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Health Benefits of the Fruit Essential Oil of Eucalyptus Citriodora: Secondary Metabolites, Radical Scavenging, Antioxidant, Anti-inflammatory, Analgesic, Antimicrobial Potential

Ololade [1], Zacchaeus S[2] and Olawore, Nureni O[2]

[1]Department of Chemistry, Faculty of Science, University of Medical Sciences, Ondo, Nigeria

[2]Department of Pure and Applied Chemistry, Faculty of Science, Ladoke Akintola University of Technology, Ogbomoso, Nigeria

[*]**Corresponding author:** Zacchaeus S, Department of Pure and Applied Chemistry, Faculty of Science, Ladoke Akintola University of Technology, Ogbomoso, Nigeria, Tel No: 2348034836546; E-mail: zacchsnatpdt@gmail.com

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Abstract

Eucalyptus essential oil is an important aroma-therapeutic agent in the treatment of diverse diseases and ailments in human and animals. The aim of this study was to determine the composition and health benefits of the fruit essential oil of E. Citriodora. The essential oil was extracted by hydro-distillation using Clevenger-type apparatus and analyzed using GC-MS. The total phenolic content, antioxidant, anti-inflammatory, analgesic and antimicrobial activities were measured by standard biochemical protocols. The GC-MS result showed the presence of twenty-two (22) organic compounds making up 98.8% of the percentage composition of the essential oil. The principal components of the oil were: politic acid (29.00%), oleic acid (10.00%), E,E,E-α-springene (9.00%), 2-ethenyl-2,5-dimethyl-4-hexen-1-ol (8.00%), 2,4-dimethylheptane (6.00%) and hexahydrofarnesyl acetone (5.00%). The TPC value was 175.84 ± 0.00 µgmg-1 GAE. The DPPH IC50 and AAI values of the essential oil were 3.00 µgml-1 and 13.30, respectively. The essential oil was capable of scavenging free radicals in a range of 67.77-71.95%, while the FRAP EC50 value was 2.00 µgml-1. The essential oil exhibited high anti-inflammatory effect with value of 43.80% and analgesic potential by inhibition of both neurogenic (41.32%) and inflammatory pains (11.11%). The zones of inhibition were between 0.80-18.00 mm. The fruit essential oil consists of useful phyto-therapeutic compounds that may be of great use in the food and pharmaceutical industries.

Keywords: Eucalyptus citriodora, Fruit essential oil, GC-MS, Phytochemicals, Pharmacological activity

Introduction

Essential oils recently received a higher interest by scientists because they have some useful therapeutic components that act synergistically for preservation and drug formulations [1-5]. Essential oils are used as flavouring and preservative substances in both food, nutraceutical and pharmaceutical industries [6,7]. Odoriferous plants possess essential oils that are very important to the plants in diverse ways such as defence mechanism against pest attacks, and covering of cell membrane [8,9]. Essential oils are valuable natural products used by man and livestock for aromatherapy, phototherapy, spices, addictive in feed stocks and nutrition [10,11]. Eucalyptus essential oils are used medicinally to treat many symptoms and ailments among human such as sore throat, cold, insomnia, cough, bronchitis, coughing, fever, flu, poor circulation, stress, depression, arthritis, pains, headaches, migraines, inflammation and dental problems [12-15]. Essential oils can have a single, synergistic or multiple targets of their activities. Essential oil as a complex mixture provides more effective activity than the activity of the single major component used alone. This means that minor constituents may be very important for the activity of essential oil and may have a synergistic effect among components [2, 16-18]. Treatment of chicken meat products with essential oils from medicinal plants improves the antioxidant property during refrigeration at very low temperature for a long period of time [19-20]. Eucalyptus citriodora Hook, commonly called lemon-scented gum is native to Australia. It is an evergreen tree 20–50 m in height. It is a tree that has different forms of leaves: the juvenile leaves and adult (phyllodes) leaves which are light green, glossy, and lanceolate. The foliage and flowers are of great interest, they are attractive to bees, and therefore they are used as beekeepers for the production of honey [21-22]. Fruit are shiny irregularly ellipsoid blackish in nature and 3–7 mm long dimension [23-25].

So far, there is scanty information on the fruit of *E.* citriodora. Therefore, this study was aimed at screening the chemical composition and pharmacological properties of the essential oil obtained from the fruit of *E.* citriodora from Nigeria.

Material and Methods

Plant Materials

The sample of the plant was collected from the Afforestation Research Station Kaduna, Nigeria and it was identified as E. citriodora Hook by Mr. Sylvester Boye of the same Institute.

