigating the potential effects of various parts (including flowers, fruits, and essential oils) of C. aurantium have been conducted. [3] Botanical classification of orange shown in Table 1.

Botanical classification of Orange (Table 1)

Kingdom	**Plantae**
Division	Magnoliophyta
Class	Magnoliopsida
Sub Class	Rosidae
Order	Sapindales
Family	Rutaceae
Sub family	Aurantoideae
Genera	Citrus
Species	C. aurantium

Chemistry of citrus Aurantium

Drug discovery is substantially benefited by ethnopharmacological approaches and, based on their traditional use; preparations obtained from Citrus aurantium L. were investigated in order to evaluate their ability to induce sedative/hypnotic, Anxiolytic and/or anticonvulsant effects in experimental models. [10]

In this study, the chemical components of the essential oil were determined, additionally antibacterial as well as antifungal activities of essential oils citrus [6].The chemical composition of C. aurantium is responsible for health-promoting effects. The chemical composition includes vitamins, minerals, phenolic compounds, and terpenoids. Among the diverse chemical components in C. aurantium, flavonoids belonging to phenolics have been recognized as important due to their physiological and pharmacological role and their health benefits. [3]

The flavonoids contained in C. aurantium can be divided into four groups, including flavones, flavanones, flavonols, and anthocyanins (only in blood oranges). Flavonoids are mainly present in Citrus fruits as glycosyl derivatives. Aglycones are mainly present in specific parts of the fruit as peel and seeds, owing to their lipophilic nature and consequently their low solubility in water. For glycoside forms, O-glycosides, C-glycosides, rutinosides, glucosides, and neohesperidosides are common. [3]

The flavones in aglycon or/and glycosidic form are the second major group of flavonoids in C. aurantium. The most commonly detected free flavones are apigenin, luteolin, and diosmetin. O-Glycosides and C-glycosides are the two main forms of flavone glycosides, and the most common linked sugar moieties include glucose, rutinose, and neohesperidose. [3]

In C. aurantium, the flavones may be present also in the methoxylated form, in which all or almost all hydroxyls are capped by methylation, as nobiletin and tangeretin. Furthermore, the C. aurantium may contain low amounts of flavonols, as kaempferol and quercetin, mainly in glycosidic form. [3]

The second class of secondary metabolites found in C. aurantium are the limonoids. The latter were considered as oxygenated triterpenoids as they contain relatively high numbers of oxygen atoms (7–11) in their structures. All components have a furan ring attached to the D-ring at C-17. Limonoids occur both in glucosidic and aglyconic form. Limonoid aglycones are water-insoluble and responsible for a bitter taste of the Citrus fruits, while limonoid glucosides are water-soluble and tasteless. Among limonoids, the most important one is limonin, known as Citrus constituent since 1841. [3]

International Journal of Pharmaceutical Research and Applications
Volume 7, Issue 1 Jan-Feb 2022, pp: 773-779 www.ijprajournal.com ISSN: 2249-7781

Limonoid glucosides are more abundant in juices and pulps, such as limonin glucoside, nomilin glucoside, obacunone glucoside, nomilinic acid glucoside, and deacetylnomilinic acid glucoside, because they are water-soluble, while limonoid aglycones such as limonin, nomilin, obacunone, ichangin, and deacetyl nomilin are water-insoluble and are present mainly in seeds and peels.[3]

Another class of compounds contained in C. aurantium are phenylethylamine alkaloids with p-synephrine being the most abundant. This compound has a hydroxyl group in the Para position on the benzene ring and has some structural similarity to ephedrine. The peel of unripe fruits is the part of the plant which has the highest level of p-synephrine.[3]

Pharmacological profile

❖ **Cytotoxic and Anticancer Effects**

Cytotoxic properties of the polysaccharides obtained from C. aurantium were examined. Cytotoxic activity on human breast cancer cells and lung cancer cells and immune-enhancement effect were examined. The results indicated that C. aurantium var. amara polysaccharides displayed good immune-enhancement activity by stimulating the production of tumor necrosis factor-α (TNF-α) and interleukin-6 (IL-6)in RAW264.7 cells and by promoting the mRNA expression levels of inducible nitric oxide synthase (iNOS), TNF-α, interleukin-1β (IL-1β), and IL-6. Moreover, phosphorylated extracellular signal-regulated kinase (ERK), phosphorylated c-Jun N-terminal kinase (JNK), phosphorylated p38, and phosphorylated p65 were significantly enhanced in C. aurantium var amara polysaccharides-treated RAW264.7 cells. [3]

