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Chemistry and Medicinal Potentials of the Seed Essential Oil of *Eucalyptus Toreliana* F. Muell Grown in Nigeria

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Abstract - E. toreliana is a distinct aromatic plant with several medicinal applications to cure many ailments. Chemistry and medicinal potentials of the seed essential oil of Eucalyptus toreliana F. Muell grown in Nigeria were examined in this study. The phytochemical composition of the seed essential oil was evaluated using multidimensional GCxGC-MS, MS and FT-IR. The seed oil was also investigated for its total phenolic content, antioxidant and acute toxicity, anti-inflammatory and antinociceptive potentials. Analyses of the seed essential oil extract resulted in the identification of 70 compounds representing 98.53 % of the oil. α-Pinene (16.0%), Copaene (10.0%), 1R-α-Pinene (8.0%), DL-Pinene (8.0%), β-trans-Ocimene (5.0%), α-Bisabolol oxide B (5.0%), Oleamide (5.0%) and Globulol (4.0%) were detected as the major components accounted for 65% of the oil. The total phenolics content of the seed oil of E. toreliana was estimated as 191.68±0.0006 μgmg^{-1} GAE. The results of DPPH and FRAP antioxidant showed that the oil possessed strong free radical scavenging and reducing potentials with IC_{50} 9.0 μgml^{-1} each in both methods. No sign of toxicity was noticed in the rats which indicate that the seed oil was relatively non-toxic and safe.

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Abstract - *E. toreliana is* a distinct aromatic plant with several medicinal applications to cure many ailments. Chemistry and medicinal potentials of the seed essential oil of *Eucalyptus toreliana* F. Muell grown in Nigeria were examined in this study. The phytochemical composition of the seed essential oil was evaluated using multidimensional GCxGC-MS, MS and FT-IR. The seed oil was also investigated for its total phenolic content, antioxidant and acute toxicity, anti-inflammatory and antinociceptive potentials. Analyses of the seed essential oil extract resulted in the identification of 70 compounds representing 98.53 % of the oil. *α*-Pinene (16.0%), Copaene (10.0%), 1R-*α*-Pinene (8.0%), DL-Pinene (8.0%), *β-trans*-Ocimene (5.0%), *α*-Bisabolol oxide B (5.0%), Oleamide (5.0%) and Globulol (4.0%) were detected as the major components accounted for 65% of the oil. The total phenolics content of the seed oil of *E. toreliana* was estimated as 191.68±0.0006 μgmg^{-1} GAE. The results of DPPH and FRAP antioxidant showed that the oil possessed strong free radical scavenging and reducing potentials with IC_{50} 9.0 μgml^{-1} each in both methods. No sign of toxicity was noticed in the rats which indicate that the seed oil was relatively non-toxic and safe. The oil at 1000 μgkg^{-1} (*p.o.*) gave 99.61% significant inhibition of paw edema. In the antinociceptive assay the oil inhibited the licking time by 88.69% and 75.90% in first phase (neurogenic pain) and second phase (inflammatory pain) respectively. These results showed that the seed essential oil of *E. toreliana* possesses antioxidant, anti-inflammatory and antinociceptive potentials, which provided an initial scientific validation of the seed essential oil as a phytotherapeutic agent against reactive oxidative, nociceptive and inflammatory processes.

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I. Introduction

Eucalyptus toreliana (*Myrtaceae*) is a tall evergreen and a dense shade plant with an irregular crown, a very hard tree with smooth, tight and grey-green bark with persistent scaly, sub-fibrous base and tessellated. The leaves has a simple, leathery, variable but usually ovate, wavy margin, green above or with a pink tint, generally pubescent when young and with a wider leaf than other *Eucalyptus.* It processes attractive flowers with large creamy white clusters and numerous stamens, the creamy fruit is large and ovoid shape and with valves well below rim of the fruit.

Globally, there is a rapid increase in screening of plants that can lead to the discovery and development of novel therapeutics. Plants from different continents have shown considerable pharmacological activities such as antioxidant, antimicrobial, anti-inflammatory, anticancer, antiviral, anti-allergic and vasodilatory properties (Rustaiyan *et al.,* 2011; Newman and Cragg, 2007).