Extraction of the Essential Oil

The fresh fruit were pulverized and the essential oil was obtained by hydro-distillation using all-glass Clevenger-type apparatus according to European pharmacopoeia [26]. The essential oil collected was then stored in vial in a refrigerator at 8°C to prevent evaporation.

GC-MS Analysis

The fruit essential oil of E. Citriodora was analysed using Shimadzu GC-MS-QP2010 Plus (Japan). The separations were carried out using a Rested Rtx-5MS fused silica capillary column (5%-diphenyl-95%-dimethylpolysiloxane) of 30 m × 0.25 mm internal diameter (di) and 0.25 mm in film thickness. The conditions for analysis were set as follows; column oven temperature was programmed from 60-280 °C (temperature at 60 °C was held for 1.0 min, raised to 180 °C for 3 min and then finally to 280°C held for 2 min); injection mode, Split ratio 41.6; injection temperature, 250 °C; flow control mode, linear velocity (36.2 cm/sec); purge flow 3.0 ml/min; pressure, 56.2 kPa; helium was the carrier gas with total flow rate 45.0 ml/min; column flow rate, 0.99 ml/min; ion source temperature, 200 °C; interface temperature, 250 °C; solvent cut time, 3.0 min; start time 3.5 min; end time, 24.0 min; start m/z, 50 and end m/z, 700. Detector was operated in EI ionization mode of 70 eV. Components were identified by matching their mass spectra data with those of the spectrometer data base using the NIST computer data bank, as well as by comparison of the fragmentation pattern with those reported in literature.

Polyphenol Content (PC) Assay: The PC of the essential oil was determined using the Folin-Ciocalteau method previously used by Baba and Malik [27]. Gallic acid was used as a reference; the index of PC was expressed as µgmg-1 gallic acid equivalent (GAE).

In vitro Antioxidant Assays

(i) In vitro 2,2'-diphenyl-1-picrylhydrazyl (DPPH) Assay: The free radical scavenging and antioxidant activities of the sample against the stable free radical 2,2'-diphenyl-1-picrylhydrazyl was measured according to the protocol previously used by Takao et al. [28]. Ascorbic acid was used as reference compound. The assay was carried out in triplicate. Scavenging effect was calculated by the percentage (I%) of faded purple 2,2'-diphenyl-1-picrylhydrazyl solution colour into yellow by the tested sample against the control.

$$\%\ Radical\ Scavenging = \frac{A_{blank} - A_{eo}}{A_{blank}}\ X\ 100$$

Where: Ablank is the absorbance of blank solution and Aeo is the absorbance of the sample. The dose-response curve was plotted and IC50 value for the sample and the standard were calculated.

Antioxidant Activity Index: The antioSSxidant activity index (AAI) was calculated using Scherer and Godoy's criteria:

$$AAI = \frac{DPPH\ Initial\ Concentration}{IC_{50}}$$

Where: weak activity < 0.5, moderate < 1.0, strong < 2.0 and very strong > 2.0 [28].

(ii) In vitro Reducing Power Assay: The reducing antioxidant capacity of fruit essential oil was determined according to the assay used by Sylvie [29]. A higher absorbance value indicates higher reducing power. Ascorbic acid was used as a standard. The assays were analysed in triplicate and IC50 of the oil value was determined.

In vivo Anti-inflammatory and Analgesic Assays

Experimental Animals

Abino rats (200 ±30 g) used in this study were kept in controlled cycles (12/12 hrs light/dark) with free access to food and water. The experiments were carried out in strict compliance with the principle of laboratory animal care [30].

In vivo Carrageenan-Induced Anti-inflammatory Assay

The anti-oedema potential of the fruit essential oil was carried out based on the previous method used by Cai et al. [31]. Results were expressed as the increase in paw volume (mm) calculated after subtraction of basal paw volume prior to carrageenan irritant injection. The inhibition percentage (I%) of the oedema was determined for each rat by comparing each group with controls and calculated by the formula below:

$$I\% = \frac{H_o - H_t}{H_o}\ X\ 100$$

Where: Ho was the average inflammation of the control group at a given time 0. Ht is the mean inflammation of the rats treated with drug at time (t).

