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Aroma Chemicals Identification by Sophisticated Technique and Their Role Against Pathogens

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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 moleculesthrough 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 areBicyclogermacrene, Isopiperitenone, Alpha eudesmol, Beta eudesmol. Antimicrobial activity of five essential oilsreports 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 aresome 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 sabinene, 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, monoterpene aldehyde/alcohol, and

sesquiterpenes group (Bora et al., 2020). Flavonoids like, naringin, narirutin, naringenin,neohesperidin, nobiletin,hesperetin, hesperidin, luteolin, and tangerinepresent 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 identifyor select the citrus fruitsis to taste or check exact matured stage of the fruit without sourness/overripenscharacter.

(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 30min. 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, Mosambiand Nagpurorange wereremoved carefully. 10-20 g of each selected citrus fruitpeel were weighed and kept in separatebeaker. 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-negativeorganisms.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 ^{o}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 flavour molecules extracted are common in *C. aurantium* (Narinja), *C. hystrix* (Gondhoajlebu), *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).

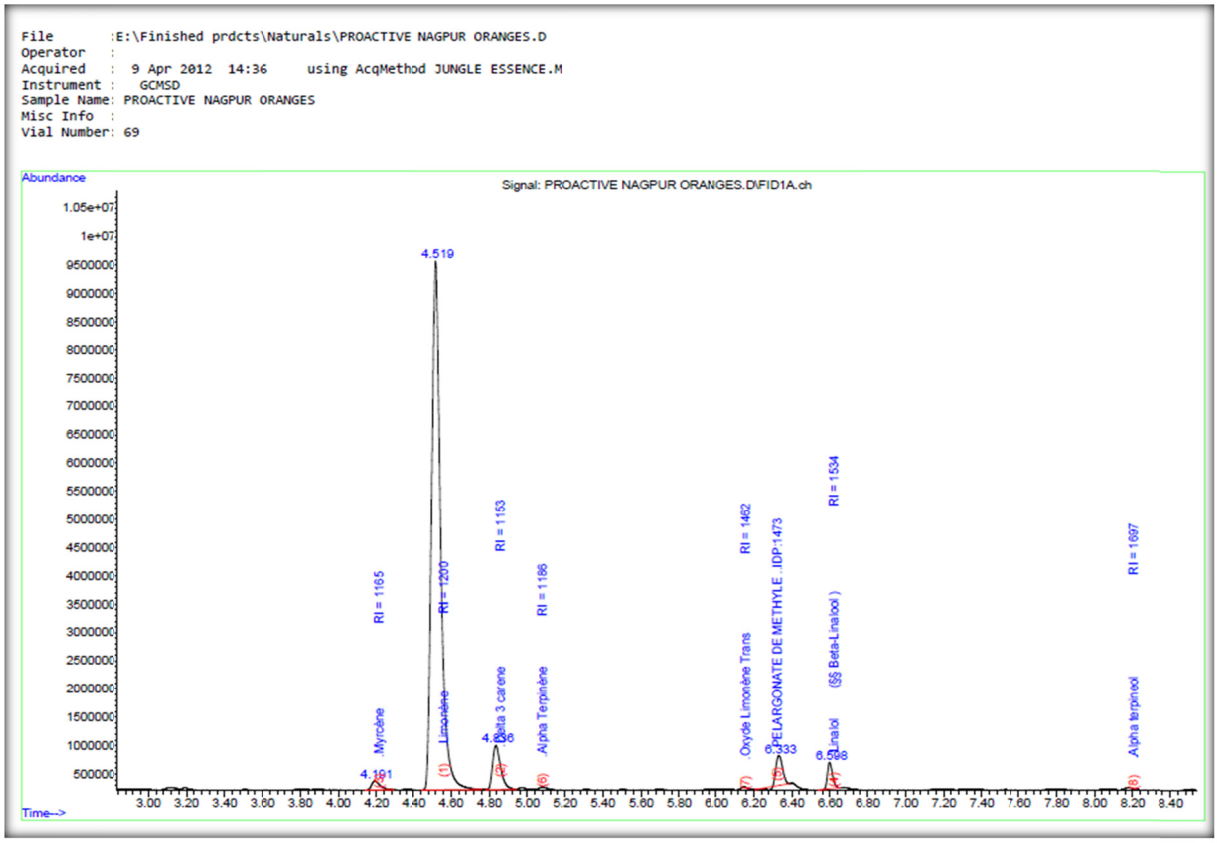


Figure 1. GCMS Analysis of Nagpur orange

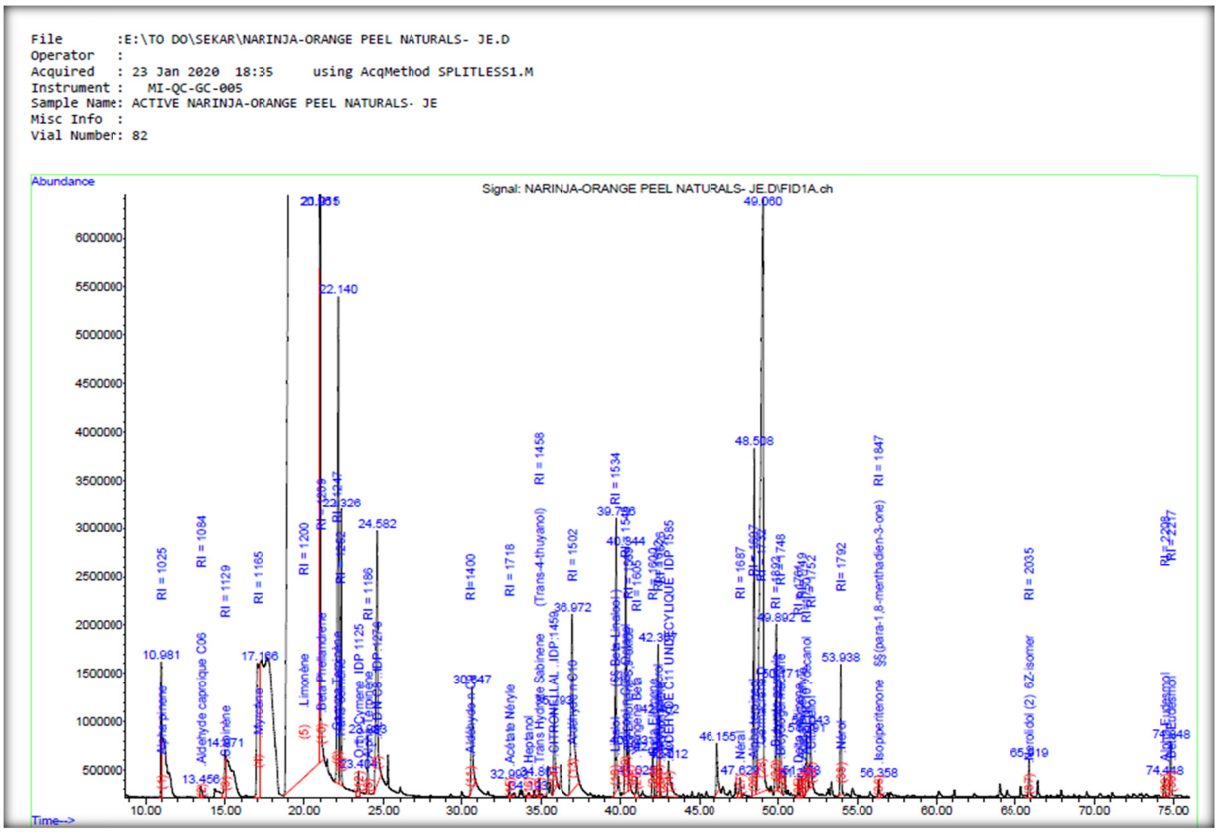


Figure 2. GCMS Analysis of Narinja orange

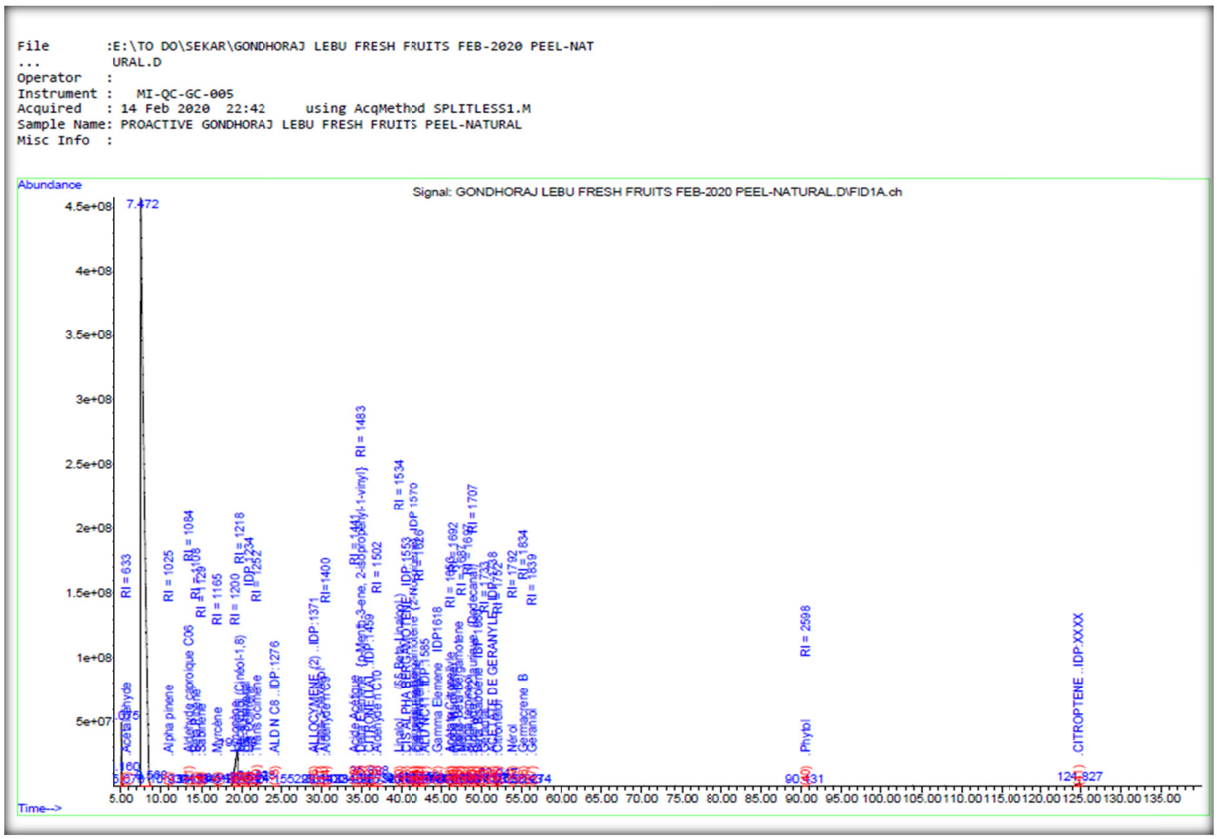


Figure 3. GCMS Analysis of Gondhoraj Lebu

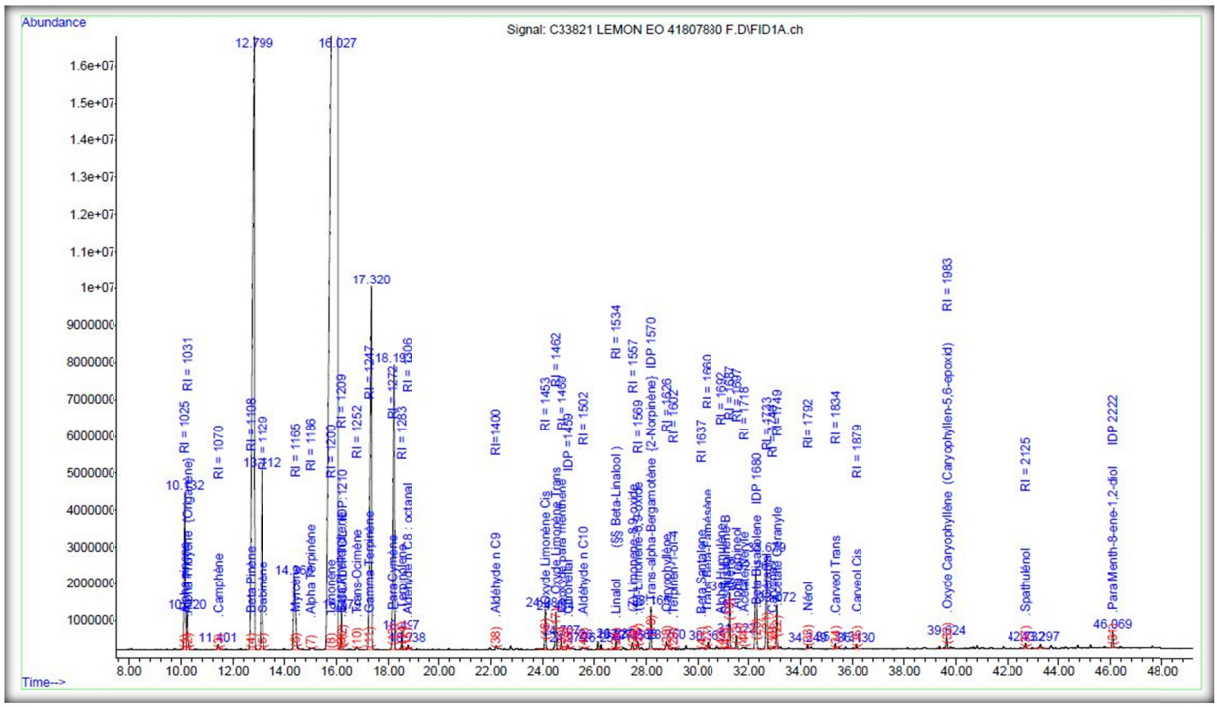


Figure 4. GCMS Analysis of Lemon

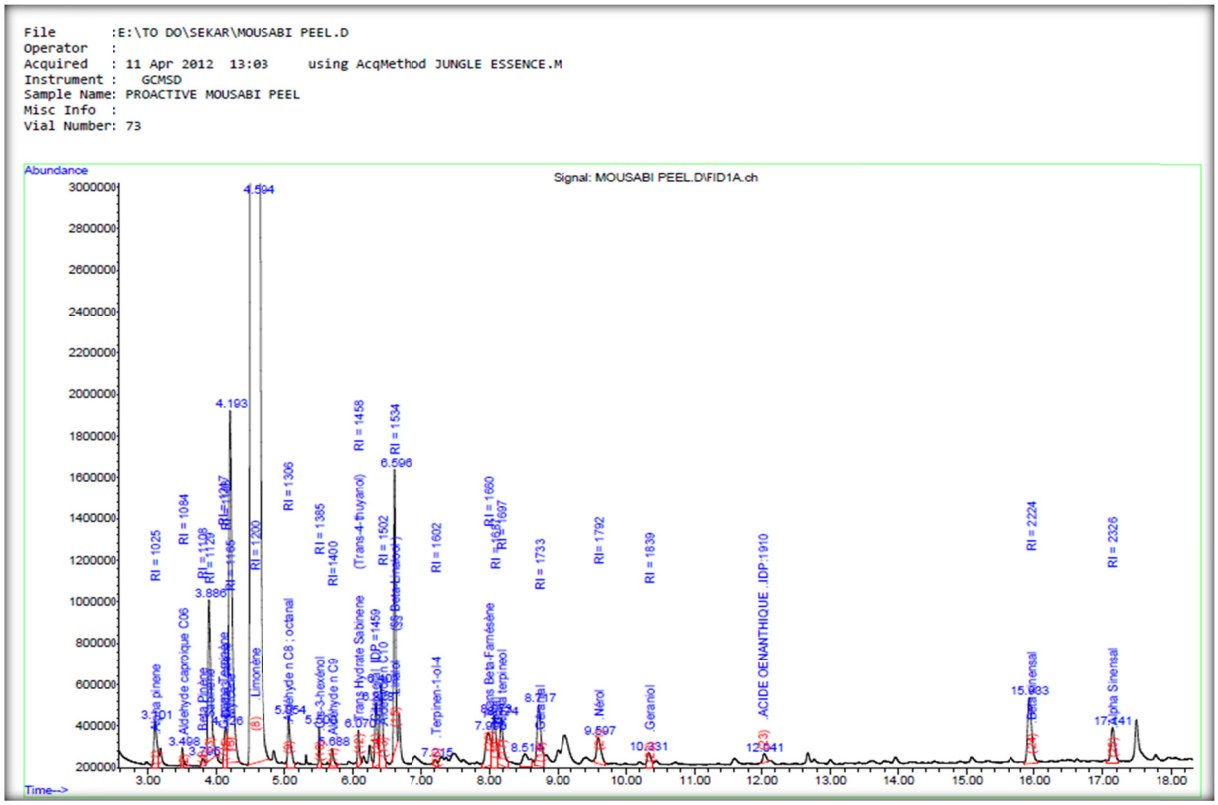
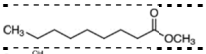
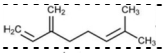
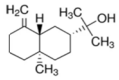
