# PHTYTOCHEMICAL ANALYSIS AND LIMONENE CONCENTRATION IN ESSENTIAL OIL AND FILTRATE OF MOSAMBI AND ORANGE PEELS

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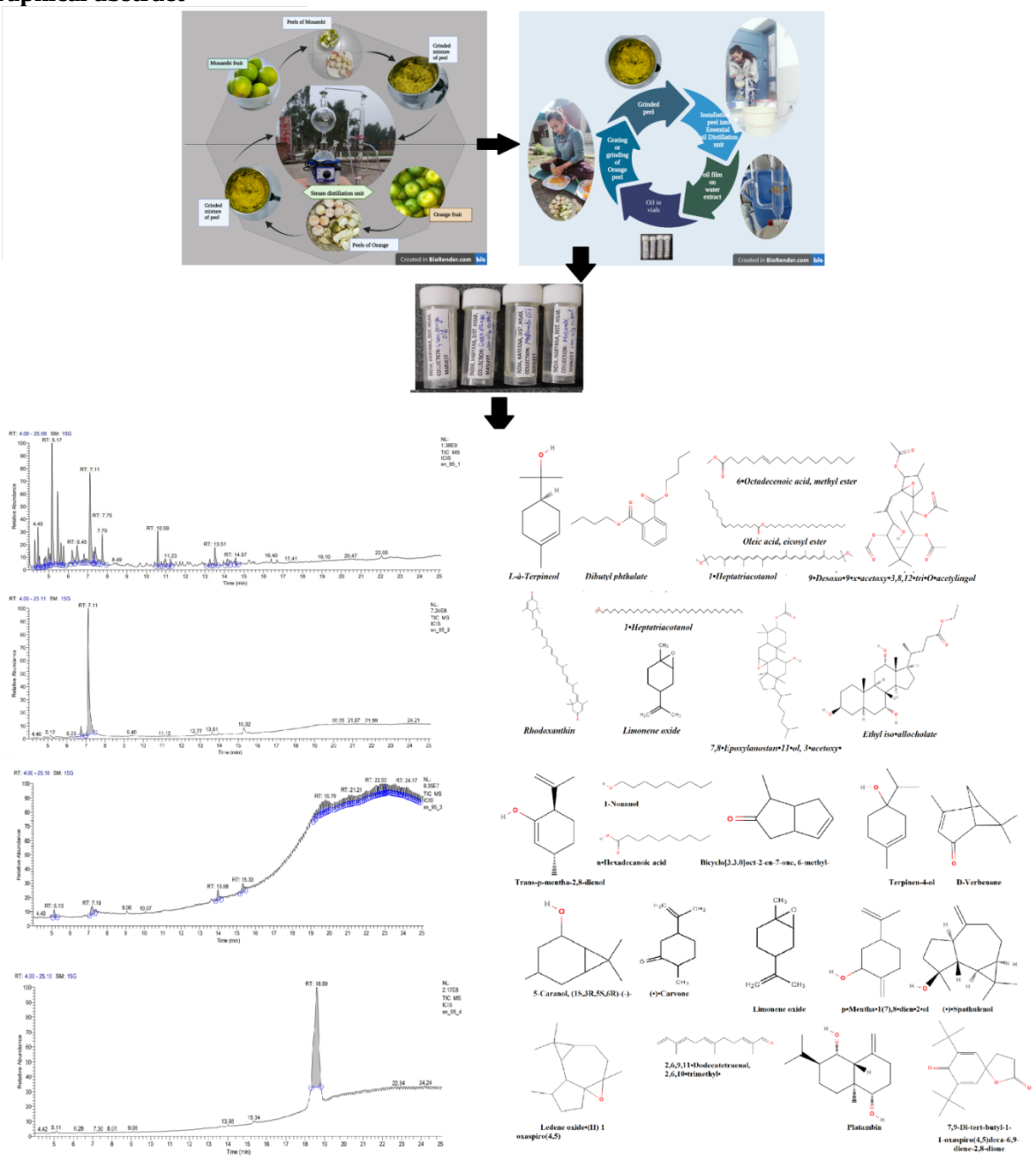
## Graphical abstract




## Abstract

Citrus fruits considered as the most commercial crop in the world and have major contribution. However, only the orange fruit has 60% participation. Citrus fruits belongs to the Rutaceae family comprises 140 genera and 1300 species. Citrus sinensis (Orange), *Citrus reticulata*, *C. grandis*, *C. limon*, *C. aurentium*, *C. paradise* and *C. medica* are the major species of Rutaceae family. Essential oils contains the volatile compounds which can be extracted from various parts of any aromatic plants. Citrus fruit essential oil has significant economic importance. In this study, citrus peel essential oils were extracted from Mosambi (*Citrus sinensis*) and Orange (*Citrus reticualta)* using the essential oil steam distillation unit. The chemical

analysis of essential oils was done by (GC-MS) Gas Chromatographic-Mass Spectroscopy. The primary goal of this research is to look for the presence of various bioactive compounds in the extracted essential oils and filtrates of citrus peels from *C. sinensis* and *C. reticualta*. The essential oil yield percentage in *C. reticualta* (4.2%) was slightly more than the *C. sinensis* (3.24%). Contrarily, *C. reticulata* produces more essential oil (7.5ml) as compare to *C. sinensis* (6.0ml). However, filtrate production is more in *C. sinensis* (163ml) as ompare to *C. reticulata* (152ml). *C. sinensis* extract contains twenty three compounds including alkene, alcohol, aldehydes, carboxylica acids, monoterpene, terpene and ether. However, *C. reticulata* contains sixteen compounds. The study determines 17.16% of Limonene present in *C. sinensis* essential oil, 92.40% in its filtrate, where 3.86% in *C. raticulata* essential oil. Essential oil can be extracted easily from any of the citrus fruits using steam distillation process/ and can be employed as the bio-pesticide against insect pests.

