

Eucalyptus camaldulensis var. *nancy* and *Eucalyptus camaldulensis* var. *petford* Seed Essential Oils: Phytochemicals and Therapeutic Potentials

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Abstract

Eucalyptus camaldulensis essential oils are aromatherapy agents in medicine. This study examined the phytochemicals and therapeutic activities of the seed essential oils of *E. camaldulensis* var. *nancy* and *E. camaldulensis* var. *petford* from Nigeria. The seed 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. GC and GC-MS analyses of the essential oils led to the identification of 23 and 15 constituents representing 99.2% and 98.5% of the oil of *E. camaldulensis* var. *nancy* and *E. camaldulensis* var. *petford*, respectively. The main components in the seed essential oils of *E. camaldulensis* var. *nancy* and *E. camaldulensis* var. *petford* was 1,8-cineole (40.8%) and (52.0%) respectively. The TPC values of the oils were 156.25 ± 0.00 and 167.93 ± 0.00 $\mu\text{g GAE/mg}$ respectively. The seed essential oils achieved optimum antioxidant potentials with IC_{50} values of $3.50 \mu\text{gml}^{-1}$. The seed essential oils have high reducing antioxidant activities, with EC_{50} values of $5.0 \mu\text{gml}^{-1}$ in the two varieties. The essential oils showed significant anti-inflammatory properties with oedema inhibitory percentages of 87.50% and 43.75% respectively and antinociceptive potential with inhibitory percentage at 70.52% and 80.52% in phase I (neurogenic), as well as 99.99% and 41.03% in phase II (anti-inflammatory). The seed essential oils showed efficient antibacterial activities with their zones of inhibitions values ranging between 13-24 mm at $1000\text{-}10 \mu\text{gml}^{-1}$. The results from this study revealed that the two seed essential oils have potentials as the therapeutic agents.

Keywords: *Eucalyptus camaldulensis*; Nancy; Petford; Seed essential oils; Chemical composition; Medicinal potentials

Introduction

Plants and their essential oils are widely used as alternative therapeutic means for the prevention or treatment of diseases [1-3]. One of the main reasons for the use of essential oils in medicine is the absence of such risks as mutagenicity, carcinogenicity and teratogenicity [4,5]. *Eucalyptus* extracts are used singly and in combination with some other plants to treat diseases [6-9]. *Eucalyptus* essential oils have been classified as non-toxic and safe [10,11]. They are used as flavouring agents and preservatives. Therefore, they are also used in a wide variety of consumer goods [12-14]. 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 the seed of these two *Eucalyptus* species so far. Hence, this present study aimed at investigating the chemical compositions and therapeutic properties of the seed essential oils of *E. camaldulensis* var. *nancy* and *E. camaldulensis* var. *petford* from Nigeria.

Experimental Methods

Plant materials and isolation of the essential oil

The seeds of the plants were collected from Kaduna, Nigeria. Fresh and air dried seeds (100 g) were subjected to hydrodistillation for 2 hours using a Clevenger-type apparatus, and the essential oils obtained were protected from light and heat by keeping it in a sealed vial and stored at 4°C to prevent evaporation [15].

GC and GC-MS analyses

The seed essential oils were analysed using Shimadzu GCMS-QP2010 Plus (Japan). The column length, diameter and thickness were (30 m \times 0.25 mm \times 0.25 μm film). 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.

Determination of Total Phenolic Content (TPC)

Total phenolic contents of the seed essential oils were determined using the Folin-Ciocalteu method. 1 ml aliquot solutions of each essential oil were mixed with 46 ml distilled water and 1 ml of Folin Ciocalteu reagent. 3 ml of 2% Na_2CO_3 solution was then added after 3 minutes and the mixture was allowed to stand for 2 hours for incubation in the dark with intermittent shaking, the absorbance of the

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reaction mixture was measured on a UV-Visible spectrophotometer at 760 nm against a blank (containing all reagents except the test sample). Gallic acid was used as a reference and for the calibration curve; results were expressed in gram of gallic acid equivalent [16].

Determination of free radical scavenging and antioxidant activities

Antioxidant activities were evaluated using two different assays; i.e., *In vitro* 2,2'-Diphenyl-1-picrylhydrazyl (DPPH) and Ferric reducing antioxidant power (FRAP).