The leaf essential oil of *E. toreliana* has been used in the treatment of lung diseases and was shown to have anti-tubercular properties (Alain *et al.*, 2012). The extracts of the leaf and stem of the plant were reported to have antibacterial and gastroprotective properties, it inhibits the growth of *Helicobacter pylori* (Adeniyi *et al.,* 2006). The leaves extracts of the plant is applied over wounds and ulcers, also used to treat gastrointestinal disorders, they decrease gastric acid production and used for the treatment of gastric ulcers, cough associated with most pulmonary diseases and medically importance for the treatment of infections caused by the non-tuberculous mycobacteria (Lawal *et al.*, 2011). The plant is also used locally in the treatment bacterial infections of the urinary tracts, respiratory tracts, inflammation of the mucous membranes and sore throat (Farah *et al.,* 2002).

Moreover, *Eucalyptus* essential oils has long history of safe use in food preservation, pharmaceuticals, phytotherapies, pesticides and have attracted extra attentions for more intensive studies (Tepe *et al.*, 2004). It was also reported that *Eucalyptus* leaf essential oil had a direct effect on the coxsakievirus B3 and ethno-pharmacologically been used to treat respiratory tract disorders such as pharyngitis, bronchitis, and sinusitis (Elaissi *et al.,* 2012).

To best our knowledge, no literature on the chemistry, phenolic content, antioxidant, anti-inflammatory and antinociceptive potentials of the seed essential oil of *E. toreliana* have been reported so far. The present research was therefore undertaken for the first time with the main objective to isolate and characterize the seed essential oils of *E. toreliana* cultivated in Nigeria for their detailed chemical constituents, pharmacological properties.

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II. Materials and Methods

a) Plant Material

Seed of *E. toreliana* were collected from Ogbomoso, Nigeria. The plant was authenticated at the Forest Research Institute of Nigeria (FRIN), Ibadan, Nigeria.

b) Extraction of the Essential Oil

Seed of *E. toreliana* was air-dried in a well ventilation place till when the moisture content reduced to a minimum suitable for grinding; the plant material was pulverized and used immediately. The crushed plant material (100 g) was subjected to hydrodistillation for 2.5 hours using a Clevenger-type apparatus (European Pharmacopoeia, 2004). The oil collected was stored in vial at low temperature.

c) Instrumentation and Analytical Conditions

i. *Multi-Dimensional GCxGC-MS Analysis*

Analysis of the seed essential oils of *E. toreliana* was performed using multi-dimensional gas chromatograph coupled with Gas Chromatography-Mass Spectrophotometer (Shimadzu, Japan) equipped with double capillary columns (25.0 m x 0.25 μm i.d., 0.25 μm df) that have different characteristics (non-polar and polar). High purity helium was used as the carrier gas at a constant flow rate of 0.99 ml/min. A total of 1 μl sample was injected (split ratio 100:1) into GCxGCMS using AOC20i auto injector for analysis. The initial temperature was set at 60 °C, heated at a rate of 3℃/minutes to 280℃ and held isothermally for 6 minutes. Ion source temperature for these analyses was set at 200℃, while the interface temperature was set at 250℃, solvent cut time was 3.0 minutes and the mass spectrometer was set to operate in electron ionization mode with an ionizing energy of 70 eV as acquisition mass range from 40-700 a.m.u. at 0.50 scan/s.

ii. *Mass Spectra Data Analysis*

MS parameters were as follows: EI mode, with ionization voltage 70 eV, ion source temperature, 180 °C. The mass spectra were generally recorded over 40-700 amu that revealed the total ion current (TIC) chromatograms. The MS fragmentation pattern was compared with those of pure compound, by matching the MS fragmentation patterns with NIST mass spectra libraries and with those given in literature.

iii. *Identification through Comparison with Reference Standards*

Identification of phytochemical organic constituents in the seed essential oil was confirmed using published electron impact-mass spectra (EI-MS) in the NIST and Shimadzu's Flavours and Fragrance of Natural and Synthetic Compounds (FFNSC) and published spectral data. The retention indices were determined based on a homologous series of *n*-alkanes of *n*-alkanes internal standard analyzed under the same operating conditions and calibrated based on the Automatic Adjustment of Compound Retention Time (AART) function of the GC-MS. Relative concentrations of the essential oil components were calculated based on GC peak area.

iv. *Fourier-Transform Infra-Red (FT-IR) Analysis*

The IR spectra in KBr pellets were recorded using a spectrophotometer. 0.25 μl of the seed essential oil was deposited in the middle of a KBr pellet and the IR spectrum was recorded at different times. The FT-IR conditions were: 4 cm^{-1} spectral resolution, 20 kHz scan speed, 128 scan co-additions and scanning range 400-4000 cm^{-1}.

