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# PHYTOANALYTICAL AND PHYTOCHEMICAL INVESTIGATION OF ESSENTIAL OILS OF LEAVES OF TWO MAJOR COMMERCIAL VARIETIES *Citrus aurantium* L. AND *Citrus sinensis* L.

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**ABSTRACT**

Plants are the fashionable foundation of drugs for the mainstream of the world people. The present study was carried out to investigate the constituents of leaves of two major commercial verities of sweet and bitter orange *Citrus aurantium* L and *Citrus sinensis* L. Alkaloids, flavonoids, steroidal glycosides, volatile oils, terpenoids, and sterols were confirmed by preliminary phytochemical studies. The physicochemical parameters like Loss on drying, Extractive values, and Ash values were also evaluated. GC-MS analysis of essential oils of *Citrus aurantium* L. contains 25 compounds and *Citrus sinensis* L. showed the presence of 30 bio-active compounds. The major components of *Citrus aurantium* are 1,3-Cyclohexadiene, trans-3-Caren-2-ol, Eucalyptol, γ-Terpinene, Linalool, 2-Cyclohexen-1-ol, 6-Octenal, Terpinen-4-ol, Benzene, α-Terpinyl acetate, 2,6-Octadien-1-ol, Cyclohexane, Caryophyllene, Tetra methyl bicyclo undeca-2,6-diene, 4-Isopropyl-3,7-dimethyloctahydro-1H-cyclopentane, Naphthalene, Caryophyllene oxide, 1-Naphthalenol, Cardinol, and Phytol. Whereas *Citrus sinensis* L essential oil shows the presence of Eucalyptol, α- Phellandrene, D-Limonene, 3-Carene, Eucalyptol, β-Ocimene, γ-Terpinene, Linalool, Isoneral, Terpinen-4-ol, 6-Octenal, 3,7-dimethyl, α-Terpineol, Citronellol, 2,6-Octadien-1-ol, Neral, Geraniol, trans-Geranic acid methyl ester, 6-Octen-1-ol, 2,6-Octadien-1-ol, 3,7-dimethyl acetate, 3,7-dimethyl-acetate, Geranyl acetate, Caryophyllene, Humulene, Caryophyllene oxide, and Phytol as the notable compounds. However, both species show similar compounds in their GC-MS analysis with little difference. This study will help us to know the similarities and differences between the closely related medicinally important species of *C.sinensis* and *C.aurantium* on their phytochemical nature.

**Keywords:** Essential Oil, *Citrus aurantium* L and *Citrus sinensis* L, Phytoconstituents, GC-MS Analysis, Sweet Orange, Bitter Orange.

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## INTRODUCTION

*Citrus aurantium* L. generally recognized as bitter Orange is a broadly available plant that belongs to the family Rutaceae. It is also known as sour Orange, Seville orange, or brigade orange.[1] *Citrus aurantium* (Khattak in Hindi, Narangam, or Narattai in Tamil) is a bush with greenish-white, glabrous sprouts cultivated in India for its fruit and used for various medicinal purposes. Bitter orange is regularly cultivated