In vitro free radical scavenging/antioxidant assay

The free radical scavenging and antioxidant activities of the seed essential oils against the stable free radical DPPH were measured. Three different concentrations (1000, 100 and 10 μgml^{-1}) of each essential oil were incubated with an absolute methanolic solution of DPPH. After 30 minutes of incubation at room temperature in the dark, the absorbance at 517 nm was measured with UV-Visible spectrophotometer. Ascorbic acid was used as the reference standard antioxidant. The percentage radical scavenging was calculated according to the formula below. The assay was carried out in triplicate and the results were expressed as mean \pm standard deviation.

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

Where: A_{blank} is the absorbance of blank solution and A_{eo} is the absorbance of the essential oil. The dose-response curves were plotted and IC_{50} value for the essential oils and the standard were calculated [17].

In vitro ferrous reduction antioxidant potential 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 the seed essential oils (1000, 100 and 10 μgml^{-1}) were 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 [$\text{K}_3\text{Fe}(\text{CN})_6$], and the resultant mixture was incubated at 50°C for 20 minutes. After addition of 2.5 ml of 10% trichloroacetic acid, the mixture was centrifuged at 3000 rpm for 10 minutes. 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. A higher absorbance value indicates higher reducing power. Ascorbic acid was used as the reference standard antioxidant. Dose response curve was plotted between the absorbance versus concentrations of the essential oils or standard. The assays were analysed in triplicate and the results were expressed as mean \pm standard deviation. Effective concentration at 50% (EC_{50}) of FRAP value is the sample concentration required to reduce Fe^{3+} to Fe^{2+} and gives an absorbance value of 0.5 [18].

Experimental animals

Healthy albino rats (200 \pm 30 g) were used for the present study. All experiments were carried out in strict compliance with the principle of laboratory animal care [19].

Anti-inflammatory activities

These were done using carrageenan induced paw oedema test model in rats. Rats of both sexes were divided into groups of 5 animals each. The test groups received 1000 μgkg^{-1} body weight, *p.o.* of the essential oils and reference group received indomethacin (1000 μgkg^{-1} , *p.o.*). After 30 minutes, 0.1 ml (1%) carrageenan suspension was

injected into the sub-planar tissue of the right hind paw. 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 each of the essential oils (1000 μgml^{-1}), using a digital vernier calliper. The difference of volumes (mm) between the basal and sequential measurements of the paw was calculated as the oedema formation.

$$\text{Anti-inflammatory activity \%} = \frac{1-D}{C} \times 100$$

Where: D represents the oedema volume after essential oil was administered to the rat and C represents the oedema volume in control group [20].

In vivo antinociceptive activities

Rats ($n=5$ per group) in cages were treated respectively with 1000 μgkg^{-1} of each seed essential oil and indomethacin. 0.05 ml (2.5%v/v) formalin was injected into the dorsal surface of the right hind paw 30 minutes after administration of the essential oils and indomethacin. The rats were observed for 30 minutes after injection of formalin and amount of time spent licking the injected paw was indicative of pain. The number of lickings from 0-5 minutes (first phase) and 15-30 minutes (second phase) of post-injection time were recorded. These phases represented neurogenic and inflammatory pain responses, respectively. The tests were 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 [21].

In vitro antimicrobial assay

The antibacterial potentials of the seed essential oils 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*, *Pseudomonas aeruginosa* and *Salmonella typhimurium*). The bacteria isolates were first sub-cultured in Nutrient agar and incubated at 37°C for 24 hours. 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 few minutes for absorption. 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 each essential oil dissolved in dimethylsulfoxide (DMSO). The plates were allowed to stand in the refrigerator for 1 hour to allow proper diffusion of the essential oils into the medium and incubated at 37°C for 24 hours before visual assessment of the inhibition zones. Antibacterial potentials of the essential oils were evaluated by measuring the clear zones of growth inhibition against the test organisms. Erythromycin (ERY) and Cefixime (CFM) were used as control [22,23].