❖ **Antidiabetic Effects**

A possible effect of the extract obtained from C. aurantium and p-synephrine on liver metabolism was evaluated. In order to measure catabolic and anabolic pathways, an isolated perfused rat liver was used. Both the extract and the compound were found to enhance glycolysis, glycogenolysis, oxygen uptake, and perfusion pressure. p-Synephrine increased the glucose out-put at 200 µm concentration. C. aurantium extract enhanced gluconeogenesis at low concentrations, however, inhibited at high concentrations. The effects of C. aurantium extract on liver metabolism was found to be similar to those of adrenergic agents, and p-synephrine could be responsible from the activity.[3]

❖ **Antiobesity Effects**

C. aurantium extract has been commonly utilized for the weight loss and as sports performance enhancer, in dietary supplements. Therefore, the use of C. aurantium extract and its constituent p-synephrine ($C_9H_{13}NO_2$), for the treatment of obesity in 360 subjects, was reviewed. More than 50% of the subjects involved in these clinical studies were overweight, and approximately two-thirds of them consumed caffeine (132–528 mg/day) and p-synephrine (10–53 mg/day). Approximately 44% of the subjects used a C. aurantium/p-synephrine product, while the remaining consumed a combination product containing multiple ingredients with p-synephrine. The results showed that C. aurantium extract alone or in combination with other ingredients did not cause significant adverse effects including an increase in heart rate or blood pressure or change in electrocardiographic data, serum chemistry, blood cell counts, or urinalysis. p-Synephrine, alone or in combination products, was demonstrated to enhance metabolic rate and energy expenditure and to promote weight loss when given for six to 12 weeks.[3]

❖ **Effect on Microorganisms**

Antimicrobial activity of C.aurantium was investigated by using several in vitro assays, The low pH of C. aurantium juice was suggested to be responsible for its antimicrobial potential along with the duration of the incubation period as well as the temperature. In another research, the antimicrobial potential of C. aurantium was investigated, and high antimicrobial activity was recorded against Bacillus subtilis and Staphylococcus aureus (among 12 microorganisms tested), with the minimum inhibition concentration (MIC) values of 2.7 mg/mL and 4.8 mg/mL. [3]

❖ **Pesticidal Effects**

Previous in vitro investigations on the essential oil of C. aurantium peels and its isolated component limonene have demonstrated their pest fumigant activity against Bemisia tabaci (silverleaf whitefly). In 24 h exposure, insect mortality between 41.00 and 47.67% was determined at 2.5 and 20.0 µL/L air concentrations. The essential oil extract from the fresh peeled ripe fruit of Citrus aurantium showed also good larvicidal effect against mosquito vector Anopheles stephensi (LC_{50} values, 31.20 ppm) the main constituent of the leaf oil was limonene (94.81). [3]

❖ **Anti ulcer activity**

The essential oil and limonene increased the production of gastric mucus. The findings revealed that C. aurantium essential oil and its main

International Journal of Pharmaceutical Research and Applications
Volume 7, Issue 1 Jan-Feb 2022, pp: 773-779 www.ijprajournal.com ISSN: 2249-7781

compound limonene can be used as a promising target for the development of a novel gastro protective drug. In another study, the gastro protective effect of β-myrcene, a monoterpene-type compound of C. aurantium, was evaluated. Experimental models of ulcer, induced by ethanol, NSAID stress, Helicobacter pylori, ischemia-reperfusion injury, and cysteamine (a drug used to treat cystinosis) was used to assess the ameliorative activity. β-Myrcene was administered at dose of 7.5 mg/kg. The results showed a potential role for β-myrcene against peptic ulcer disease. β-Myrcene contributed to the maintenance of integrity of thegastric mucosa with a significant decrease of ulcerative lesions, attenuating lipid peroxidative damage and preventing depletion of GSH, GR, and GPx. [3]

❖ **Antioxidant effects**

DPPH, ABTS, and ferric-reducing antioxidant power assays were used for the determination of the antioxidant potential of the macerate of the albedo layers of C.aurantium fruits obtained by protopectinase-SE (produced by Geotrichum klebahnii and hydrolysed selectively the intercellular protopectin of plant tissues). More-over, the levels of total phenols, reducing sugars, vitamin C, total flavonones, naringin, and galacturonic acid and total acidity were detected. Antioxidant activity, vitamin C, and total flavonone levels were found to be the highest with the greatest degree of tissue maceration. In vivo and in vitro antioxidant activities of polysaccharide fractions from C. aurantium were evaluated. The most active fraction was subjected to ion exchange and gel-filtration chromatography to obtain four purified polysaccharides. Upon the evaluation of their antioxidant effect, it was found that C.aurantium can be utilized as an antioxidant in the food and medical industries. [3]