**Keywords:** Citrus fruits, essential oil, bioactive compounds, limonene.

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## Introduction

Along with the United States, China, and Brazil, India accounts for roughly half of global orange production. India ranked sixth in the world in citrus fruit production where bananas (33%), mango (21%), papaya (6%), guavava (4%), grapes (3%), and apples (3%) (Chavan et al., 2018). Orange, lemon, and sweet lime were the most difficult fruits to obtain due to their pleasant flavours (Sikdar et.al, 2017). The sweet orange (*Citrus sinensis*) was the most widely cultivated tree fruit in the world, accounting for roughly 60% of all citrus produced globally (Hegazy & Ibrahium, 2012; Morton 1987; Erukainure 2016). The ectocarp or peel, of citrus fruits contains numerous oil glands that contain citrus oil (Sikdar et al., 2017). Citrus peel scraps can be used to create essential oils rather than being discarded. Additionally, you can use the leftover peel residue as rat compost (Sikdar & Baruah 2017). Citrus peel contains protein (9%), lignin (8%), flavinoids (4.5%), cellulose (20-40%), hemicelluloses (8-11%), pectin (13-23%), and essential oils (up to 4%) (Brahmi& Coworkers 2021). Essential oils carry volatile compounds which obtained from various aromatic plant parts (Edogbanya et al., 2019). The wax layer of insect's respiratory system can be damage by limonene carrying essential oil that can be used as bio-insecticide (Mursiti et al., 2019). About 90% of the citrus crude oil made up of the terpene (limonene) a significant pesticide compound, trepene alcohol linalool, and another significant pesticide compound (Amusan et al., 2005). Mandarin, bitter orange, sweet orange, and grapes contains 65.3% to 95.9% of d-liomene in their essential oils (Dosoky and Setzer 2018). Reusing citrus fruit peels reduces the risks to human health and the environment (Simas et al., 2017). According to previous researches essential oils play effective role against pests like bacteria, fungi, and insects (Sikdar and Baruah 2017). Citrus fruit essential oils and filtrates may be the best choice because of their insecticidal, larvicidal, biodegradable, effective, and afford-able qualities (Bailão et al., 2022).

The chemical compounds such as limonene (monoterpene), verbenol (terpene), carene (bicyclic monoterpene), carvol, citronellal, caryophyllene etc. were detected in the filtrate and essential oil of the citrus peels.The purpose of this study was to look into the types of chemical compounds found in the essential oils and filtrate of *Citrus sinensis* and *Citrus reticulata* which is least studied. This data will be helpful to know the comparative study of citrus fruits and eco-friendly uses of essential oil as well as filtrates as bio-pesticides.

## Material and Method

**Study area:** The study was performed in Hisar district, Haryana, India. Hisar (29°09'N, 75°42'E) is a well-known semi-arid district close to Rajasthan, North India. The current study attempted to quantify the extraction and chemical analysis of essential oil and filtrate from peels of citrus fruits. Fruits were collected from the local market, Patel Nagar of Hisar district located at 29° 7'54.70" N -75° 43'27.37" E (Google Earth) (Figure 1).


**Figure 1** Citrus fruit (*C. sinensis* and *C. raticulata*) collection site (29° 7'54.70" N -75° 43'27.37" E (Google Earth).

**Material:** Essential oil steam distillation unit with two necked round bottom flask, burette stand, condenser, heater, and measuring cylinder.

**Raw Material:** 175g peels of *Citrus reticualta*, *Citrus sinensis*, and 500ml dstilled water.

**Plant material:** In this research citrus fruits *Citrus sinensis* (Mosambi), *Citrus reticualta* (Orange), belonging to Rutacae family were selected to extract essential oil (Figure 2, Table1). The peels of all fruits were firstly washed to remove impurities. Separated peels and distilled water (500ml) finally added to a grinding machine.


**Figure 2** Peels of citrus fruits (*C. sinensis* and *C. reticulata*).

**Table 1**Citrus fruits used to extract the essential oil.

| Citrus fruits (scientific name) | Common name | Local name (Harayana) |
|---|---|---|
| Family-Rutacae | | |
| *Citrus sinensis* | Sweet orange | Mosambi |
| *Citrus reticualta* | Mandarin orange | Orange |

**Isolation of essential oil by steam distillation**

The essential oil extraction procedure began with peeling off the citrus fruits (Figure 2**).** The essential oil was extracted using the grinded citrus peels of *Citrus sinensis* and *Citrus reticualta* via essential oil steam distillation unit (Prasad et al., 2017). Initially, 175g of citrus peels added to 500 ml of distilled water. The entire grinded mixture was poured into a 1000ml round bottom distillation flask and then placed on the heater**.** The heating process began with boiling it at constant temperature (50°C). As the mixture begins evaporate, the vapors start moving to the condenser. The collecting funnel carries the condensed extract towards the beaker. On the surface of the extract, a clear oil layer can be seen. At last collect the oil film from the filtrate and poured it into a vial (Figure 4).


**Figure 4** Process of essential oil extraction from peels of citrus fruits.

**Separation of essential oil from filtrate**

On top of the hydrosol a clear layer of citrus oil can be easily separated from the surface because of hydrophobic nature. The yield percentage was calculated using the formula:

$$Yield = \frac{Amount\ of\ citrus\ oil\ extracted \times 100\%}{Total\ amount\ of\ grated\ citrus\ peels}$$

**Chemical analysis of essential oil:**

**GC-MS Analysis**: The present study reflected the GC-MS (Gas Chromatography-Mass Spectroscopy) analysis of steam distillated citrus peels essential oils and their filtrates samples. For the sample analysis, samples were sent to the Department of Science and Technology, SAIF, Punjab University, Chandigarh.

