Greener Journal of Biological Sciences
ISSN: 2276-7762 ICV: 5.99
Vol. 8(1), pp. 001-009, February, 2018

http://gjournals.org/GJBS

Research Article (DOI: http://doi.org/10.15580/GJBS.2018.1.011818006)

Phytochemical Characterization, Antioxidant, Anti-Inflammatory, Anti-Nociceptive and Antimicrobial Properties of the Seed Essential Oil of *Eucalyptus tereticornis*

Ololade Z.S.[1,2*] and Olawore N.O.[1]

[1]Department of Pure and Applied Chemistry, Ladoke Akintola University of Technology, Ogbomoso, Nigeria
[2]Department of Chemical and Food Sciences, Bells University of Technology, Ota, Nigeria

E-mail: zsololade@ bellsuniversity.edu.ng*; noolawore@ lautech.edu.ng[1]

ARTICLE INFO

Article No.: **011818006**
DOI: *10.15580/GJBS.2018.1.011818006*

Submitted: *18/01/2018*
Accepted: *27/01/2018*
Published: *12/02/2018*

Corresponding Author
Ololade, Z.S.
E-mail: *zsololade@ bellsuniversity.edu.ng*
Phone:*+2348034836546*

Keywords: *Eucalyptus tereticornis, seed essential oil, 1,8-cineole, α-selinene, α-pinene, phytochemicals, therapeutic*

ABSTRACT

The seed essential oils of *Eucalyptus tereticornis* possess strong medicinal properties. The aim of the current research study was to determine the phytochemicals and medicinal properties of the seed essential oil of *E. tereticornis* from Nigeria. The essential oil was extracted by hydrodistillation and analyzed using GC and 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. The GC and GC-MS analyses revealed the presence of 33 phytochemicals making up 97.2% of the total percentage composition of the essential oil. The most abundant component were *α*-selinene (15.5%), *α*-pinene (13.0%), 1,8-cineole (10.5%), *β*-pinene (8.0%), *α*-limonene (8.0%), *α*-terpineol (7.0%) ledol (5.0%) and palmitic acid (5.0%). The TPC was 206.68±0.00 μgmg^{-1} GAE. The DPPH IC_{50} and AAI values of the essential oil were 2.0 μgml^{-1} and 20.0. The essential oil was capable of scavenging free radicals in a range of 62.7-67.6%, while the reduction antioxidant potential was EC_{50}: 5.0 μgml^{-1}. The essential oil gave high anti-inflammatory with value of 99.76% and antinociceptive properties by inhibition in both neurogenic (57.81%) and inflammatory pain (47.86%). The essential oil was active against all the tested bacteria with high zones of inhibition (11.0-30.0 mm). The seed essential oil of *E. tereticornis* has potential medicinal compounds that may be of great use for the development of natural drugs.

INTRODUCTION

Natural products such as essential oils have been the object of growing interest because of their medicinal properties; they provide unlimited opportunities for novel phytochemicals and good additives and drug treatments because of their unmatched range of chemical diversity (Chikezie *et al*., 2015; Paul *et al*., 2015; Sadgrove and Jones, 2015; Chouhan *et al*., 2017). Essential oils have received much attention in the prevention and treatment of diseases as well as for preventing oxidative damage by reactive oxygen and nitrogen species, essential oil inhalation helps in mental stability and relaxation effect in human organs (Varela-Lopez *et al*., 2015; Dzialo *et al*., 2016; Murillo and Fernandez, 2016).

Eucalyptus essential oils are very useful aromatherapeutic agents because of their various phytochemicals with several medicinal properties (Sadlon *et al*., 2010; Hamid *et al*., 2011; Nazzaro *et al*., 2013; Dagli *et al*., 2015). *Eucalyptus* oils rejuvenate the mind of people suffering from some disorders by stimulating mental activity and increase blood flow to the brain (Ali *et al*., 2015). They increase the blood flow around the body and brain by relaxing the blood vessels and allowing more blood to circulate due to their vasodilatory properties. They are also employed in form of causal aromatherapy to increase students' performance. *Eucalyptus* essential oils help reduce blood sugar level (Elaissi *et al*., 2012; Jaradat *et al*., 2016; Ozkan *et al*., 2016). They are used in the pharmaceutical and food industries as excipients, preservatives and flavouring agents which are used to improve the odour and taste of drugs and foods (Jaradat *et al*., 2016; Chauhan, 2017).

To the best of our knowledge, there is dearth of information on the phytochemical, total phenolic content, free radical scavenging, antioxidant, anti-inflammatory, antinociceptive and antimicrobial potentials of the seed of this *E. tereticornis* so far. Therefore, the present study was aimed at looking into the chemical and pharmacological properties of the seed essential oils of *E. tereticornis* from Nigeria.

2. MATERIAL 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 as *E. tereticornis* Smith and the fresh seeds were pulverized and the essential oil was extracted by hydrodistillation using all-glass clevenger-type apparatus according to European pharmacopoeia, 2004. The essential oil was then stored in vial at low temperature to prevent evaporation.

GC and GC-MS Analyses

The seed essential oil of *E. tereticonis* was analysed 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 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 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 (Ololade *et al*., 2014).

