

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

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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- $\alpha$ -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  $175.84 \pm 0.00$   $\mu\text{gmg}^{-1}$  GAE. The DPPH IC<sub>50</sub> and AAI values of the essential oil were 3.00  $\mu\text{gml}^{-1}$  and 13.30, respectively. The essential oil was capable of scavenging free radicals in a range of 67.77-71.95%, while the FRAP EC<sub>50</sub> value was 2.00  $\mu\text{gml}^{-1}$ . 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

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.

## 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

## 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-Ciocalteu method previously used by Baba and Malik [27]. Gallic acid was used as a reference; the index of PC was expressed as µmg-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.

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

Where:  $A_{\text{blank}}$  is the absorbance of blank solution and  $A_{\text{eo}}$  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 antioxidant activity index (AAI) was calculated using Scherer and Godoy's criteria:

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

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:

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

Where:  $H_o$  was the average inflammation of the control group at a given time 0.  $H_t$  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:

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

Where:  $J_o$  represents the vehicle treated control group value for each phase while  $J_t$  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 °C for 16-24 h before visual assessment of the zones of inhibition (ZI). Ofloxacin (OFL) and Cefuroxime (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-  $\alpha$ -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%),  $\beta$ -citronellol (14.88%) and  $\delta$ -cadinene (6.32%) [34] while the leaves essential oil of *E. citriodora* from Egypt 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
$\alpha$ -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- $\alpha$ -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 $\pm$ 0.00  $\mu$ mg-1 GAE. The sample had a high value for phenolic compound(s) when

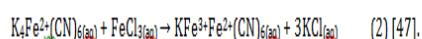
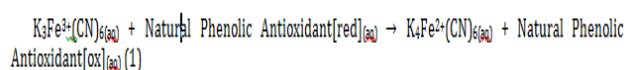
compared with the previous study on the related species such as the leaf oil of *E. globulus* from Greece with  $10.50 \pm 0.30$  mgg-1 GAE [36]. Moreover, literatures showed that the TPC for the commercial Eucalyptus leaf extract from Japan was 11.90 mgg-1 GAE [37,38]. This study indicates that there 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

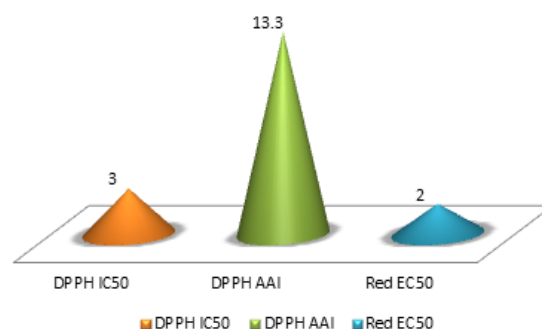
**DPPH Free Radical Scavenging and Antioxidant Activities.** The essential oil scavenges the radicals using DPPH. in a concentration dependent manner with the percentage inhibitions as  $71.95 \pm 0.00$ ,  $68.08 \pm 0.00$  and  $67.77 \pm 0.00$  % respectively; while the DPPH IC50 and AAI values of the essential oil were  $3.00 \mu\text{gml}^{-1}$  and 13.30, respectively (Figure 2), in comparison to ascorbic acid which gave IC50 value of  $9.00 \mu\text{gml}^{-1}$ . The antioxidant IC50 value of the fruit essential oil was lower than the leaf essential oil of *E. globulus* from Pakistan (IC50 =  $15.27 \pm 1.77$ ) [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  $1536.30 \pm 40.50$ , fruits  $441.10 \pm 12.70$ , immature flowers  $1270.40 \pm 33.40$  of *E. oleosa* from south Tunisia with IC50:  $1,536.30 \text{ mg}^{-1}$  [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:  $2.00 \mu\text{gml}^{-1}$ ) was 82% higher in reducing antioxidant potential than ascorbic acid ( $11.00 \mu\text{gml}^{-1}$ ). The oil was more active than the leaf essential oil of *E. citriodora* from South-East Asia with reduction value of  $95.80 \mu\text{M}$  at the concentration of  $50.00 \mu\text{l}$  [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 ( $\text{K}_3\text{Fe}^{3+}(\text{CN})_6$ ) to form potassium ferrocyanide ( $\text{K}_4\text{Fe}^{2+}(\text{CN})_6$ ) (1), which then reacts with ferric chloride to form a ferric-ferrous complex (2) that has an absorption maximum at 700 nm [47].



