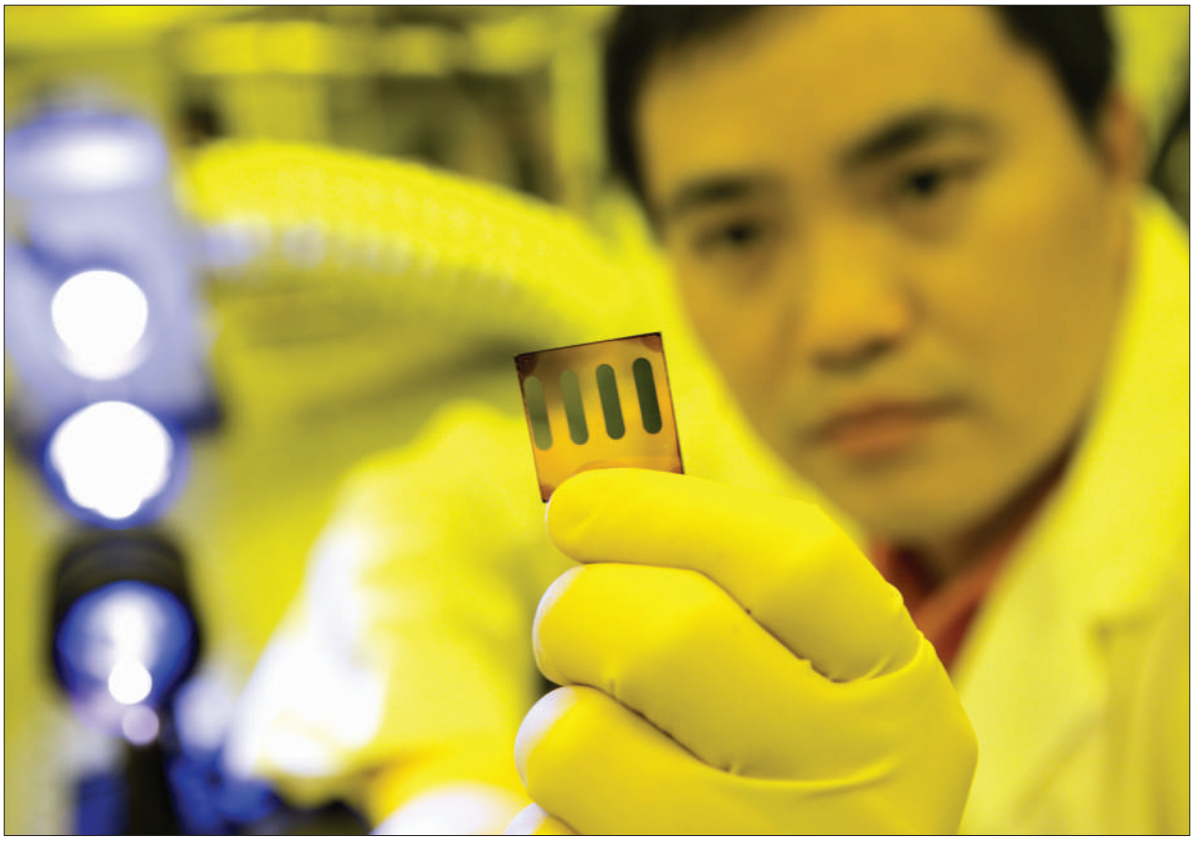

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Literature Survey on Extraction Procedure, Chemical Constituents and Pharmacological Activities of Volatile Oils and Other Extract from Citrus Plants

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ABSTRACT

The plants providing citrus fruits are rich in different types of volatile oils. The volatile oil so obtained is intended for different pharmacological activities. The essential oils from different parts like leaves, fruits and bark has been reported several time in different amount by using different extraction procedures. The volatile oils were evaluated for their pharmacological activities. On the basis of their reported pharmacological activities these essential oils are being used to manufacture several medicinal preparations. As several researches has been reported their findings on the volatile oils from such plants for different purposes so such researches are extremely needed to be brought in knowledge of every concern researchers. The present study is therefore focused on the literature review on such researches on volatile oil extraction procedures, reported chemical constituents and pharmacological properties.