**Result**

**Yield of essential oil:**

The essential oil were extracted from two types of citrus fruits, Mosambi (*Citrus sinensis*) and Orange (*Citrus reticulata*), each 175g. *Citrus reticulata* peels contains more oil glands as compare to *Citrus sinensis* as shown in Figure 3.

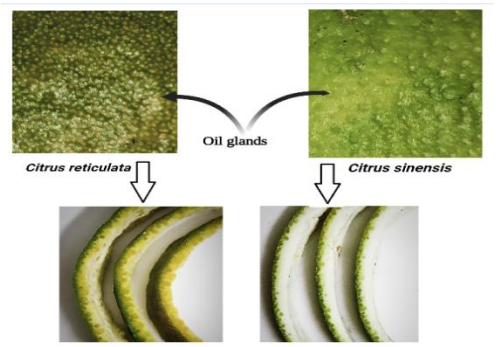


**Figure 3** Oil glands in peels of *C. raticulata* and *C. sinensis*.

After a single distillation of 120 to 135 minutes of 1.2ml/min. evaporation rate, 175g of each citrus peel produces 6 to 7.5ml of essential oil. Yields of total extract were 75ml/hr and 70.8ml/hr, yields of essential oil extracted were 2.6ml/hr and 3.3ml/hr, yields of filtrate were 72.4ml/hr and 67.5ml/hr. The data revealed, as time passes, the oil yield of *C. sinensis* grows first then falls (after each 45-minute) but it diminishes constantly in the case of *C. reticulata* (Table 2). However, essential oil and filtrate yield percentages (at 50°C) of *C. sinensis* and *C. raticulta* were 3.24% and 4.2% respectively, which was more in *C. reticulata* as compare to *C. sinensis* **(**Table 3).

**Table 2** Essential oil and filtrate production at different time duration (50°C).

| | *Citrus sinensis* | | | |
|---|---|---|---|---|
| | Time (min.) | Time interval (Min.) | Oil Extracted (ml) | Filtrate (ml) |
| Initial time | 4:00 pm | 00 | 00 | 00 |
| Evaporation start | 5:00 pm | 60 | 00 | 00 |
| 1st extraction | 5:45 pm | 45 | 2.0 | 55 |
| 2nd extraction | 6:30 pm | 45 | 3.5 | 55 |
| 3rd extraction | 7:15 pm | 45 | 0.5 | 53 |
| total | | | 6.0 | 163 |
| | *Citrus raticulata* | | | |
| Initial time | 10:50 pm | 00 | 00 | 00 |
| Evaporation start | 11:50 pm | 60 | 00 | 00 |
| 1st extraction | 12:35 pm | 45 | 6.0 | 50 |
| 2nd extraction | 1:20 pm | 45 | 1.0 | 50 |
| 3rd extraction | 2:05 pm | 45 | 0.5 | 52 |
| Total | | | 7.5 | 152 |

**Table 3**Total utilization and production of essential oil and filtrate from citrus fruit peel.

| | Mosambi (*Citrus sinensis*) | Orange (*Citrus reticulata*) |
|---|---|---|
| Amount of Citrus peel used (g) | 175 | 175 |
| Volume of distilled water (ml) | 500 | 500 |
| Average evaporation rate (ml/hr) | 1.2 | 1.2 |
| Yield of total extract (ml/hr) | 75.0 | 70.8 |
| Yield of essential oil extracted (ml/hr) | 2.6 | 3.3 |
| Yield of filtrate (ml/hr) | 72.4 | 67.5 |
| Yield at 50°C (%) | 3.42 | 4.2 |

The total essential oil output from 175g of citrus fruit peels were 6ml and 7.5ml, respectively which was comparatively greater in *C. reticulata*(Table 4).The extracted essential oil and filtrate from the *C. sinensis* and *C. reticulata* peels were initially collected into separate vials before being analyzed by GC-MS.

**Table 4** Overall peel portion and yield of essential oil from citrus plants at 50°C (2.25 hrs.).

| Cultivar | Peel portion (g) | Essential oil yield (ml) | Filtrate (ml) | Total extract (ml) |
|---|---|---|---|---|
| *Citrus sinensis* | 175g | 6.0 | 163 | 169.0 |
| *Citrus raticulata* | 175g | 7.5 | 152 | 159.5 |

We can see the peaks of RT values of different compound including their m/z values as in Figure 5(a); Figure 5(b); Figure 5(c) and Figure 5(d).