Results and Discussion

Compositional profiles of *E. camaldulensis* var. *nancy* and *E. camaldulensis* var. *petford*

The GC and GC-MS analyses of the seed essential oils led to the identification of 23 and 15 constituents representing 99.2% and 98.5% of the total oil of *E. camaldulensis* var. *nancy* and *E. camaldulensis* var. *petford*, respectively. The compound, retention indices, percentage composition were given in Table 1, where the identified components were listed in order of their retention indices. The main components

Compound	Retention Index	Percentage Composition	
		<i>nancy</i>	<i>petford</i>
α -pinene	948	1.8	2.5
α -phellandrene	969	2.0	-
<i>trans</i> - β -ocimene	976	2.7	4.0
β -pinene	978	-	1.0
2,2,3-trimethylmethylenecyclopropane	1003	20.8	-
<i>p</i> -mentha-1,3,8-triene	1029	0.5	-
1,8-cineole	1059	40.8	52.0
α -terpineol	1150	1.0	2.0
terpinen-4-ol	1175	1.4	-
9-oxabicyclo[6.1.0]non-6-en-2-one	1280	3.6	-
<i>p</i> -mentha-[1(7),8]diene-2-hydrperoxide	1335	-	2.5
α -gurjunene	1410	1.0	-
α -farnesene	1458	-	0.5
β -selinene	1491	-	2.5
caryophyllene	1494	0.5	-
ledol	1530	1.5	1.5
1-ethylideneoctahydro-7a-methyl-1H-indene	1540	-	2.0
anozol	1551	1.5	-
germacrene D	1562	-	1.5
2-methyl-3-(3-methyl-but-2-enyl)-2-(4-methyl-pent-3-enyl)-oxetane	1577	-	13.0
n-hexadecane	1600	-	2.0
β -eudesmol	1650	0.4	1.5
aromadendrene	1668	2.4	11.0
<i>cis</i> -vaccenic acid	1687	0.6	-
n-heptadecane	1700	0.3	-
n-octadecane	1800	0.4	-
methyl-14-methylpentadecanoate	1814	2.0	-
(+)-ledene	1943	2.0	-
palmitic acid	1968	1.0	-
methyl-laidate	2085	7.0	-
methyl-linolelaidate	2093	4.0	-
Percentage Total		99.2	98.5

Table 1: Composition of the Seed Essential Oils of *E. camaldulensis* var. *nancy* and *E. camaldulensis* var. *Nancy*.

in the seed essential oil of *E. camaldulensis* var. *nancy* were 1,8-cineole (40.8%), 2,2,3-trimethylmethylenecyclopropane (20.8%) and methylelaidate (7.0%) while 1,8-cineole (52.0%), 2-methyl-3-(3-methyl-but-2-enyl)-2-(4-methyl-pent-3-enyl)-oxetane (13.0%) and aromadendrene (11.0%) were the most abundant components in the seed essential oil of *E. camaldulensis* var. *petford*. Monoterpenoids (50.2, 63.5%) constituted the major components while the proportions of sesquiterpenoids were 8.2, 31.0% in the two seed essential oils respectively, though the percentage sesquiterpenoids was very low in the seed essential oil of variety *petford* compared to *nancy*. Reviewing the existing literature, nothing was found concerning the qualitative and quantitative analyses of the seed essential oils of *E. camaldulensis* var. *nancy* and *E. camaldulensis* var. *petford*. The seed essential oil of *E. camaldulensis* var. *nancy* has a compositional profile that was like that of seed essential oil of *E. camaldulensis* var. *petford*; however, there were some minor differences. There are compositional similarities between the components of seed essential oils of *E. camaldulensis* var. *petford* and *E. camaldulensis* var. *nancy* as stated in Table 1, but both varieties contained more monoterpenoids than sesquiterpenoids. These seed essential oils have components that were like essential oils obtained from the leaf of *E. camaldulensis* [24-27]. The composition of the leaves essential oils of *E. camaldulensis* var. *petford* from Nigeria indicated the predominance of monoterpenes, with 1,8-cineole occurring as the single most abundant constituent [25]. It was reported that variation in the yield of monoterpenes 1,8-cineole, α -pinene, β -pinene and

limonene in the oil of the leaves of *E. camaldulensis* var. *petford* up to five months of age, were significantly affected not only by genotype, but also by external forces such as prolonged drought and water logging [28].