In vivo Analgesic (Antinociceptive Formalin Licking Assay): This test was based on the method described by Onifer et al. [32] with slight modification. The first 5 min of post formalin injection is referred to as the early phase and the period between 15 and 30 min as the late phase. The test was performed at room temperature and strict actions were taken to exclude environmental disturbances that might interfere with the animals' response. The percentage inhibition (I%) of pain was calculated as the reduction in the number of licking compared to the control using the formula below:

$$I\% = \frac{J_o - J_t}{J_o}\ X\ 100$$

Where: Jo represents the vehicle treated control group value for each phase while Jt represents the treated group value for each phase.

In vitro Antimicrobial assay

This assay was carried out according to the agar-well diffusion method used by Nkechukwu [33] against multi-drug resistance Gram-positive organism (Streptococcus agalactiae and Staphylococcus aureus), and Gram-negative organisms (Escherichia coli, Klebsiella pneumoniae and Pseudomonas aeruginosa). The plates were allowed to stand in the refrigerator for 1 hr to allow proper diffusion of the sample into the medium and incubated at 37 oC for 16-24 h before visual assessment of the zones of inhibition (ZI). Ofloxacin (OFL) and Cefuroxine (CRX) were used as reference drugs.

Results and Discussion

Chemical Composition of the Fruit Essential Oil

The GC-MS result of fruit essential oil showed that the sample consist of twenty-two (22) components, representing 98.80% of the total components identified (Table 1.). The major phytochemical in the oil were: palmitic acid (29.00%), oleic acid (10.00%), E,E,E- α-springene (9.00%), 2-ethenyl-2,5-dimethyl-4-hexen-1-ol (8.00%), 2,4-dimethylheptane (6.00%), hexahydrofarnesyl acetone (5.00%), geranyl butanoate (4.00%), farnesol (4.00%), geranylgeraniol (4.00%) and trans-2-methyl-2-(4-methyl-3-pentenyl)-cyclopropanecarboxaldehyde (4.00%). Previous studies on the leaf essential oil of E. citriodora from Brazil showed that the major components found in E. citriodora essential oil were citronellal (29.31%), geraniol (27.63%), β-citronellol (14.88%) and δ-cadinene (6.32%) [34] while the leaves essential oil of E. citriodora from Eygpt mainly comprised of 3-hexen-1-ol (31.26%), cis-geraniol (19.66%), citronellol acetate (13.68%), 5-hepten-1-ol, 2,6-dimethyl (13.14%) and citronellal (9.36%) [35].

Compound	Percentage Composition
2,4-dimethylheptane	6.0
α-pinene	4.0
p-cymene	0.4
1,8-cineole	0.5
phthalic acid, di(1-hexen-5-yl) ester	1.0
palmitic acid	29.0
oleic acid	10.0
geranyl butanoate	4.0
hexahydrofarnesyl acetone	5.0
2,4-dibromopentane	0.4
2,4-dimethyl-2,4-heptadiene	0.05
levomenol	1.0
E,E,E- α-springene	9.0
2-ethenyl-2,5-dimethyl-4-hexen-1-ol	8.0
5-bromo-n-pentanol-cyclohexyl ether	2.0
threo-2,3-dibromopentane	2.0
farnesol	4.0
lavandulol	1.0
1,1'-bicyclooctyl	1.0
geranylgeraniol	4.0
(E,E)-geranyl linalool	2.0
trans-2-methyl-2-(4-methyl-3-pentenyl)-cyclopropanecarboxaldehyde	4.0
Percentage Total	98.8

Table 1: Chemical composition of the fruit essential oil of e citriodora

Polyphenol Content (PC)

The PC of the essential oil was 175.84±0.00 µgmg-1 GAE. The sample had a high value for phenolic compound(s) when

compared with the previous study on the related species such as the leaf oil of E. globulus from Greece with 10.50 ± 0.30 mgg-1 GAE [36]. Moreover, literatures showed that the TPC for the commercial Eucalyptus leaf extract from Japan was 11.90 mgg-1 GAE [37,38]. This study indicates that there is a correlation between the TPC, antioxidant and therapeutic activities of the essential oil. Polyphenols are very useful phytochemicals in food and pharmaceutical industries due to their beneficial effects on human health [39,40].

