Aroma Chemicals Identification by Sophisticated Technique and Their Role Against Pathogens

N. Sekhar¹, M. Srimannarayana¹ & N. Deepika²

¹ Department of Chemistry, GITAM School of Science, Hyderabad, India
² Sweet Creation Mane India Pvt. Ltd., Hyderabad, India

Correspondence: N. Sekhar, Department of Chemistry, GITAM School of Science, Hyderabad, India. E-mail: nadiminti_sekhar@live.com

Received: September 27, 2023      Accepted: October 25, 2023      Online Published: November 15, 2023
doi:10.5539/jas.v15n12p92          URL: https://doi.org/10.5539/jas.v15n12p92

Abstract

Citrus fruits and their essential oils play a vital role in every aspect of human life. Essential oils derived from citrus peel are rich in polyphenols and act as secondary metabolites to treat various diseases and they can be used as insecticide or pesticide. These citrus oil derivatives are much popular in flavour and fragrance industry and FMCG sector. In present research work five variants of citrus fruits; C. aurantium (Narinja), C. hystrix (Gondhoajlebu), C. limon (Lemon), C. limetta (Mosambi) and Citrus reticulata Blanco (Nagur Orange) were selected from different regions of India; Telangana, Andhra Pradesh, Kolkata and Maharashtra. The collected samples were further studied using SPME-GCMS analysis to identify the specific molecules which are not in common. Most identified molecules through GCMS analysis are Limonene, Alpha pinene, Myrcene, Delta-carene, Sabinene etc. Each molecule has a significant aroma and used in many Flavour & Fragrance industry. The chemical molecules identified in Narinja (C. aurantium) citrus fruit are specific and not identified in any of the selected fruits they are Bicyclogermacrene, Isopiperitenone, Alpha eudesmol, Beta eudesmol. Antimicrobial activity of five essential oils reports Narinja oil has potent activity on E. coli, Staphylococcus aureus and Bacillus followed by lemon oil and orange oil on E. coli and Bacillus, Mosambion Bacillus and Gondhorajlebu on E. coli. This data reveals that there are some specific molecules in C. aurantium to be considered for further research for their medicinal aspects, as mosquito repellent or in F&F industry.

Keywords: essential oils, GCMS, antimicrobial activity, extraction, aroma chemicals

1. Introduction

Citrus fruits were one of the most refreshing and nutrient rich fruits in the world. There are many different varieties of citrus fruits all over the world in tropical, sub-tropical and temperate regions. Citrus fruits include Oranges, Lemon, Lime, Mandarin, Grapefruit, Pomelos, Tangerines etc. They belong to Rutaceae family and rich in Vitamin C (Abobatta, 2019a). Citrus fruits have good nutritional value and they have been cultivated since 4000yrs ago. Citrus fruit production especially sweet oranges are mostly produced from Brazil, followed by USA and other countries. Important crops like oranges, lemon, limes, grapefruit, and pomelos are prominently grown around the world but its origin is from Southern Eastern Asian region.

Citrus fruit production has huge demand globally. In 2016 the production of citrus fruits was around 124.25 million tons from China which was followed by Brazil, India, USA, Spain, Mexico etc. (FAO, 2017). Among all the variants navel orange, blood orange and Valencia variety considered as sweet orange—Citrus sinensis L., Citrus aurantium is named as bitter orange and it was sour in taste. Lemon (Citrus limon) and lime (Citrus aurantifolia) can be found all over the year in most of the places. There are citrus fruits like Mandarin (Citrus reticulate) harvested from August to September which can be peeled easily (Abobatta, 2019b). Most of the citrus fruits pulp is consumed and remaining 50% of the fruit is peel which cannot be consumed but it consists of oil glands to store the citrus essential. Cold pressed citrus oils have good aroma, and it can be used in food and beverage industries (Kapsaski-Kanelli et al., 2017).

Citrus essential oils can be used for various health benefits due to the presence of secondary metabolites like sabine, α/β-pinene, β-myrcene, linalool, d-limonene, α-terpineoland α-humulene. They act as antioxidant, anti-inflammatory agent and anti-cancer agent and belong to monoterpenes, monoterpenoid alcohol, and
sesquiterpenes group (Bora et al., 2020). Flavonoids like, naringin, narirutin, naringenin, neohesperidin, nobiletin, hesperetin, hesperidin, luteolin, and tangerine present in citrus fruits act as anti-microbial agents and used in quality and food safety system to inhibit the microbial growth or as preservative (Damián-Reyna et al., 2016).