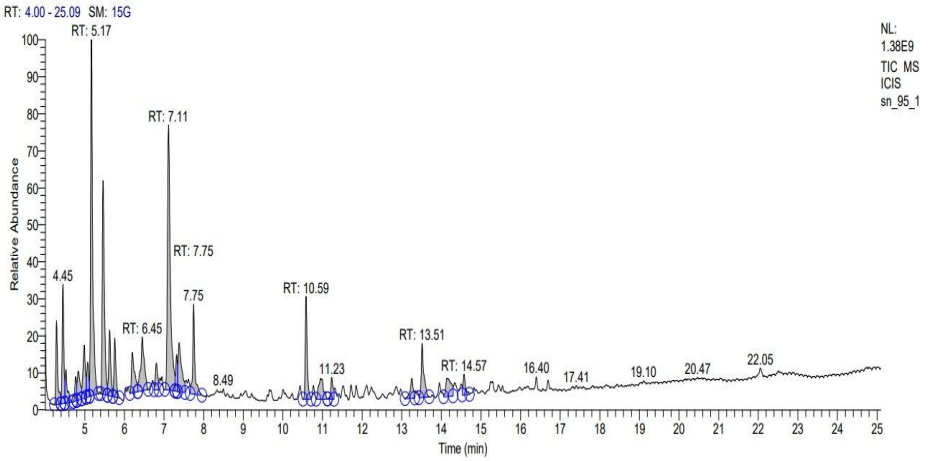


**Figure 5(a)** GC-MS Chromatogram of extracted essential oil (*Citrus sinensis*)

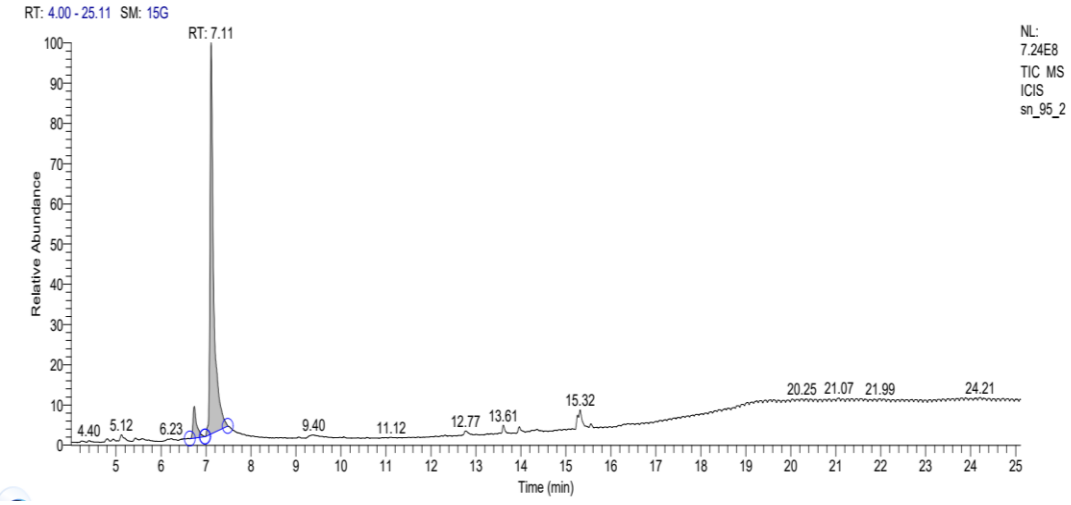


**Figure 5(b)** GC-MS Chromatogram of extracted filtrate *(C. sinensis*).

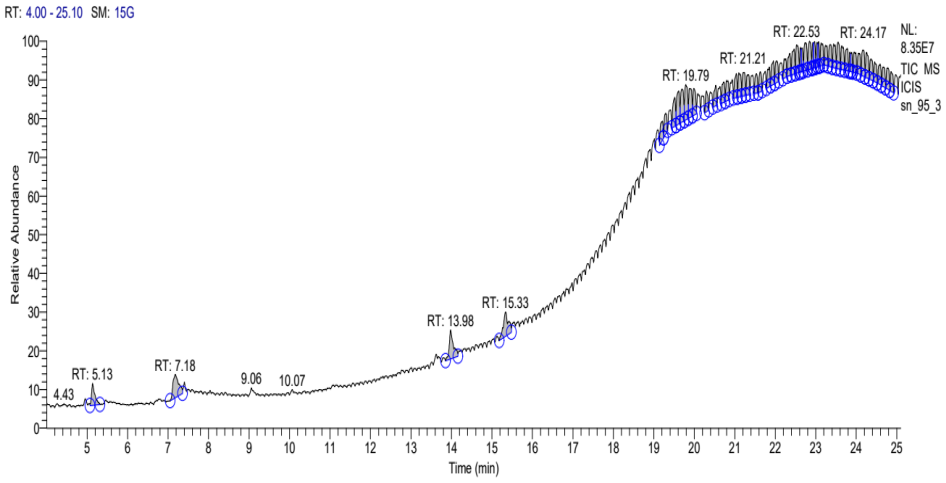


**Figure 5(c)** GC-MS Chromatogram of extracted essential oil (*Citrus raticulata*).

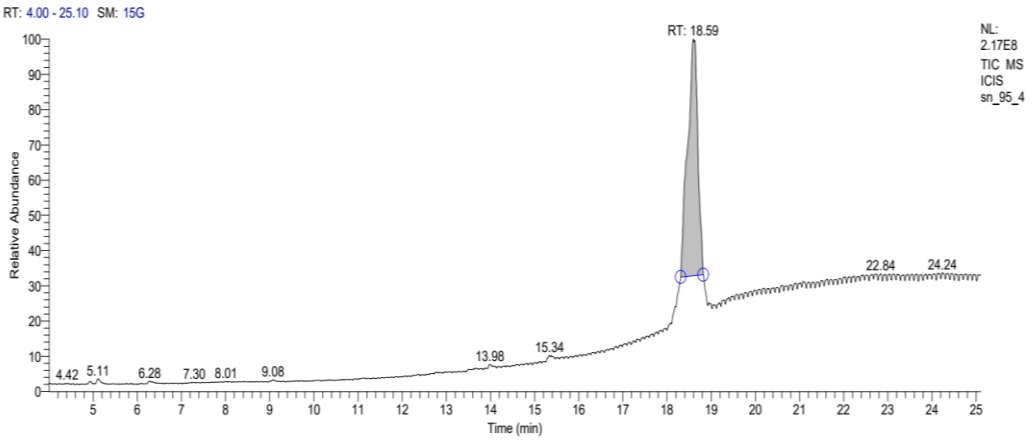


**Figure 5(d)** GC-MS Chromatogram of extracted filtrate (*Citrus raticulata*).

The citrus fruit *C. sinensis* carry *trans-p-mentha-2,8-dienol; 1-Nonanol; Bicyclo[3.3.0]oct-2-en-7-one, 6-methyl-; Terpinen-4-ol; D-Verbenone; 5-Caranol, (1S,3R,5S,6R)-(-)-; trans-3(10)-Caren-2-ol; (-)-Carvone; Ethanone,1-(6-methyl-7-oxabicyclo[4.1.0]hept-1-yl)-; Limonene oxide, cis-; 2,6-Octadien-1-ol, 3,7-dimethyl-, (Z)-; n-Decanoic acid; p-Mentha-1(7),8-dien-2-ol; (-)-Spathulenol; Ledene oxide-(II) 1; 2,6,9,11-Dodecatetraenal, 2,6,10-trimethyl-; 1,4-dihydroxy-p-menth-2-en; Platambin; 7,9-Di-tert-butyl-1-oxaspiro(4,5)deca-6,9-diene-2,8-dione; n-Hexadecanoic acid; 2,5-Cyclohexadiene-1,4-dione, 2,6-bis(1,1-dimethylethyl)-; 2-Furanmethanol, 5-ethenyltetrahydro-alpha,alpha,5-trimethyl-, cis-* and *Limonene oxide, cis-*compounds in its extracted essential oil and filtrate. However, *C. reticulata* species carry *L-alpha-Terpineol; Limonene oxide, cis-; Dibutyl phthalate; 6-Octadecenoic acid, methyl ester; 9-Desoxo-9-x-acetoxy-3,8,12-tri-O-acetylingol; 9,12,15-Octadecatrienoic acid, 2,3-bis[(trimethylsilyl)oxy]propyl ester, (Z,Z,Z)-; 1-Monolinoleoylglycerol trimethylsilyl ether; Oleic acid, eicosyl ester; .psi.,.psi.-Carotene, 1,1',2,2'-tetrahydro-1,1'-dimethoxy-;Carda-4,20(22)-dienolide,3-[(6-deoxy-3-O-methyl-alpha-D-allopyranosyl)oxy]-1; Rhodoxanthin; 1-Heptatriacotanol7,8-Epoxylanostan-11-ol, 3-acetoxy-; Ethyl iso-allocholate*and*17alpha-Hydroxypregnenolone, dimethyl ether* (Table 5; Figure 6(a); Figure 6(b).