It was also reported that the main components of the leaf essential oil of *E. camaldulensis* var. *petford* were 1,8-cineole, α -pinene, β -pinene, α -terpineol, globulol, borneol, aromadendrene, eudesmol and terpinen-4-ol [29]. Leaf essential oils of *E. camaldulensis* Dehn contained *p*-cymene (68.43%), 1,8-cineole (13.92%), α -pinene (3.45%) and limonene (2.84%), 1,8-cineole (43.00%), α -pinene (5.50%), β -pinene (3.40%), *p*-cymene (5.2%), terpinen-4-ol (3.1%), and globulol (4.1%) as the main constituents [30], while [31] in another investigation reported ethanone (25.36%), 1,8-cineole (13.73%), β -caryophyllene (11.55%) and carvacrol (9.05%) as the main component of leaf essential oils of *E. camaldulensis*. The essential oil of *E. camaldulensis* obtained by supercritical carbon dioxide extraction had higher amounts of *allo*-aromadendrene and globulol, but has lower quantity of 1,8-cineole, α -pinene, β -pinene, and terpinen-4-ol [27]. Two groups of *E. camaldulensis* essential oils have been distinguished: those that contain 1,8-cineole as the main compound [32], and those that contain spathulenol, *p*-cymene and cryptone as main compounds and small quantities of 1,8-cineole [33]. Therefore, these seed essential oils of *E. camaldulensis* from Nigeria could be classified in the chemotype with high 1,8-cineole.

Total Phenolic Contents (TPC) in the essential oils

The TPC of *E. camaldulensis* var. *nancy* and *E. camaldulensis* var. *petford* were 156.25 ± 0.00 and 167.93 ± 0.00 $\mu\text{g GAE/mg}$ respectively. The results demonstrated significant levels of phenolic compounds of the two essential oils. The seed essential oils gave higher TPC when compared with the previous studies on the related species such as the *E. gillii* extracts which gave the following TPC values: ethanol extract ($143.4 \text{ GAE gKg}^{-1}$), methanol extract ($143.2 \text{ GAE gKg}^{-1}$), acetone ($53.7 \text{ GAE gKg}^{-1}$) and petroleum ether extract (4.5 GAE gKg^{-1}) [34]. Phenolic compounds contribute to good quality, nutritional value and health beneficial effects of plants. They also serve as defence mechanisms to counteract reactive oxygen species [35]. Natural phenolic compounds play an important role in disease prevention and treatment. Various therapeutic activities of phenolic compounds are responsible for their chemopreventive properties and regulating carcinogen metabolism and ontogenesis expression, inhibiting DNA binding and cell adhesion, migration, proliferation or differentiation, and blocking signaling pathways [36].

In vitro free radical scavenging and antioxidant activities

The percentage free radical inhibitions of the two seed essential oils at various concentrations (1000, 100 and $10 \mu\text{gml}^{-1}$) were 90.67 ± 0.00 , 79.11 ± 0.00 , $68.53 \pm 0.00\%$ respectively for variety *nancy* and 79.50 ± 0.03 , 60.05 ± 0.00 and $60.00 \pm 0.00\%$ respectively for variety *petford*; while the ascorbic acid gave 96 ± 0.00 , 69 ± 0.00 and $54 \pm 0.00\%$ as the percentage inhibitions with IC_{50} value of $9.00 \mu\text{gml}^{-1}$. The two seed essential oils achieved optimum values of radical scavenging with lower IC_{50} value of $3.50 \mu\text{gml}^{-1}$, which signified higher efficiency as free radical scavenger and antioxidant than ascorbic acid with higher value IC_{50} of $9.00 \mu\text{gml}^{-1}$ (Table 2), which were two and half folds (61%) higher in antioxidant activities in comparison with ascorbic acid. From the result of IC_{50} values, the seed essential oil of this *Eucalyptus* species investigated at very low concentrations were more effective than leaf essential oil of other related species such as *E. oleosa* with $\text{IC}_{50} > 1000$ [37]. The presence of some major terpenoids, besides phenolic compounds in the seed essential oils were suggested to contribute to their high antioxidant properties, compared with ascorbic acid, which contained a single compound.

In vitro ferric reduction and antioxidant potentials (FRAP)

The seed essential oils have significantly high reduction antioxidant activities ($5.0 \mu\text{gml}^{-1}$) in the two varieties of *Eucalyptus* investigated than ascorbic acid ($11.0 \mu\text{gml}^{-1}$) (Table 3). The seed essential oils have about two times higher reducing ion potentials than ascorbic acid. It was observed that the essential oils of the two varieties showed higher iron (III) reducing power than the synthetic antioxidant. Antioxidant activities from the FRAP assay of these essential oils were in close agreement with the total phenolics. The seed essential oils investigated were more effective than the leaf essential oil of *E. sideroxylon* with FRAP antioxidant potential as $130.5 \mu\text{M}$ at concentration of $50 \mu\text{l}$ [38]. The presence of terpenoids and phenolic compounds in the essential oils contributed to their higher FRAP values than that of ascorbic acid since these compounds are able to chelate with Fe^{2+} ion. Phenolics with ion reducing ability diminish the possibility of hydroxyl radical's formation path from superoxide anion radicals and additionally inhibit enzymes due to their abilities to chelate metal at the active site [39]. Therefore, reducing power indicates compounds that are electron donors, which can act as primary and secondary antioxidants. The ability to reduce Fe^{3+} may be attributed to hydrogen donation from phenolic compound which is also related to the presence of reducing agent. In addition, the

