

# Characterization of essential oil from the seed of *Eucalyptus cloeziana* and evaluation of its modes of medicinal potentials

Z. S. Ololade, N. O. Olawore

## ABSTRACT

**Aims:** Different parts of *Eucalyptus* plants are widely used in medicine for the prevention and treatment of diseases. This study examined the phytochemicals and medicinal properties of the seed essential oil of *E. cloeziana* from Nigeria. **Methods:** The essential oil was extracted by hydrodistillation and analyzed using gas chromatography (GC) and gas chromatography-mass spectrometry (GC-MS). TPC, antioxidant, anti-inflammatory, antinociceptive and antimicrobial activities were measured by Folin-Ciocalteu's, DPPH, FRAP, carrageenan, formalin and agar-well diffusion methods respectively. **Results:** The GC and GC-MS analyses revealed the presence of 33 phytochemicals making up 99.7% of the total percentage composition of the essential oil. The most abundant component was 1, 8-cineole (23.8%). The TPC was  $199.18 \pm 0.0 \mu\text{mg}^{-1}$  gallic acid equivalents. The antioxidant  $\text{IC}_{50}$  value of the essential oil was  $2.8 \mu\text{gml}^{-1}$  and it was capable of scavenging free radicals in a range of 62.6–70.3%, the seed essential showed strong antioxidant activity with AAI higher than

that of ascorbic acid. The reduction antioxidant potential of the essential oil was  $\text{EC}_{50}$ :  $1.5 \mu\text{gml}^{-1}$ . The essential oil gave high anti-inflammatory with value of 68.8% and antinociceptive properties by inhibition in both neurogenic (81.3%) and inflammatory pain (80.3%). The essential oil was active against all the tested bacteria with high zones of inhibition (8.0–20.0 mm). **Conclusion:** Therefore, the seed essential oil of *E. cloeziana* is a sustainable and promising source of natural product with good therapeutic properties.

**Keywords:** *Eucalyptus cloeziana*, Medicinal properties, Phytochemicals, Seed essential oil

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## INTRODUCTION

Natural products have, for decades, been contributing to the development of modern therapeutic drugs. The focus on the therapeutic properties of secondary metabolites have increased due to their great benefits for human being and animals; many phytochemicals are now receiving particular attention from industries because they are important sources of a wide variety of commercially useful base products [1]. Essential oils are

important natural products used as raw materials in many fields; one of such is aromatherapy [2]. Aromatherapy is a system in which the healing effects are due to the phytochemicals in essential oils. Thus, essential oils are used to prevent and treat diseases, they are very useful in several healing systems such as treatment of orthopedic (bone, joint, and soft tissue) infections. They also have diaphoretic, disinfectant, antimalarial, antiseptic, analgesic, antipyretic, anti-inflammatory, antimicrobial, expectorant and antioxidant properties. Application of essential oils by either vapor inhalation or oral route provides benefit for both purulent and non-purulent respiratory problems. They are used as remedies for the symptoms of respiratory tract disorders such as cold, flue, pharyngitis, bronchitis, sinusitis, asthma and other respiratory diseases. Essential oils can also be used as quick and effective mood enhancers, for increasing energy, alertness or reducing stress and promoting relaxation [3–7]. Essential oils are good natural preservatives and flavoring agent in food and drug because they improve their shelf-life, better than the synthetic preservatives [8, 9].

*Eucalyptus cloeziana* F. Muell, known as Gympie messmate tree, is one of the numerous species of the genus *Eucalyptus*. Essential oils from *Eucalyptus* plants are known for their medicinal properties. They are used in medicine to treat pains and respiratory infections [10–12]. *Eucalyptus* oil is included in products used as sealers and solvents for root canal fillings in treating dental problems [13, 14]. They are known as potential natural drugs with their potential applications for the treatment of diseases [15, 16]. *Eucalyptus* essential oils have been classified as non-toxic and safe for human being and animals [17–21]. Previous studies on *E. cloeziana* were mainly focused on the chemical composition of the essential oil extracted from the leaves [22, 23]. To the best of our knowledge, there is paucity of information on the phytochemical, total phenolic content, free radical scavenging, antioxidant, anti-inflammatory, antinociceptive and antimicrobial potentials of *E. cloeziana* so far. Therefore, the present study was aimed at looking into the characterization of essential oil from the seed of *E. cloeziana* and evaluation of its modes of medicinal properties.

## MATERIALS AND METHODS

### Plant materials and isolation of the essential oil

The seeds of the plant were collected from Afforestation Research Station Kaduna, Nigeria and it was authenticated by Mr Sylvester Boye of the same institution as *Eucalyptus cloeziana* F. Muell. Fresh seeds (100 g) were pulverized and the essential oil was obtained by hydrodistillation using all-glass Clevenger-type apparatus [24]. The essential oil was then stored in vial at 5°C temperature to prevent evaporation.