**Table 5** GC-MS analyzed chemical compounds present in essential oil and filtrate of the *Citrus sinensis* and *Citrus raticulalta* peels.

| ***Essential oil (Citrus sinensis)*** | | | | | | |
|---|---|---|---|---|---|---|
| S.NO. | Name of Chemical compound | RT | Molecular Formula | Area % | Molecular weight | Cas# |
| 1. | Trans-p-mentha-2,8-dienol | 4.29 | C10H16O | 3.47 | 152.23 | NA |
| 2. | 1-Nonanol | 4.77 | C9H20O | 0.87 | 144.25 | 143-08-8 |
| 3. | Bicyclo[3.3.0]oct-2-en-7-one, 6-methyl- | 4.83 | C9H12O | 1.88 | 136.19 | NA |
| 4. | Terpinen-4-ol | 4.99 | C10H18O | | | 562-74-3 |
| 5. | D-Verbenone | 5.08 | C10H14O | 1.51 | 150.22 | 18309-32-5 |
| 6. | 5-Caranol, (1S,3R,5S,6R)-(-)- | 5.17 | C10H18O | 16.47 | 154.25 | 6909-21-3 |
| 7. | trans-3(10)-Caren-2-ol | 5.46 | C10H16O | 10.28 | 152.23 | NA |
| 8. | (-)-Carvone | 5.76 | C10H14O | 2.56 | 150.22 | 6485-40-1 |
| 9. | Ethanone, 1-(6-methyl-7-oxabicyclo[4.1.0]hept-1-yl)- | 6.81 | C9H14O2 | 1.42 | 154.21 | 15120-94-2 |
| 10. | Limonene oxide, cis- | 7.11 | C10H16O | 17.16 | 152.23 | 13837-75-7 |
| 11. | 2,6-Octadien-1-ol, 3,7-dimethyl-, (Z)- | 7.32 | C10H18O | 1.3 | 154.24 | 106-25-2 |
| 12. | n-Decanoic acid | 7.38 | C10H20O2 | 3.95 | 172.26 | 334-48-5 |
| 13. | p-Mentha-1(7),8-dien-2-ol | 4.45, 5.63 | C10H16O | 3.07, 4.36 | 152.23 | 35907-10-9 |
| 14. | (-)-Spathulenol | 10.59 | C15H24O | 2.3 | 220.35 | 77171-55-2 |
| 15. | Ledene oxide-(II) 1 | 10.95 | C15H24O | 2.3 | 220.35 | NA |
| 16. | 2,6,9,11-Dodecatetraenal, 2,6,10-trimethyl- | 11.23 | C15H22O | 1.04 | | 4955-32-2 |
| 17. | 1,4-dihydroxy-p-menth-2-en | 6.2, 6.45 | C10H18O2 | 5.84 | 170.25 | NA |
| 18. | Platambin | 13.25 | C15H26O2 | 1.35 | 238.36 | 58556-80-2 |