Antioxidant Potential

DPPH Free Radical Scavenging and Antioxidant Activities. The essential oil scavenges the radicals using DPPH. in a concentration dependent manner with the percentage inhibitions as 71.95±0.00, 68.08±0.00 and 67.77±0.00 % respectively; while the DPPH IC50 and AAI values of the essential oil were 3.00 µgml-1 and 13.30, respectively (Figure 2), in comparison to ascorbic acid which gave IC50 value of 9.00 µgml-1. The antioxidant IC50 value of the fruit essential oil was lower than the leaf essential oil of E. globulus from Pakistan (IC50 = 15.27±1.77) [41]. The lower the IC50 value, the higher the antioxidant potential. The result obtained in this study was found to be five times more active than that of leaf essential oil of E. globulus. Likewise, the essential oil investigated in this study was more active than the extracts of different parts (stems > 10000, adult leaves 1536.30±40.50, fruits 441.10±12.70, immature flowers 1270.40 ± 33.40 of E. oleosa from south Tunisia with IC50: 1,536.30 mgl-1 [42]. The essential oil had a very strong AAI value of 13.30 (Table 2), indicating that the presence of terpenoids played an active roles in the antioxidant potential of the fruit essential oil.

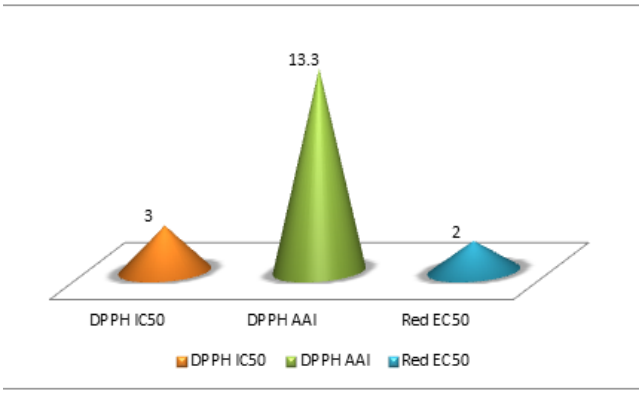
Reduction Antioxidant Potential

According to figure 2, the reduction antioxidant activity of the essential oil (EC50: 2.00 µgml-1) was 82% higher in reducing antioxidant potential than ascorbic acid (11.00 µgml-1). The oil was more active than the leaf essential oil of E. citriodora from South-East Asia with reduction value of 95.80 µM at the concentration of 50.00 µl [43]. The presence of terpenoids in the oil contributed to its higher reduction antioxidant effect since these compounds are known to form chelate metal ions [44-46]. The reducing ability assay is based on the principle that substances, which have reduction potential, react with potassium ferricyanide (K3Fe3+(CN)6) to form potassium ferrocyanide (K4Fe2+(CN)6) (1), which then reacts with ferric chloride to form a ferric-ferrous complex (2) that has an absorption maximum at 700 nm [47].

$$K_3Fe^{3+}(CN)_{6(aq)} + \text{Natural Phenolic Antioxidant}[red]_{(aq)} \rightarrow K_4Fe^{2+}(CN)_{6(aq)} + \text{Natural Phenolic Antioxidant}[ox]_{(aq)} \quad (1)$$

$$K_4Fe^{2+}(CN)_{6(aq)} + FeCl_{3(aq)} \rightarrow KFe^{3+}Fe^{2+}(CN)_{6(aq)} + 3KCl_{(aq)} \quad (2)\ [47].$$

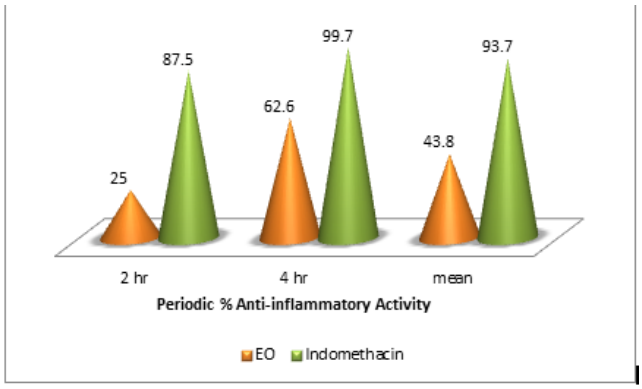
Figure 2: Antioxidant IC50 and AAI of the Fruit Essential Oil



Anti-Inflammatory Potential

The essential oil investigated has a moderate anti-inflammatory property of 43.80% at 1000 µg (Figure 3). The oil has a comparable activity with 2% isolates of E. globulus from India which caused inhibition of inflammation between 47.58-49.18% [48]. This study has shown that the oil possessed a moderate and dose dependent anti-inflammatory effect on paw oedema induced by carrageenan due to the presence of terpenoids in the essential oil.