### Gas chromatography and gas chromatography-mass spectrometry analyses

The seed essential oil was analyzed using Shimadzu GC-MS-QP2010 Plus (Japan). The separations were carried out using a Restek Rtx-5MS fused silica capillary column (5%-diphenyl-95%-dimethylpolysiloxane) of 30×0.25 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 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 the literature [25].

### Determination of total phenolic content

Total phenolic content (TPC) in the seed essential oil was determined using Folin-Ciocalteu method according. Gallic acid was used as a standard phenolic compound. 1 ml of Folin-Ciocalteu reagent was added to 1 ml of the sample solution, then the entire solution was diluted with 46 ml distilled water and the content was mixed thoroughly. After 3 min, 3 ml of 2% Na<sub>2</sub>CO<sub>3</sub> was added and then the mixture was allowed to stand in dark for 2 h with intermittent shaking. The absorbance was measured at 760 nm. The index of TPC in the essential oil determined as µgmg<sup>-1</sup> of gallic acid equivalent (GAE) using an equation obtained from the calibration curve of gallic acid [26].

### Determination of free radical scavenging and antioxidant activities

**In vitro DPPH assay:** The free radical scavenging and antioxidant activities of the seed essential oil against the stable free radical DPPH were measured. Briefly, three different concentrations (1000, 100 and 10 µgml<sup>-1</sup>) of the essential oil in methanol were incubated with a methanolic solution of DPPH. After 30 min. of incubation at room temperature in the dark, the absorbance at 517 nm was measured with ultraviolet-visible spectrophotometer. 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 DPPH solution color into yellow by the tested sample against the control (DPPH solution only). The IC<sub>50</sub> of DPPH assay

represents the concentration of the tested sample needed to reduce the DPPH by 50%.

$$I\% = [(A_{\text{blank}} - A_{\text{eo}}) / A_{\text{blank}}] \times 100$$

where:  $A_{\text{blank}}$  is the absorbance of blank solution and  $A_{\text{eo}}$  is the absorbance of the essential oil. The dose-response curve was plotted and IC<sub>50</sub> value for the essential oil and the standard were calculated [25].

## Antioxidant activity index

The antioxidant activity index (AAI) was calculated as:

$$AAI = [\text{DPPH initial concentration } (\mu\text{gml}^{-1}) / \text{IC}_{50} (\mu\text{gml}^{-1})]$$

Antioxidant activity index was classified as weak, when AAI ranged < 0.5, moderate, when AAI ranged between 0.5–1.0. It is strong, when AAI ranged between 1.0–2.0, and very strong, when AAI > 2.0 [27].

**In vitro FRAP assay:** The sample at different concentrations in distilled water were mixed with phosphate buffer (2.5 ml, 0.2 M, pH 6.6) and potassium ferricyanide [ $\text{K}_3\text{Fe}(\text{CN})_6$ ] (2.5 ml, 1%) and incubated at 50°C for 20 min. Then, 2.5 ml trichloroacetic acid (10%) and 0.5 ml of  $\text{FeCl}_3$  (0.1%) were added to the reaction mixture. The increases in the absorbance were spectrophotometrically measured at 700 nm as an indication of reducing capacity. The activity of ascorbic acid was used as a reference drug over the same concentrations. The assay was carried in triplicate and the results were expressed as mean  $\pm$  standard deviation. Effective concentration at 50% (EC<sub>50</sub>) of FRAP value is the sample concentration required to reduce  $\text{Fe}^{3+}$  to  $\text{Fe}^{2+}$  [28].

## Experimental animals

Healthy albino rats (200 $\pm$ 30g) were used for this study. All experiments were carried out in strict compliance with the principle of laboratory animal care [29].

## Carrageenan-induced anti-inflammatory assay

Anti-inflammatory activity was assessed on the basis of inhibition of paw edema induced by the injection of 0.1 ml of 1% carrageenan into the subplantar region of the right hind paw of the rat. Three groups of five animals each were used. Carrageenan is known to result in at least neutrophil linked edematous inflammation. The essential oil solution was subjected at a dose of 0.1 ml each of 1000  $\mu\text{gkg}^{-1}$ , postoperatively were administered orally 30 min before carrageenan injection. Indomethacin 1000  $\mu\text{gml}^{-1}$  was used as reference drug. Control group received the vehicle only (10 mlkg<sup>-1</sup>). Rat paw volume was measured immediately (0 hour) before the injection of the “irritant” substance and at regular selected time intervals (2 and 4 hours) after injection of the essential oil solution (1000  $\mu\text{gml}^{-1}$ ) or equivalent volume of vehicle, using a digital vernier caliper. Results were expressed as the increase in paw volume (mm) calculated after subtraction of basal

paw volume prior to carrageenan irritant injection [30]. The inhibition percentage of the inflammatory reaction was determined for each rat by comparing each group with controls and calculated by the formula below:

$$I\% = 1 - [dt/dc] \times 100$$

where I% = percentage inhibition and  $dt$  is the difference in paw volume in the drug-treated group and  $dc$  is the difference in paw volume in control group [31].