d) Total Phenolic Content (TP) and Antioxidant Capacity

Total phenolic content of the seed oil of *E. toreliana* was analysed by the Folin-Ciocalteu method (Wua and Ng, 2008). A solution of the seed oil (0.2 ml) containing 1000 μgml^{-1} of the oil in methanol was pipetted into a 50 ml volumetric flask, 46 ml distilled water and 1ml Folin-Ciocalteu's phenol reagent were added, and the opaque flask was thoroughly shaken. After 3 minutes, 3 ml of (2% w/v) Na_2CO_3 solution was added and the mixture was allowed to stand for 2 hours for incubation in dark with intermittent shaking. Absorbance values of the clear supernatants were measured at 760 nm against a blank (0.5 ml Folin-Ciocalteu's reagent + 1 ml Na_2CO_3) on UV-Visible spectrophotometer. The same procedure was repeated for all the standard gallic acid solutions (0-1000 μg/0.1 ml) and a standard curve obtained with the following equation:

$$\text{Absorbance} = 0.0008 \times \text{gallic acid } (\mu g) + 0.0068$$

Calculation of percentage total phenols content was based on Gallic Acid Equivalents (GAE).

e) Pharmacological Assays

i. *In vitro DPPH Free Radical Scavenging Assay*

The antioxidant activity of the seed oil extract was measured using the stable radical 2,2´-diphenyl-1-picrylhydrazyl (DPPH). 1.0 ml of the seed *E. toreliana* essential oils (10, 100 and 1000 μgml^{-1}) in methanol was added to 1.0 ml of a 0.004% w/v methanol solution of DPPH. The mixture was shaken vigorously and the absorbance was monitored at 517 nm after 30 minutes of incubation, when the reaction reached a steady state. Ascorbic acid was used as reference compound. Assays were carried out in triplicate. The inhibition percentage (%) of radical scavenging activity was calculated by using following formula (Ololade *et al.*, 2012).

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

Where A_{blank} is the absorbance value of the control reaction (containing all reagents except the test compound) and A_{sample} is the absorbance values of the test compounds.

ii. *In vitro Ferrous Reducing Power Assay (FRAP)*

The reducing power of the seed oil was determined by the method of (Saeed *et al*., 2012). An aliquot of the sample (1.0 ml) at various concentrations (10, 100 and 1000 μgml^{-1}) were mixed with phosphate buffer (0.2 M, pH 6.6, 2.5 ml) and 1% potassium ferricyanide (2.5 ml). The mixture was incubated at 50℃ for 20 minutes. After adding 10% Trichloroacetic acid (2.5 ml), the mixture was centrifuged at 1000 rpm for 10 minutes. The supernatant (2.5 ml) was mixed with distilled water (2.5 ml) and 0.1% $FeCl_3$ (0.5 ml) and the absorbance was measured at 700 nm using an appropriate blank. Assays were carried out in triplicate. Ascorbic acid was used as a reference. The average values were plotted to obtain the half maximum effective concentration (EC_{50}) of Fe^{3+} reduction.

iii. *In vivo Anti-inflammatory Assay*

Healthy rats (200 ±30 g) acclimatized to laboratory hygienic conditions were housed in polycarbonated clean cages under standard conditions of temperature (25 ± 2^0C) and RH was % 55-60, 12 hours light/dark cycle were maintained in the quarantine and were fed with standard pellet diet and water *ad libitum*. The handling and uses of animals were in accordance to the institutional guidelines. The *in vivo* toxicity of seed essential oil *E. toreliana* was also observed during and after the experiment (Santin *et al*., 2011).

In vivo antiinflammatory assay of the seed oil of *E. toreliana* was studied in rat paw edema. The rats of were divided into three groups of five animals each and the rats were fasted for 12 hours in order to avoid food interference with substance absorption, ensure uniform hydration and minimize variability in edematous response. 1% carrageenan (0.1 ml) was injected into the plantar surface of the rat hind paw 30 minutes after oral administration of the test compounds or vehicle. Indomethacin (25 $mgkg^{-1}$) was used as reference drug. Paw volume was determined immediately after the injection of the phlogistic agent and again 2 and 4 hours later by means of a digital vernier calliper. The anti-inflammatory activity of the seed oil was expressed as the percentage of inhibition calculated from the difference between the responses of the treated and the control groups. The inhibition percentage of the inflammatory reaction which was calculated by the formula given in equation below was determined for each rat by the comparison of each group with controls (Sousa *et al.,* 2010).

$$I\ \% = 1-(dt/dc) \times 100$$

Where:

I % = Percentage inhibition

'd*t*' is the difference in paw volume in the drug-treated group and 'd*c*' is the difference in paw volume in control group.