in Khasi hills and Catcher. Decoction of leaves in oral charity for cold, coughs, and bronchitis in Rodrigues Islands. Warm water extract of *C.aurantium* var. *Tachibana* leaves is used to manage inflammation in customary medicine as early as South Korea. Extract of garden-fresh leaves used for ear aches in East Africa. Water extracts of *C. Aurantium* garden-fresh leaves are used for dysentery orally.[2] *C. Aurantium* is customarily known for the management of extensive pane of illnesses like cough, cold, bronchitis, stomach ache, vomiting, blood pressure, fever, earache, diarrhea, dysentery, and abdominal pain. Barks are utilized for Urinary Tract Infections. Infusion of the dried-up flower is used for anti-spasmodic, influenza, insomnia, cold, sedative, digestive, and as a cardiovascular analeptic in the oral route. Roots are used to manage boils and urinary tract infections.[3-5] *Citrus sinensis* L. commonly known as sweet Orange is a trifling, shallow, perennial bush or tree rising around 6 to 13m in altitude with surrounded tapering greater and mostly spiny branches.[6] It is well-known for its fruits, commonly available in countries around the globe. The tree is commonly cultivated for its fruit in temperate, subtropical, and tropical zones.[7] It desires a noticeable change in the periods and so is not so suited to the tropics, where it is grown up additional as a garden bush, but is widely full-grown commercially in the subtropics.[8] The water-distilled new leaves contain α-Pinene, cis-Sabinene hydrate, Citral, Lavandulol, l-Limonene, Perillyl alcohol, Perillaldehyde, and tert-Butyl benzoate. The ethanolic extract of leaves reported the presence of terpenoids, alkaloids, tannins, saponins, and flavonoids. It is rich in flavonoids, Vitamin-C, phenolic compounds, and volatile oils. The fruit is an appetizer and blood purifier. The fruit juice was used for the treatment of bilious affections and bilious diarrhea. The inner rind of the fruit is carminative and tonic. The fresh rind is scrubbed on the face to cure acne. The dried peel is useful in the treatment of anorexia, coughs, and colds.[9-10] The leaves with salt in the form of the decoction are taken vocally for gastrointestinal tract sicknesses, nerve disorders, pyrexia, blood pressure, asthma, vomiting, and general fatigue. The crumpled leaves or fruit sap are kneaded on the skin for itch relief. Fruit sap or leaf decoction with or without sugar is taken vocally for cold and loss of appetite, while crumpled leaf decoction as a steam bath discharges headache and rheumatism. Macerated root, leaves, or fruits mesoderm is taken vocally for urethritis. Leaf oil shows carminative stuff, antispasmodic and sedative properties.[11] Medicinal plants are a noteworthy part of intrinsic medical systems all over the world due to the existence of significant bioactive pharmaceutical metabolites. Improvement of novel and profound techniques to perceive biologically dynamic natural products to isolate, purify and structurally characterize these active constituents are highly inspiring in the current era.[12] The current study is dedicated to the examination of phytochemical constituents found in leaves essential oils of *Citrus aurantium* L and *Citrus sinensis* L using Gas chromatography and Mass spectroscopic (GC-MS) methodology along with preliminary phytochemical screening and physicochemical analysis to study the similarities and dissimilarities.

## EXPERIMENTAL

### Plant Collection and Authentication

The leaves of the healthy plants of *Citrus aurantium* L. and *Citrus sinensis* L. selected for the research were composed from Azhagar hills, Madurai District, Tamil Nadu, and India on the month of January 2020 and were authenticated by a taxonomist, in Madurai.

### Macroscopic analysis

Macroscopic analysis of the plants was done by observing their exterior topographies. Their contour, magnitude, outward characters, consistency, colour, odour, and palate are acknowledged and compared with reference according to the standard protocol.[13]

### Analytical Parameters

Ash values, extractive values, and loss on drying were performed for the study of the physical possessions of the herbal medicines for their quality control. The ash of any organic material is composed of non-volatile inorganic components. Ash values are helpful in determining the quality and purity of the crude drug, especially in the powdered form. Meticulous burning of herbal drugs results in ash consisting of an inorganic material such as sand, silica, earthy, and mud matter along with inorganic metallic salt. This value varies within a wide range and is, therefore a significant constraint for purpose of estimation of crude drugs. In certain remedies, the percentage variation of the weight of ash from drug to drug is very small and any

noticeable change indicates a change in quality. The ash value can be determined by three different methods to measure the total ash, the acid insoluble ash, and water-soluble ash.[14-16]

**Determination of Total Ash Value**
Accurately weigh about 5gm of the air-dried powdered drug in a tarred silica pot and burned by gradually increasing the temperature to make it dull red until it is free from carbon. Then it is chilled and evaluated to calculate the ratio of total ash with orientation to air-dried drug.

**Determination of Acid Insoluble Ash Value**
Boil the total ash with 25ml of 2M Hydrochloric acid for five minutes, the unsolvable material is collected in a silica pot or on an ash-less filter paper and it is splashed with hot water. Burn and cool in a desiccator and weigh the residue. Calculate the percentage of acid-insoluble ash with orientation to the air-dried sample.

**Determination of Water-soluble Ash Value**
Water-soluble ash is the part of the total ash content which is soluble in water. It is good a pointer of either previous extraction of the water-soluble salts in the drug or incorrect preparation. To the silica pot containing the total ash, add 25ml of distilled water and boil for five minutes. The insoluble matter is collected in a sintered glass silica pot or on an ashless filter paper. Wash the ash obtained with hot water and ignite it in a crucible for 1 minute at a temperature not exceeding 450ºC. Deduct the weighed residue in mg from the weight of the total ash. The content of water-soluble ash is calculated in mg/g of the air-dried sample and the percentage of water-soluble ash is find out with reference to air dried sample taken.

**Determination of Sulphated Ash Value**
Heat the silica pot to redness for 10 minutes; allow the silica pot to cool in a desiccator and weighed it. Unless it is specified in the individual monograph, transfer to the silica pot 1gm of the substance under examination and weigh the silica pot and the contents accurately. Ignite gently at first until the substance is thoroughly burned. Cool, moisten the residue with 1 ml of sulphuric acid, and heat gently until the white fumes are no longer evolved and burn at 800ºC ± 25ºC until all black particles have vanished. Conduct the ignition in a place protected from air currents.