III. EXTRACTON

Extraction of orange (Citrus aurantium) peel oil by using various methods, After the orange peels were obtained, they were washed, cleaned with water and sundried for five days. The dried peels were then pulverized before being used in each of the extraction methods investigated in this work. **Preparation of orange peel powder**: The collect sample of orange peels is cleaned and pith is manually separated from the outer coloured part of the peels. That is because of the reason that the majority of the oil in oil sac present in them. After the orange peels were obtained, they were washed, cleaned with water and chopped, placed on blotting paper, spread out and dried under shade at room temperature for 5 days. The shade dried orange peels was grinded to give consistent and fine powder using electrical grinder. The powdered orange peels was then stored in well labelled airtight container at ambient temperature and protected from sunlight for further use. [4]

1. Extraction of Oil by Steam Distillation

The Distillation set up is arranged as show in Figure.1.It consists of distillation flask, Basket heater, horizontal condenser and a conical flask.

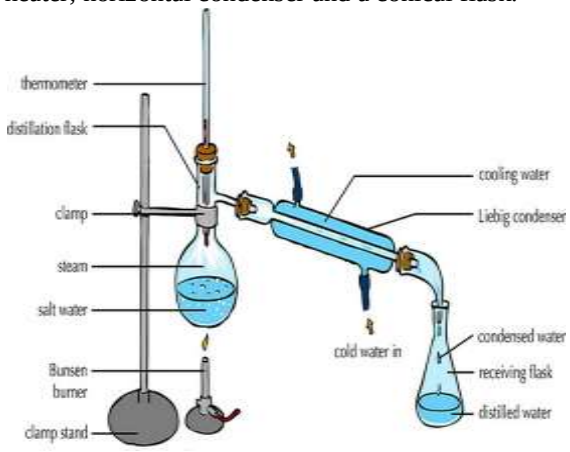

Figure.1.Steam distillation unit

100g of pre-treated orange peels sample is taken in a distillation flask. To that 200ml of water is added. Heat is supplied to the distillation unit by temperature controlled basket heater. At the initial stage, experiment is carried out at a Temperature of 88C for 60 min. time period. The distillate is collected in a conical flask. This distillate has two layers, one dense layer and other less dense layer. This is then separated using a separating funnel. The less dense upper layer is the citrus oil. This oil is then stored in a glass bottles. [5]

2. Extraction of essential Oil by hydro Distillation

Water or hydro distillation is one of the oldest and easiest methods. It consists of distillation flask, Basket heater, horizontal condenser and a conical flask. About 150 g of the orange peel powder was weighed using digital weighing balance and then transferred into a round bottom flask with large amount of water added to cover the peels. The flask was connected to the still column which was connected to the condenser. Heat is supplied to the distillation unit by temperature controlled basket heater. The experiment is carried out at different Temperature and time period. The distillate is collected in a conical flask. This distillate has two

International Journal of Pharmaceutical Research and Applications
Volume 7, Issue 1 Jan-Feb 2022, pp: 773-779 www.ijprajournal.com ISSN: 2249-7781

layers, one dense layer and other less dense layer. This is then separated using a separating funnel. [4]

3. Solvent Extraction

Essential oil extraction from orange peels was done using the Soxhlet method. The orange peels were pureed using a blender. A round bottom flask was washed, and oven dried and cooled in a desiccator. To carry out this procedure, the ground peels were sieved using a standard 0.6 mm particle size sieve. A dried mass of 10 g of the orange peel powder was weighed, and the weight recorded. The weighed sample was dropped in the Soxhlet extractor apparatus as shown in Figure-2. The extraction was carried out using normal hexane, methanol, and petroleum as the extraction solvent. In the Soxhlet apparatus, the solvent in the round bottom flask was heated from the heating mantle to become evaporated and got condensed down through the sample where it was able to extract the oil along, thereby, giving a mixture of oil and solvent. [4]