Key words: Citrus, volatile oil, pharmacological activities

INTRODUCTION

The volatile oils also known as ethereal oils and aetherolea are liquid hydrophobic principle of fragrance bearing plants commonly known as 'Aromatic plants'. Not necessarily, the plants bearing fragrance are medicinally important but those plants which are having volatile oils with disagreeable taste and odor may bear pharmacologically active principles. Volatile oils from different parts of plant has been reported by researchers like from rhizome of *Stephania glabra* and from leaves of *Woodfordia fruticosa* (Hemraj *et al*., 2012). If separately find about the literature for volatile oils from citrus fruits, plants and leaves then clearly the number of researches comes in front in different fields like in 'Ayurveda', medicine, agriculture and many more. Citrus oil is used frequently in aromatherapy to uplift mood and relieve stress. Also the volatile oil is used as an antiseptic, antifungal and antibacterial (Vashist *et al*., 2014). The extraction of volatile oil from citrus fruits has been reported by hydrodistillation from citrus fruit peels (Vashist and Sharma, 2013). Number of active principles such as citral, limonene, hesperidins, geraniol, citronellal, aurantiamarin and aurantiamaric acid has been isolated from different parts of citral plants. By noting the importance of citrus oil, the present study has been focused on the literature survey on citrus volatile oil:

- Hydrodistillation (a) Extraction of volatile oil by hydrodistillation has been reported from the peels of *Citrus latifolia*. They extracted 60 g of lime peels by hydrodistillation using a modified

clevenger apparatus. Higher concentrations of oxygenated compound were reported by using the extraction time of 1-8 h from the whole and milled parts of plants (Atti-Santos *et al.*, 2005), (b) The hydrodistillation of orange peel has been reported in different ways. Here the dried grinded peel (30 g) was extracted by using clevenger apparatus oil for lighter than water. Extraction was carried for 3 h at 75°C (Vashist and Sharma, 2013)

- The supercritical fluid extraction has also been reported (Atti-Santos *et al.*, 2005). Here the researchers used Hewlett Packard 7680 extraction module to perform all the experiments. They loaded the material into a thick-walled stainless steel thimble self-sealing extraction cell of 7.0 mL internal volume of capacity. They used 1.0 g of whole lime peels sample for extraction with supercritical carbon dioxide according to the procedure described. Temperature (40-60°C) and pressure (90 to 110 bars) were used as the parameters to be evaluated. All other variables were kept constant using milled material, with 2.0 mL min^{-1} (CO_2) flow, 10 min equilibrium time, 30 min extraction time. The best conditions established for temperature and pressure were used in the next series of experiments, changing the CO flow and the extraction time. They deposited extracts in an internal trap and rinsed off into a vial with 1 mL of hexane and the oil yields were determined in each case

CHEMICAL CONSTITUENTS

A report on the chemical constituent from hydrodistillation of oven dried peels of three species of citrus fruits viz., *Citrus reticulata* (*C. reticulata*), *Citrus sinensis* (*C. sinensis*) and *Citrus paradisi* (*C. paradisi*) by using the process of GC and GC/MS. The findings from their report have been depicted in Table 1 (Kamal and Jawaid, 2011). The chemical constituents of mandarin peel oil by cold expression by using GC-MS have been reported. Limonene as more abundant monoterpene, α-pinene and α-tujene were reported and was observed abundantly in the oil whereas α-copaene, trans-α-bergamotene and β-farnesene were reported as the sesquiterpenoid (Kostadinovic *et al.*, 2011). Similarly, the chemical constituents of volatile oils from the peels of *Citrus sinensis* by Gas Chromatography-Flame Ionization Detector (GC-FID) and Gas Chromatography-Mass Spectrometry (GC-MS) were reported. Limonene, myrcene, sabinene and α-pinene were reported as the main component in cold expression extracted volatile oil (Azar *et al.*, 2011). Erucylamide, limonene and citral in essential oil from leaf and peel of *Citrus medica* analyzing by Gas Chromatography Mass Spectroscopy (GC-MS) has been reported (Bhuiyan *et al.*, 2009).