**Extractive Values**
Extractive value is used to decide the number of therapeutic metabolites in medicinal plant material when it is extracted with appropriate solvents. This method is employed for medicinal plant material for which no chemical or biological assay is unavailable.

**Determination of Alcohol Soluble Extractive**
5gm of coarsely powdered and air-dried drug is macerated with 100ml of absolute alcohol in a stoppered flask for 24 hours, pulsating often during the first 6 hours, and permitted to stand for the remaining 18 hours. Then filter immediately taking safety measures against loss of absolute alcohol. From this 25ml of filtrate is evaporated to dryness in a calibrated flat-bottomed narrow dish, dry the residue at 105ºC in an oven, and weighed. The percentage of ethanol soluble extractive value with reference to the air-dried sample has been calculated.[17-19]

**Determination of Water-Soluble Extractive**
The water-soluble extractive value plays an important role in the evaluation of crude drugs. 5gm of the air-dried and coarsely powdered drug must be macerated with 100 ml of water/chloroform water of the specified strength in a closed flask for 24 hours, shaking frequently during the first 6 hours and allowing stand for 18 hours. Thereafter, the filter rapidly takes precautions against loss of water. Evaporate 25ml of the filtrate to dryness in a tarred flat-bottomed shallow dish, dry at 105ºC, and weighed. The percentage of water-soluble extractive value with reference to the air-dried drug.

**Loss on Drying**
Loss on drying is the loss of weight communicated as a percentage w/w. It governs both water and volatile matter present in crude drugs. It can able to be done either by heating at 100ºC-105ºC or keeping in

desiccators over dehydrating agents such as phosphorus pentoxide under controlled or reduced pressure at room temperature for a specified period of time.[20-22] Procedure: About 5 gm of the powdered crude drug was taken in a tarred china dish which was before dried at 105ºC in a hot air oven to a persistent weight and then weighed. The percentage of loss on drying with reference to the air-dried sample has been calculated to find out the result.[23]

**Isolation of Volatile Oils**

The specified quantity of fresh leaves of *C.aurantium* and *C.sinensis* were subjected to hydro distillation method using the clevenger apparatus for the determination of volatile oil. In this process, the clevenger oil armrest was fixed with a condenser over which chilled water was dispersed to avert low volatiles from absconding. The oil acquired upon isolation from the drug was dried over anhydrous sodium sulphate and kept in scaled glass bottles and stored in a fridge freezer.[24]

**Preliminary Phytochemical Screening**

Phytochemical screening tests were carried out on the dehydrated sample using customary procedures based on the procedures of Edeoga *et al.,* 2005; Harborne,1973 and Software,1993 to identify the various Phytoconstituents present in them.[25-27]

**GC-MS Analysis**

GC-MS investigation of the volatile isolated from leaves of *Citrus aurantium* L and *Citrus sinensis* L has been executed using an Agilent 8890 system comprising an AOC-20i auto-sampler and a Gas Chromatograph connected to a Mass Spectrometer (GC-MS) fitted out with an Elite-5MS (5% diphenyl /95% dimethyl polysiloxane) glued a capillary column (30 × 0.25µm ID × 0.25µm df). For GC-MS finding, an electron ionization system was worked in electron stimulus mode and with ionization energy of 70 eV. Helium gas (99.99%) was applied as a carrier gas at an unceasing flow rate of 1 ml/min, and an injection volume of 2µl was engaged (a split ratio of 10:1). The injector temperature was preserved at 250°C, the ion-source temperature was 200°C, the oven temperature was planned from 110°C (isothermal for 2 min), with an escalation of 10°C/min to 200°C, then 5°C/min to 280°C, ending with a 9 min isothermal at 280°C. Mass spectra were booked at 70 eV; a scan intermission of 0.5 sec. and fragments from 45 to 450 Da. The solvent delay was 0 to 2 min, and the total GC/MS running time was 36 min. The comparative proportion quantity of each constituent was calculated by equating its average peak area to the total areas. The mass-detector applied in this examination was Turbo-Mass Gold-Perkin–Elmer, and the software implemented to knob mass spectra and chromatograms was a Turbo-Mass ver-5.2.[28-29]

## RESULTS AND DISCUSSION

**Macroscopic Analysis**

The macro-morphological characters of the leaves of *C.aurantium* and *C.sinensis* were studied and the results were documented in Table-1.