Figure 3: Result of the Anti-inflammatory Activities



Analgesic Potential

The essential oil showed a moderate analgesic property by inhibition of both neurogenic (41.32%) and inflammatory pain (11.11%) induced by injection of formalin (Figure 4 and 5). This indicates the presence of analgesic phytochemical(s) in the essential oil. The fruit oil investigated in this study had a lower analgesic activity at the concentration used compared to the leaf essential oil of E. citriodora from Brazil at concentration of 0.10 mgkg-1 which caused inhibition of neurogenic pain by 57% [49]. This result showed that the oil was able to block both phases of the formalin response although, the effect was more pronounced in the first phase (neurogenic). The analgesic activity of the essential oil was in a correlation with its antioxidant activity.

Figure 4: Time of Licking by the Abino Rats

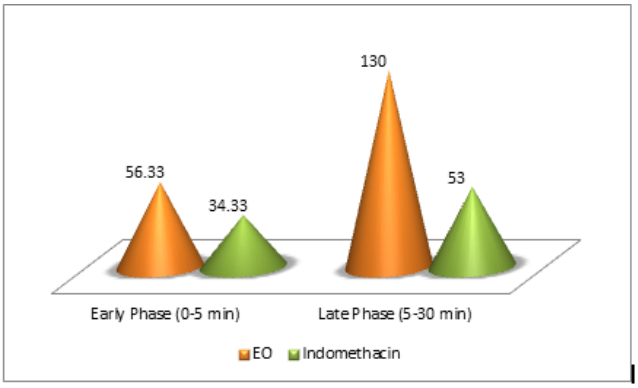
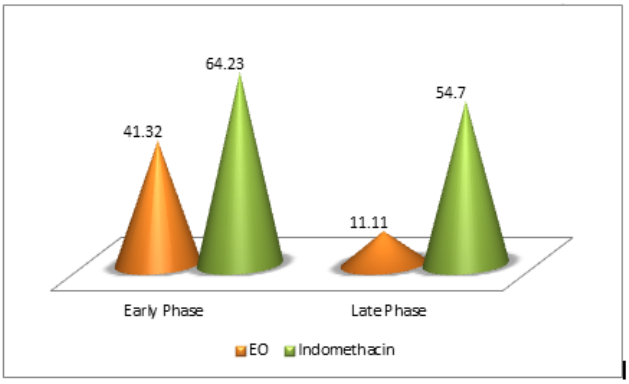


Figure 5: Percentage Biting Inhibition



Antibacterial Potentials

The antibacterial activities of the fruit essential oil were determined against five bacteria, including Gram-positive and Gram-negative bacteria (E. coli, K. pneumoniae, P. aeruginosa, S. agalactiae, and S. aureus) were shown in figure 6 and 7. The sample showed different potentials against the bacteria. The highest inhibitory effect of the oil was observed against E. coli (18 mm), P. aeruginosa (15.00 mm), S. aureus (15.00 mm), S. agalactiae (10.00 mm) and K. pneumoniae (13.00 mm) (Figure 6). The bacteria were found to be sensitive to Ofloxacin (OFL) and some are resistant to Cefuroxine (CRX) conventional antibiotics (Figure 7).

It was observed that some of the organisms were sensitive (9–14 mm) to the essential oil activity while some were very sensitive (15–19 mm) and ultrasensitive (>20 mm), respectively. The antibacterial properties of the oil were higher than that of essential oils of other Eucalyptus species such as leaf essential oil of E. globulus from Australia which showed no or very low ZI with on S. aureus, B. subtilis, L. monocytogenes, E. coli and S. typhi ranging between 2.20–10.10 mm, but resistant to S. typhi [41]. The high antibacterial activities of the essential oil was most likely due to the presence of terpenoids which have antimicrobial properties, particularly, 1,8-cineole [50].