Determination of Total Phenolic Content

Total phenolic content of the seed essential oil of *E. tereticornis* was determined using the Folin-Ciocalteau reagent. 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, then 3 ml of (2% w/v) Na_2CO_3 solution was added after 3 mins and the mixture was allowed to stand for 2 hrs for incubation in dark with intermittent shaking, the absorbance was then measured at 760 nm using SM 7504 UV-vis spectrophotometer. Gallic acid was used as a reference; the index of TPC was expressed as μgmg^{-1} gallic acid equivalents (Mnayer *et al*., 2014).

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^{-1}) of the essential oil in methanol were incubated with a methanolic solution of DPPH. After 30 minutes of incubation at room temperature in the dark, the absorbance at 517 nm was measured with SM 7504 UV-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 colour into yellow by the tested sample against the control (DPPH solution only). The IC_{50} of DPPH assay represents the concentration of the tested sample needed to reduce the DPPH by 50% where the value obtained from linear regression graph

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

Where: A_{blank} is the absorbance of blank solution and A_{eo} is the absorbance of the essential oil. The dose-response curve was plotted and IC_{50} value for the essential oil and the standard were calculated (Oloade *et al.*, 2014).

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

$$AAI = [DPPH\ initial\ concentration]/[IC_{50}]$$

where the AAI depending on whether the essential oil showed weak antioxidant activity (AAI < 0.5), moderate antioxidant activity (AAI, between 0.5 and 1.0), strong antioxidant activity (AAI, between 1.0 and 2.0) and very strong antioxidant activity when AAI > 2.0 (Foe *et al.*, 2016).

***In vitro* FRAP assay:** This method was based on the reduction of the Fe(III)/ferricyanide complex to the ferrous form by one-electron-donating antioxidant. Different concentrations of *E. tereticornis* seed essential oils (1000, 100 and 10 μgml^{-1}) was dissolved in 1.0 ml of distilled water, followed by the addition of 2.5 ml of 0.2 M sodium phosphate buffer (pH 6.6) and 2.5 ml of 1% w/v potassium ferricyanide [$K_3Fe(CN)_6$], and the resultant mixture was incubated at 50 ^oC for 20 mins. After addition of 2.5 ml of 10% trichloroacetic acid, the mixture was centrifuged at 3000 rpm for 10 mins. The upper layer (2.5 ml) was mixed with 2.5 ml of deionised water and a freshly prepared 0.5 ml of 0.1% ferric chloride, and the absorbance was measured at 700 nm using SM 7504 UV-vis spectrophotometer. The activity of ascorbic acid was used as a standard over the same concentrations. The assays were analysed in triplicate and the results are expressed as mean ± standard deviation. Effective concentration at 50% (EC_{50}) of FRAP value is the sample concentration required to reduce Fe^{3+} to Fe^{2+} (Omoregie *et al.*, 2014).

Experimental Animals

Healthy albino rats (200 ±30 g) were used for the present study were kept in controlled cycles (12/12 hrs light/dark) with free access to food and water. All experiments were carried out in strict compliance with the principle of laboratory animal care (OECD, 2001).

Carrageenan-Induced Anti-inflammatory Assay

This test was carried out on the basis of inhibition of paw oedema induced by the injection of 0.1 ml of freshly prepared 1% carrageenan (an oedematogenic agent) into the subplantar region of the right hind paw of the rat. Three groups of five animals each were used. The 10% seed essential oil was subjected at a dose of 0.1 ml each of 1000 μgkg^{-1} and was administered orally 30 mins before carrageenan injection. Indomethacin 1000 μgml^{-1} was used as reference drug. Control group received the vehicle only. The paw size was measured before and immediately after the administration of carrageenan using a digital vernier calliper. Paw sizes were measured at time intervals of 0-4 hrs. Increases in the linear diameter of the hind paws were taken as an indication of paw oedema. 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 inflammatory reaction was determined for each rat by comparing each group with controls and calculated by the formula below:

$$I\% = A_o\text{-}A_t/A_o x100$$

where, A_o was the average inflammation (hind paw oedema) of the control group at a given time 0. A_t is the average inflammation of the drug treated (i.e., essential oil or reference indomethacin) rats at time (t) (Iroanya *et al.*, 2010).

Formalin Licking *In vivo* Antinociceptive Activity

This test was based on the method described by Sofidiya *et al.* (2014) with slight modification. Rats (n = 5 per group) were treated respectively with 1000 μgkg^{-1} of the seed essential oil and 1000 μgkg^{-1} of indomethacin. 30 min 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 time (sec) spent in licking the paw and the biting responses of the injected paw were taken as an indicator of pain response. The mice were observed for 30 min after the injection of formalin and the amount of time spent licking the injected hind paw was recorded. The first 5 min post formalin injection is referred to as the early (neurogenic) phase and the period between 15 and 30 mins as the late (inflammatory) phase. 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%) of pain was calculated as the reduction in the number of licking compared to the control using the formula below:

$$I\% = B_o\text{-}B_t/B_o x100$$

where, B_o represents the vehicle treated control group value for each phase and B_t represents the treated groups value for each phase.