iv. *In vivo Antinociceptive Assay*

In vivo antinociceptive activity of the seed oil of *E. toreliana* was studied in rats according to (Ouedraogo *et al*., 2011). The rats of were divided into three groups of five animals each and the rats were fasted for 12 hours in order to avoid food interference with substance absorption, ensure uniform hydration and minimize variability in response. The rats were treated respectively with 1000 μgkg^{-1} of *E. toreliana* seed essential oil or indomethacin. Thirty minutes later, the pain was induced by injecting 0.05ml 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 amount of time spent in licking the injected paw was monitored and was considered as an indicative of pain and frequency of the injected paw were recorded for 30 minutes. The number of lickings from 0-5 minutes (first phase) and 15-30 minutes (second phase) were counted after injection of formalin.

The percentage inhibition (I) was calculated accordingly.

III. Results and Discussion

a) Identification and Quantification of the Essential Oil

Exhaustive hydrodistillation of the seed of *E. toreliana* of afforded light-cream coloured oil with 1.30 % v/w yield per 100g of dried seed sample and possessed a distinct sharp aromatic scent. The analyses of the seed essential oil were carried out using GCxGC-MS, MS and FT-IR systems. The percentage composition and retention index are given in Table 1. Seventy compounds were identified from the seed essential oil of *E. toreliana* amounting to 98.53 % of the oil. The seed oil was dominated by *α*-Pinene (16.0%), Copaene (10.0%), 1R-*α*-Pinene (8.0%), DL-Pinene (8.0%), *β-trans*-Ocimene (5.0%), *α*-Bisabolol oxide B (5.0%), Oleamide (5.0%), 2-fluoro-*β*-3,4-trihydroxy-N-isopropyl-Benzeneethanamine (4.0%) and Globulol (4.0%). None of these principal compounds has ever been detected in the leaves extracts of *E. toreliana* that had been investigated before except *α*-Pinene. Monoterpenes (43.40%) dominated the seed essential oil, because of the remarkable proportions of Pinene derivatives. The percentage composition of monoterpenoid was very low (3.50%), while the level of sesquiterpene was relatively high (20.70%), sesquiterpenoids constituted (10.50%), but few diterpenoids (2.30%) were also available in the seed oil of *E. toreliana*. We also identified some new phytocompounds which are not reported in the previous study on the leaf essential oil of this plant. Most of the principal components present in the seed oil were not available in the leaf oils and many components identified in the current study have not been previously identified in the leaf oil of the same species. Apart from the main compounds, other newly identified

phytochemicals in the seed oil of *E. toreliana* are: compound 1, 3, 4, 12, 13, 14, 15, 16, 21, 25, 26, 27, 29, 30, 31, 40, 49, 59, 60, 61, 62, 63, 64 and 65.

The result shows that the percentage of eucalyptol is lower in the seed oil *E. toreliana* compared to the leaf oil, but more components were present in the seed oil investigated than in the leaf oil results presented in the literatures. 45 and 41 components were detected in the leaf oils by Ogunwande *et al.*, 2011; Coffi *et al.*, 2012 respectively.

As shown in Table 1, the main constituents of *E. toreliana* seed oil were different from that of the same species investigated in the leaves from Nigeria (30%, 4.2%) (Ogunwande *et al.*, 2011), Ethiopia (44%, 7%) (Dagne *et al.*, 2000), Congo-Brazzaville (78%, 1%) (Loumouamou *et al.*, 2009), Brazil (40%, 55%; 24%, 7%), Morocco (14%, 64%) (Coffi *et al.*, 2012), Republic of Benin (38, 18 and 14%) (Alain *et al.,* 2012; Sohounhloue *et al.*, 1996), Mali (Chalchat *et al.*, 2000).