## In vivo Antinociceptive Potential

Formalin test was used to determine the antinociceptive property of the essential oil [32]. Rats ( $n = 5$  per group) were treated respectively with 1,000  $\mu\text{gkg}^{-1}$  of the essential oil solution, 1000  $\mu\text{gkg}^{-1}$  of indomethacin. 30 minutes later, the pain was induced by injecting 0.05 ml of 2.5% v/v formalin (formaldehyde) in distilled water into the sub-plantar right hind paw of rat, immediately placed in a transparent plastic cage separately. The licking time and frequency of the injected paw were recorded for 30 min. The amount of time spent licking the injected paw was indicative of pain. The number of lickings from 0–5 min (first phase) and 15–30 min (second phase) of post-injection time was recorded. These phases represented neurogenic and inflammatory pain responses, respectively. The test was performed at room temperature and strict actions were taken to exclude environmental disturbances (high temperature, noise and excessive movement) that might interfere with the animal’s response. The percentage inhibition (I) (analgesic activity %) was calculated by:

$$I\% = [A_o - A_t / A_o] \times 100$$

Where  $A_o$  = Average number of stretching of control per group and  $A_t$  = Average number of stretching of test per group [31].

## In vitro antimicrobial activities

The antibacterial activities of the seed essential oil were evaluated by agar-well diffusion method against multi-drug resistance gram-positive bacteria (*Streptococcus agalactiae* and *Staphylococcus aureus*) and gram-negative bacteria (*Escherichia coli*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa* and *Salmonella typhimurium*). Tested bacteria were cultured on Mueller Hinton Broth media plates at 37°C for 24 h. The turbidity of the bacteria were adjusted to match 0.5 McFarland standard. The bacteria suspensions were used to inundate sterile plates containing nutrient agar. Wells of 6 mm diameter each were bored in the plates with the help of a cork-borer. Different concentration of the essential oil solutions in DMSO were incubated at 37°C for 24 h. The antibacterial activities of the essential oil were compared with synthetic antibiotics i.e., gentamicin (GEN) and cloxicillin (CXC). Antibacterial potentials of different concentrations of the essential oil solutions were evaluated by measuring the clear zones of growth inhibition against the test organisms [33].

## RESULTS

The seed essential oil of *E. cloeziana* analyzed showed that 33 components, representing 99.7% of the seed essential oil were identified (Table 1). The major component of seed essential oil is 1, 8-cineole (23.8%). The other main compounds identified were cis-oleic acid (18.0%),  $\alpha$ -pinene (14.5%) and palmitic acid (9.0%).

Table 1: Chemical composition of the seed essential oil of *E. cloeziana*

Compound	Retention Index	Percentage Composition
$\beta$ -pinene	943	1.9
$\alpha$ -pinene	948	14.5
$\gamma$ -terpinene	998	0.5
<i>o</i> -cymene	1042	1.0
Sulfurous acid, decyl-2-pentyl ester	1057	1.0
1,8-cineole	1059	23.8
(+)-citronellal	1125	0.9
1-terpinen-4-ol	1137	0.3
$\beta$ -citronellol	1182	0.9
L-isopulegol	1196	0.3
$\alpha$ -cubebene	1344	1.4
Methyleugenol	1361	0.5
(+)-aromadendrene	1386	1.0
10s,11s-himachala-3(12),4-diene	1389	1.7
<i>cis</i> -caryophyllene	1490	0.8
D-valencene	1495	2.0
[s-E,E]-germacrene D	1515	0.6
$\alpha$ -curcumene	1524	0.3
<i>cis</i> -1-ethylideneoctahydro-7 $\alpha$ -methyl-1H-indene	1544	1.7
L-globulol	1578	1.7
2-methylene-4,8,8-trimethyl-4-vinylbicyclo[5.2.0]nonane	1619	1.0
1-butylheptylbenzene	1636	2.0
$\alpha$ -eudesmol	1650	2.5
n-heptadecane	1700	1.0
1-hexylheptylbenzene	1730	0.9
1-pentylheptylbenzene	1731	1.0
1-butylloctylbenzene	1736	1.0
1-propylonylbenzene	1747	1.0
1-butylonylbenzene	1836	0.5
1,3-bis-(2-cyclopropyl,2-methylcyclopropyl)-but-2-en-1-one	1893	2.0
palmitic acid	1955	9.0
n-heneicosane	2100	3.0
<i>cis</i> -oleic acid	2175	18.0
Percentage total		99.7

## Total phenolic content

TPC analysis revealed the presence of high quantity phenolic compounds in the seed essential oil. This was found to be  $199.18 \pm 0.00 \mu\text{gmg}^{-1}$  gallic acid equivalents.