Determination of *In vitro* Antimicrobial Potentials

The antibacterial potentials of the seed essential oil were evaluated by agar-well diffusion method against representative multi-drug resistance Gram-positive organism (*Streptococcus agalactiae* and *Staphylococcus aureus*), Gram-negative organisms (*Escherichia coli*, *Klebsiella pneumoniae*, *Proteus mirabilis* and *Pseudomonas aeruginosa*). The bacteria isolates were first sub-cultured in Nutrient agar and incubated at 37 ^oC for 24 hrs. All the bacteria cultures were adjusted to 0.5 McFarland standards, 20 ml of

sterilized Nutrient agar medium was poured into each Petri dish aseptically and plates were then swabbed with inocula of the test organisms, and kept for 15 mins for adsorption. Using sterile cork borer of 6 mm diameter wells were bored into the seeded agar plates, and these were loaded with 10 µl of different concentrations (1000, 100 and 10 μgml^{-1}) of the essential oil in dimethylsulfoxide (DMSO). The plates were allowed to stand in the refrigerator for 1 hr to allow proper diffusion of the essential oil into the medium and incubated at 37 ^{o}C for 24 hrs before visual assessment of the inhibition zones. Antibacterial potential of the essential oil was evaluated by measuring the clear zones of growth inhibition against the test organisms. Amxoxicillin (AMX), Augmentin (AUG) and Cefixime (CEF) were used as control (Opawale *et al*., 2014).

3. RESULTS AND DISCUSSION

Chemical Constituents of the Essential Oil

The seed essential oil of *E. tereticornis* analysed showed 33 components, representing 97.2% of the seed essential oil were identified (Table 1.). The major component of seed essential oil was *α*-selinene (15.5%), The other main compounds identified were *α*-pinene (13.0%), 1,8-cineole (10.5%), *β*-pinene (8.0%), *α*-limonene (8.0%), *α*-terpineol (7.0%) ledol (5.0%) and palmitic acid (5.0%). Previous studies on the *E. tereticornis* leaf essential oil from Nigeria showed that *α*-pinene (21.4%) was the major constituent but 1,8 cineole was absent (Ogunwande *et al*., 2003; Ghaffar *et al*., 2015). It was also reported that leaf essential oil of *E. tereticornis* from Brazil showed that the composition was dominated by 1,8-cineole (54.8%) (Silva *et al*., 2006). Moreover, the leaf essential oil of *E. tereticornis* from India analyzed by Kaur *et al*., (2011) gave α-Pinene (32.5%) and 1,8-cineole (22.4%) as the two major constituents. *E. tereticornis* leaf essential oil from Pakistan showed 24 components with 1,8-cineole (15.2%), *α*-pinene (12.1%), myrtenal (8.1%), linalool, (7.4%) and paraldehyde nitrile (7.1%) as the most abundant (Ghaffar *et al*., 2015). The leaf essential oil of *E. tereticornis* from Benin and Argentina were characterized by the presence of *p*-cymene, cryptone, spathulenol, caryophyllene oxide with low percentage of 1,8-cineole (Alitonou *et al*., 2004; Lucia *et al*., 2008; Toloza *et al*., 2008; Bossou *et al*., 2013), Likewise, the major constituents of the leaf essential oil of *E. tereticornis* from Algeria, Ethiopia, India and Pakistan were 1,8-cineole and *α*-pinene (Dagne *et al*., 2000; Benayache *et al*., 2001; Singh *et al*., 2009; Ghaffar *et al*., 2015).

Table 1: Chemical Composition of the seed Essential Oil of *Eucalyptus tereticornis*

Compound	Retention Index	Percentage Composition
pentacosane	395	1.0
octacosane	442	1.0
o-xylene	907	1.0
camphene	933	0.5
β-pinene	943	8.0
α-pinene	948	13.0
γ-terpinene	998	1.0
α-limonene	1018	8.0
o-cymene	1021	1.0
m-cymene	1042	1.0
1,8-cineole	1059	10.5
pinocarvone	1114	0.4
fenchol	1125	1.0
L-*trans*-pinocarveol	1131	1.0
endo-borneol	1138	1.0
α-terpineol	1143	7.0
myrtenol	1191	1.0
2-(1,1-dimethylethyl)-1,4-dimethoxybenzene	1386	1.0
n-tetradecane	1400	1.0
allo-aromadendrene	1458	4.0
(+)-ledene	1505	1.0
α-selinene	1513	15.5
germacrene D	1515	0.5
spathulenol	1528	2.0
ledol	1530	5.0
1-pentylheptylbenzene	1731	0.5
1-butyloctylbenzene	1736	0.5
1-propylnonylbenzene	1747	1.0
1-pentylocylbenzene	1822	0.5
1-butylnonylbenzene	1825	0.3
palmitic acid	1968	5.0
n-hexadecanoic acid	1975	1.0
oleic acid	2175	1.0
Percentage Total		**97.2**

Total Phenolic Content (TPC)

Total phenolic content analysis revealed the presence of high quantity phenolic compounds in the seed essential oil. This was found to be 206.68±0.00 μgmg^{-1} gallic acid equivalents. The essential oil gave a higher TPC, when compared with the previous study on the related species such as the leaf extract of *E. globulus* from Portugal with 67.92 ± 2.39 mgg^{-1} gallic acid equivalents which was found to contain a relatively low concentration of phenolic compound compared with the seed essential oil of *E. tereticornis* investigated in this study (Pombal *et al*., 2014). Moreover, literatures showed that the TPC for the commercial *Eucalyptus* leaf extract from the Japan Food Additive Association was 11.9 mgg^{-1} GAE (Amakura *et al*., 2002; Hasssine *et al*., 2012). The seed essential oil of *E. tereticornis* exhibited the high TPC due to the presence of low molecular mass terpenoid and phenolic compounds. This report indicates that TPC is directly proportional to antioxidant and pharmacological properties of the seed part of the plant. Phenolic compounds have aroused considerable interest recently because of their potential beneficial effects on human health (Lincy *et al*., 2017).