Citrus fruits are rich in polyphenols, vitamins, carotenoids, flavonoids, coumarins etc and they help to build up our immune system. Essential oils from citrus fruits can prevent food spoilage caused by food borne pathogens like Escherichia coli, Salmonella, Shigella, Vibrio cholera etc. and these oils are considered as preservatives or natural antimicrobial agents to overcome spoilage of food. Antimicrobial assay was performed to study the activity of essential oils against pathogens (Hasija et al., 2015).

2. Methods

2.1 Sample Collection

Minimum 5 to 6 kg of matured Citrus fruit variant were collected from (fruit market/orchids) different locations. Selection is mainly based on the color, texture of the skin and taste. Important parameter to identify or select the citrus fruits is to check exact matured stage of the fruit without sourness/overripen character.

(1) Narinja (C. aurantium) orange collected from Makkinavarigudem, Andhra Pradesh.
(2) Gondhorajlebu (C. hystrix) collected from Kolkata.
(3) Lemon (C. limon) collected from Telangana.
(4) Mosambi (C. limetta) collected from Telangana.
(5) Nagpur orange (C. reticulata Blanco) collected from Maharastra.

Collected fruits were carefully bought to the lab and cleaned with sterile water and kept for air drying for 30 min. After drying they are analyzed to identify the flavor molecules by performing GCMS.

2.2 Extraction of Flavor Molecules From Different Citrus Fruits by GCMS Analysis

Wash the fruits and keep them drying in room temperature before extraction. Peel of the selected citrus fruits like Narinja, Gondhorajlebu, lemon, Mosambi, Nagpur orange were removed carefully. 10-20 g of each selected citrus fruit peel were weighed and kept in separate beaker. The peel of each fruit is mixed with cellulose powder. In next step transfer 60-70 ml of prepared sample into glass tube and fill the tube with floran liquid gas. Shake the contents using orbital shaker at 200 rpm for 2 hrs. After 2 hrs transfer the liquid gas into Dichloro methane solution and allow to stand for some time until liquid gas evaporates. After evaporation of liquid gas inject the sample into GCMS port and run the analysis.

2.3 Antimicrobial Activity of Citrus Essential Oils

Essential oils from selected citrus fruits are extracted through steam distillation and tested for their antimicrobial activity against both Gram-positive and Gram-negative organisms. Nutrient agar media was prepared as per the composition and poured in petri plates and allowed for solidification. After solidification spread plate technique was performed using E. coli, Pseudomonas, Bacillus and Staphylococcus aureus. Then incorporate the discs impregnated in essential oils for overnight and labeled as S1, S2, S3, S4, S5 and one blank disc was placed for comparison studies. After placing the discs in their respective places, the plates were incubated at 37 °C for 24-48 hrs. Zone of clearance or antimicrobial activity of selected citrus oils were measured after incubation.

3. Results

3.1 Identification of Flavor Molecules by GCMS Analysis

Essential oils extracted from all the citrus fruits are subjected to GCMS analysis and observed that few of the flavor molecules extracted are common in C. aurantium (Narinja), C. hystrix (Gondhorajlebu), C. limon (Lemon), C. limetta (Mosambi) and Citrus reticulata Blanco (Nagur Orange) and few are unique or different from other extracted molecules. Major components identified in Nagpur orange (Figure 1) are Limonene (84.26%), Carene delta (6.26%), Methyl pelargonate (4.0%), Linalool (2.6%) and Myrcene (1.29%).