number and position of hydroxyl group of phenolic compounds also contributed to their antioxidant activities [40,41].

In vivo anti-inflammatory properties

The potentials of the seed essential oils investigated in acute inflammations were in the following order: *E. camaldulensis* var. *nancy* (87.50%) > *E. camaldulensis* var. *petford* (43.75%); while indomethacin (reference drug) was observed to give anti-inflammatory power value of 93.75% (Table 4). The seed essential oil of variety *nancy* investigated was more effective than the leaf essential oil of *E. globulus* at concentration of 100 mgkg^{-1} which caused inhibition of inflammation by 76% [42]. The results obtained from the carrageenan-induced paw oedema showed that paw oedema was markedly inhibited by the oral administration of the seed essential oils of the two varieties of *E. camaldulensis* in dose-response relationship. The effect observed, which was time-dependent, lasted for at least 4 h with the doses. The inflammatory response is usually quantified by increase in paw size (oedema) which is maximal around 5 h post-carrageenan injections. The inhibitory values of oedema indicated that the essential oils were orally active at doses used and can inhibit acute inflammatory processes. Phytochemicals have been shown to modulate various points in these inflammatory processes. These modulations serve as controlling points where the amplification of the inflammatory processes can be disconnected and thereby reduce subsequent diseases risk [43].

In vivo antinociceptive properties

The seed essential oils investigated displayed strong antinociceptive activities; the effects of essential oils were particularly noteworthy (Table 5). The trend of the antinociceptive potentials in the first (neurogenic) phase is: *E. camaldulensis* var. *petford* (80.52%) > *E. camaldulensis* var. *nancy* (70.52%); while in the second (inflammatory) phase the order is *E. camaldulensis* var. *nancy* (99.99%) > *E. camaldulensis* var. *petford* (41.03%). The seed essential oils investigated were more effective than the leaf essential oil of *E. globulus* at concentrations of 100 mgkg^{-1} which caused inhibition of neurogenic pain by 53% respectively [42]. The formalin test is different from most models of pain, as it can assess the way animals respond to moderate, continuous pain generated in injured tissue. The test provides a more valid and reliable model for clinical pain than other tests of nociception. It is a very useful method not only for assessing antinociceptives, but also for elucidating the mechanism of pain and analgesia, whether the site of action is central and/or peripheral [44,45]. The formalin test consists of two distinct phases, possibly reflecting different types of pain. The early (neurogenic) phase is due to direct chemical stimulation of nociceptors. This phase can be inhibited by centrally acting antinociceptives. The late (inflammatory) phase seems to be due to the combination of an inflammatory response in the peripheral tissue, partly mediated by prostaglandins, and functional changes in the dorsal horn of the spinal cord that are initiated by a C fiber barrage during the early phase. This phase can be inhibited by non-steroidal, anti-inflammatory drugs (NSAIDs) and steroids, as well as centrally acting drugs. Experimental results have indicated that histamine, serotonin and bradykinin are also involved in the late phase [46].

Antimicrobial properties

The antimicrobial activities of the two seed essential oils of *E. camaldulensis* varieties against *E. coli*, *K. pneumoniae*, *P. aeruginosa*, *S. typhimurium*, *S. aureus* and *S. agalactiae* were shown in Table 6. The seed essential oils showed variable activities against tested bacteria. The highest inhibitory effect of the seed essential oil of *E. camaldulensis* var.

Essential Oils and Reference Compound	DPPH IC ₅₀ µgml ⁻¹
<i>E. camaldulensis</i> var. <i>nancy</i>	3.5
<i>E. camaldulensis</i> var. <i>petford</i>	3.5
Ascorbic acid	9.0

Table 2: IC₅₀ of the DPPH Antioxidant Properties of the Seed Essential Oils and Reference Drug.