***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 μgml^{-1}) were 67.60±0.09, 64.89±0.03 and 62.71±0.06 % respectively; while the IC_{50} value was found to be 2.0 μgml^{-1} in comparison to ascorbic acid which gave 96±0.00 69±0.00 and 54±0.00, as the percentage inhibitions with IC_{50} value of 9.0 μgml^{-1}. The percentage of free radical scavenging of the seed essential oil was similar to what was reported for the leaf essential oil of *E. tereticornis* from Pakistan (81.8%) (Ghaffar *et al*., 2015). The DPPH radical scavenging capacity of the seed essential oil of *E. tereticornis* was higher than that of ascorbic acid. The free radical scavenging and antioxidant properties of the seed essential oil were found to be four times more active than the synthetic antioxidant (ascorbic acid). Moreover, the seed essential oil of *E. tereticornis* inhibited the DPPH free radicals than the leaf essential oil of *E. tereticornis* from India with IC_{50}: 146.3 μgml^{-1} (Kaur *et al*., 2011) and the leaf extract of *E. globulus* from Portugal with IC_{50}: 426.8 μgml^{-1} (Pombal *et al*., 2014).

Antioxidant Activity Index (AAI)

The seed essential oil had a very strong AAI value of 20.0 (Table 2), indicating that the presence of phenolic compounds and terpenoids increasing the antioxidant potential of the seed essential oil. AAI by the DPPH method is considered appropriate for comparing extracts and pure compounds. There used to be no difference in AAI values when different solutions of DPPH and concentrations of the compounds or extracts were used (Scherer and Godoy, 2009; Takao *et al*., 2015).

***In vitro* Reduction Antioxidant Potential**

Reduction antioxidant potential of the seed essential oil of *E. tereticornis* (EC_{50}: 5.0 μgml^{-1}) was 55% higher in reducing antioxidant 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 (Shahwar *et al*., 2012). The presence of terpenoid and phenolic compounds in the seed essential oil of *E. tereticornis* contributed to its higher FRAP value since these compounds are known to chelate metal ions (Ololade and Olawore, 2017; Sivaramakrishnan *et al*., 2017).

Table 2: IC_{50} of the Antioxidant Properties of *E. tereticornis* Seed Essential Oil

Essential Oil and Reference Drug	DPPH IC_{50} μgml^{-1}	DPPH AAI	FRAP IC_{50} μgml^{-1}
E. tereticonis	2.0	20.0	5.0

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

Anti-Inflammatory Potential

The seed essential oil of *E. tereticornis* investigated has a very high percentage anti-inflammatory property of 99.76% at 1000 µg, this showed that it has a comparative properties as indomethacin (93.7%), but it was more effective than the leaf essential oil of *E. tereticornis* at concentration of 100 $mgkg^{-1}$ which caused inhibition of inflammatory by 80% (Silva *et al*., 2003). This study has shown that the seed essential oil of *E. tereticornis* investigated possessed a significant anti-oedematogenic effect on paw oedema induced by carrageenan due to the presence of terpenoids and phenolics compounds in the essential oil. It is generally accepted that tissue injury associated with inflammation is attributed to infiltration of neutrophils and macrophages followed by the release of proinflammatory mediators such as eicosanoids, toxic radical species and lytic enzymes. Therefore, inhibition of the function of the macrophages and neutrophils participates on the mechanism of action of a number of anti-inflammatory drugs (Dinarello, 2010; Dalbeth *et al*., 2014; Cruz *et al*., 2017). The seed essential oil significantly inhibited some of the functions of these cells, which may be implicated in the anti-inflammatory action. The essential oil was able to scavenge free radicals; therefore, they could also act as anti-inflammatory agents, because one of the inflammatory

responses is the oxidative burst that occurs in diverse cells (monocytes, neutrophils, eosinophils, and macrophages) (Juergens *et al*., 2003; Miguel, 2010; Nagpal *et al*., 2010; Sa *et al*., 2013). This essential oil can ease pain and discomfort, reduce swelling, and relieve sore muscles. Therefore provides natural relief for disorders such as muscular pains of fibromyalgia, bursitis, fibromyositis, rheumatism, inflammation, osteoarthritis and phlogistic actions alleviates fatigue and aids healing (Sumpton and Moulin, 2008, Bote *et al*., 2013; Dolan *et al*., 2016).