Table-1: Macro-morphological Characters of Leaves of *C.aurantium* and *C.sinensis* L. (Rutaceae)

| S. No. | Parameters | *C. Aurantium* | *C. Sinensis* |
|---|---|---|---|
| 1. | Colour | Whitish green | Green |
| 2. | Odour | Strongly scented | Strong characteristic citrus |
| 3. | Taste | Bitter and astringent | Bitter and astringent |
| 4. | Texture | Hard and rough | Hard and papery |
| 5. | Size | 4-10 cm long | 4-12 cm long and 6 cm wide |
| 6. | Shape | Foliate, elliptic | Elliptical, oblong to oval, |
| 7. | Surface features | The shape of blades ranges from elliptical, bluntly serrated | The shape of blades ranges from elliptical, oblong to oval, bluntly toothed |
| 8. | Leaf margin | Serrated margin | Crenulated margins |
| 9. | Apex | Acuminate | Acute to obtuse |
| 10. | Base | Symmetrical | More or less symmetrical |
| 11. | Petiole | Winged | Narrowly winged |

**Analytical Parameters**

The phyto-analytical parameters were examined and stated as Total Ash Value (6.30% w/w, 5.24±1.04), Acid Insoluble Ash value (2.60%, 0.98±0.08), Aqueous Soluble Ash Value (1.20% w/w, 1.82±0.10), Sulphated Ash Value (2.90% w/w, 2.12±0.04), Alcohol Soluble Extractive Value (12.60% w/w, 14.15±1.14), Water Soluble Extractive Value (12.75% w/w, 16.42±1.18), and Loss on drying (8.90% w/w, 5.50±0.10) for *C.aurantium* and *C.sinensis* respectively. These studies were empowered to recognize the plant material for forthcoming exploration and form a significant aspect of drug research (Table-2, 3).

Table-2: Determination of Ash Values of Leaf Powder of *C. aurantium* L. and *C. sinensis* L.

| Ash values | *C. aurantium* (w/w) | *C. sinensis* (w/w) |
|---|---|---|
| Total Ash | 6.30±0.12 | 5.24±1.04 |
| Water soluble Ash | 1.20±0.32 | 1.82±0.10 |
| Acid insoluble Ash | 2.60±0.14 | 0.98±0.08 |
| Sulphated Ash | 2.90±0.10 | 2.12±0.04 |

Values are represented as Mean ±S.D, where n=3.

Table-3: Determination of Extractive Values and Moisture Content Leaf of Powder of *C. aurantium* L. and *C. sinensis*. L.

| Extractive values | *C. aurantium* (w/w) | *C. sinensis* (w/w) |
|---|---|---|
| Alcohol soluble Extractive Value | 12.60±1.22 | 14.15±1.14 |
| Water soluble Extractive Value | 12.75±1.30 | 16.42±1.18 |
| Loss on drying | 8.90±0.22 | 5.50±0.10 |

Values are represented as Mean ±S.D, where n=3.

**Phytochemical Screening**

In the current study, preliminary phytochemical screening of the leaves of *Citrus aurantium* L. and *Citrus sinensis* L. publicized the existence of various bioactive components such as carbohydrates, proteins, amino acids, saponins, cardiac glycosides, steroids, flavonoids, triterpenoids, and phenolic compounds. This study will help to understand the chemical nature of the drug under investigation (Table-4).

Table-4: Phytochemical Screening of Leaf Parts of Volatile Oil *Citrus aurantium* L and *Citrus sinensis* L

| Phytocontituents | *C. aurantium* | *C.sinesis* |
|---|---|---|
| Carbohydrates | + | + |
| Fixed oils and fats | - | - |
| Proteins and amino acids | + | + |
| Saponins | + | + |
| Steroids | + | + |
| Alkaloids | - | - |
| Glycosides | + | + |
| Flavonoids | + | + |
| Tannins | - | - |
| Gums and mucilage | - | - |
| Triterpenoids | + | + |
| Phenolic compounds | + | + |

(+) indicates positive and (-) indicates negative for the test.

**GC-MS Analysis of the Extracts**

In the GC-MS analysis of 25 compounds, were identified from the volatile oils of *Citrus aurantium* L whereas 30 compounds were identified from the volatile oils of *Citrus sinensis* L. The approval of Phytoconstituents is identified by the peak area, molecular weight, and molecular formula. The chromatogram of *Citrus aurantium* L. represents 7 notable peaks and *Citrus sinensis* L represents 12 notable peaks (Fig.-1,2) authorized the presence of Phyto-components with diverse retention times as concisely represented by Table-5-8. The mass spectrometer analysis of the compounds eluted at different

times to recognize the nature of the compounds, molecular weight, and molecular structure by presenting compound fragments at different m/z ratios.