Components extracted and identified in Narinja fruit (Figure 2) are Limonene (93.7%), Myrcene (0.31%), Germacrene D (1.39%) and Beta eudesmal (1.39%). In Gondhorajlebu (Figure 3) major molecules like Limonene (64.97%), Citronellal (12%), Citroptene (7.23%) Citronellol 90% (3.6%), Bisabolene (1.62%), Caryophellene (1.15%), Myrcene and Alpha bergamotene (1%) are identified. In Lemon molecules (Figure 4) identified through GCMS are Limonene (66%), Pinene Beta (12.7%), Terpinene Gamma (5.52%), Paracymene (3.37%), Citral (1.71%), Bisabolene (1.13%), Sabinene (1.18%) and Geranyl acetate extra (0.48%) etc. Limonene
(92.75%) and Myrcene (1.68%) are the major chemical components identified in Mosambi/sweet lime (Figure 5) by GCMS analysis. Comparative studies were performed to identify uniques molecules among the extracted components (Table 1).
Figure 2. GCMS Analysis of Naringia orange

Figure 3. GCMS Analysis of Gondhoraj Lebu
Figure 4. GCMS Analysis of Lemon

Figure 5. GCMS Analysis of Mosambi
Table 1. Comparative studies of Citrus molecules by GCMS analysis

<table>
<thead>
<tr>
<th>S.No.</th>
<th>Name of the molecule identified</th>
<th>Structure</th>
<th>Aroma</th>
<th>Narinj</th>
<th>Nagpur Orange</th>
<th>Gondhrajlehu</th>
<th>Lemon</th>
<th>Mosambi</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Limonene</td>
<td>Citrus, Juicy, lemon, orange</td>
<td>93.72</td>
<td>84.26</td>
<td>64.97</td>
<td>66</td>
<td>92.75</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>Carene delta</td>
<td>Citrus, herbal, terpenic</td>
<td>-</td>
<td>6.26</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>Methyl pelargonate</td>
<td>Fruity pear/green</td>
<td>-</td>
<td>4.01</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>Linalool</td>
<td>Citrus, Floral, soapy</td>
<td>-</td>
<td>2.60</td>
<td>-</td>
<td>-</td>
<td>0.83</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>Terpenic green, Raw mango</td>
<td>0.31</td>
<td>-</td>
<td>1.29</td>
<td>1.07</td>
<td>-</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>Geracrene d</td>
<td>Mild chemical note</td>
<td>1.39</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>Beta eudesmal</td>
<td>Woody green</td>
<td>0.05</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>Citronellal</td>
<td>Citrus, floral, rose</td>
<td>-</td>
<td>-</td>
<td>12.06</td>
<td>-</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>9</td>
<td>Citroptene</td>
<td>Less aromatic with chemical note</td>
<td>-</td>
<td>-</td>
<td>7.23</td>
<td>-</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>Citronellol</td>
<td>Citrus, floral, rose</td>
<td>-</td>
<td>-</td>
<td>3.6</td>
<td>-</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>11</td>
<td>Bisabolene</td>
<td>Citrus, fruity, balsamic</td>
<td>-</td>
<td>-</td>
<td>1.62</td>
<td>1.13</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>12</td>
<td>Caryophyllene</td>
<td>Woody, spicy, clove</td>
<td>-</td>
<td>-</td>
<td>1.15</td>
<td>-</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>13</td>
<td>Alpha bergamotene</td>
<td>Woody, herbal, green</td>
<td>-</td>
<td>-</td>
<td>1.07</td>
<td>-</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>14</td>
<td>Terpinene gamma</td>
<td>Woody, terpenic, citrus</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>5.52</td>
<td></td>
</tr>
<tr>
<td>15</td>
<td>Paracymene</td>
<td>Citrus, spicy, terpenic</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>3.37</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>16</td>
<td>Citral</td>
<td>Citrus, lemon, juicy</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>1.71</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>17</td>
<td>Sabinene</td>
<td>Citrus, woody, terpenic</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>1.98</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>18</td>
<td>Geranyl acetate</td>
<td>Floral, rose, green</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>0.48</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>19</td>
<td>Pinene beta</td>
<td>Woody, pine, eucalyptus</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>12.75</td>
<td>-</td>
<td></td>
</tr>
</tbody>
</table>
3.2 Antimicrobial Activity of Essential Oils

After 24-48 hrs of incubation the zone of inhibition is calculated for the essential oils selected to confirm their activity or to inhibit the growth of major pathogens from both Gram positive (Bacillus and Staphylococcus aureus) and Gram negative (Pseudomonas and E. coli) bacteria. Based on the zone of inhibition it has been noticed Narinja oil has great microbial activity against E. coli (7 mm), Bacillus (7 mm), Staphylococcus aureus (3 mm) and Pseudomonas (2 mm), followed by Lemon oil, it exhibited zone of inhibition on E. coli (4 mm), Bacillus (4 mm), Nagpur orange oil actively suppressed E. coli (3 mm), Bacillus (3 mm), Gondhoraj leu oil showed its antagonistic activity only to E. coli (3 mm) (Figure 6, Table 2).