Figure 6: Antibacterial Activity of the Essential Oil against Bacteria Isolates

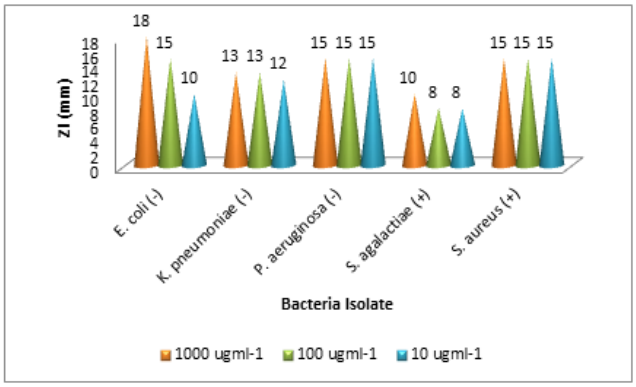
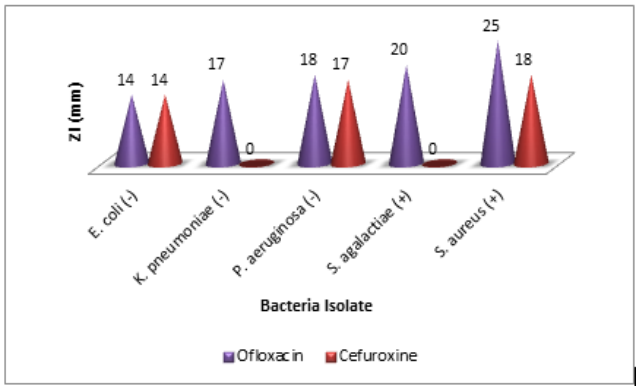


Figure 7: Antibacterial Activity of the Standard Antibiotic Discs against Bacteria Isolates



Conclusion

This study showed that the fruit essential oil of E. citriodora could be considered as natural antioxidant and antibiotic agent. The synergistic activity of the phytochemicals in the oil would be of benefit in health and industrial applications. The terpenoids in the oil could lower the risk of serious health disorders. It can also be a good flavouring and preservative agents in the food and pharmaceutical industries for the production of drugs that can be used to treatment of pathogenic and reactive oxygen species related diseases.

Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

References

1. Hyldgaard M, Mygind T, Meyer RL (2012) Essential oils in food preservation: mode of action, synergies, and interactions with food matrix components. Front Microbiol 3: 1-24.
2. Chouhan S, Sharma K, Guleria S (2017) Antimicrobial activity of some essential oils-present status and future perspectives. Medicines 4: 1-21.
3. Nazzaro F, Fratianni F, Coppola R, De-Feo V (2017) Essential oils and antifungal activity. Pharmaceuticals 10: 1-20.
4. Sharif ZIM, Mustapha FA, Jai J, Yusof NM, Zaki NAM, et al. (2017) Review on methods for preservation and natural preservatives for extending the food longevity. Chem Eng Res Bull 19: 145-153.

5. Blowman K, Magalhaes M, Lemos MFL, Cabral C, Pires IM, et al. (2018) Anticancer Properties of Essential Oils and Other Natural Products. Evidence-Based Complementary and Alternative Medicine 18: 1-13.

6. Burt S (2004) Essential oils: their antibacterial properties and potential applications in foods-a review. Int J Food Microbiol 94: 223-253.

7. Nieto G (2017) Biological Activities of Three Essential Oils of the Lamiaceae Family. Medicines 4: 1-10.

8. Dosoky NS, Setzer WN (2018) Biological Activities and Safety of Citrus spp Essential Oils. Int J Mol Sci 19: 1-23.

9. Ghazoul J (2005) Pollen and seed dispersal among dispersed plants. Biological Reviews 80: 413–443.

10. Luis A, Duarte AP, Pereira L, Domingues F (2017) Chemical Profiling and Evaluation of Antioxidant and Anti-Microbial Properties of Selected Commercial Essential Oils: A Comparative Study. Medicines 4: 1-16.

11. Stevanovic ZD, Bosnjak-Neumuller J, Pajic-Lijakovic I, Raj J, Vasiljevi M, et al. (2018) Essential Oils as Feed Additives-Future Perspectives. Molecules 23: 1-20.

12. Jun YS, Kang P, Min SS, Lee JM, Kim HK, et al. (2013) Effect of Eucalyptus Oil Inhalation on Pain and Inflammatory Responses after Total Knee Replacement: A Randomized Clinical Trial. Evidence-Based Complementary and Alternative Medicine 13: 1-8.

13. Ali B, Al-Wabel NA, Shams S, Ahamad A, Khan SA, et al. (2015) Essential oils used in aromatherapy: A systemic review. Asian Pacific Journal of Tropical Biomedicine 5: 601–611.

14. Dagli N, Dagli R, Mahmoud RS, Baroudi K (2015) Essential oils, their therapeutic properties, and implication in dentistry: A review. J Int Soc Prev Community Dent 5: 335-340.

15. Sharifi-Rad J, Sureda A, Tenore GC, Daglia M, Sharifi-Rad M, et al. (2017) Biological Activities of Essential Oils: From Plant Chemoecology to Traditional Healing Systems. Molecules 22: 1-55.