## In vitro free radical scavenging and antioxidant potentials

The essential oil was able to inhibit the formation of DPPH radicals in a concentration dependent manner. The percentage inhibitions of the essential oil at various concentrations (1000, 100 and  $10 \mu\text{gml}^{-1}$ ) were  $70.25 \pm 0.10$ ,  $68.42 \pm 0.01$  and  $62.60 \pm 0.00\%$  respectively; while the IC<sub>50</sub> value was found to be  $2.80 \mu\text{gml}^{-1}$  in comparison to ascorbic acid which gave  $96 \pm 0.00$ ,  $69 \pm 0.00$  and  $54 \pm 0.00$  as the percentage inhibitions with IC<sub>50</sub> value of  $9.0 \mu\text{gml}^{-1}$ . Table 2 the seed essential oil of *E. cloeziana* showed very strong AAI > 2, while ascorbic acid also showed a strong AAI > 2 [27].

Table 2: IC<sub>50</sub> and AAI of the antioxidant properties of the seed essential oil of *E. cloeziana*

Essential Oil and Reference Drug	DPPH IC <sub>50</sub> $\mu\text{gml}^{-1}$	AAI
<i>E. cloeziana</i>	2.8	14.3
Ascorbic acid	9.0	4.4

Data are presented as the mean value  $\pm$  S.D. of triplicate

## In vitro Reduction Antioxidant Potential

Reduction antioxidant potential of the seed essential oil of *E. cloeziana* (EC<sub>50</sub>:  $1.5 \mu\text{gml}^{-1}$ ) was seven times higher than ascorbic acid ( $11.0 \mu\text{gml}^{-1}$ ) (Table 3).

Table 3: EC<sub>50</sub> of the FRAP antioxidant properties of the seed essential oil of *E. cloeziana*

Essential Oil and Reference Drug	EC <sub>50</sub> $\mu\text{gml}^{-1}$
<i>E. cloeziana</i>	1.5
Ascorbic acid	11.0

Data are presented as the mean value  $\pm$  S.D. of triplicate

## Anti-Inflammatory Potential

The seed essential oil of *E. cloeziana* investigated has a very high percentage anti-inflammatory value of 68.8% at 1000  $\mu\text{g}$ , this showed that it has comparative properties as indomethacin (93.7%) (Table 4).

Table 4: In vivo anti-inflammatory activities of the seed essential oil of *E. cloeziana*

Essential Oil and Reference Drug	% I (2 Hrs)	% I (4 Hrs)	Mean % I
<i>E. cloeziana</i>	75.0	87.5	68.8
Indomethacin	87.5	99.7	93.7

Data are presented as the mean value  $\pm$  S.D. of triplicate

## Antinociceptive potential

The seed essential oil of *E. cloeziana* showed a very active antinociceptive properties by inhibition in both neurogenic (81.25%) and inflammatory pain (80.34%) induced by intraplantar injection of formalin (Table 5).

## Antibacterial potentials

The antimicrobial activities of the seed essential oil of *E. cloeziana* against *E. coli*, *K. pneumonia*, *P. aeruginosa*, *S. typhimurium*, *S. aureus* and *S. agalactiae* were given in Table 6. The essential oil showed variable activities against tested bacteria. The essential oil was effective on all the bacteria tested. The highest inhibitory effect of the seed essential oil of *E. cloeziana* was observed against *S. aureus* (20 mm), *E. coli* (19 mm), *K. pneumonia* (16 mm), *P. aeruginosa* (15 mm), *S. agalactiae* (10 mm) but resistant to *S. typhimurium*. The tested bacteria were found to be resistant to cloxicillin (CXC) while some were sensitive to gentamicin (GEN) synthetic antibiotics.