Zone of inhibition was incorporated in formula π². The π² value for 4mm radius of inhibition it is 50.24 mm², for 3mm the value was 28.26 mm², for 7 mm the value was 153.86 mm² and for 2mm the value was 12.56 mm². Based on all essential oils Narinja oil exhibited pronounced antimicrobial activity on three microbes compared to other oils.

![Figure 6. Antimicrobial activity of essential oils on gram positive and gram negative bacteria](image)

Table 2. Zone of inhibition in radius

<table>
<thead>
<tr>
<th>S.No.</th>
<th>Name of oil</th>
<th>Pseudomonas</th>
<th>E. coli</th>
<th>Bacillus</th>
<th>S. aureus</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>Control</td>
<td>No zone</td>
<td>No zone</td>
<td>No zone</td>
<td>No zone</td>
</tr>
<tr>
<td>S1</td>
<td>Mosambi</td>
<td>No zone</td>
<td>No zone</td>
<td>3mm</td>
<td>No zone</td>
</tr>
<tr>
<td>S2</td>
<td>Lemon</td>
<td>No zone</td>
<td>4mm</td>
<td>4mm</td>
<td>No zone</td>
</tr>
<tr>
<td>S3</td>
<td>Gondhorajlebu</td>
<td>No zone</td>
<td>3mm</td>
<td>No zone</td>
<td>No zone</td>
</tr>
<tr>
<td>S4</td>
<td>Narinja</td>
<td>2mm</td>
<td>7mm</td>
<td>7mm</td>
<td>3mm</td>
</tr>
<tr>
<td>S5</td>
<td>Nagpur Orange</td>
<td>No zone</td>
<td>3mm</td>
<td>3mm</td>
<td>No zone</td>
</tr>
</tbody>
</table>

4. Discussion

Essential oils of citrus fruits have excellent properties to overcome anxiety and act as stress relief with fresh aroma. Citrus oils are used in perfumery, food, and flavour industry in various applications to serve human. Most of the citrus essential oils are extracted through cold pressed method or steam distillation from peel (Grassmann & Elstner, 2003). Essential oils of citrus family comprise complex molecules offerpenic hydrocarbons, alcohols, aldehydes, and esters. Citrus fruits structure specifically determines that the outer peel or colored layer consists of numerous glands or sacs which stores the essential oil (Arce & Soto, 2008). History reveals that essential oils are extracted through hydro or steam distillation process from various aromatic plants. Currently other extraction procedures like cold pressing, CO₂ extraction process are in progress to extract essential oils (Palazzolo, Laudicina, & Germanà, 2013).

A sophisticated equipment GCMS was used to identify the active aromatic molecules present in citrus essential oils. In current research aroma molecules in orange peel were Limonene (84.62%), Carene delta (6.26%), Methyl pelargonate (4.0%), Linalool (2.6%) and Myrcene (1.29%), in Narinja peel Limonene (93.7%), Myrcene (0.31%), Germacone D (1.39%) and Beta eudesmal (1.39%) were extracted, Limonene (64.97%), Citronellal (12%), Citroptene (7.23%) Citronellol 90% (3.6%), Bisabolene (1.62%), Caryophellene (1.15%), Myrcene and Alpha bergamotene (1%) were identified in gondhorajlebu peel, Limonene (66%), Pinene Beta (12.7%), Terpinene Gamma (5.52%), Paracymene (3.37%), Citral (1.71%), Bisabolene (1.13%), Sabinene (1.18%) and Geranyl
acetate extra (0.48%) identified in lemon peel and from mosambi peel Limonene (92.75%) and Myrcene (1.68%) are key components detected. Comparative studies significantly states that Limonene is commonly identified compound in all the five citrus variants. Based on GCMS analysis report Limonene (90%), Valencene (2.18%), Eicosane (2.01%), Tetrahydro linalool (2.02%), α-Cadinene (1.425%), Nonanal (1.31%) and Dodecanol (1.06%) are major constituents identified in Citrus paradisi (Yadav, Lawrence, & Dar, 2017). Research has been done by Anupama and team on C. maxima and identified 28.26% of Citronellol in leaf oil and 89.04% of D-limonene in the rind of the fruit through GCMS analysis (Prasad et al., 2016).