16. Bassole IHN, Juliani HR (2012) Essential Oils in Combination and Their Antimicrobial Properties. Molecules 17: 3989-4006.

17. Zengin H, Baysal AH (2014) Antibacterial and Antioxidant Activity of Essential Oil Terpenes against Pathogenic and Spoilage-Forming Bacteria and Cell Structure-Activity Relationships Evaluated by SEM Microscopy. Molecules 19: 17773-17798.

18. Dhifi W, Bellili S, Jazi S, Bahloul N, Mnif W (2016) Essential Oils' Chemical Characterization and Investigation of Some Biological Activities: A Critical Review. Medicines 3: 1-16.

19. Christaki E, Bonos E, Giannenas I, Florou-Paneri P (2012) Aromatic Plants as a Source of Bioactive Compounds. Agriculture 2: 228-243.

20. Sarmento-Neto JF, do-Nascimento LG, Felipe CFB, de-Sousa DP (2016) Analgesic Potential of Essential Oils. Molecules 21: 1-29.

21. Aslan CE, Liang CT, Galindo B, Hill K, Topete W, ey al. (2016). The Role of Honey Bees as Pollinators in Natural Areas. Natural Areas 36: 478- 488.

22. Ejigu K, Tesfa A, Dagnew D (2017) Identification and Characterization of Honeybee Flora in Western Amhara Region, Ethiopia. Journal of Natural Sciences Research 7: 36-42.

23. Brooker MIH, Kleinig DA (2006) Field Guide to Eucalyptus.1. South-eastern Australia, 3rd edition. Bloomings, Melbourne.

24. Orwa C, Mutua A, Kindt R, Jamnadass R, Anthony S, et al. (2009) Agroforestree Database: a tree reference and selection guide version 4: 10.

25. Rahul SJ, Falaq N, Barkha S, Safiya K, Mohammad A, et al. (2012) Effect of Eucalyptus citriodora extract on hsp70 expression and tissue damage in the third instar larvae of transgenic Drosophila melanogaster (hsp70-lacZ) Bg9. Phytopharmacology 3: 111-121.

26. European Pharmacopoeia Commission (2008) Sage leaf (Salvia officinalis). European Pharmacopoeia. 6 edition. Strasbourg, France: European Directorate Quality Medicine 2853.

27. Baba SB, Malik SA (2015) Determination of total phenolic and flavonoid content, antimicrobial and antioxidant activity of a root extract of Arisaema jacquemontii Blume. J Taibah Univ Sci 9: 449–454.

28. Takao LK, Imatomi M, Gualtieri SCJ (2015) Antioxidant activity and phenolic content of leaf infusions of Myrtaceae species from Cerrado (Brazilian Savanna). Braz J Biol 75: 948-952.

29. Sylvie DD, Anatole PC, Cabral BP, Veronique PB (2014) Comparison of in vitro antioxidant properties of extracts from three plants used for medical purpose in Cameroon: Acalypha racemosa, Garcinia lucida and Hymenocardia lyrata. Asian Pac. J Trop Biomed 4: 625-632.

30. OECD (2001) Organization for Economic cooperation and Development Guideline for Testing of Chemicals-Acute Oral Toxicity-Fixed Dose Procedure 1-14.

31. Cai C, Chen Y, Zhong S, Ji B, Wang J, et al. (2014). Anti-Inflammatory Activity of N-Butanol Extract from Ipomoea stolonifera In Vivo and In Vitro. PLoS ONE 9: 1-9.

32. Onifer SM, Reed WR, Sozio RS, Long OR (2015) Antinociceptive Effects of Spinal Manipulative Therapy on Nociceptive Behavior of Adult Rats during the Formalin Test. Evidence-Based Complementary and Alternative Medicine 15: 1-9.

33. Nkechukwu IM, Onyeka AL, Calistus DN, Ruth AA, Okechukwu EC, et al. (2014) Antimicrobial Activity of Selected Medicinal Plants of South-Eastern Nigeria on Pseudomonas species Expressing Extended Spectrum Beta Lactamase (ESBL). European Journal of Medicinal Plants 4: 1367-1377.

34. Costa V, Pinheiro PF, de-Queiroz VT, Rondelli VM, Marins AK, et al. (2015) Chemical Composition of Essential Oil from Eucalyptus citriodora Leaves and Insecticidal Activity Against Myzus persicae and Frankliniella schultzei. Journal of Essential Oil Bearing Plants 18: 374-381.