| | | | | | | |
|---|---|---|---|---|---|---|
| 19. | 7,9-Di-tert-butyl-1-oxaspiro(4,5)deca-6,9-diene-2,8-dione | 13.51 | C17H24O3 | 3.19 | 276.37 | 82304-66-3 |
| 20. | n-Hexadecanoic acid | 14.14 | C16H32O2 | 2.25 | 256.42 | 57-10-3 |
| 21. | 2,5-Cyclohexadiene-1,4-dione, 2,6-bis(1,1-dimethylethyl)- | 14.57 | C14H20O2 | 1.11 | 220.31 | 719-22- |
| | ***Filtrate (Citrus sinensis)*** | | | | | |
| 22 | 2-Furanmethanol, 5-ethenyltetrahydro-alpha,alpha,5-trimethyl-, cis- | 6.74 | C10H18O2 | 7.60 | 170.25 | 5989-33-3 |
| 23 | Limonene oxide, cis- | 7.11 | C10H16O | 92.40 | 152.23 | 13837-75-7 |
| | ***Essential oil (Citrus raticulata)*** | | | | | |
| 1. | L-alpha-Terpineol | 5.13 | C10H18O | 2.09 | 154.25 | 10482-56-1 |
| 2. | Limonene oxide, cis- | 7.18 | C10H16O | 7.18 | 152.23 | 13837-75- |
| 3. | Dibutyl phthalate | 13.98 | C16H22O4 | 3.41 | 278.34 | 84-74-2 |
| 4. | 6-Octadecenoic acid, methyl ester | 15.33 | C19H36O2 | 3.55 | 296.49 | 52355-31-4 |
| 5. | 9-Desoxo-9-x-acetoxy-3,8,12-tri-O-acetylingol | 19.17 | C28H40O10 | 1.59 | 536.61 | NA |
| 6. | 9,12,15-Octadecatrienoic acid, 2,3-bis[(trimethylsilyl)oxy]propyl ester, (Z,Z,Z)- | 19.61 | C27H52O4Si2 | 2.79 | | 55521-22-7 |
| 7. | 1-Monolinoleoylglycerol trimethylsilyl ether | 19.69 | C27H54O4Si2 | 2.84 | | 54284-45-6 |
| 8. | Oleic acid, eicosyl ester | 19.79 | C38H74O2 | 2.92 | 562.99 | 22393-88-0 |
| 9. | 9-Octadecenoic acid, 1,2,3-propanetriyl ester, (E,E,E)- | 19.88 | C57H104O6 | 2.5 | 885.43 | 537-39-3 |
| 10. | .psi.,.psi.-Carotene, 1,1',2,2'-tetrahydro-1,1'-dimethoxy- | 19.99 | C42H64O2 | 2.07 | 600.95 | 13833-01-7 |
| 11. | Carda-4,20(22)-dienolide, 3-[(6-deoxy-3-O-methyl-alpha-D-allopyranosyl)oxy]-1, | 20.53 | C30H44O9 | 1.38 | 548.66 | 56701-08-7 |
| 12. | Rhodoxanthin | 22.02 | C40H50O2 | 1.67 | 562.82 | 116-30-3 |
| 13. | 1-Heptatriacotanol | 22.74 | C37H76O | | 537 | 105794-58-9 |
| 14. | 7,8-Epoxylanostan-11-ol, 3-acetoxy- | 19.27, 19.52 | C32H54O4 | 3.77 | 502.77 | NA |
| 15. | Ethyl iso-allocholate | 19.52, 20.30, 20.70, 20.80, 21.04, 21.10, 21.21, 21.34, 21.63, 21.85, 22.22, 22.53 | C26H44O5 | 24.82 | 436.62 | NA |
| | ***Filtrate (Citrus raticulata)*** | | | | | |
| 16 | 17alpha-Hydroxypregnenolone, dimethyl ether | 18.59 | C23H36O3 | 100 | 360.53 | NA |