From the mass spectrometry analysis, Compound **5, 7** and **8** are pinene derivatives, they are bicyclic monoterpenes with molecular ion peak 136, the relatively low abundance of the molecular ion peak is consistent with the view that the molecular structure of the compound is crowded; the base peak m/z 93 corresponds to the loss of 43 mass units and relatively abundance of the ion m/z 41 is about one quarter of the base peak. A point of distinction between the isomers arises from the abundance of the ion m/z 29 and 39 in 1S-α-pinene which is not feature in α-pinene and 1R-α-pinene. The failure to detect the isopropyl ion strengthens that the loss of 43 mass units is not an entity. Therefore, the groups elided may be obtained by the breaking of two tertiary bonds with the removal of or concomitant hydrogen migration. The occurrence of gem dimethyl group as a part of ring system is common feature of many monoterpenes. Compound **10** a monocyclic monoterpene, with molecular formula of $C_{10}H_{16}$ (m/z 136) is β-*trans*-Ocimene, m/z 43 ($C_3H_7^+$) which was due to the detachment of isopropyl [$(H_3C)_2$ C-] group attached to the quaternary carbon of the compound and weak bond is broken to give a fragment at m/z 93 as the base peak. Compound **24** was obtained as Copaene a bicyclic sesquiterpene with molecular formula of $C_{15}H_{24}$ (m/z 204) and base peak at m/z 161 due to loss of m/z 43 ($C_3H_7^+$) which occurred as a result of propyl fragment from compound, other prominent peaks observed in Copaene occurred at m/z 93, 104 and 120. Compound 34 is Globulol, a tricyclic oxygenated sesquiterpenoid of alkanol family, the prominent peaks observed in this compound are m/z 43 ($C_3H_7^+$) which occurred due to detachment of propyl fragment from the compound and m/z 41 ($C_3H_5^+$) while molecular ion peak is m/z 222. Compound **54** also a bicyclic oxygenated sesquiterpenoid with molecular formula $C_{15}H_{26}O_2$ is α-Bisabolol oxide B, m/z 238 as molecular ion peak, the molecular ion peak of the mass spectrum indicates that there are no heteroatoms in the molecule other than oxygen, the even m/z indicates absence of nitrogen atom and that the compound has a molecular weight of 238amu. Base peak was observed at m/z 43 ($C_3H_7^+$) in the compound, which is due to the formation of $(H_3C)_2C^+H$ or $CH_3C{=}O$, this is due to α-cleavage at C_3 of the compound. Other prominent peaks are m/z 41due to ($C_3H_5^+$) or $((H_3C)_2{=}C^+$, m/z 59 which is due to the formation of $(H_3C)_2{}^+COH$, fragmentation of weak bond at tertiary carbon atom and cleavage of C-C bond next to hetero atom with the elimination of largest group of the molecule confirms that the compound is tertiary alkanol, m/z 70 calculated for C_5H_{11} (m/z 71) due to formation of pentenyl fragment from compound. m/z 161 which is due to the loss of propyl [$(H_3C)_2$C-] group attached to the quaternary carbon of the compound and weak bond is broken to give a fragment at m/z 43.

The FT-IR spectra of the seed oil of *E. toreliana* revealed some prominent peaks especially in the regions around 3700-2933 cm^{-1}, 2300-1447 $cm^{-1}$and 1440-1090 cm^{-1} the oil showed a peak. The band at 3500-3400 cm^{-1} was due OH stretching vibration, 3600 (sharp) was due to unassociated OH, while 3400 cm^{-1} (broad) was due to associated (hydrogen bonded) OH; both bands frequency present alkanol spectra; bands at 3400-3200 are due to N-H stretching vibrations, 3400 (sharp) was due to free N-H, while 3200 cm^{-1} (broad) was due to associated N-H; Peaks at 1750 and 1447 cm^{-1} were attributed to >C=O stretch and -C=C- stretch and can be used as an indicative for the presence of unsaturated bonds in the oil. These functional groups detected by FT-IR are futures of the compounds found in the seed essential oil.

Table 1 : Chemical Compositions of the Seed Essential Oil of *Eucalyptus toreliana*

Compounds	Percentage Composition	Retention Index
3-Methylene-1,7-octadiene	0.3	863
D-Sabinene	0.4	897
9-Oxabicyclo[6.1.0]non-6-en-2-one	2.0	908
1R-α-pinene	8.0	937
1S-α-pinene	0.4	941