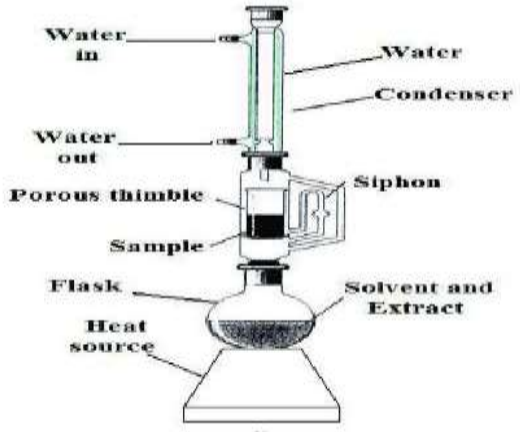

Figure.2.soxhlet apparatus set-up

Separation of essential oil

Method-1

When the mixture is poured into a Separatory funnel, the oil and water separate into two distinct layers as shown in figure 3. since water is denser than oil, it is collected at the bottom of the funnel. After this the funnel tap is opened and the liquid at the bottom of the funnel is transferred into a container. [4]

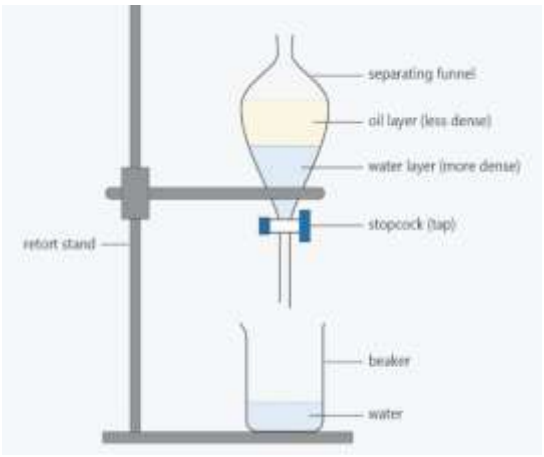

Figure.3.Separating funnel

Method-2

The oil was obtained from fresh material (C. aurantium and C. Limon) by steam distillation in a Clevenger-type trap (Figmay). Two fractions were obtained: an insoluble distillate separated by decantation and a soluble one that was extracted with diethyl ether. The oil was dried over anhydrous sodium sulphate and stored at 4ºC. [7]

Confirmation test for Limonene

Citrus oil is extracted from orange peels by cold press method and subjected to conformation test for limonene content in the oil. Bromine test: A dilute Bromine-water solution is prepared and taken in a test tube. To that citrus oil extracted from orange peels is added. If limonene is present in the oil extracted, the colour of the Bromine - water gets changes from red brown to pale yellow. This is because of the fact that the Bromine present in the Bromine – water solution occupies the space between the two double bonds present in limonene. The conformation test for Limonene. [5]

IV. ANTI MICROBIAL ACTIVITY

Antimicrobial activities of the essential oil were carried out against Gram-positive bacteria (Staphylococcus aureus ATCC 6538 and Bacillus cereus) and Gram negative (Salmonella typhi, Escherichia coli ATCC 739, Pseudomonas spp, and Shigella spp) and the fungal cultures (Aspergillus niger, Aspergillus flavus, Aspergillus Parasiticus) using well diffusion technique. [6]

- **Microorganisms and their growth conditions**

Microbial strains including Salmonella typhi, Escherichia coli ATCC 739, Staphylococcus aureus ATCC6538, Bacillus cereus, Pseudomonas spp, Shigella spp, Aspergillus niger, Aspergillus flavus, Aspergillus parasiticus were obtained from Food Microbiology Laboratory PCSIR Laboratories

International Journal of Pharmaceutical Research and Applications
Volume 7, Issue 1 Jan-Feb 2022, pp: 773-779 www.ijprajournal.com ISSN: 2249-7781

Complex Jamrud Road Peshawar Pakistan. All bacterial and mould strains were cultivated in Nutrient Agar and Potato Dextrose Agar for 48hrs at 37ºC (bacteria) and 25ºC for 3 days (mould) respectively following refrigeration storage at 4°C until required for sensitivity testing. [6]

Antimicrobial Activity Evaluation of Essential Oil

- **Antibacterial Susceptibility Assay**

The antimicrobial activity of essential peel oil was determined by agar well in nutrient broth at 37°C for 24h. Each test bacterium was spread with the help of sterile spreader on to a sterile Muller-Hinton Agar plate so as to achieve a confluent growth. The plates were allowed to dry and a sterile cork borer (6.0mm diameter) was used to bore wells in the agar. Subsequently,10µL, 25µL and 50µL volume of the essential oil was introduced in triplicate wells of the agar plates. The plates were allowed to stand for 1h or more for diffusion to take place and then incubated at 37°C for 24h. The zone of inhibition was recorded to the nearest size in mm. The results concerning In vitro antimicrobial activities of sour orange essential oils with the inhibition zone (mm) values are shown in Table2. [6]