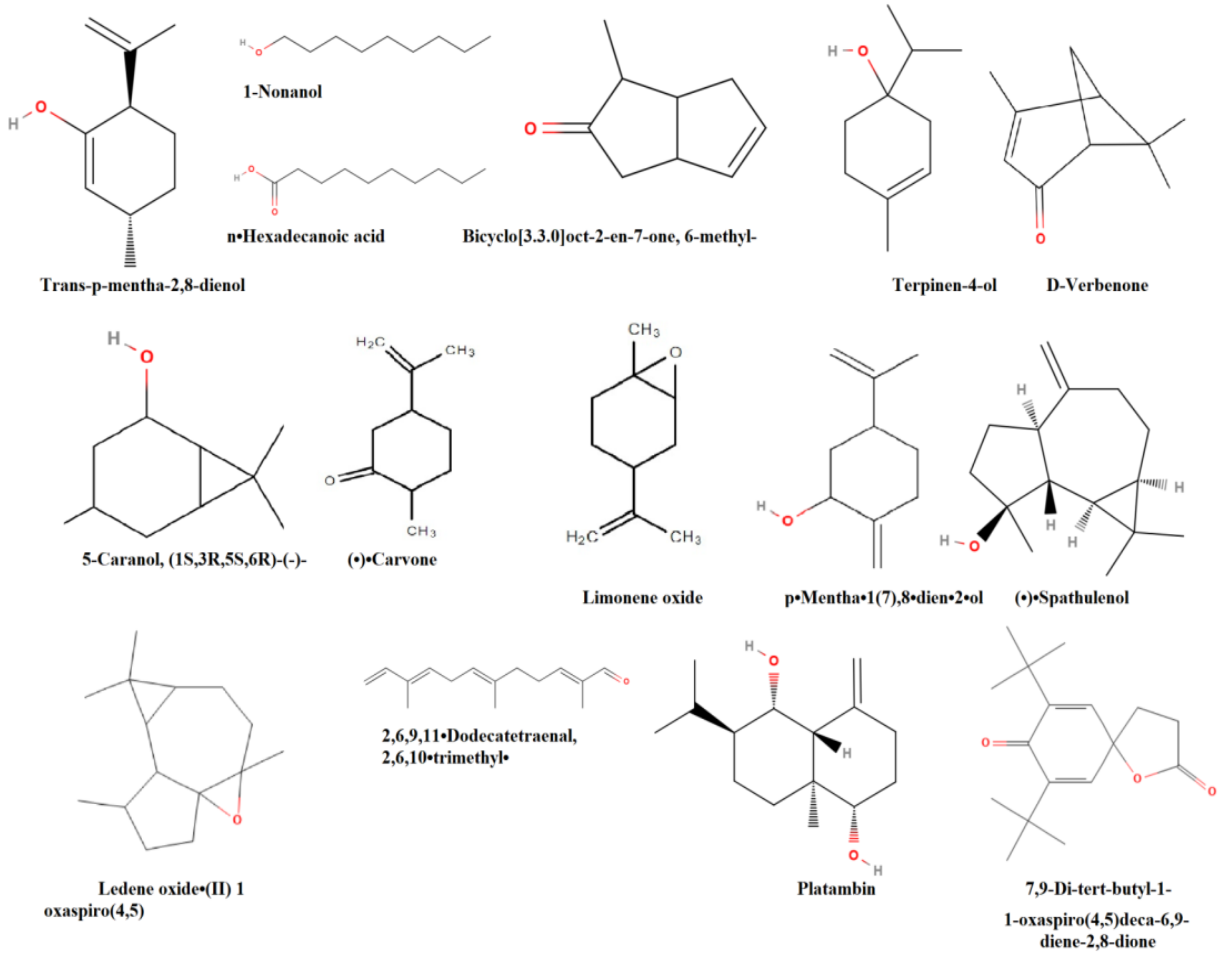


**Figure 6(a)** Chemical structures of phytochemical compounds present in peels of *C. sinensis.*

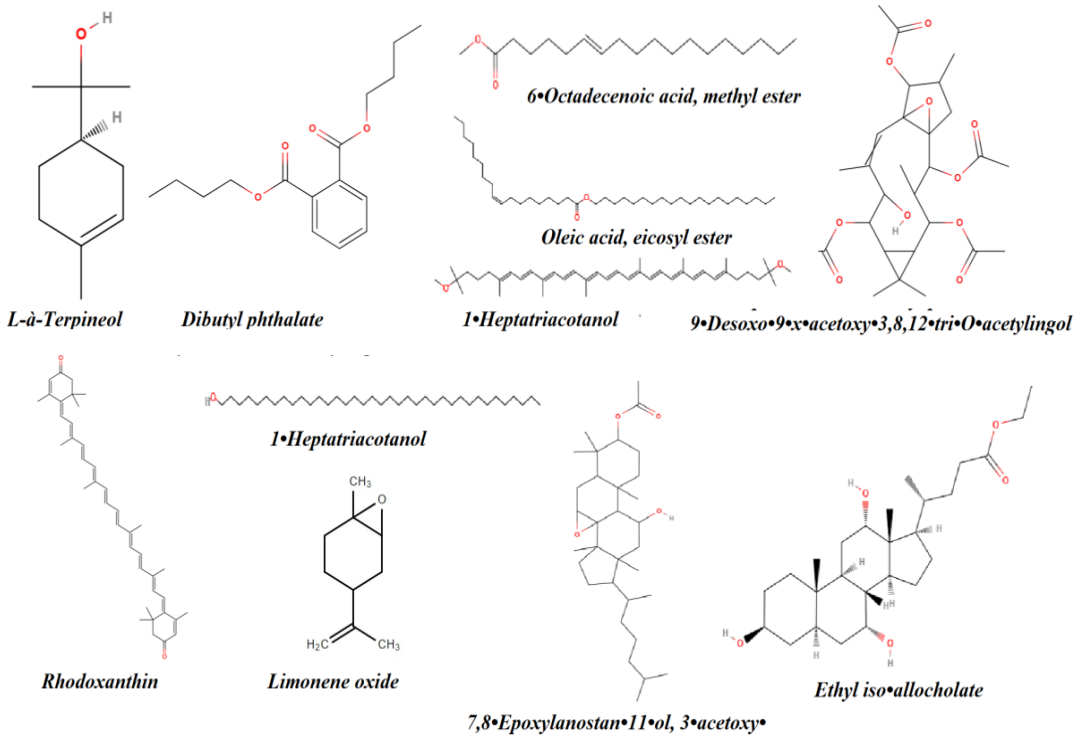


**Figure 6(b)** Chemical structures of phytochemical compounds present in peels of *C. raticulata.*

A total of 39 compounds were discovered in the extracts of citrus peels. Out of these compounds 16 were identified in *C. raticlata* while 23 compounds in *C. sinensis* (Figure 7) (Adapted from https://molview.org/**)**.

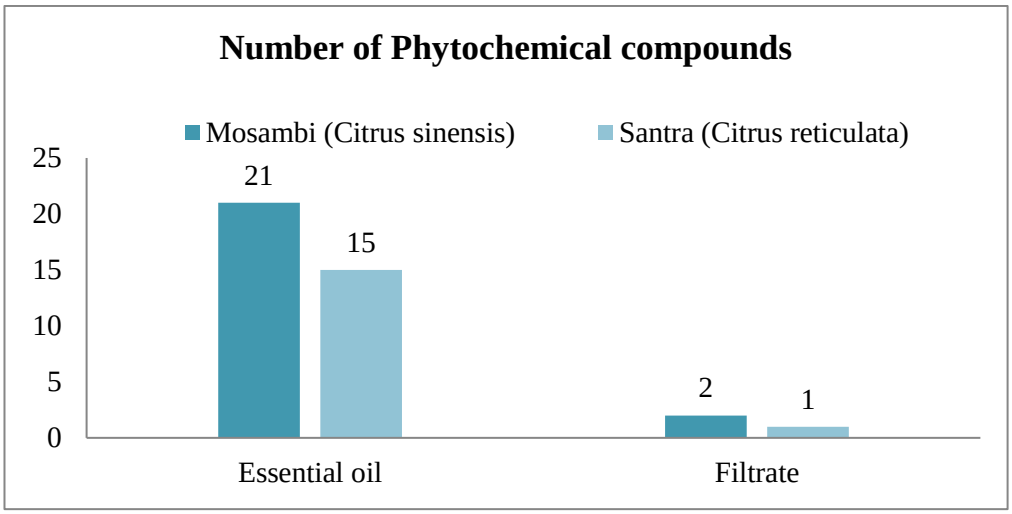


**Figure 7** Proportion of chemical compounds present in the extract of citrus fruits.

**T**he study revealed 17.16% (in essential oil), 92.40% (in filtrate) of limonene present in *C. sinensis* and 3.86% (in essential oil) in *C. raticulata* (Figure 8). Nutshell, limonene was the most dominant compound found in both of the citrus plant species.