1R-α-pinene	8.0	937
1S-α-pinene	0.4	941
DL-pinene	8.0	943
α-pinene	16.0	948
β-pinene	2.0	970
trans-β-Ocimene	8.0	976
L-β-pinene	0.5	978
Ethyl-2,2,3,3-tetramethylcyclopropanecarboxylate	0.3	1002
2-fluoro-β-3,4-trihydroxy-N-isopropylbenzeneethanamine	4.0	1100
5-Methylsulfanyl-2H-[1,2,4]-triazol-3-ylthiophen-2-ylmethyla- mine	0.3	1102
1,5-Dimethyl-1,5-cyclooctadiene	0.3	1103
3,7-dimethyl-(*Z*)-1,3,6-Octatriene	0.1	1029
Limonene epoxide	0.3	1031
D-*trans*-Limonene oxide	0.4	1039
cis-Sabinenhydrate	0.6	1041
Eucalyptol	1.0	1059
DL-Lavandulol, trifluoroacetate	0.2	1124
4-Caranol	0.5	1125
p-Menth-8-en-2-ol	0.5	1196
Cycloisolongifolene	0.5	1197
Copaene	10.0	1221
2*E*-1-Methoxy-3,7-dimethylocta-2,6-diene	0.3	1222
2-Methylene-4,8,8-trimethyl-4-vinylbicyclo[5.2.0]nonane	0.5	1301
4,8-dimethyl-3,7-Nonadien-2-ol	1.0	1329
α-Cubebene	1.0	1344
4,4-Dimethyl-1-[2*E*]-2,7-octadienyl]-1-cyclobutene	0.2	1380
2-Bromotetradecane	0.2	1401
5*E*,9*E*-12-Methyl-1,5,9,11-tridecatetraene	0.2	1404
β-*trans*-Caryophyllene	0.2	1418
Caryophyllene	1.0	1428
5-Phenylnonane	0.4	1437
allo-aromadendrene	1.0	1445
α-Selinene	0.2	1474
γ-Gurjunene	0.5	1476
β-*cis*-Caryophyllene	0.2	1494
α-Farnesene	0.4	1496
1-Methylene-2b-hydroxymethyl-3,3-dimethyl-4b-3-methylbut-2-enylcyclohexane	0.3	1501
α-Bulnesene	1.0	1508
[s-*E*,*E*]-Germacrene D	0.5	1515
(+)-δ-Cadinene	2.0	1517
Ledane	0.4	1530
α-Muurolene	0.5	1546
L-Globulol	0.5	1578
(+)-Viridiflorol	0.4	1587
Globulol	4.0	1591
2-Ethyl-2-methyl-tridecanol	0.3	1601
D-Dihydrocarveol	0.6	1611
5-phenyldecane	0.3	1633
trans-Longipinocarveol	0.3	1634
3-Phenylundecane	0.3	1646
α-Bisabolol oxide B	5.0	1655
n-Heptadecane	0.2	1700
6-Phenyldodecane	0.1	1718
7-Phenyltridecane	0.03	1818
1-pentyloctylbenzene	0.2	1829
2*E*,6*E*-Farnesyl acetate	0.4	1843
4,6-Diisopropylidene-8,8-dimethylbicyclo[5.1.0]octan-2-one	0.4	1883

Z-5,17-Octadecadien-1-ol acetate	1.0	2082
Sulfurous acid, cyclohexylmethyltetradecylester	0.3	2100
Farnesyl-β-D-Mannofuranoside	0.2	2102
Geranylgeraniol	0.3	2201
Oleamide	5.0	2397
n-Heptacosane	0.2	2700
n-Octacosane	0.2	2800
n-Hentriacontane	0.2	3100
Percentage Total	98.53	

b) Phenolic Content and Antioxidant Property

The seed essential oil of *E. toreliana* is highly rich in phenoloids. Based on the absorbance value of the seed oils solution reacting with Folin-Ciocalteu phenol reagent and compared with the absorbance values of standard solutions of Gallic acid, total phenolics content of the seed oil of *E. toreliana* was estimated as 191.68±0.0006 μgmg^{-1} of GAE. This might be due to the presence of low molecular mass phenolic compound like 2-fluoro-β-3,4-trihydroxy-N-isopropyl-Benzeneethanamine (4.0%) as revealed by GCxGC-MS and others which might be available among other unidentified compounds in the essential oil. The Folin-Ciocalteu assay relies on the transfer of electrons in alkaline medium from phenolic compounds to phosphomolybdic or phosphotungstic acid complexes to form blue complexes.

Folin-Ciocalteu measured both total phenolics and antioxidation strength base on the nature of its chemistry, to prevent inhibitory effects due to the oxidants competing with Folin-Ciocalteu reagent and/or air oxidation after the sample is made alkaline, the Folin-Ciocalteu reagent is added before the alkali (Singleton *et al.*, 1999).

c) Pharmacological Activities

i. *In vitro Antioxidant Activity by DPPH Radical Scavenging Method*

The free radicals scavenging activity of the seed oil of *E. toreliana* was estimated by DPPH and FRAP´S assays. DPPH involves single electron transfer and hydrogen atom transfer reactions (Prior *et al.*, 2005). The 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 (10, 100 and 1000 μgml^{-1}) are 50.07±0.0006, 62.48±0.0006 and 81.14±0.0006 % respectively; while the IC_{50} values was found to be 9.0 μg/ml in comparison to ascorbic acid which gave 54.37±0.00, 84.51±0.001 and 95.50±0.00 as the percentage inhibitions with IC_{50} value of 9.0 μgml^{-1}. The DPPH radical scavenging capacity of the seed oil of *E. toreliana* is at same concentration as observed for ascorbic acid as shown in Table 2.