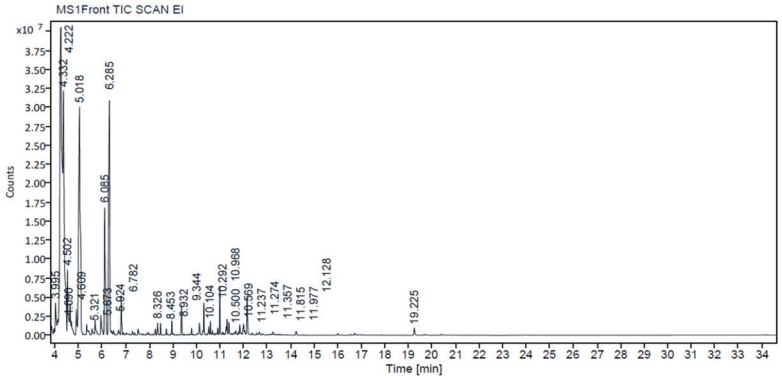


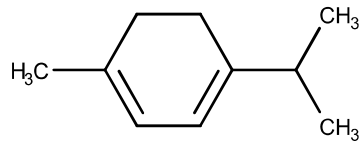
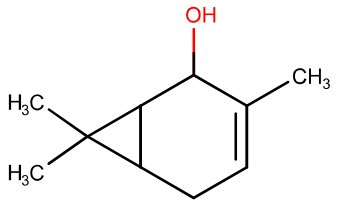
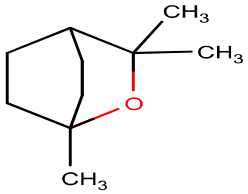
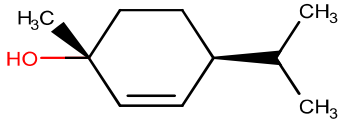
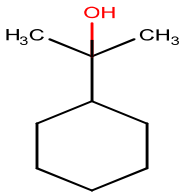
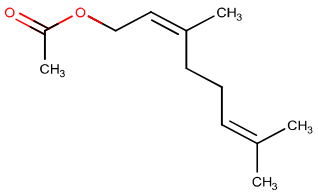
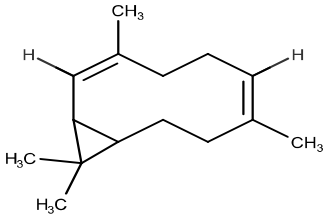

Fig.-1: GC-MS Chromatogram of *Citrus aurantium* L.

Table-5: Chemical Constituents Present in the *Citrus aurantium* L. Determined by GC-MS