Table 2.Anti microbial activity of sour orange peel essential oil

Tested Microorganism	Used volume of essential oil Zone of inhibition in mm		
	10 µL	25 µL	50 µL
Salmonella typhi	12mm	14mm	17mm
Escherichia coil	10mm	12mm	15mm
Pseudomonas spp	12mm	13mm	14mm
Staphylococcus aureus	11mm	13mm	15mm
Bacillus cereus	10mm	14mm	16mm
Aspergillus niger	13mm	15mm	17mm

V. CONCLUSION

Orange is a fruit which is easily available in all over the world, whole parts of orange plant is used for the various number of medicinal purpose such as anti-bacterial, anti-fungal, anti- diabetic, cardio- protective, anti-cancer, anti-arthritic, anti-inflammatory, anti-oxidant, anti-tubercular, anti-asthmatic and anti-hypertensive, and also extracted essential oil of orange peel has medicinal properties such as anti-microbial activity, skin care purpose ,oral care, etc. Various number of orange species in the world in which there are Number of researches are done and also there are number of developed products based on oranges. Now a days also there are lot of ongoing researches based on various species of orange plant, so in future there is development of number of products regarding to various therapeutic application and cosmetic application of orange.

REFERENCES

[1]. Parle Milind, Chaturvedi Dev, 2012, "Orange: range of benefits," International research journal of Pharmacy,vol.3, no.7, pp. 59-63.

[2]. Saidat Olanipekun Giwa, Mahmood Muhammad, Abdulwahab Giwa, 2018, "Utilizing orange peels for essential oil production,"APRN Journal of engineering and applied science, vol.13,no.1, pp. 17-27.

[3]. Ipek Suntar, Haroon Khan, Seema Patel, Rita Celano, Luca Rastrelli, 2018, "An overview on citrus aurantium L.: its function as food ingredient and therapeutic agent," Oxidative medicine and cellular longevity, pp .1-12.

[4]. TsegayeFekadu, Tesfaya Seifu, Abambagade Abera, 2020, " Extraction of Essential Oil from peel using Different Methods and Effect of Solvents, Time, Temperature to Maximize Yield," International Journal of Engineering Science and Computing,vol.9,no.12, pp.24300-24308.

[5]. D.C.Sikdar5, Rohan Menon, Karan Duseja, Piyush Kumar, Priksha Swami, 2016, "Extraction of citrus oil from orange (citrus sinensis) peel by steam distillation and its characterization," International Journal of Technical Research and Applications, vol.4,no.3,pp.341-346.

[6]. Javid Ali, Asfandyar khan, Naseem Ullah, Muhammad Amin, Zia-ur-Rahman, Muhammad Umar Khayam, Farhat Ali Khan, Arshad Hussain, Muhammad Khurram,

Muhammad Nasimul Allah Quarashi, 2012, " Essential oil chemical composition and Antimicrobial activity of sour orange (citrus aurantium) peels," Journal of Pharmacy Research,vol.5,no.3, pp.1690-1693.

[7]. Emilio Villafane, Diego Tolosa, Alicia Bardon, Adriana Neske, 2011, "Toxic effects of citrus aurantium and C. limon essential oils on spodopters frugiperda (Lepidoptera: Noctuidae)," Natural Product Communications, vol.6, no.9, pp.1389-1392.

[8]. Iness Jabri-Karoui, Brahim Marzouk, 2014, "Bioactive Compounds, Antioxidant Activities and Heat Stability of Corn oil Enriched with Tunisian Citrus aurantium L. peel Extract," Journal of the American Oil Chemists' Society, pp.1-9.

[9]. Reza Farahmandfar, Behraad Tirgarian, Bahre Dehghan, Azita Nemati, 2019, "Comparison of different drying methods on bitter orange (citrus aurantium L.) peel waste : changes in physical (density and color) and essential oil (yield,composition,antioxidant and antimicrobial) properties of powder," Journal of Food Measurement and Characterization,pp.1-14.

[10]. Maria Isabel Roth Carvalho-Fretitas, Mirtes Costa, 2002, "Antiolytic and sedative of extracts and essential oil from citrus aurantium.L," Pharmaceutical biology, vol.25, no.12, pp.1629-1633.