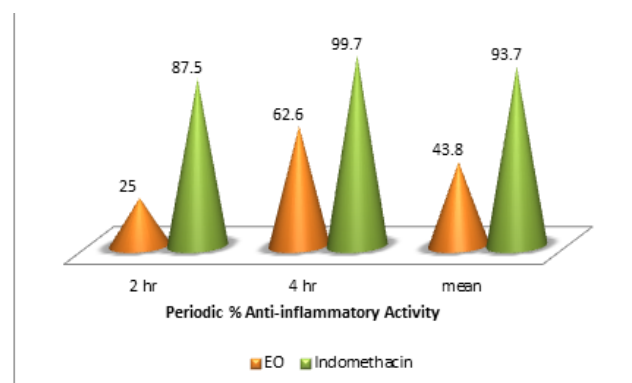
**Figure 2:** Antioxidant IC50 and AAI of the Fruit Essential Oil



## Anti-Inflammatory Potential

The essential oil investigated has a moderate anti-inflammatory property of 43.80% at  $1000 \mu\text{g}$  (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.

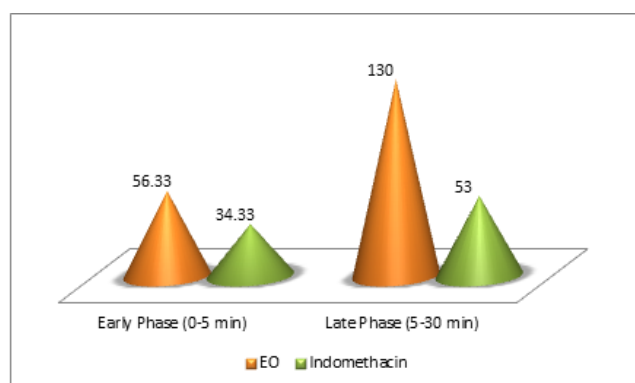
**Figure 3:** Result of the Anti-inflammatory Activities



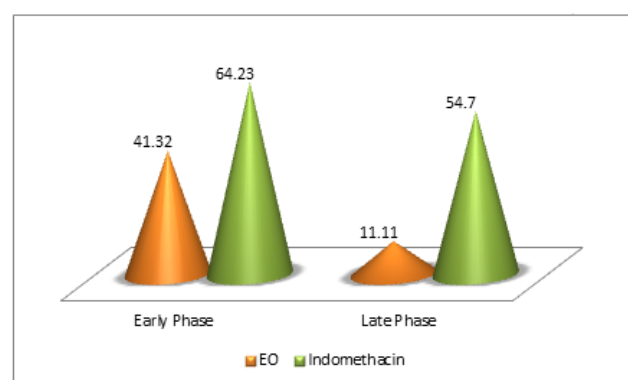
## Analgesic Potential

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  $0.10 \text{ mgkg}^{-1}$  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

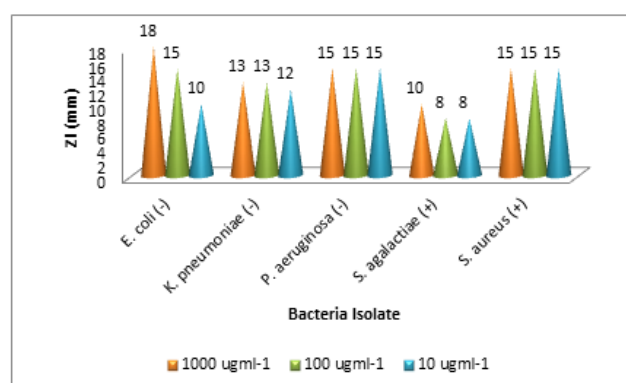


## Antibacterial Potentials

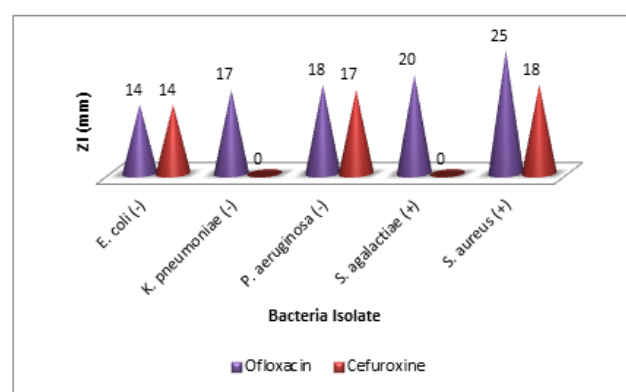
The antibacterial activities of the fruit essential oil were determined against five bacteria, including Gram-positive and Gram-negative bacteria (*E. coli*, *K. pneumoniae*, *P. aeruginosa*, *S. agalactiae*, and *S. aureus*) were shown in figure 6 and 7. The sample showed different potentials against 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 Cefuroxime (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.

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