The result of reducing power (FRAP) of the seed oil of *E. toreliana* in comparison with ascorbic acid as a reference antioxidant is also shown in Table 2. FRAP involves single electron transfer (Prior *et al.*, 2005). The reducing power of ascorbic acid used as standard in this study was EC_{50}: 20.00. The seed oil exhibited the high of reducing power value at concentrations of 10, 100 and 1000 μg/ml with effective dose value at (EC_{50}: 9.00 μgml^{-1}). Reducing power of *E. toreliana* oil increases from 0.641±0.008 at 10 μgml^{-1} to 1.016±0.02 at 100 μgml^{-1} and finally appreciated to 1.566±0.004 at 1000 μgml^{-1} in a concentration dependent manner. At tested concentrations the oil possessed the ability to reduce Fe^{3+}. It was observed that the seed oil of *E. toreliana* showed higher Fe^{3+} reducing power comparable to Ascorbic acid activity. The reducing power of the seed oil increased with concentrations in a strongly linear manner. The reducing power assay measures the electrondonating ability of antioxidants using the potassium ferricyanide reduction method. Antioxidants cause the reduction of the Fe^{3+}/ferricyanide complex to the ferrous form and activity is measured as the increase in the absorbance at 700 nm.

Overall, IC_{50} values of the seed essential oils of the *E. toreliana* examined was more effective than oils of some other related species: *E. toreliana* (leaf from Republic of Benin) IC_{50}: 2.90 gl^{-1} (Alain *et al.*, 2012), *E. oleosa* with IC_{50}: >1000 (Marzoug *et al.*, 2011), *E. globulus*(leaf) IC_{50} : 57.00 μgml^{-1} (Noumi *et al.*, 2011). These findings in DPPH and FRAP assays are in agreement with Vardar-Unlu *et al.*, 2003, who reported that the entire essential oil showed greater antioxidant activity than individual components, indicating the possible synergistic interaction of the essential oil constituents. These results showed that the seed oil of *E. toreliana* potentially exert its radical scavenging effects at a much lower concentration. This observed effect is certainly associated with high phenolic content and sesquiterpenoids components in the oil. The results clearly showed that the seed oil of *E. toreliana* possesses strong antioxidant activity and can be considered as good sources of natural antioxidants for medicinal purposes such as reactive oxygen species ailments including chronic inflammatory joint disease such as rheumatoid arthritis. Studies have showed that the electron donating capacity, reflecting the reducing power of bioactive compounds, is associated with antioxidant activity.

Table 2 : IC_{50} of the DPPH and FRAP Antioxidant Activity of the Seed Essential Oil of *E. toreliana*

Oil and Reference Compound	DPPH IC_{50} μgml^{-1}	FRAP EC_{50} μgml^{-1}
E. toreliana	9.00	9.00
Ascorbic acid	9.00	20.00

Data are presented as triplicate of the mean ± S.E.M

ii. *In vivo Anti-inflammatory Activity*

The anti-inflammatory effects of the seed oil of *E. toreliana* on carrageenan induced oedema in rats hind paws is presented in Table 3. The anti-inflammatory activity of oil was found to have effect in time manner. There was a significant decrease in oedema paw volume of rats in the test group. However, there was no reduction in inflammation found in case of control group. The results showed that the seed oil of *E. toreliana* causes significant reduction in inflammation i.e. 99.61% (1000 μgkg^{-1} *p.o*), while the standard anti-inflammatory drug indomethacin gave 93.75% (25 $mgkg^{-1}$).

The seed oil of *E. toreliana* proves its anti-inflammatory potential in *in vivo* study by controlling biphasic inflammatory events induced by carrageenan. The carrageenan induced oedema shows to be a multi-mediated phenomenon that liberates diversity of mediators which could be in two phases. Degree of inflammatory immune responses is controlled by involvement of inflammatory cells into inflammatory lesions (Solanki and Jain, 2010). The early phase (one hour) of the inflammation is due to the release of serotonin, histamine and related substances. The later phase (over one hour) is mediated by prostaglandins, proteases and lysosome (Ayoola *et al.*, 2009). The seed oil extract promptly controlled both the phases of inflammation.