## DISCUSSION

This study examined the phytochemicals, total phenolic content, antioxidant, anti-inflammatory, antinociceptive and antimicrobial properties of the seed essential oil of *E. cloeziana* from Nigeria. The seed

Table 5: *In vivo* antinociceptive activities of the seed essential oil of *E. cloeziana*

Seed Essential Oil and Reference Drug	Time of Licking and Biting Percentage Inhibition			
	Early Phase (0–5) min.	% Inhibition	Late Phase (5–30) min.	% Inhibition
<i>E. cloeziana</i>	18.0±0.6	81.3	75.0±8.9	80.3
Indomethacin	34.3±2.1	64.2	53.0±2.1	54.7

Data are presented as the mean value±S.D. of triplicate

Table 6: Zones of Inhibition (mm) showing the antimicrobial properties of the seed essential oil of *E. cloeziana*

Conc. (µgml <sup>-1</sup> ) Organisms	Seed Essential Oil			GEN	CXC
	1000	100	10	10 µg	5 µg
<i>E. coli</i>	19	19	10	17	-
<i>K. pneumonia</i>	16	13	-	15	-
<i>P. aeruginosa</i>	15	15	12	11	-
<i>S. agalactiae</i>	10	10	-	-	-
<i>S. aureus</i>	20	08	-	-	-
<i>S. typhimurium</i>	-	-	-	18	-

Key note: Resistant (--), not sensitive (< 8 mm), sensitive (8–14 mm), very sensitive (15–19 mm) and ultrasensitive (>20 mm)

essential oil of *E. cloeziana* contained medicinally active phytochemicals (Table 1). Previous studies on the leaf oil of *E. cloeziana* from Nigeria showed that the composition was dominated by  $\alpha$ -pinene (46.6%) and 1,8-cineole (15.4%) [22]. Moreover, the leaf essential oil from Brazil gave  $\alpha$ -pinene (74.9%) as their main component [23]. It is worthy to note that the percentage of 1,8-cineole is higher in the seed essential oil while the percentage of  $\alpha$ -pinene is low in the seed essential of *E. cloeziana* compared to the leaf essential oil of this plant [22, 23].

The essential oil gave a higher TPC, when compared with the previous study on the related species such as the leaf essential oil of *E. globulus* from Greece with 10.5±0.3 mgg<sup>-1</sup> gallic acid equivalents which was found to contain a relatively low concentration of phenolic compound compared with the seed essential oil of *E. cloeziana* investigated in this study [34]. This report indicates that total phenolic content is directly proportional to antioxidant and pharmacological properties of the seeds of the plant. Phenolic compounds have aroused considerable interest recently because of their potential beneficial effects on human health [35].

The DPPH radical scavenging capacity of the seed essential oil of *E. cloeziana* was higher than that of ascorbic acid [25]. The free radical scavenging and antioxidant properties of the essential oil were found to be three times more active than the synthetic antioxidant as given in Table 2. As the IC<sub>50</sub> concentration and the antioxidant capacity have inversely proportional values, *E. cloeziana* was established to have the high antioxidant capacity. Moreover, the seed essential oil of *E. cloeziana* inhibited the DPPH free radicals than extracts of other related species such as *E. oleosa var. obtuse* (Iran) with the following IC<sub>50</sub>: polar (H<sub>2</sub>O) extract from leaves IC<sub>50</sub>: 39.8 µgml<sup>-1</sup>, non-polar (chloroform) extract from leaves IC<sub>50</sub>: 217.8 µgml<sup>-1</sup>, polar (H<sub>2</sub>O) extract from flowers IC<sub>50</sub>: 18.2 µgml<sup>-1</sup> and non-polar (chloroform) extract from flowers IC<sub>50</sub>: 264.2 µgml<sup>-1</sup> [36]. The antioxidant activity has been related to the number and position of free hydroxyl groups in terpenoids and phenolic compounds, which could be a result of their hydrogen donating ability. The reduction in the number of DPPH molecules can be correlated with the number of available hydroxyl groups [37]. The seed essential oil has the AAI value of 14.3 and could therefore be classified as a very strong antioxidant substance.

The seed essential oil of *E. cloeziana* had seven times higher metal reducing ion potential than ascorbic acid. The seed essential oil investigated were more effective than the leaf essential oil of *E. sideroxylon* which FRAP antioxidant potentials as 130.5 µM [38]. The presence of terpenoid and phenolic compounds in the seed essential oil of *E. cloeziana* are likely to contribute to its higher FRAP value than that of ascorbic acid since these compounds are known to chelate metal ions [39].

The anti-inflammatory activity of the seed essential oil was more effective than the leaf essential oil of *E. globulus* at concentration of 100 mgkg<sup>-1</sup> which caused inhibition of

inflammatory by 76% [40]. This study has shown that the seed essential oil of *E. cloeziana* investigated possessed a significant antiedematogenic effect on paw edema induced by carrageenan due to the presence of secondary metabolites in the seed essential oil. The nerve damage is caused by inflammation; Inflammation occurs when the body's own immune cells attack the nervous system [41]. Phenolic compounds have been suggested to be beneficial for the treatment of neurodegenerative diseases [42].