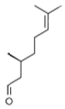
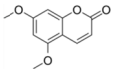
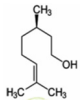
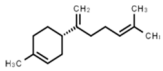
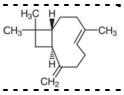
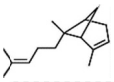
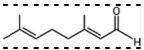
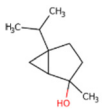
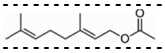


Figure 5. GCMS Analysis of Mosambi

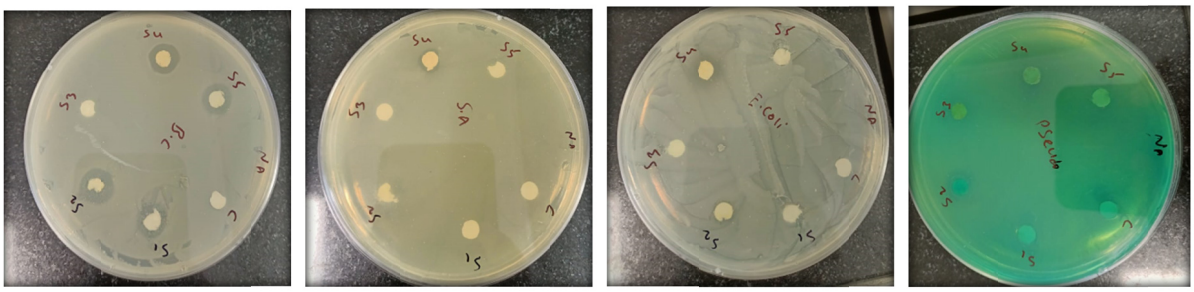
Table 1. Comparative studies of Citrus molecules by GCMS analysis