Three major chemotypes has distinguished from lemon peel oil viz., Limonene, Limonene/β-pinene/γ-terpene and limonene/Linalyle acetate/Linalool whereas two chemotypes were identified for lemon leaf oils: Limonene/-pinene/geranial/neral and linalool/linalyl acetate/R-terminal. Again, four chemotypes viz. limonene; limonene/-terpinene; limonene/-pinene/-terpinene and limonene/-terpinene/-pinene/oxygenated products in lime peel oils have been reported. Among this they reported four others for lime leaf oils like β-pinene/limonene, limonene/geranial/neral and limonene/sabinene/citronellal/linalool (Lota *et al.*, 2002).

Fourteen different components constituting approximately 99% has been identified from citrus oil. The major components reported were dl-limonene (89.089%), β-myrcene (2.933%) and (±)-linalool (2.927%), a-pinene (0.865%), (E) citral (0.749%) (Vasudeva and Sharma, 2012).

PHARMACOLOGICAL ACTIVITIES

Antimicrobial activity by disc diffusion method: Antibacterial activity was reported by using two bacterial strains of major human and animal pathogens, *E. coli* and *S. enteritidis* by disc

Table 1: Chemical constituents from the extract of *Citrus reticulate*, *Citrus sinensis* and *Citrus paradisi*

Hydrocarbons	*Citrus reticulate*	*Citrus sinensis*	*Citrus paradisi*
Monoterpene hydrocarbons	α-pinene	α-pinene	α-pinene
	Sabinene β-pinene,β-myrcene	Sabinene	Sabinene
	Limonene	Aphyllandrene	β-myrcene
	Z-β-ocimeneγ-terpinene	β-myrcene	γ-terpinene
		Δ^3-carene	Limonene
		Limonene	
		Z-β-ocimene4-carene1,	
		3,8-p-menthatriene	
Oxygenated monoterpene	1-octanol	Linalool oxide	Linalool oxide
hydrocarbons	Linalooloxide	Linalool	Linalool
	Linalool	tras-p-2,8-menthadien-1-ol	trans-p-2,8-menthadien-1-ol
	Menthadien-1-oltrans-p-1,8-dienol	Limonene oxidea-terpinenol	Limonene oxide Citronellal
	Citronellal	Decanal	4-terpineol
	α-terpineol	Z-carveol	4-carvon menthenola-terpineola-
			terpinenol
		Citronellol	Decanal
		d-carvone	Z-carveol
		Isophorone	Citronellol
		4-vinyl guaiacol	E-carveol
		PiperitenoneEugenol	Isophoroned-carvone
			Geraniol
			Citral4-vinyl guaiacol
			Carvyl acetate
			Eugenol
			Geranyl butyrate
			Geranyl acetate
Sesquiterpene	α-cubebeneCopaeneAllyl	α-cubebene	α-copaene
hydrocarbons	isovalerate,β-cubebene	Valencene	β-cubebene
	β-caryophylleneGermacarene	d-cadinene	β-caryophyllene
	α-farnesene		Germacarene D
	γ-munrolened-cadinene		Valencene, α-panasinsend-
			cadinene
			β-gurjuneneγ-gurjunene
Oxygenated sesquiterpene	Dodecanal	3-furanacetic acid	Dodecanal
hydrocarbons	Elemol	β-sinensal	Elemol
	γ-eudesmol, α-cadinol β-sinensal	α-sinensal	Nerolidol
	Farnesol, α-sinensal	Nikkol	β-caryophyllene oxideβ-
	Nootkatone		inensal, Farnesol
			α-sinensalSantolina

diffusion method. The essential oils of *Citrus limettioides* are used for the experiment. In this study, both microbes were cultured on agar media. Collected volatile oil was dissolved in methanol to make a solution of sample. The disc of sterilized whatman filter paper were cut in 6 mm in diameter and impregnated with the sample and kept on plates of bacterial culture and incubated at 37°C for 24 h (Nam *et al.*, 2006).