Table 3 : In vivo Anti-inflammatory Activity the Seed Essential Oil of *E. toreliana*

Oil and Reference Compound	2 Hour	% I (2 Hr)	4 Hour	% I (4 Hr)	Mean Paw Diameter (mm)	Mean % I
E. toreliana	4.5±0.50	99.50	4.5±0.12	99.88	4.50±0.39	99.61
Indomethacin (Standard)	4.7±0.21	87.50	4.6±0.35	99.65	4.65±0.29	93.75
10% DMSO (Control)	5.5±0.07	-	5.5±0.00	-	5.50±0.31	-

Data are presented as triplicate of the mean with standard deviation

iii. *In Vivo Antinociceptive Activity*

The antinociceptive activity of the seed oil of *E. toreliana* measured on abino rat by using injection of formalin solution is shown in Table 4. The extracts exhibited significant dose related reduction of hind paw licking caused by formalin. Interestingly, the seed oil at the concentration of 1000 μgkg^{-1} exhibited high inhibitory effect 88.69 and 75.90% in early and late phases respectively, while the standard anti-inflammatory drug indomethacin gave 64.23 and 54.70% in first and second phase respectively. The results showed that the seed oil is more active than the synthetic drug (indomethacin) commonly used in pain and inflammatory problems. Drugs that act primarily on the central nervous system inhibit both phases equally while peripherally acting drugs inhibit the late phase. The early phase is probably a direct result of stimulation of nociceptors in the paw which reflects centrally mediated pain while the late phase is due to inflammation with a release of serotonin, histamine, bradykinin and prostaglandins. These phases represented neurogenic and inflammatory pain responses, respectively (Chen *et al.*, 1995).

Table 4 : In Vivo Analgesic Activity the Seeds Essential Oil of E. toreliana

Oil and Reference Compounds	Time of Licking and Biting Percentage Inhibition			
	Early Phase (0-5) min	Percentage Inhibition	Late Phase (5-30) min	Percentage Inhibition
E. toreliana	11.20±0.00	88.69	28.20±3.61	75.90
Indomethacin (Standard)	34.33±2.121	64.23	53.00±2.12	54.70
10% DMSO (Control)	96.00	-	117	-

Data are presented as triplicate of the mean ± S.E.M

During and after the *in vivo* experiment, no apparent behavioural side effects were observed in the animals; they were very active. This shows that the seed oil was relatively non-toxic and safe. This is in agreement with the report on the *Eucalyptus* leaf essential oil (Silva *et al.*, 2003).

Generally, phytocompounds found in the seed essential oil are very useful for various pharmacological

purposes such immunoinhibition; pinene derivatives are used as antioxidant, anticancer, antiinflammatory, antinociceptive, antibacterial, antifungal, antinoxious, insecticides, fungicides, inhibitors in breast cancer (Mercier *et al.*, 2009). Oleamide commonly use as analgesic, mood and sleeping agent, while 2-fluoro-*β*-3,4-trihydroxy-N-isopropylbenzeneethanamine which is a fluoro-substituted Ethylnorepinephirine (Trihydroxyl-subtituted 2-fluoroamphitamine) drugs used in the treatment of asthma, chronic bronchitis, emphysema and relaxing the smooth muscle in the lungs and dilates airways to improve breathing. Therefore the seed oil extract could have sympathomimetic, bronchodilating, analgesic and anorexiant potentials to handle problem such as arthritis, tumour, goitre and cancer. Bisabolol was also reported to have spasmolytic effects on intestinal smooth muscle, anti-inflammatory, antipyretic, ulcer protective, anti-inflammatory, anti-allergic, anti-pruritic, healing, decongestive and antispasmodic properties (Presibella *et al.*, 2010; Alves *et al.*, 2010). Eucalyptol which is one of the principal components of the seed oil and by far the most known naturally occurring oxide as it is the most common in essential oils as an oxygenated monoterpenoid has can readily penetrate tissue, one of the reasons for its efficacy in various decongestants and pain relief products and has anticatarrhale, mucolytic, antimicrobial, antiviral and as a stimulating expectorant in cases of chronic bronchitis (Ben-Hadj *et al.*, 2011; Caballero-Gallardo *et al.*, 2011).

IV. Conclusion

This research represents the first comprehensive study of the seed essential oil *E. toreliana*. The analysis of the seed essential oil from the plant indicates terpenes, terpenoids and phenoloids are the major constituents of this medicinal plant. Pharmacological activities of the oil may be due to the synergetic effects of these chemical constituents. Therefore, the seed essential oil of *E. toreliana* can be used as natural therapeutic product that may serve as leads for the development of new pharmaceuticals that can handle many health problems.

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