Table 3: Anti-inflammatory Activities of *E. tereticornis* Seed Essential Oil

Essential Oil and Reference Drug	% I (2 Hr)	% I (4 Hr)	Mean % I
E. tereticornis	20.00	99.97	99.76
Indomethacin	87.50	99.70	93.70

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

Antinociceptive Potential

The seed essential oil of *E. tereticornis* showed a moderate antinociceptive properties by inhibition in both neurogenic (57.81%) and inflammatory pain (47.86%) induced by injection of formalin. The seed 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 of the seed essential oil investigated had a higher antinociceptive activity as the leaf essential oil of *E. tereticornis* at concentration of 10 $mgkg^{-1}$ caused inhibition of neurogenic pain by 50% (Silva *et al*., 2003). This result indicate antinociceptive and anti-inflammatory properties of the seed essential oil mediated via inhibition of prostaglandin synthesis and other peripherally pathway. The seed essential oil inhibited both phases of the formalin-induced nociception because they act mainly centrally, but its effect was more prominent in the second phase.

Table 4.0: Antinociceptive Activities of *E. tereticornis* Seed Essential Oil

Essential Oil and Reference Drug	Time of Licking and Biting Percentage Inhibition			
	Early Phase (0-5) min.	% Inhibition	Late Phase (5-30) min.	% Inhibition
E. tereticornis	40.50±1.41	57.81	61.00±7.59	47.86
Indomethacin	34.33±2.12	64.23	53.00±2.12	54.70

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

Antibacterial Potentials

The antimicrobial potential of the seed essential oil of *E. tereticornis* was tested against five bacteria (Table 5). The essential oil showed variable activities against tested bacteria. The highest inhibitory effect of the seed essential oil of *E. tereticornis* was observed against *E. coli* (30 mm), *S. aureus* (20 mm), *S. agalactiae* (19 mm) *K. pneumoniae* (18 mm) and *P. aeruginosa* (11 mm). The tested bacteria were found to be resistant to Cefixime (CEF), but some were sensitive to Amxoxicillin (AMX) and Augmentin (AUG) synthetic antibiotics (Table 6.0). 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 leaves essential oils of *Eucalyptus* species (*E. bicostata*, *E. cinerea*, *E. exerta*, *E. gigantea*, *E. gunnii*, *E. macarthurii*, *E. macrorrhyncha*, *E. maidenii*, *E. odorata*, *E. pauciflora*, *E. sideroxylon*, *E. tereticornis*, *E. viminalis*, *E. cladocalyx*, *E. citriodora*, *E. diversicolor*, *E. fasciculosa*, *E. grandis* and *E. ovata* all from Tunisia and *E. botryoides* var. *botryoides* from Morocco and Italy) showed low inhibitions to *E. coli*, *P. aeruginosa*, *E. faecalis* and *S. aureus* between 6.3–14.4 mm, which are very low compared to the antibacterial potentials of the seed essential oil investigated in this study (Elaissi *et al*., 2011). The high antimicrobial potentials were most likely due to the presence of compounds which have antimicrobial properties, particularly, 1,8-cineole which has very high percentage in the seed essential oil investigated, and which is known to have relatively strong antimicrobial properties against many important pathogens and respiratory and spoilage organisms including *S. aureus*, *E. coli* and *B. subtilis* (Santos *et al*., 2004; Sonboli *et al*., 2006; Rosato *et al*., 2007; Hassine *et al*., 2012; Sa *et al*., 2013).

Table 5.0: Zones of Inhibition (mm) showing the Antimicrobial Properties of *E. tereticornis* Seed Essential oil

	Seed Essential Oil			AMX	AUG	CEF
Conc. (µgml^{-1}) / Organisms	1000	100	10	25 µg	30 µg	5 µg
E. coli	30	30	30	-	-	-
K. pneumoniae	18	18	18	10	07	-
P. aeruginosa	11	11	11	15	10	-
S. agalactiae	19	19	17	-	-	-
S. aureus	20	20	20	-	-	-

Key note: Resistant (–), not sensitive (<8 mm), sensitive (9–14 mm), very sensitive (15–19 mm) and ultrasensitive (>20 mm)

CONCLUSION

The present study on the seed essential oil of *E. tereticornis* contains potential medicinal compounds that may be of great use for the development of natural drugs as therapies against various diseases. The seed essential oil of *E. tereticornis* possesses marked antioxidant, anti-inflammatory, analgesic and antimicrobial potentials. All these activities might be attributed to terpenoids and phenolic compounds present in seed essential oil of *E. tereticornis*. Further, the potential of these plants must be explored more and more, in order to develop an alternate therapy for the treatment of infections caused by reactive oxygen species (ROS) and antibiotic multi-resistant bacteria. Based on our findings we recommend the seed essential oil for clinical trial to uncover the mechanism of above mentioned activities.