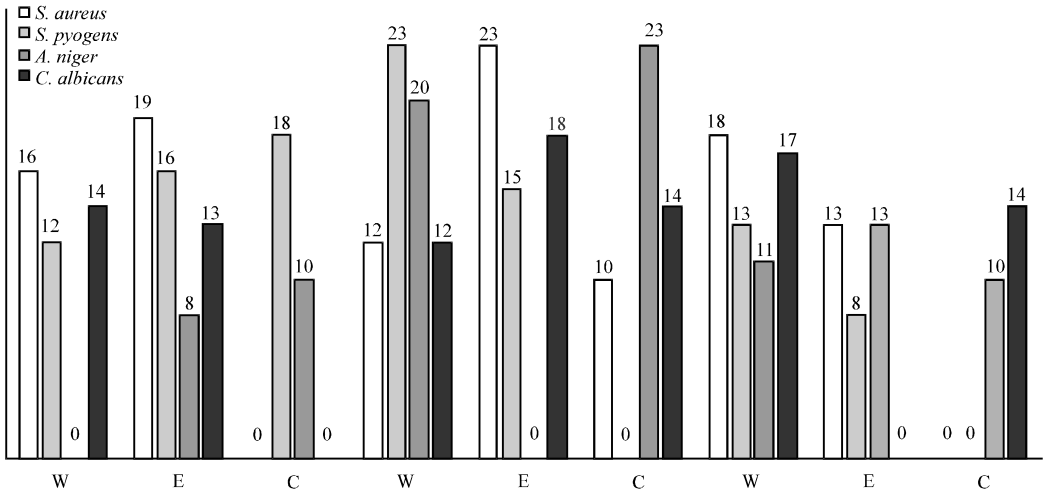

Fig. 1: Zone of inhibition from water, ethanolic and chloroform extracts of kinnow, orange and haddock against *S. aureus*, *S. pyogenes*, *A. niger* and *C. albicans*

MIC determination by using micro-dilution broth: For this experiment, researchers used serial dilution of EO over a range of 3.25-100 μL mL^{-1} in nutrient broth, potato dextrose broth media, for all bacterial strains like *Micrococcus luteus*, *Staphylococcus epidermidis*, *Enterobacter aerogenes*, *Enterococcus faecalis*, *Lactobacillus acidophilus*, *Propionibacterium acnes*, *Salmonella typhi*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Alcaligenes faecalis* were used except *Propionibacterium acnes*, fungal strains and *Propionibacterium acnes*, respectively. Suspension culture of each microbial strain was used in a concentration of 1-2×10^3 cell mL^{-1} of bacterial and fungal strains and then was added to their respective media. After this they incubated the bacterial strains at 37±1°C for 24 h. While propionibacterium acnes blood agar base media plates were incubated at 35°C for 48 h in anaerobic conditions in anaerobic jars and plates with fungal strains were incubated at 29±1 for 7 days. The MIC was defined as the lowest concentration of the essential oil at which the microorganism did not demonstrate any visible growth (Vasudeva and Sharma, 2012).

Tube dilution methods: Both the bacteria were cultured in test tube in broth media. Different concentrations of volatile oils in methanol were added to 10 mL bacteria culture tube and incubated at 37°C for 12, 24, 36, 48 and 72 h. Bacterial growth at each incubation time was measured using a UV spectrophotometer at 620 nm (Nam *et al*., 2006).

Antibacterial activities: The antibacterial activities of citrus pulp rather than its volatile was reported on pure cultures of *Aspergillus niger*, *Candida albicans*, *S. aureus* and *S. pyogenes* by using nutrient agar medium. In this study aqueous extract of pulp of kinnow and orange was found to possess maximum antimicrobial activity whereas aqueous peel extract of shaddock was reported to possess more antimicrobial activity against *S. aureus* in comparison to aqueous peel extract of kinnow and orange. Again it was reported that ethanolic pulp extract of kinnow and orange showed similar antimicrobial activity against *S. aureus* in comparison to ethanolic pulp extract of shaddock. The chloroform pulp extract of kinnow showed maximum antimicrobial activity (Fig. 1). The aqueous extract of the same was found to be most potent antimicrobial agent against *S. pyogenes* than that of aqueous extract of pulp of orange and shaddock (Mathur *et al*., 2011).