35. El-Mageed AAA, Osman AK, Tawfik AQ, Mohammed HA (2011) Chemical Composition of the Essential Oils of four Eucalyptus Species (Myrtaceae) from Egypt. Phytochemistry 5: 115-122.

36. Proestos C, Lytoudi K, Mavromelanidou OK, Zoumpoulakis, Sinanoglou, et al. (2013) Antioxidant Capacity of Selected Plant Extracts and Their Essential Oils. Antioxidants 2: 11-22.

37. Hassine DB, Abderrabba M, Yvon Y, Lebrihi A, Mathieu F, et al. (2012). Chemical Composition and in Vitro Evaluation of the Antioxidant and Antimicrobial Activities of Eucalyptus gillii Essential Oil and Extracts. Molecules 17: 9540-9558.

38. Ololade ZS, Olawore NO (2018) Phytochemical Characterization, Antioxidant, Anti-Inflammatory, Anti-Nociceptive and Antimicrobial Properties of the Seed Essential Oil of Eucalyptus tereticornis. Greener Journal of Biological Sciences 8: 1-9.

39. Lin D, Xiao M, Zhao J, Li Z, Xing B, et al.(2016) An Overview of Plant Phenolic Compounds and Their Importance in Human Nutrition and Management of Type 2 Diabetes. Molecules 21: 1-19.

40. Baiao DD, De-Freitas CS, Gomes LP, Da-Silva D, Correa, et al. (2017) Polyphenols from Root, Tubercles and Grains Cropped in Brazil: Chemical and Nutritional Characterization and Their Effects on Human Health and Diseases. Nutrients 9: 1-29.

41. Dezsi S, Badarau AS, Bischin C, Vodnar DC, Silaghi-Dumitrescu R, et al. (2015) Antimicrobial and antioxidant activities and phenolic profile of eucalyptus globulus labill and Corymbia ficifolia (F. Muell.) K.D. Hill & L.A.S. Johnson Leaves. Molecules 20: 4720-4734.

42. Naceur H, Romdhane M, Lebrihi A, Mathieu F, Couderc F, et al. (2010). Eucalyptus (gracilis, oleosa, salubris, and salmonophloia) essential oils: Their chemical composition and antioxidant and antimicrobial activities. Journal of Medicinal Food 13: 1005-1012.

43. Shahwar D, Raza MA, Bukhari S, Bukhari G (2012) Ferric reducing antioxidant power of essential oils extracted from eucalyptus and curcuma species. Asian Pac J Trop Biomed S1633-S1636.

44. Moukette BM, Pieme AC, Biapa PCN, Njimou JR, Stoller M, et al. (2015). In Vitro Ion Chelating, Antioxidative Mechanism of Extracts from Fruits and Barks of Tetrapleura tetraptera and Their Protective Effects against Fenton Mediated Toxicity of Metal Ions on Liver Homogenates. Evidence-Based Complementary and Alternative Medicine 15: 1-14.

45. Ololade ZS, Olawore NO (2017) Characterization of essential oil from the seed of eucalyptus cloeziana and evaluation of its modes of medicinal potentials. Edorium Journal of Infectious Diseases 3: 1-8.

46. Sivaramakrishnan T, Swain S, Saravanan K, Kiruba SR, Roy SD, et al. (2017). In Vitro Antioxidant and Free Radical Scavenging Activity and Chemometric Approach to Reveal Their Variability in Green Macroalgae from South Andaman Coast of India. Turkish Journal of Fisheries and Aquatic Sciences 17: 639-648.

47. Adjimani JP, Asare P (2015) Antioxidant and free radical scavenging activity of iron chelators. Toxicol Rep 2: 721-728.

48. Gomase, PV, Shire PS, Nazim S (2011) Phytochemical Evaluation and Anti-Inflammatory Studies of Polyherbal Formulation. IJPI'S Journal of Pharmacognosy and Herbal Formulations 1: 63-72.

49. Silva J, Abebe W, Sousa SM, Duarte VG, Machado MIL, et al.(2003) Analgesic and anti-inflammatory effects of essential oils of Eucalyptus. J Ethnopharmacol 89: 277-283.

50. Hendry ER, Worthington T, Conway BR, Lambert PA (2009) Antimicrobial efficacy of eucalyptus oil and 1,8-cineole alone and in combination with chlorhexidine digluconate against microorganisms grown in planktonic and biofilm cultures. J Antimicrob Chemother 64: 1219 –1225.