S.No.	Name of the molecule identified	Structure	Aroma	Narinja	Nagpur Orange	Gondhorajlebu	Lemon	Mosambi
				---------- % ----------				
1	Limonene		Citrus, Juicy, lemon, orange	93.72	84.26	64.97	66	92.75
2	Carene delta		Citrus, herbal, terpenic	-	6.26	-	-	-
3	Methyl pelargonate		Fruity pear/green	-	4.01	-	-	-
4	Linalool		Citrus, Floral, soapy	-	2.60	-	-	0.83
5	Myrcene		Terpenic green, Raw mango	0.31	1.29	1.07	-	1.68
6	Geracrene d		Mild chemicalnote	1.39	-		-	-
7	Beta eudesmal		Woody green	0.05	-		-	-
8	Citronellal		Citrus, floral, rose	-	-	12.06	-	-
9	Citroptene		Less aromatic with chemical note	-	-	7.23	-	-
10	Citronellol		Citrus, floral, rose	-	-	3.6	-	-
11	Bisabolene		Citrus, fruity, balsamic	-	-	1.62	1.13	-
12	Caryophyllene		Woody, spicy, clove	-	-	1.15	-	-
13	Alpha bergamotene		Woody, herbal, green	-	-	1.07	-	-
14	Terpinene gamma		Woody, terpenic, citrus	-	-	-	5.52	-
15	Paracymene		Citrus, spicy, terpenic	-	-	-	3.37	-
16	Citral		Citrus, lemon, juicy	-	-	-	1.71	-
17	Sabinene		Citrus, woody, terpenic	-	-	-	1.98	-
18	Geranyl acetate		Floral, rose, green	-	-	-	0.48	-
19	Pinene beta		Woody, pine, eucalyptus	-	-	-	12.75	-

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 (*Bacillius* 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), *Staphylococcu aureus* (3 mm) and *Pseudomonas* (2 mm), followed by Lemon oil, itexhibited 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 oilshowed its antagonistic activity only to *E.coli* (3 mm) (Figure 6, Table 2).

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



Bacillius *Staphylococcus aureus* *E. coli* *Pseudomonas*

Figure 6. Antimicrobial activity of essential oils on gram positive and gram negative bacteria

Table 2. Zone of inhibition in radius

S.No.	Name of oil	*Pseudomonas*	*E. coli*	*Bacillus*	*S. aureus*
Control	Control	No zone	No zone	No zone	No zone
S1	Mosambi	No zone	No zone	3mm	No zone
S2	Lemon	No zone	4mm	4mm	No zone
S3	Gondhorajlebu	No zone	3mm	No zone	No zone
S4	Narinja	2mm	7mm	7mm	3mm
S5	Nagpur Orange	No zone	3mm	3mm	No zone

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 ofterpenic 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, CO_2 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 wereLimonene (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%), Germacrene 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 reportLimonene (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 paradisii* (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 avital 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 (*Bacillius* and *Staphylococcus aureus*) and Gram negative (*Pseudomonas* and *E. coli*) bacteria when compared to other oils. Antimicrobial activity of Narinja oil through πr^2 formula states that on *E. coli* the value was 153.86 mm^2, *Bacillus* was 153.86 mm^2, *Staphylococcus aureus* was 28.26 mm^2 and *Pseudomonas* was 12.56 mm^2. The values state that it was more effective on *E. coli* and *Bacillus*.

The πr^2 applied to check the antimicrobial activity of Lemon oil, itexhibited zone of inhibition on *E. coli* with πr^2 of 50.24 mm^2, *Bacillus* was 50.24 mm^2 and there is no activity was found on *Staphylococcus aureus* and *Pseudomonas*. The values for Nagpur orange oil in πr^2 on *E. coli* was 28.26 mm^2, *Bacillus* was 28.26 mm^2 and antagonistic activity of Gondhorajlebu oil showed only on *E. coli* and the value was 28.26 mm^2. 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 *Microcous 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. hystrix* (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 exploreand to study the selected citrus oils in medical, flavour and fragrance industry.

References

Abobatta, W. F. (2019a). Citrus varieties in Egypt: An impression. *International Research Journal of Applied Sciences, 1*, 63-66.

Abobatta, W. F. (2019b). Nutritional benefits of citrus fruits. *Am. J. Biomed. Sci. Res., 3*, 303-306. https://doi.org/10.34297/AJBSR.2019.03.000681

Arce, A., & Soto, A. (2008). Citrus essential oils: Extraction and deterpenation. *Tree and Forestry Science and Biotechnology, 2*(1), 1-9.