The antimicrobial potential of citrus peel and citrus leaf volatile oil has been reported by using cup-plate method of Woods and Washington (1995). For this experiment they selected *Staphylococcus aureus* as gram positive bacteria *Pseudomonas aeruginosa, Klebsiella pneumonia* and *Escherichia coli* as gram negative bacteria and *Aspergillus niger, Candida albicans* as fungal strains. Nutrient agar and sabouraud's agar were used as nutrient media for bacteria and fungi, respectively. Total 100 µL of fruit peel and leaf oils were separately dissolved in 500 µL dimethyl formamide (DMF) and used it as sample. They compared the results obtained from 100 µL of fruit peel and leaf oil sample with Amoxicillin 500 µg mL^{-1} and amphotericin B 500 µg mL^{-1} as standard antibacterial and antifungal drug. Antibacterial activity ranged from 50-66% of total activity of Amoxicillin and 82.6%% total activity of Amphotericin B (Hamdan *et al*., 2013).

Bacteriostatic and bacteriocidal activity: The bacteriostatic and bacteriocidal activity of essential oils was tested from different citrus fruits along with anise, calamint, celery, coriander, cornmint, cumin, dill, fennel, laser, laurel, rosemary, sage, savory, tarragon, thyme, wild thyme and *Ziziphora* against *Aerobacter aerogenes, Bacillus subtilis, Escherichia coli, Proteus vulgaris, Pseudomonas aeruginosa, Staphylococcus albus* and *Staphylococcus aureus*. Here the citrus oils were reported sensitive to different extent (Kivanc and Akqul, 1996).

Anti-fungal: Antifungal Activity the antifungal activity in terms of zone of inhibition and minimum inhibitory concentration of EO of *Citrus limettioides* was tested against ten fungal strains viz. *Alternaria alternata, Rhizoctonia solani, Curvularia lunata, Fusarium oxysporum, Helminthosporium oryzae, Aspergillus fumigatus, Aspergillus niger, Aspergillus terreus, Cladosporium herbarum* and *Trichoderma viride*. The EO of *Citrus limettioides* exhibited varying antifungal activity against the various test strains. The highest antifungal activity of this essential oil was observed against *Fusarium oxysporum* (zone of inhibition of 10.23 mm) followed by *Aspergillus niger, Aspergillus fumigatus* with zone of inhibition of 9.9 and 9.32 mm, respectively. The zone of inhibition shown by EO against *Curvularia lunata, Aspergillus terreus* and *Trichoderma viride* was moderate (zone of inhibition 8.63, 8.65 and 8.56 mm, respectively). However, *Rhizoctonia solani, Helminthosporium oryzae* and *Cladosporium herbarum* were resistant to the EO. The minimum inhibitory concentrations were observed in the range from 6.25-25 µg mL^{-1} *Aspergillus niger* showed maximum susceptibility to the investigated EO with MIC value of 6.25 µg mL^{-1}. Weak inhibitory effect of the essential oil was observed against *Aspergillus terreus* with MIC value of 25 µg mL^{-1}. *Alternaria alternata, Curvularia lunata, Fusarium oxysporum, Aspergillus fumigatus, Trichoderma viride* showed similar susceptibility to the investigated essential oil with MIC of 12.5 µg mL^{-1}. In accordance with the results from disc diffusion method, *Rhizoctonia solani, Helminthosporium oryzae* and *Cladosporium herbarum* were resistant to the peel EO of *Citrus limettioides*. Dimethylsulfoxide (DMSO) was used as standard (Fig. 2). The chemical compounds like linalool, a-pinene, a-terpineol have antifungal and antibacterial activity which are found in appreciable amounts in *C. limettioides* oil. Citral one of the components of EO of *C. limettioides* acts as a fungicidal agent because it is able to form a charge transfer complex with an electron donor to fungal cells which results in fungal death (Nam *et al*., 2006; Vasudeva and Sharma, 2012).

Antifungal activity: Antifungal action of essential oils from *Citrus sinensis, C. aurantium, C. deliciosa, C. paradise, C. limon* on *Penicillium digitatum* and *Penicillium italicum* has been reported significantly (Caccioni *et al*., 1998).