| No | Name of the compound | Retention time | Molecular weight | Molecular formula | Peak area | Peak area (%) |
|---|---|---|---|---|---|---|
| 1 | 1,3-Cyclohexadiene1-methyl-4-(1-methylethyl), | 4.090 | 136.23 | $C_{10}H_{16}$ | 4279031.977 | 0.53 |
| 2 | trans-3-Caren-2-ol | 4.222 | 152.23 | $C_{10}H_{16}O$ | 240731423.566 | 29.71 |
| 3 | Eucalyptol | 4.219 | 154.24 | $C_{10}H_{18}O$ | 136450699.329 | 16.84 |
| 4 | 5-Isopropyl-2-methylbicyclo hexan-2-ol | 4.331 | 154.22 | $C_{10}H_{17}O$ | 16587843.637 | 2.05 |
| 5 | γ-Terpinene | 4.500 | 136.23 | $C_{10}H_{16}$ | 6208510.133 | 0.77 |
| 6 | Linalool | 5.019 | 154.24 | $C_{10}H_{18}O$ | 2913096.192 | 0.36 |
| 7 | 2-Cyclohexen-1-ol,1-methyl-4-(1-methylethyl) trans | 5.319 | 154.24 | $C_{10}H_{18}O$ | 4980212.943 | 0.61 |
| 8 | 6-Octenal 3,7-dimethyl | 5.675 | 154.24 | $C_{10}H_{18}O$ | 7239181.942 | 0.89 |
| 9 | Cyclo hexane methanol, α, α-dimethyl -4-methylene | 5.925 | 222.36 | $C_{15}H_{26}O$ | 50878890.800 | 6.28 |
| 10 | Terpinen-4-ol | 6.088 | 154.25 | $C_{10}H_{18}O$ | 103401524.131 | 12.76 |
| 11 | Benzene,2-methoxy-4-methyl-1-(1-methylethyl) | 6.782 | 164.24 | $C_{11}H_{16}O$ | 3238923.207 | 0.40 |
| 12 | α-Terpinyl acetate | 8.326 | 196.29 | $C_{12}H_{20}O_2$ | 2646814.818 | 0.33 |
| 13 | 2,6-Octadien-1-ol, 3,7-dimethyl-, acetate | 8.451 | 196.28 | $C_{12}H_{20}O$ | 3268685.121 | 0.41 |
| 14 | Cyclohexane,1-ethenyl-1-methyl-2,4-bis(1-methylethenyl)-,[1S(1α,2β,4β)] | 8.932 | 204.35 | $C_{15}H_{24}$ | 6764747.866 | 0.83 |
| 15 | Caryophyllene | 9.345 | 204.35 | $C_{15}H_{24}$ | 3075210.977 | 0.38 |
| 16 | 8-Isopropyl-1-methyl-3-methylenetricyclo[4.4.0.02,7]decane | 10.101 | 220.35 | $C_{15}H_{24}O$ | 7117907.622 | 0.88 |
| 17 | 3,7,11,11Tetramethylbicyclo[8.1.0]undeca-2,6-diene | 10.289 | 204.35 | $C_{15}H_{24}$ | 2155726.599 | 0.27 |
| 18 | 4-Isopropyl-3,7-dimethyloctahydro-1H-cyclopentane | 10.501 | 222.37 | $C_{15}H_{26}O$ | 2846261.295 | 0.35 |
| 19 | Naphthalene,1,2,4a,5,8,8a-hexahydro-4,7-dimethyl-1-(1-methylethyl) | 10.570 | 204.35 | $C_{15}H_{24}$ | 9732684.296 | 1.20 |
| 20 | Dodecatrien-3-ol,3,7,11-trimethyl | 10.970 | 222.37 | $C_{15}H_{26}O$ | 1797350.371 | 0.22 |
| 21 | Cycloprop[e]azulen-7-ol, decahydro-1,1,7-trimethyl-4-methylene | 11.276 | 220.35 | $C_{15}H_{24}O$ | 3335442.313 | 0.41 |
| 22 | 1-Naphthalenol, decahydro-1,4a-dimethyl-7-(1-methylethylidene) | 11.814 | 222.37 | $C_{15}H_{26}O$ | 3539872.718 | 0.44 |
| 23 | tau-Cadinol | 11.976 | 204.35 | $C_{15}H_{24}$ | 11712657.711 | 1.45 |

| | | | | | | |
|---|---|---|---|---|---|---|
| 24 | 2-Naphthalenemethanol,decahydro-α,α,4a-trimethyl-8-methylene | 12.126 | 222.36 | $C_{15}H_{26}O$ | 2286482.182 | 0.28 |
| 25 | Phytol | 19.228 | 296.53 | $C_{20}H_{40}O$ | 2155726.599 | 0.43 |

Table-6: Chemical Structures of the Most Prevalent Compounds of *Citrus aurantium* L

| Name of compound | Chemical structure of the compound |
|---|---|
| 1,3-Cyclohexadiene,1-methyl-4-(1-methylethyl) |  |
| Trans-3-Caren-2-ol |  |
| Eucalyptol |  |
| 2-Cyclohexen-1-ol,1-methyl-4-(1-methylethyl) trans |  |
| Cyclo hexane methanol, α, α-dimethyl -4-methylene |  |
| 2,6-Octadien-1-ol, 3,7-dimethyl-, acetate |  |
| 3,7,11,11Tetramethylbicyclo[8.1.0]undeca-2,6-diene |  |

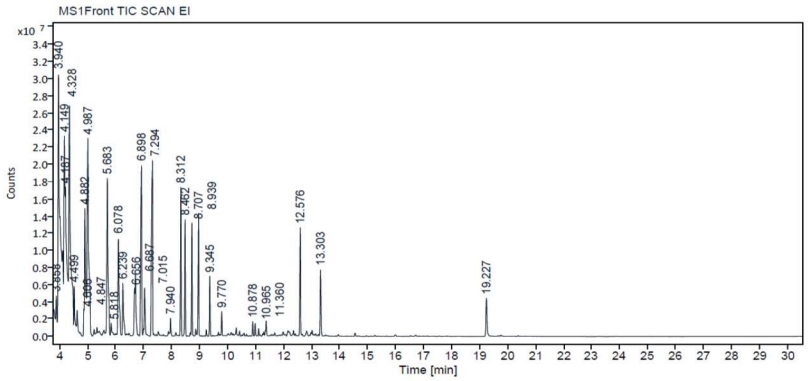



Fig.-2: GC-MS Chromatogram of *Citrus sinensis* L

Table-7: Chemical Constituents Present in the *Citrus Sinensis* L Determined by GC-MS