In current era processed food and packed food was in high demand and to avoid contamination of food products people prefer natural ingredients as preservatives rather than synthetic chemicals. Among all the natural ingredients citrus essential oils play a vital role to preserve foods. Citrus essential oils contain bioactive compounds which effectively inhibits or kills the pathogens. Present study states that selected citrus essential oils like Narinja oil exhibit high antimicrobial activity on Gram positive (Bacillus and Staphylococcus aureus) and Gram negative (Pseudomonas and E. coli) bacteria when compared to other oils. Antimicrobial activity of Narinja oil through r² formula states that on E. coli the value was 153.86 mm², Bacillus was 153.86 mm², Staphylococcus aureus was 28.26 mm² and Pseudomonas was 12.56 mm². The values state that it was more effective on E. coli and Bacillus.

The r² applied to check the antimicrobial activity of Lemon oil, it exhibited zone of inhibition on E. coli with r² of 50.24 mm², Bacillus was 50.24 mm² and there is no activity was found on Staphylococcus aureus and Pseudomonas. The values for Nagpur orange oil in r² on E. coli was 28.26 mm², Bacillus was 28.26 mm² and antagonistic activity of Gondhorajlebu oil showed only on E. coli and the value was 28.26 mm². Grapefruit oil and lemon oil has excellent antimicrobial activity against Leuconostoc mesenteroides MS1, Escherichia coli ATCC 25922 and Lactobacillus plantarum ES147 and ATCC 8014 (Raspo et al., 2020). Citrus oil components terpineol and terpeneless fractions has more antagonistic activity on both Gram-positive and Gram-negative bacteria. Citrus essential oils and terpene derivatives has great antimicrobial activity on bacteria and candida strains (Mancuso et al., 2019). Death or Inhibition of microorganisms due to citrus oils was due to cytoplasmic membranedestruction (Kong et al., 2008).

There are reports stating citrus essential oils like finger citron oil exhibit antimicrobial activity on microbes like S. aureus with an inhibition zone 19.2±2.1 mm, on Bacillus subtilis the inhibition zone was 16.3±1.3 mm, for Micrococcus luteus it was, 16.1±0.4 mm and on E. coli it was 11.2±0.9 mm (Li et al., 2019). Two main components of lemon oil like limonene and β-pinene exhibit high microbial activity on both Gram-positive and Gram-negative bacteria (Ben Hsouna et al., 2017). When we compare the above data with current results Limonene is major content in all citrus oils and other molecules in combination with Limonene exhibit great antimicrobial activity against Gram-positive and Gram-negative microbes.

5. Conclusions

In present research the oils extracted from citrus oils like C. aurantium (Narinja), C. hystryx (Gondhorajlebu), C. limon (Lemon), C. limetta (Mosambi) and Citrus reticulata Blanco (Nagur Orange) reveals they contains various aroma chemicals and has great potential activity to inhibit pathogenic microorganisms. Further research is to explore and to study the selected citrus oils in medical, flavour and fragrance industry.

References


Acknowledgments
We would like to thank Mr. Sumit Dasgupta, Managing Director and R. Raghuraman, QC head, Mane India Pvt Ltd and Dr. Jaya Madhuri Ravuri, Professor, Department of Microbiology, Sri Padmavathi Mahila Visvavidyalayam for their great support for analytical work to perform the study.

Authors Contributions
All authors read and approved the final manuscript.

Funding
Not applicable.

Competing Interests
The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Informed Consent
Obtained.

Ethics Approval
The Publication Ethics Committee of the Canadian Center of Science and Education.

The journal’s policies adhere to the Core Practices established by the Committee on Publication Ethics (COPE).
Provenance and Peer Review
Not commissioned; externally double-blind peer reviewed.

Data Availability Statement
The data that support the findings of this study are available on request from the corresponding author. The data are not publicly available due to privacy or ethical restrictions.

Data Sharing Statement
No additional data are available.

Open Access
This is an open-access article distributed under the terms and conditions of the Creative Commons Attribution license (http://creativecommons.org/licenses/by/4.0/).

Copyrights
Copyright for this article is retained by the author(s), with first publication rights granted to the journal.