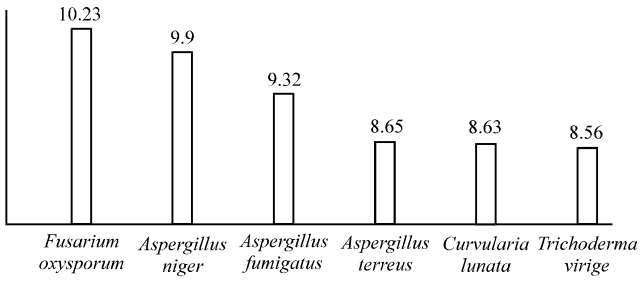

Fig. 2: Zone of inhibition in millimeter as compare to DMSO (8 mm) against *Fusarium oxysporum*, *Aspergillus niger*, *Aspergillus fumigates*, *Aspergillus terreus*, *Curvularia lunata* and *Trichoderma viride*

Antifungal effect of citrus fruits: A significant antifungal effect of citrus fruits has been reported on some plant pathogen fungi viz., *Alternaria alternate*, *Rhizoctonia solani*, *Curvularia lunata*, *Fusarium oxysporum* and *Helminthosporium oryzae* (Chutia *et al*., 2009).

Antimicrobial activity of plants: Antimicrobial activity on *Citrus aurantium* and *Cympogon nardus* essential oil has been reported significantly along with essential oils from 50 other plants on microbial strains like *Acinetobacter baumanii*, *Aeromonas veronii* biogroup *sobria*, *Candida albicans*, *Enterococcus faecalis*, *Escherichia coli*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Salmonella enterica* subsp. *Enterica serotype typhimurium*, *Serratia marcescens* and *Staphylococcus aureus* using an agar dilution method (Hammer *et al*., 1999).

Antimicrobial potential of plants: Antimicrobial potential of *C. odorata*, *C. citrates* and along with other essential oils from 9 other plants has reported against *Candida albicans*, *Rhodotorula glutinis*, *Schizosaccharomyces pombe*, *Saccharomyces cerevisiae*, *Yarrowia lypolitica* (Sacchetti *et al*., 2005).

Antibacterial potential of essential oils: The antibacterial potential of essential oils from lemon, lime, orange were evaluated along with oil from 18 other plants by disc diffusion method. Gram positive (*Bacillus subtilis* and *Staphylococcus aureus*) and Gram negative (*Escherichia coli*, *Klebsiella pneumoniae*), *Pseudomonas aeruginosa*, *Proteus vulgaris*) bacterial strain was used for the purpose. In result all the citrus plant essential oil has been reported to have significant antibacterial potential (Prabuseenivasan *et al*., 2006).

Anti-aflatoxin activity: Aflatoxins are extremely toxic secondary metabolite produced by *Aspergillus* species especially *Aspergillus flavus* and *Aspergillus parasiticus* which are found worldwide in air and soil and are also found in biologically contaminated food and feed. Aflatoxin are strong hepatotoxic and are highly toxic and caustic to liver which play sinister role in liver and extra liver cancer. Here researchers ground the experimental samples (Table 2) to pass through a 2 mm screen using a Wiley Mill. Then they sterilized it at 121°C for 15 min finely they dissolved citral essential oil in methanol to give final concentrations of 0.028, 0.056 and 0.112 g kg^{-1} and sprayed gently onto feed samples. Humidity was maintained between 78 and 83%. They used the samples treated with 0.05 g kg^{-1} antifungal agent, containing 62% propionic acid, 5% acetic acid,

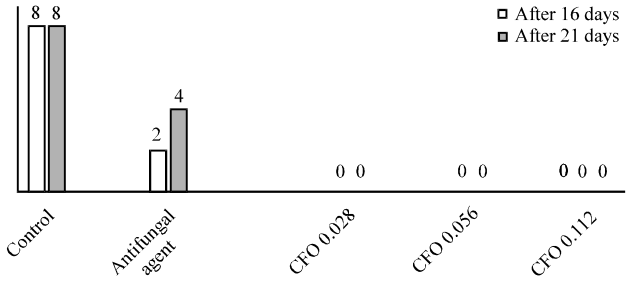

Fig. 3: Effect of Citrus Essential Oil (CEO) on aflatoxin reduction as compared to antifungal agent after 16 and 21 days