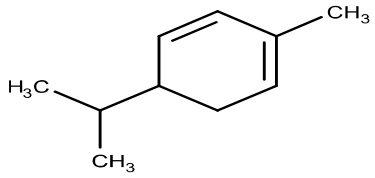
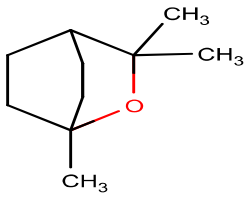
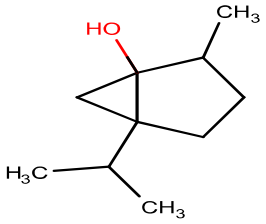
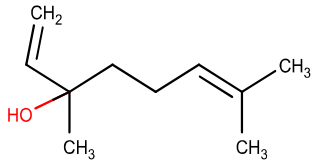
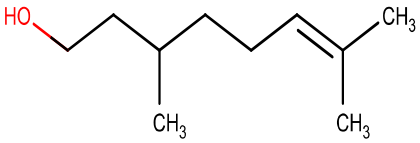
| No | Name of the compound | Retention time | Molecular weight | Molecular formula | Peak area | Peak area (%) |
|---|---|---|---|---|---|---|
| 1 | α-Phellandrene | 3.856 | 136.23 | $C_{10}H_{16}$ | 107839364.895 | 12.56 |
| 2 | 3-Carene | 3.937 | 136.24 | $C_{10}H_{16}$ | 36916623.787 | 4.30 |
| 3 | D-Limonene | 4.150 | 136.23 | $C_{10}H_{16}$ | 38974406.367 | 4.54 |
| 4 | Eucalyptol | 4.187 | 154.24 | $C_{10}H_{18}O$ | 87224843.016 | 10.16 |
| 5 | β-Ocimene | 4.331 | 136.24 | $C_{10}H_{16}$ | 8080356.756 | 0.96 |
| 6 | γ-Terpinene | 4.500 | 136.23 | $C_{10}H_{16}$ | 6208510.133 | 0.77 |
| 7 | 5-Isopropyl-2-methylbicyclo[3.1.0]hexan-2-ol | 4.606 | 154.22 | $C_{10}H_{17}O$ | 3966191.430 | 0.46 |
| 8 | Cyclohexene,3-methyl-6-(1methylethylidene)- | 4.881 | 136.23 | $C_{10}H_{16}$ | 37998861.666 | 4.43 |
| 9 | Linalool | 5.681 | 154.24 | $C_{10}H_{18}O$ | 55389455.324 | 6.45 |
| 10 | 6-Octenal, 3,7-dimethyl-, (S) | 5.819 | 154.24 | $C_{10}H_{18}O$ | 2839065.438 | 0.33 |
| 11 | Isoneral | 6.081 | 152.23 | $C_{10}H_{16}O$ | 33643546.715 | 3.92 |
| 12 | Terpinen-4-ol | 6.238 | 154.25 | $C_{10}H_{18}O$ | 14743274.393 | 1.72 |
| 13 | α-Terpineol | 6.656 | 154.25 | $C_{10}H_{18}O$ | 9256221.309 | 1.08 |
| 14 | Citronellol | 6.688 | 156.27 | $C_{10}H_{20}O$ | 17102637.546 | 1.99 |
| 15 | 2,6-Octadien-1-ol, 3,7-dimethyl-, (Z)- | 6.900 | 196.28 | $C_{12}H_{20}O$ | 51458412.387 | 5.99 |
| 16 | Neral | 7.013 | 152.24 | $C_{10}H_{16}O$ | 11636681.517 | 1.36 |
| 17 | Geraniol | 7.294 | 154.25 | $C_{10}H_{18}O$ | 57915013.159 | 6.75 |
| 18 | 2,6-Octadienal, 3,7-dimethyl-, (E)- | 7.938 | 196.28 | $C_{12}H_{20}O$ | 4043086.048 | 0.47 |
| 19 | trans-Geranic acid methyl ester | 8.313 | 182.25 | $C_{11}H_{18}O$ | 31514649.708 | 3.67 |
| 20 | 6-Octen-1-ol, 3,7-dimethyl-, acetate | 8.463 | 198.30 | $C_{12}H_{22}O_2$ | 23202606.167 | 2.70 |
| 21 | Geranyl acetate | 8.707 | 196.29 | $C_{12}H_{20}O_2$ | 23890334.639 | 2.78 |
| 22 | Cyclohexane, 1-ethenyl-1-methyl-2,4-bis(1-methylethenyl)-, | 8.938 | 204.35 | $C_{15}H_{24}$ | 11067841.889 | 1.29 |
| 23 | Caryophyllene | 9.344 | 204.36 | $C_{15}H_{24}$ | 4526890.461 | 0.53 |
| 24 | Humulene | 9.769 | 204.35 | $C_{15}H_{24}$ | 2694929.309 | 0.31 |
| 25 | Cyclohexanemethanol, 4-ethenyl-α,α,4-trimethyl-3-(1-methylethenyl) | 10.876 | 264.40 | $C_{17}H_{28}O2$ | 2359506.093 | 0.27 |