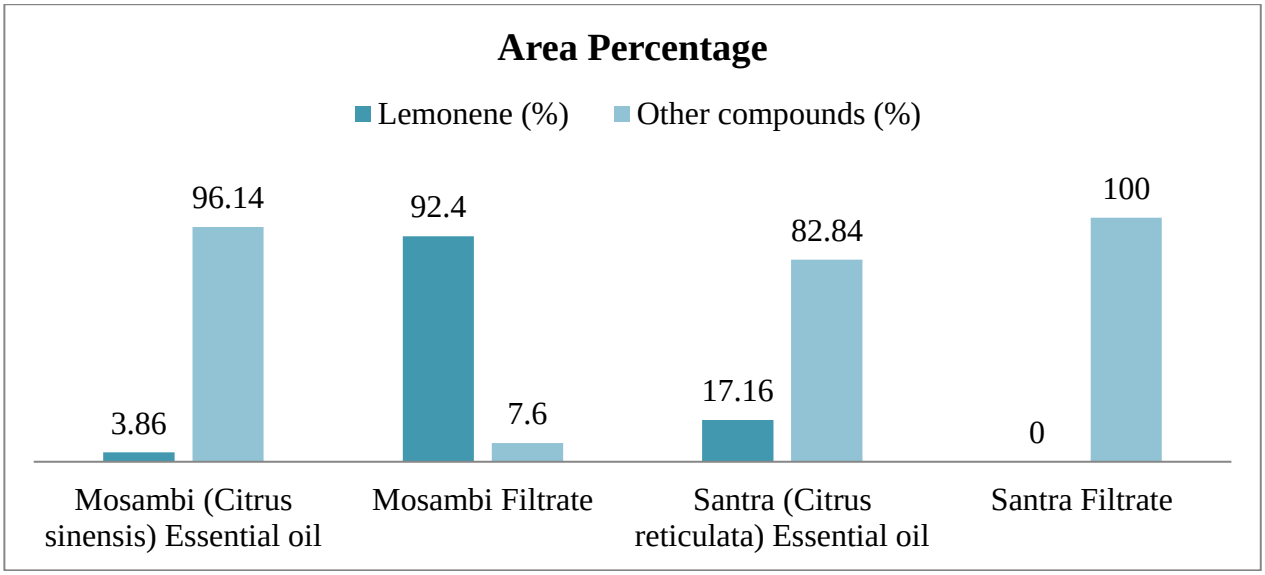


**Figure 8** Area% of Limonene present in the essential oil and filtrate of citrus fruits.

**Discussion**

Various plants species had different concentrations of d-Limonene. The Citrus essential oil carries about 83-97% w/w d-Limonene. Every ton of orange fruits contains approx 5.346kg of essential oil inside their peels in which about 90% of d-Limonene present (Negro et al., 2016). It can be extracted at a varied concentration ranging from 2.64% to 81.85% from plants of Lauraceae, Myrtaceae, Poaceae, Piperaceae and Rutaceae families (Tripathi et al., 2003). Grapefruit essential oil contained 70.10% to 73.96 % d-Limonene. Grapefruit essential oil untreated worker ants can move flexibly and efficiently with a normal walking rate i.e more than 90%. However, grapefruit essential oil-treated worker ants walked slower with walking rates of 55% to 65% (Zhang et al., 2021). Limonene shows safe pesticide property towards animals. Although 1% of limonene solution was responsible for damaging insect pests, some ferns and delicate flowers but caused no effect on the plants with waxy leaves like palm (Hollingsworth 2005). d-Limonene behave as an ovipositor

deterrent when its concentration rises. Where, 2.14 mg/cm$^2$ of d-Limonene showed 92.3% of ovipositor deterrent, and 0.60 mg/cm$^2$ concentration decreased the ovipositor upto 48.4%. As the concentration of d-Limonene increased, the ovipositor of *T. castaneum* adults was decreased (Tripathi et al., 2003). All these findings show the percentage of limonene compound in various plants species and bio-pesticide effect of their essential oils on various insects. Nutshall, *C. sinensis* and *C. raticulata* citrus plant species contains limonene and other bioactive compounds such as monoterpene and terpenes. According to our findings, limonene was most dominant compound in filtrate of *C. sinensis* as compare to its essential oil as well as essential oil of the *C. reticulata* fruit peels. However, filtrate of *C. reticulata* don't have limonene compound.

## Conclusion

The complete study reveals that essential oil and filtrate concentrations in *C. sinensis* and *C. raticulata*. *C. raticulata* peels contain more oil glands than the peels of *C. sinensis*. Due to which *C. raticulata* species shows high essential oil extraction rate as compare to *C. sinensis* species. Maximum no. of chemical compounds has been determined in *C. raticulata*. As limonene is more toxic compound for insects and can be used as bio-pesticides, present in the filtrate of the *C. sinensis* followed by its essential oil and *C. raticulata* essential oil. The present study may provide information about the bioactive compounds in the citrus peel essential oil as well as in their filtrate which can be used as the data to check their beneficial effects for human and most of animals but toxic as bio-pesticides against the harmful insects.

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## Conflict of Interest and Disclosure Statement

All of the authors state they do not have no proprietary, monetary, professional, or other type of personal interest in any organization that could be considered to be a conflict of interest that could have impacted the opinions stated in this manuscript.

## Authors' contributions

All the authors have read and approved the final version of the manuscript.

**Data availability**: Data will be available from the corresponding author upon good scientific reason and request.

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