Table 2: Content of swine feed checked for aflatoxin presence

Name	Quantity (g)
Yellow corn	65.16
Rice hull	8.00
Soybean meal	19.00
Lupin seeds	3.20
Animal fat	3.00
Molasses	3.00
Limestone	0.90
Salt	0.30
Hog premix	0.30
Methionine	0.01
Lysine	0.04

Table 3: Pharmacological activity of *Citrus aurantium, Citrus limon is* essential oil on mice (Costa *et al*., 2013)

Name of drug	Extract used	Method of evaluation	Uses and proposed mechanism of action
Citrus aurantium	Fruit extract	Light/dark box procedure	Anxiolytic, through serotonergic system (5-HT (1A) receptors)
		Neurochemical evaluation	Cholesterol level decreased but no alterations in neurotransmitter levels in the cortex, the pons and the hypothalamus
Citrus limonia	Leaves extract	Open-field observation	Decrease in crossings, grooming and rearing
		Elevated-plus-maze	Increased in time of permanence and the number of entrances in the open arms
		Rota rod	No effect on motor coordination
		Phenobarbital induced sleeping time test	Increase in sleeping time
		Swimming test	No sedative effect

1% sorbic acid, 1% benzoic acid and 1% phosphoric acid, as positive controls and samples treated with methanol as negative controls. After 16 and 21 days of storage they collected samples of feed and measured aflatoxin concentrations by using Aflatoxin kits. Finally in their result they found that in comparison to uninterrupted control group no aflatoxin contents were observed in Citrus Essential Oil (CEO) treated swine feed. Similarly few absence of aflatoxin was observed as compared to other group which is treated with antifungal agent (Fig. 3) (Nam *et al*., 2006) the pharmacological activity of essential oils of citrus shown in Table 3.

Insecticide activity: Insecticide activity has been reported for essential oils from orange peels. This was performed in combination with camphor and kerosene. For this purpose researchers first

crushed camphor into powder. Then mixed 60 mL kerosene and 20 mL orange peel essential oil in a beaker of 250 mL capacity. To this mixture they added crushed powder of camphor gradually to make saturated mixture and stored the mixture in an air tight container. The mixture so obtained was tested on mosquitoes, ants, flies, cockroaches and spiders. All insects were killed after 1-5 min of contact. Here researchers also reported that orange peel can serve as a source of pectin. For this purpose they diluted citric acid with distilled water to get a pH value of 1.5. Orange peel residue weighing 100 g was dipped into 1000 mL of the citric acid solution and heated at 100°C for 20 min. After cooling the solution, they filtered it by using cloth filter. The solution was then filtered using cloth filter. Exact 250 mL ethanol solution was then added to the filtrate to facilitate filtration of pectin. The solution was filtered using fine filter cloth to separate jelly pectin which was then sun dried.

Anti-inflammatory activity: The anti-inflammatory activity of *Cleopatra mandarin* (*Citrus reshni*) oils was determined relative to its ability to inhibit both TNF-α and NO production (Hamdan *et al*., 2013).

- Anti-inflammatory activity of Bergamot (BO) essential oil has reported by using carrageenan induced rat paw edema model using six different groups. Three different doses of BO: 0.025, 0.05 and 0.10 mL kg^{-1} were used. Indomethacin was used as a reference agent. The oil reduced the inflammation upto 95.70% with indomethacin, 27.56% with 0.025 mL kg^{-1} BO, 30.77% with 0.05 mL kg^{-1} BO and 63.39% with 0.10 mL kg^{-1} BO (Karaca *et al*., 2005)
- The significant anti-inflammatory activity of *Citrus paradisi* fruit extract has been reported combination with *Citrus paradise*. Similarly the anti-inflammatory activity of citrus flavonoids has been reported (Benavente-Garcia and Castillo, 2008)

Acute effect of bergamot oil: Saiyudthong and Marsden (2011) has reported the acute effect Bergamot oil on anxiety related behavior whereas Wiebe (2000) has reported importance of citrus oil in aromapathy to lower the anxiety.