Moreover, the essential oil inhibited the two phases of the formalin response. This indicates the presence of analgesic phytochemical(s) in the seed essential oil. The antinociceptive activities of the seed essential oil investigated were more effective than the leaf essential oil of *E. globulus* at concentration of 100 mgkg<sup>-1</sup> which caused inhibition of neurogenic pain by 53% [40]. The formalin test has an advantage over other frequently used tests as it involves a biphasic response with an early and a late phase representing respectively neurogenic and inflammatory pain and agents can be screened for activities in these two phases models of pain. This is of interest considering that both phases are sensitive to centrally acting drugs, such as opioids [43]. However, the second phase is also sensitive to NSAIDs (non-steroidal, anti-inflammatory drugs) and corticosteroids [44].

The antibacterial properties of this essential oil were more active than that of leaf essential oils of other *Eucalyptus* species such as leaves essential oils of eight species of the *Eucalyptus* from Tunisia (*E. bicostata*, *E. cinerea*, *E. maidenii*, *E. odorata*, *E. sideroxylon*, *E. astringens*, *E. lahmarii* and *E. leucoxylon*) showed low inhibitions against *H. influenzae*, *K. pneumoniae*, *P. aeruginosa*, *S. aureus*, *S. agalactiae*, *S. pneumoniae* and *S. pyogenes* between 6.0–14.5 mm, which are very low compared to the antibacterial activities of the seed essential oil investigated in this study except for *E. maidenii* (*S. aureus* = 22.8 mm and *S. pyogenes* = 15.5 mm) *E. odorata* (*H. influenzae* = 19.2 mm, *S. aureus* = 27.4 mm, *S. agalactiae* = 19.4 mm, *S. pneumoniae* = 17.4 and *S. pyogenes* = 19.0 mm), *E. leucoxylon* (*S. aureus* = 16.4 mm), *E. bicostata* (*S. aureus* = 15.6 mm) and *E. astringens* (*S. aureus* = 15.5 mm) which showed better inhibitions against few bacteria [45].

## CONCLUSION

The seed essential oil of *Eucalyptus cloeziana* showed good therapeutic properties. The results of the total phenolic content, free radical scavenging, antioxidant, antiinflammatory, antinociceptive and antimicrobial properties of the part of the plant investigated in this study was thought to be basically due to the synergic effects of the phytochemical constituents in the seed essential oil. The present study suggested that the seed essential oil could be used for the development of naturally occurring antioxidants, anti-inflammatory, analgesic, antibiotics

and other forms of drugs. Further studies should be carried out on other medicinal properties of the seed essential oil.

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## Author Contributions

Ololade Z.S. – Substantial contributions to conception and design, Acquisition of data, Analysis and interpretation of data, Drafting the article, Revising it critically for important intellectual content, Final approval of the version to be published

Olawore N.O. – Substantial contributions to conception and design, Analysis and interpretation of data, Drafting the article, Revising it critically for important intellectual content, Final approval of the version to be published

## Guarantor

The corresponding author is the guarantor of submission.

## Conflict of Interest

Authors declare no conflict of interest.

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## REFERENCES

1. Pan SY, Zhou SF, Gao SH, et al. New perspectives on how to discover drugs from herbal medicines: CAM's outstanding contribution to modern therapeutics. *Evid Based Complement Alternat Med* 2013;2013:627375.
2. Prasad K, Moulekhi K, Bisht G. Chemical composition of the essential oil of *Pavetta indica* L. leaves. *Research Journal of Phytochemistry* 2011;5(1):66–9.
3. Hamid AA, Aiyelaagbe OO, Usman LA. Essential oils: Its medicinal and pharmacological uses. *International Journal of Current Research* 2011;33(2):86–98.
4. Shaaban HAE, El-Ghorab AH, Shibamoto T. Bioactivity of essential oils and their volatile aroma components: Review. *Journal of Essential Oil Research* 2012;24(2):203–12.
5. Andrade BF, Braga CP, Dos Santos KC, et al. Effect of inhaling *Cymbopogon martinii* essential oil and geraniol on serum biochemistry parameters and oxidative stress in rats. *Biochem Res Int* 2014;2014:493183.
6. Raj RA. A recent technology in drug discovery and development. *International Journal of Innovative Drug Discovery* 2012;2(1):22–39.
7. Bellik Y, Boukraâ L, Alzahrani HA, et al. Molecular mechanism underlying anti-inflammatory and anti-allergic activities of phytochemicals: An update. *Molecules* 2012 Dec 27;18(1):322–53.