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. Amakura, Y., Uminoa, Y., Tsujia, S., Itob, H., Hatanob, T., Yoshidab, T. (2002). Constituents and their antioxidative effects in *Eucalyptus* leaf extract used as a natural food additive. *Food Chemistry*, 77, 47-56.
2. Ali, B., Al-Wabel, N.A., Shams, S., Ahamad, A., Khan, S.A. and Anwar, F. (2015). Essential oils used in aromatherapy: A systemic review, Asian Pacific Journal of Tropical Biomedicine, 5(8), 601–611.
3. Alitonou G, Avlessi F, Wotto VD, Ahoussi E, Dangou J, Sohounhloue, D.C.K. (2004). Composition chimique, propriétés antimicrobiennes et activités sur les tiques de l'huile essentielle d'Eucalyptus tereticornis Sm. C R Chimie, 7, 1051–1055.
4. Benayache S, Benayache F, Benyahia S, Chalchat J-C, Garry R-P (2001): Leaf Oils of some *Eucalyptus* Species Growing in Algeria. J Essent Oil Res, 13:210–213.
5. Bossou, A.D., Mangelinckx, S., Yedomonhan, H., Boko, P.M., Akogbeto, M.C., Kimpe, N.D., Avlessi, F. and Sohounhloue, D.C.K. (2013). Chemical composition and insecticidal activity of plant essential oils from Benin against *Anopheles gambiae* (*Giles*), Parasites and Vectors, 6, 1-17.
6. Bote, M.E., Garcia, J.J., Hinchado, M.D. and Ortega, E. (2013). Fibromyalgia: Anti-Inflammatory and Stress Responses after Acute Moderate Exercise. PLoS ONE 8(9), 1-6.
7. Chikezie, P.C., Ibegbulem, C.O. and Mbagwu, F.N. (2015). Medicinal Potentials and Toxicity Concerns of Bioactive Principles. Med Aromat Plants 4: 202.
8. Chouhan, S., Sharma, K. and Guleria, S. (2017). Antimicrobial Activity of Some Essential Oils-Present Status and Future Perspectives, 4, 1-21.
9. Chauhan, R. (2017). Taste Masking: A Unique Approach for Bitter Drugs. *Journal of stem cell Biology and Transplantion*, 1(2), 1-6.
10. Cruz, F.F., Rocco, P.R.M. and Pelosi, P. (2017). Anti-inflammatory Properties of Anaesthetic Agents, *Critical Care*, 21, 1-7.
11. Dagli, M., Dagli, R., Mahmoud, R.S. and Baroudi, K. (2015). Essential oils, their therapeutic properties, and implication in dentistry: A review, *Journal of International Society of Preventive and Community Dentistry*, 5(5), 335–340.
12. Dagne, E., Bisrat, D., Alemayehu, M., Worku, T. (2000). Essential oils of Twelve *Eucalyptus* species from Ethiopia. *Journal of Essential Oil Research*, 12:467-470.
13. Dalbeth, N., Lauterio, T.J. and Henry R. Wolfe, H.R. (2014). Mechanism of Action of Colchicine in the Treatment of Gout, *Clinical Therapeutics*, 36(10), 1465-1479.
14. Dinarello, C.A. (2010). Anti-inflammatory Agents: Present and Future, Cell, 140(6): 935–950.
15. Dolan, L., Tung, L.L.D. and Raizada, S.R. (2016). Fibromyalgia in the Context of Rheumatoid Arthritis: A Review. Fibrom Open Access 1, 1-5.
16. Działo, M., Mierziak, J., Korzun, U., Preisner, M., Szopa, J. and Kulma, A. (2016). The Potential of Plant Phenolics in Prevention and Therapy of Skin Disorders, *International Journal of Molecular Sciences*, 17, 1-41.
17. Elaissi, A., Rouis, Z., Salem, N.A.B., Mabrouk, S., Salem, Y.B., Salah, K.B.H., Aouni, M., Farhat, F., hemli, R., Harzallah-Skhiri, F. and Khouja, M.L. (2012). Chemical composition of 8 *Eucalyptus*