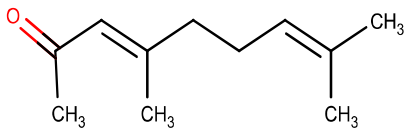
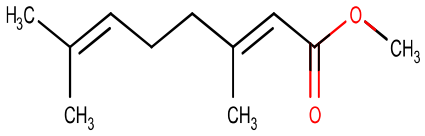
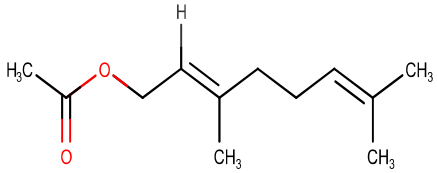
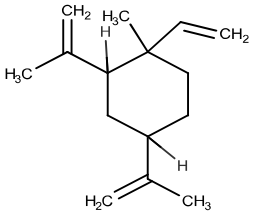
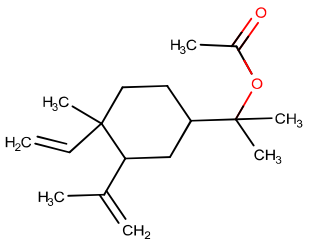
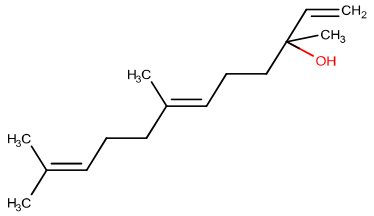
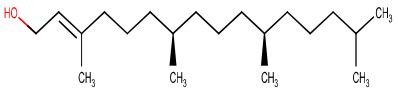
| | | | | | | |
|---|---|---|---|---|---|---|
| 26 | 1,6,10-Dodecatrien-3-ol, 3,7,11-trimethyl | 10.963 | 222.36 | $C_{15}H_{26}O$ | 2928968.352 | 0.34 |
| 27 | Caryophyllene oxide | 11.357 | 220.35 | $C_{15}H_{24}O$ | 23591025.417 | 2.75 |
| 28 | 2,6,11-Dodecatrienal,2,6-dimethyl-10-methylene | 12.576 | 218.33 | $C_{15}H_{22}O$ | 15417267.606 | 1.80 |
| 29 | 2,6,9,11-Dodecatetraenal, 2,6,10-trimethyl | 13.301 | 218.33 | $C_{15}H_{22}O$ | 12807274.027 | 1.49 |
| 30 | Phytol | 19.227 | 296.53 | $C_{20}H_{40}O$ | 2155726.599 | 0.43 |

## CONCLUSION

The current study revealed the morphological nature of the two closely related species *Citrus aurantium* L and *Citrus sinensis* L. The physio-chemical constants, Phyto-analytical parameters, and preliminary chemical screening would serve as a fingerprint for standardizing the two medicinally important species. The presence of various bioactive compounds of *Citrus aurantium* L and *Citrus sinensis* L was confirmed using GC-MS analysis.

Table-8: Chemical Structures of the Most Prevalent Compounds of *Citrus sinensis* L

| Name of compound | Chemical structure of the compound |
|---|---|
| α-Phellandrene |  |
| Eucalyptol |  |
| 5-Isopropyl-2-methylbicyclo[3.1.0]hexan-2-ol |  |
| Linalool |  |
| Citronellol |  |

| | |
|---|---|
| 2,6-Octadienal, 3,7-dimethyl-, (E)- |  |
| trans-Geranic acid methyl ester |  |
| Geranyl acetate |  |
| Cyclohexane,1-ethenyl-1-methyl-2,4-bis(1-methylethenyl)-, |  |
| Cyclohexanemethanol, 4-ethenyl-α,α,4-trimethyl-3-(1-methylethenyl |  |
| 1,6,10-Dodecatrien-3-ol, 3,7,11-trimethyl-, |  |
| Phytol |  |

There were up to 55 volatile compounds detected from both oils and about 18 important components were detected as biologically active in nature. However, the phytochemical isolation of separate compounds and showing of the oils in appropriate biological action tests will undoubtedly offer more meaningful results and will open a new area of investigation of different components and their potential pharmacological use from the leaves of *Citrus aurantium* L and *Citrus sinensis* L.

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