8. Tongnuanchan P, Benjakul S. Essential oils: Extraction, bioactivities, and their uses for food preservation. *J Food Sci* 2014 Jul;79(7):R1231–49.
9. Bachir RG, Benali M. Antibacterial activity of the essential oils from the leaves of *Eucalyptus globulus* against *Escherichia coli* and *Staphylococcus aureus*. *Asian Pac J Trop Biomed* 2012 Sep;2(9):739–42.
10. Pereira V, Dias C, Vasconcelos MC, Rosa E, Saavedra MJ. Antibacterial activity and synergistic effects between *Eucalyptus globulus* leaf residues (essential oils and extracts) and antibiotics against several isolates of respiratory tract infections (*Pseudomonas aeruginosa*). *Ind Crops Prod* 2014;52:1–7.
11. Dezsi Ş, Badarau AS, Bischin C, et al. 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* 2015 Mar 16;20(3):4720–34.
12. Boulekbache-Makhlouf L, Slimani S, Madani K. Total phenolic content, antioxidant and antibacterial activities of fruits of *Eucalyptus globulus* cultivated in Algeria. *Ind Crops Prod* 2013;41:85–9.
13. Nagata H, Inagaki Y, Tanaka M, et al. Effect of *Eucalyptus* extract chewing gum on periodontal health: A double-masked, randomized trial. *Journal of Periodontology* 2008;79(80):1378–85.
14. Ercan N, Erdemir EO, Ozkan SY, Hendek MK. The comparative effect of propolis in two different vehicles: Mouthwash and chewing-gum on plaque accumulation and gingival inflammation. *Eur J Dent* 2015 Apr–Jun;9(2):272–6.
15. Djenane D, Yangüela J, Amrouche T, Boubrit S, Boussad N, Roncalés P. Chemical composition and antimicrobial effects of essential oils of *Eucalyptus globulus*, *Myrtus communis* and *Satureja hortensis* against *Escherichia coli* O157:H7 and *Staphylococcus aureus* in minced beef. *Food Sci Technol Int* 2011 Dec;17(6):505–15.
16. Ben Hassine D, Abderrabba M, Yvon Y, et al. Chemical composition and *in vitro* evaluation of the antioxidant and antimicrobial activities of *Eucalyptus gillii* essential oil and extracts. *Molecules* 2012 Aug 9;17(8):9540–58.
17. Takahashi T, Kokubo R, Sakaino M. Antimicrobial activities of *Eucalyptus* leaf extracts and flavonoids from *Eucalyptus maculata*. *Lett Appl Microbiol* 2004;39(1):60–4.
18. Batish DR, Singh HP, Kohli RK, Kaur S. *Eucalyptus* essential oil as a natural pesticide. *Forest Ecology and Management* 2008;256:2166–74.
19. Silva SM, Abe SY, Murakami FS, Frensch G, Marques FA, Nakashima T. Essential oils from different Plant Parts of *Eucalyptus cinerea* F. Muell. ex Benth. (Myrtaceae) as a source of 1,8-cineole and their bioactivities. *Pharmaceuticals (Basel)* 2011 Nov 25;4(12):1535–50.
20. Boukhatem MN, Amine FM, Kameli A, Saidi F, Walid K, Mohamed SB. Quality assessment of the essential oil from *Eucalyptus globulus* Labill of blida (Algeria) origin. *International Letters of Chemistry, Physics and Astronomy* 2014;17(3):303–15.
21. Amakura Y, Umino Y, Tsujia S, Itob H, Hatanob T, Yoshidab T. Constituents and their antioxidative effects in *Eucalyptus* leaf extract used as a natural food additive. *Food Chemistry* 2002;77:47–56.
22. Ogunwande IA, Olawore NO, Kasali AA, Ekundayo O. Volatile constituents from the leaves of *Eucalyptus cloeziana* F. Muell and *Eucalyptus propinqua* Deane & Maiden from Nigeria. *Flavour and Fragrance Journal* 2005;20:637–9.
23. Silva PHM, Brito JO, Junior FGS. Potential of eleven *Eucalyptus* species for the production of essential oils. *Sci Agric (Piracicaba, Braz.)* 2006;63(1):85–9.
24. European Pharmacopoeia Commission. Sage leaf (*Salvia officinalis*). *European Pharmacopoeia*. 6ed. Strasbourg, France: European Directorate Quality Medicine; 2008. p. 2853.
25. Ololade ZS, Fakankun OA, Alao FO, Udi OU. *Ocimum basilicum* var. *purpureum* floral essential oil: Phytochemicals, phenolic content, antioxidant, free radical scavenging, antimicrobial potentials. *Global Journal of Science Frontier Research* 2014;14(7):31–8.
26. Elmastas M, Cinkilic S, Aboul-Enein HY. Antioxidant capacity and determination of total phenolic compounds in daisy (*Matricaria chamomilla*, Fam. Asteraceae). *World Journal of Analytical Chemistry* 2015;3:9–14.
27. Ndoye Foe FM, Tchinnang TF, Nyegue AM, et al. Chemical composition, *in vitro* antioxidant and anti-inflammatory properties of essential oils of four dietary and medicinal plants from Cameroon. *BMC Complement Altern Med* 2016 Apr 7;16:117.
28. Tohma H, Köksal E, Kiliç Ö, et al. RP-HPLC/MS/MS analysis of the phenolic compounds, antioxidant and antimicrobial activities of *Salvia* L. species. *Antioxidants (Basel)* 2016 Oct 21;5(4). pii: E38.
29. Organization for economic cooperation and development. Guideline for testing of chemicals-acute oral. *Toxicity-Fixed Dose Procedure* 2001;1–14.
30. Ajayi AM, Tanayen JK, Ezeonwumelu J, et al. Anti-inflammatory, anti-nociceptive and total polyphenolic content of hydroethanolic extract of *Ocimum gratissimum* L. leaves. *Afr J Med Med Sci* 2014 Sep;43(Suppl 1):215–24.
31. Iroanya O, Okpuzor J, Mbagwu H. Anti-nociceptive and anti-phlogistic actions of a polyherbal decoction. *International Journal of Pharmacology* 2010;6:31–6.
32. de Sousa OV, Vieira GD, de Jesus R G de Pinho J, Yamamoto CH, Alves MS. Antinociceptive and anti-inflammatory activities of the ethanolic extract of *Annona muricata* L. leaves in animal models. *Int J Mol Sci* 2010 May 6;11(5):2067–78.
33. Ololade ZS, Fakankun OA, Alao FO, Udi OU. Phytochemical and therapeutic studies of the fruit essential oil of *Thuja orientalis* from Nigeria. *Global Journal of Science Frontier Research* 2014;14(7):15–20.
34. Proestos C, Lytoudi K, Mavromelanidou OK, Zoumpoulakis P, Sinanoglou VJ. Antioxidant capacity of selected plant extracts and their essential oils. *Antioxidants (Basel)* 2013 Jan 4;2(1):11–22.
35. Ololade ZS, Olaoye OO, Oyelese OJ, Olatunde OZ. *Callitris columellaris* var. *campestris* Silba leaf extract: Phytochemicals, phenolic contents, free radical scavenging, antioxidant and antimicrobial potentials. *Nature and Science* 2014;12(9):73–9.
36. Safaei-ghomi J, Ghadamib M, Batooli H. Bioactivity of methanol extracts of *Eucalyptus sargentii* Maiden