species' essential oils and the evaluation of their antibacterial, antifungal and antiviral activities, *BMC Complementary and Alternative Medicine*, 12:81, 1-15.
18. Elaissi, A., Salah, K.H., Mabrouk, S., Larbi, K.M., Chemli, R., Harzallah-Skhiri, F. (2011). Antibacterial activity and chemical composition of 20 *Eucalyptus* species'essential oils, *Food Chemistry*, 129, 1427–1434.
19. European Pharmacopoeia Commission (2008). Sage leaf (*Salvia officinalis*). European Pharmacopoeia. 6 edition. Strasbourg, France: *European Directorate Quality Medicine*, 2853.
20. Foe, F.M.C.N., Tchinang, T.F.K., Nyegue, A.M., Abdou, J.P., Yaya, A.J.G., Tchinda, A.T., Essame, J.L.O. and Etoa, F.X. (2016). Chemical composition, in vitro antioxidant and anti-inflammatory properties of essential oils of four dietary and medicinal plants from Cameroon, *BMC Complementary and Alternative Medicine*, 16:117, 1-12.
21. Hamid, A.A., Aiyelaagbe, O.O. and Usman, L.A. (2011). Essential Oils: Its Medicinal and Pharmacological Uses, *International Journal of Current Research*, 33 (2), 86-98.
22. Iroanya, O., Okpuzor, J. and Mbagwu, H. (2010). Anti-Nociceptive and Anti-Phlogistic Actions of a Polyherbal Decoction. *International Journal of Pharmacology, 6*, *31-36.*
23. Jaradat, N.M., Al-Zabadi, H., Rahhal, B., Hussein, A.M.A., Mahmoud, J.S., Mansour, B., Khasati, A.I. and Issa, A. (2016). The effect of inhalation of Citrus sinensis flowers and Mentha spicata leave essential oils on lung function and exercise performance: a quasi-experimental uncontrolled before-and-after study, *Journal of the International Society of Sports Nutrition*, 13, 1-8.
24. Juergens, U.R., Dethlefsen, U., Steinkamp, G., Gillissena, R.R. and Vetter, H. (2003). Anti-inflammatory activity of 1.8-cineol (eucalyptol) in bronchial asthma: A double-blind placebo-controlled trial, *Respiratory Medicine*, 97, 250–256.
25. Ghaffar, A., Yameen, M., Kiran, S., Kamal, S., Jalal, F., Munir, B., Saleem, S., Rafiq, N., Ahmad, A., Saba, I. and Jabbar, A. (2015). Chemical Composition and in-Vitro Evaluation of the Antimicrobial and Antioxidant Activities of Essential Oils Extracted from Seven *Eucalyptus* Species, *Molecules*, 20, 20487–20498. .
26. Hassine, D.B., Abderrabba, M., Yvon, Y., Lebrihi, A., Mathieu, F., Couderc, F. and Bouajila, J. (2012). Chemical Composition and *in Vitro* Evaluation of the Antioxidant and Antimicrobial Activities of *Eucalyptus gillii* Essential Oil and Extracts, *Molecules*, 17, 9540-9558.
27. Kaur, S., Singh, H.P., Batish, D.R. and Kohli, R.K. (2011). Chemical characterization and allelopathic potential of volatile oil of *Eucalyptus tereticornis* against *Amaranthus viridis*, *Journal of Plant Interactions*, 6(4), 297-302..
28. Lincy, J., Mathew, G. and Leena, P.N. (2017). Evaluation of Hepatoprotective Activity of *Clerodendron paniculatum* Linn Root Extract, *World Journal of Pharmaceutical and Medical Research*, 3(7), 149-151.
29. Lucia, A., Licastro, S., Zerba, E., Masuh, H. (2008). Yield, chemical composition, and bioactivity of essential oils from 12 species of *Eucalyptus* on *Aedes aegypti* larvae. Entomologia Experimentalis et Applicata, 129, 107–114.
30. Miguel, M.G. (2010). Antioxidant and Anti-Inflammatory Activities of Essential Oils: A Short Review, *Molecules*, 15, 9252-9287.
31. Mnayer, D., Fabiano-Tixier, A.S., Petitcolas, E., Hamieh, T., Nehme, N., Ferrant, C., Fernandez, X. and Chemat, F. (2014). Chemical Composition, Antibacterial and Antioxidant Activities of Six Essentials Oils from the *Alliaceae* Family, *Molecules*, 19, 20034-20053. .
32. Murillo, A.G. and Fernandez, M.L. (2016). Potential of Dietary Non-Provitamin A Carotenoids in the Prevention and Treatment of Diabetic Microvascular Complications, *Advances in Nutrition*, 7, 14–24.
33. Nagpal, N., Shah, G., Arora M.N., Shri, R. and Arya, Y. (2010). Phytochemical and Pharmacological Aspects of *Eucalyptus* Genus, *International Journal of Pharmaceutial Sciences and Research*, 1(12), 28-36.
34. Nazzaro, F., Fratianni, F., Martino, L.D., Coppola, R. and Vincenzo De Feo, V.D. (2013). Effect of Essential Oils on Pathogenic Bacteria, *Pharmaceuticals*, 6, 1451-1474.
35. OECD 2001. Organization for Economic cooperation and Development Guideline for Testing of Chemicals-Acute Oral Toxicity-Fixed Dose Procedure, 1-14.
36. Ogunwande, I. A., Olawore, N. O., Adeleke, K. A. and Konig, W. A. (2003). Chemical composition of the essential oils from the leaves of three *Eucalyptus* species growing in Nigeria, *Journal of Essential Oil Research*, 15, 297-301.
37. Omoregie, E.S., Oriakhi, K., Oikeh,E.I., Okugbo, O.T. and Akpobire, D. *(*2014). Comparative Study of Phenolic Content and Antioxidant Activity of Leaf Extracts of *Alstonia boonei* and *Eupatorium odoratum*, *Nigerian Journal of Basic and Applied Science*, 22, 91-97.
38. Ololade, Z.S., Fakankun, O.A., Alao, F.O. and Udi, O.U. (2014). *Ocimum basilicum* var. *purpureum* Floral Essential Oil: Phytochemicals, Phenolic Content, Antioxidant, Free Radical Scavenging, Antimicrobial Potentials, *Global Journal of Science Frontier Research*, 14(7), 31-38.
39. Ololade, Z.S., Fakankun, O.A., Alao, F.O. and Udi, O.U. (2014). Phytochemical and Therapeutic Studies of the Fruit Essential Oil of *Thuja orientalis* from Nigeria, *Global Journal of Science Frontier Research*, 14(7), 15-20.
40. Ololade, Z.S. and Olawore, N.O. (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.
41. Opawale, B.O., Onifade, A.K. and Ogundare, A.O. (2015). Evaluation of Antimicrobial, Antioxidant