Anticancer property: The anticancer property on the citrus limonoid (limonene) has been reported in mice (Jacob *et al*., 2000):

- Benavente-Garcia and Castillo (2008) has reported the anticancer potential of citrus flavonoids whereas Silalahi (2002) reported anticancer and health protective properties of citrus fruit components
- Maliheh *et al*. (2009) reported the antimutagenicity and anticancer effect of citrus medica fruit juice by performing vital capacity test on *Salmonella typhimurium*. The comparative study was performed by using fully ripe and half ripe fruit juice. In the result they reported that half ripen fruit extract than fully ripe fruit
- Anticancer activity of *Citrus reticulate* and *Pelargonium graveolens* essential oil has been reported by using human leukemia promyelocytic cell lines on *in vitro* study. The volatile oil from both the plants was significantly found to be effective against these cell lines
- The antitumor activity of *Citrus maxima* (Burm.) Merr. leaves has reported in Ehrlich's Ascites Carcinoma (EAC) cell-Treated Mice. They administered orally the extract at the doses of

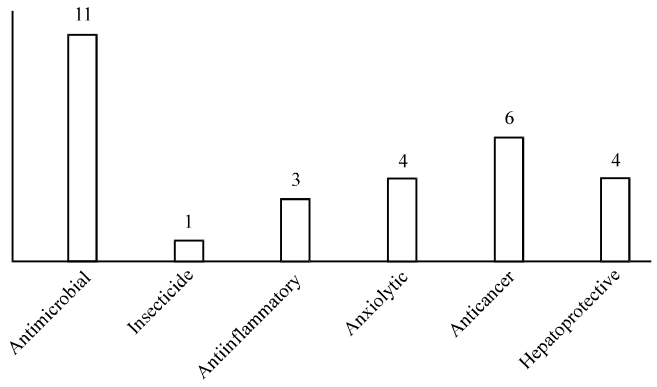

Fig. 4: Intensity of total pharmacological activities studied during literature survey

200 and 400 mg kg^{-1}. They reported significant decrease in tumor parameters such as tumor volume, viable tumor cell count and increased body weight, hematological parameters and life span in respect of the EAC control mice (KunduSen *et al.*, 2011)

- Similarly the anticancer activity of *Citrus paradisi* and *Citrus sinensis* has been reported (Samman *et al.*, 1998; Wilcox *et al.*, 1999)

Hepatoprotective Activity: Hepatoprotective activity of Bergamot Orange (BO) essential oil has been evaluated on carbon tetrachloride-induced hepatotoxicity in rats. From this study it was reported that the BO has very poor hepatoprotective effect on carbon tetrachloride-induced hepatotoxicity (Karaca *et al.*, 2005).

- Significant hepatoprotective effect of *Citrus reticulata* on isoniazid hepatotoxicity in Wistar rats has been reported. A dose of 200 mg kg^{-1}, p.o. was administered in rate for 30 days (Kangralkar *et al.*, 2010)
- Presence of monoterpenes, a major component of the plant essential oil of *Citrus sinensis* leaves has been reported to have hepatoprotective property on albino rats (Soji-Omoniwa *et al.*, 2014)
- Bergamot essential oil was reported for its hepatoprotective effect on carbon tetrachloride-induced hepatotoxicity in rats. Six different groups were used for the purpose. In the report significant decrease in serum alanine transaminases level was reported when compared to CCl group while it did not affect the serum aspartate level. They absorbed no significant difference between the BEO and CCl group (Karaca *et al.*, 2005)

CONCLUSION

From the above studied literature it is clear that the citrus extracts as essential oil or as other extraction contain number of valuable chemical constituents from different parts of these plants. Number of pharmacological activities has been studied by different researchers with the gigantic expectations to be a better alternate with synthetic drugs for number of ailments. Antimicrobial activities were studied several times (Fig. 4) by researchers which shows that different extracts from citrus plants could be a better topical as well as systemic antimicrobial agents.

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