Essential Oils and Reference Drug	EC ₅₀ µgml ⁻¹
<i>E. camaldulensis</i> var. <i>nancy</i>	5.00
<i>E. camaldulensis</i> var. <i>petford</i>	5.00
Ascorbic acid	11.00

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

Table 3: Reduction Power of the Seed Essential Oils and Reference Drug.

Essential Oils and Reference Drug	% I (2 Hr)	% I (4 Hr)	Mean % I
<i>E. camaldulensis</i> var. <i>nancy</i>	87.50	87.50	87.50
<i>E. camaldulensis</i> var. <i>petford</i>	37.50	50.00	43.75
Indomethacin	87.50	99.65	93.70

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

Table 4: *In vivo* Anti-inflammatory Activities of the Seed Essential Oils of *Eucalyptus*.

Essential Oils and Reference Drug	Time of Licking and Biting Percentage Inhibition			
	Early Phase (0-5) min.	% Inhibition	Late Phase (5-30) min.	% Inhibition
<i>E. camaldulensis</i> var. <i>nancy</i>	28.30	70.52	0.00	99.90
<i>E. camaldulensis</i> var. <i>petford</i>	18.70	80.52	69.00	41.03
Indomethacin	34.33	64.23	53.00	54.70

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

Table 5: *In vivo* Antinociceptive Activities of the Seed *Eucalyptus* Essential Oils.

Conc. (µg.ml ⁻¹)	Seed Essential Oils						ERY	CFM
	<i>nancy</i>			<i>petford</i>				
	1000	100	10	1000	100	10	10 µg	30 µg
<i>E. coli</i>	18	18	14	10	10	10	22	-
<i>K. pneumoniae</i>	19	17	17	24	23	19	21	07
<i>P. aeruginosa</i>	20	18	18	13	13	13	20	10
<i>S. agalactiae</i>	-	-	-	15	10	-	-	-
<i>S. aureus</i>	20	13	12	20	20	20	-	-
<i>S. typhimurium</i>	-	-	-	-	-	-	21	-

Key note: - : Resistant; 6-9 mm: Low inhibition; 10-14 mm: Moderate inhibition; ≥ 15 mm: High inhibition

Table 6: Zones of Inhibition (mm) showing the Antimicrobial Properties of the Seed Essential oils of the two varieties of *E. camaldulensis*.

nancy was observed against *P. aeruginosa* (20 mm) and *S. aureus* (20 mm), followed by *K. pneumoniae* (19 mm), *E. coli* (18 mm) but resistant to *S. agalactiae* and *S. typhimurium* while the inhibitory effects of *E. camaldulensis* var. *petford* were observed against *K. pneumoniae* (24 mm) followed by *S. aureus* (20 mm), *S. agalactiae* (15 mm), *P. aeruginosa* (13 mm) and *E. coli* (10 mm) but resistant to *S. typhimurium*. The tested bacteria were found to be resistant to the synthetic antibiotics used. The antibacterial properties of the two seed essential oils from Nigeria showed higher activities than that of the leaves essential oils of five species of the *Eucalyptus* from Nigeria (*Eucalyptus alba*, *E. deglupta*, *E. saligna*, *E. camaldulensis* var. *catharine*, *E. camaldulensis* var. *mysore*) which gave lower inhibitions against *B. cereus*, *E. coli*, *P. aeruginosa*, *S. aureus* at the range between 9.0-14.0 mm, except for *E. coli* (16-24 mm) [47]. It was reported that the antimicrobial actions of terpenoids are due to the fact that they diffuse into the cell membrane structures and damage them [48,49]. The antimicrobial properties of such molecules

also depend on their presence in gaseous form which facilitates their solubilization in cell membranes [50]. Globally, antibacterial resistance is becoming an increasing public health problem. A variety of reasons, including inappropriate and excessive use of synthetic antibiotics, has led to the emergence of pathogenic bacteria strains that are highly resistant to most antibiotics. Thus, there is a significant need for discovery of new types of antimicrobials from natural source to treat infections and diseases caused by resistant organisms [51,52].

Conclusion

This study revealed that the essential oils from seeds of the two varieties of *E. camaldulensis* showed qualitative differences in respect to identified components. The overall results demonstrate that the seed essential oils possess secondary metabolites that could be very useful for human, animals and industries. Therefore, these essential oils could be considered as promising substances for development of new drugs.

Conflict of Interest

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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