and Cytotoxic Activity of *Lovoa trichiliodes* Extracts and Essential Oils. Med Aromat Plants 5:222, 1-6.
42. Ozkan, G., Kamiloglu, S., Ozdal, T., Boyacioglu, D. and Capanoglu, E. (2016). Potential Use of Turkish Medicinal Plants in the Treatment of Various Diseases, *Molecules*, 21, 1-32.
43. Paul C. Chikezie, Chiedozie O. Ibegbulem and Ferdinand N. Mbagwu, (2015). Bioactive Principles from Medicinal Plants. *Research Journal of Phytochemistry*, 9, 88-115.
44. Pombal, S., Rodilla, J., Gomes, A., Silva, L. and Rocha, P. (2014). Evaluation of the antibacterial activity of the essential oil and antioxidant activity of aqueous extracts of the Eucalyptus globulus Labill. Leaves, *Global Advanced Research Journal of Agricultural Science*, 3(11), 356-366.
45. Rosato, A., Vitali, C., de Laurentis, N., Armenise, D. and Milillo, M. (2007). Antibacterial effect of some essential oils administered alone or in combination with Norfloxacin, *Phytomedicine*, 14, 727–732.
46. Sa, R.C.S., Andrade, L.N. and Sousa, D.P. 2013. A Review on Anti-Inflammatory Activity of Monoterpenes, *Molecules*, 18, 1227-1254.
47. Sadlon, A.E. and Lamson, D.W. (2010). Immune-modifying and antimicrobial effects of *Eucalyptus oil* and simple inhalation devices, *Alternative Medicine Review*, 15(1), 33-47.
48. Sadgrove, N. and Jones, G. (2015). A Contemporary Introduction to Essential Oils: Chemistry, Bioactivity and Prospects for Australian Agriculture, *Agriculture*, 5, 48-102.
49. Santos, F.A., Silva, R.M., Campos, A.R., Araujo, R.P., Junior, R.C.P.L. and Rao, V.S.N. (2004). 1,8-cineole (*Eucalyptol*), a monoterpene oxide attenuates the colonic damage in rats on acute TNBS-colitis, *Food and Chemical Toxicology*, 42, 579-584.
50. Scherer, R. and Godoy, H.T., (2009). Antioxidant activity index (AAI) by the 2,2-diphenyl-1-picrylhydrazyl method. *Food Chemistry*, 112 (3), 654-658.
51. Shahwar, D., Raza, M.A., Bukhari S. and Bukhari, G. (2012). Ferric reducing antioxidant power of essential oils extracted from *Eucalyptus* and *Curcuma* species, *Asian Pacific Journal of Tropical Biomedicine*, S1633-S1636.
52. Silva, J. Abebe, W., Sousa, S.M., Duarte, V.G., Machado, M.I.L. and Matos, F.J.A. (2003). Analgesic and anti-inflammatory effects of essential oils of *Eucalyptus*, *Journal of Ethnopharmacology*, 89, 277-283.
53. Silva, P.H.M., Brito, J.O. and Junior, F.G.S. (2006). Potential of Eleven *Eucalyptus* Species for the Production of Essential Oils, *Science and Agriculture* (Piracicaba, Braz.), 63 (1), 85-89.
54. Singh, H.P., Mittal, S., Kaur, S., Batish, D.R. and Kohli, R.K. (2009). Characterization and antioxidant activity of essential oils from fresh and decaying leaves of Eucalyptus tereticornis. *Journal Agriculture and Food Chemistry*, 57, 6962–6966.
55. Sofidiya, M.O., Imeha, E., Ezeani, C., Aigbe, F.R. and Akindele, A.J. (2014). Antinociceptive and anti-inflammatory activities of ethanolic extract of *Alafia barteri*, *Brazilian Journal of Phamacognosy*, 24, 348-354.
56. Sonboli, A., Babakhani, B. and Mehrabian, A.R. (2006). Antimicrobial activity of six constituents of essential oil from Salvia, *Zeitschrift Naturforschung C*, 61, 160–164.
57. Sivaramakrishnan, T., Swain, S., Saravanan, K., Kiruba S.R., Roy, S.D., Biswas, L. and Shalini, B. (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.
58. Sumpton, J.E. and Moulin, D.E. (2008). Fibromyalgia: Presentation and management with a focus on pharmacological treatment, *Pain Res Manage*, 13(6), 477-483.
59. Takao, L.K., Imatomi, M. and Gualtieri, S.C.J. (2015). Antioxidant activity and phenolic content of leaf infusions of *Myrtaceae* species from Cerrado (Brazilian Savanna), *Brazillian Journal Biology*, 75(4), 948-952.
60. Toloza, A.C., Lucia, A., Zerba, E., Masuh, H. and Picollo, M.I. (2008). Interspecific hybridization of *Eucalyptus* as a potential tool to improve the bioactivity of essential oils against permethrin-resistant head lice from Argentina. Bioresource Technology, 99,7341–7347.
61. Varela-Lopez, A., Bullon, P., Giampieri, F., Jose L. and Quiles, J.L. (2015). Non-Nutrient, Naturally Occurring Phenolic Compounds with Antioxidant Activity for the Prevention and Treatment of Periodontal Diseases, *Antioxidants*, 4, 447-481.

Cite this Article: Ololade ZS and 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): 001-009, http://doi.org/10.15580/GJBS.2018.1.011818006.