- cultivated in Iran. Digest Journal of Nanomaterials and Biostructures 2010;5(4):859–63.
37. Vidyamadhavi K, Chandrashekhar GJ, Manjunath HM, Nivya MT, Anand D, Raju NG. Evaluation of in-vitro antioxidant, anti-inflammatory properties of aerial parts of zanthoxylum rhesta. Research Journal of Pharmaceutical, Biological and Chemical Sciences 2014;5(5):997–1003.
  38. Shahwar D, Raza MA, Bukhari S, Bukhari G. Ferric reducing antioxidant power of essential oils extracted from *Eucalyptus* and *Curcuma* species. Asian Pacific Journal of Tropical Biomedicine 2012;S1633–S6.
  39. Karim AA, Azlan A, Ismail A, et al. Phenolic composition, antioxidant, anti-wrinkles and tyrosinase inhibitory activities of cocoa pod extract. BMC Complementary and Alternative Medicine 2014;14:381.
  40. Silva J, Abebe W, Sousa SM, Duarte VG, Machado MI, Matos FJ. Analgesic and anti-inflammatory effects of essential oils of *Eucalyptus*. J Ethnopharmacol 2003 Dec;89(2–3):277–83.
  41. Laube M, Kniess T, Pietzsch J. Development of antioxidant cOX-2 inhibitors as radioprotective agents for radiation therapy: A hypothesis-driven review. Antioxidants (Basel) 2016 Apr 19;5(2). pii: E14.
  42. Trebatická J, Duracková Z. Psychiatric disorders and polyphenols: Can they be helpful in therapy? Oxid Med Cell Longev 2015;2015:248529.
  43. Guimaraes AG, Melo MS, Bonfim RR, et al. Antinociceptive and anti-inflammatory effects of the essential oil of *Eugenia candolleana* DC., Myrtaceae, on mice. Rev Bras Pharmacogn 2009;19(4):883–7.
  44. Melo MS, Sena LC, Barreto FJ, et al. Antinociceptive effect of citronellal in mice. Pharm Biol 2010 Apr;48(4):411–6.
  45. Elaissi A, Rouis Z, Salem NA, et al. Chemical composition of 8 *Eucalyptus* species' essential oils and the evaluation of their antibacterial, antifungal and antiviral activities. BMC Complement Altern Med 2012 Jun 28;12:81.

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