Ben Hsouna, A., Ben Halima, N., Smaoui, S., & Hamdi, N. (2017). Citrus lemon essential oil: Chemical composition, antioxidant and antimicrobial activities with its preservative effect against Listeria monocytogenes inoculated in minced beef meat. *Lipids in Health and Disease, 16*, 1-11. https://doi.org/10.1186/s12944-017-0487-5

Bora, H., Kamle, M., Mahato, D. K., Tiwari, P., & Kumar, P. (2020). Citrus essential oils (CEOs) and their applications in food: An overview. *Plants, 9*(3), 357. https://doi.org/10.3390/plants9030357

Damián-Reyna, A. A., González-Hernández, J. C., & Chávez-Parga, M. (2016). Current procedures for extraction and purification of citrus flavonoides. *Revista Colombiana de Biotecnología, 18*(1), 135-147. https://10.15446/rev.colomb.biote.v18n1.57724

FAO. (2017). *FAO Statistical Bulletin*. Retrieved from http://www.fao.org/docrep/018/mi317e/mi317e.pdf

Grassmann, J., & Elstner, E. F. (2003). *Encyclopedia of Food Sciences and Nutrition* (2nd ed.).

Hasija, S., Ibrahim, G., & Wadia, A. (2015). Antimicrobial activity of *Citrus sinensis* (Orange), *Citrus limetta* (Sweet lime) and *Citrus limon* (lemon) peel oil on selected food borne pathogens. *Int J Life Sci Res, 3*(3), 35-9.

Kapsaski-Kanelli, V. N., Evergetis, E., Michaelakis, A., Papachristos, D. P., Myrtsi, E. D., Koulocheri, S. D., & Haroutounian, S. A. (2017). "Gold" pressed essential oil: an essay on the volatile fragment from citrus juice industry by-products chemistry and bioactivity. *BioMed Research International, 2017*. https://doi.org/10.1155/2017/2761461

Kong, M., Chen, X. G., Liu, C. S., Liu, C. G., & Meng, X. H. (2008). Antibacterial mechanism of chitosan microspheres in a solid dispersing system against *E. coli*. *Colloids and Surfaces B: Biointerfaces, 65*(2), 197-202. https://doi.org/10.1016/j.colsurfb.2008.04.003

Li, Z. H., Cai, M., Liu, Y. S., Sun, P. L., & Luo, S. L. (2019). Antibacterial activity and mechanisms of essential oil from *Citrus medica* L. var. sarcodactylis. *Molecules, 24*(8), 1577. https://doi.org/10.3390/molecules 24081577

Mancuso, M., Catalfamo, M., Laganà, P., Rappazzo, A. C., Raymo, V., Zampino, D., & Zaccone, R. (2019). Screening of antimicrobial activity of citrus essential oils against pathogenic bacteria and Candida strains. *Flavour and Fragrance Journal, 34*(3), 187-200. https://doi.org/10.1002/ffj.3491

Palazzolo, E., Laudicina, V. A., & Germanà, M. A. (2013). Current and potential use of citrus essential oils. *Curr. Org. Chem., 17*(24), 3042-3049. https://doi.org/10.2174/13852728113179990122

Prasad, D. A., Prasad, B. R., Prasad, D. K., Shetty, P., & Kumar, K. S. (2016). GC-MS compositional analysis of essential oil of leaf and fruit rind of *Citrus maxima* (Burm.) Merr. from Coastal Karnataka, India. *Journal of Applied Pharmaceutical Science, 6*(5), 068-072. https://doi.org/10.7324/JAPS.2016.60511

Raspo, M. A., Vignola, M. B., Andreatta, A. E., & Juliani, H. R. (2020). Antioxidant and antimicrobial activities of citrus essential oils from Argentina and the United States. *Food Bioscience, 36*, 100651. https://doi.org/10.1016/j.fbio.2020.100651

Yadav, R., Lawrence, R. S., & Dar, M. S. (2017). GC/MS analysis, antioxidant and antibacterial activities of essential oil extracted from *Citrus paradisii* macfad fruit peels. *Int. J. Sci. Eng., 8*(7), 653-660.

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