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Chemical composition, antioxidant and cytotoxic effects of *Eucalyptus globulus* grown in north-central Nigeria

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ABSTRACT

The investigation was designed to determine the chemical composition, antioxidant and cytotoxic effects of the leaf essential oil of Eucalyptus globulus Labill grown in Nigeria. Fresh leaves of E. globulus on steam distillation yielded 0.96 % (v/w) of essential oil. Investigation of the oil on GC/MS resulted in the identification of 16 compounds, the bulk of the oil was constituted by oxygenated monoterpenes (46.5 %) with terpinen-4-ol (23.46 %) as the most abundant constituent. Other notable compounds include γ-terpinene (17.01 %), spathulenol (8.94 %), ρ-cymene (8.10 %) and ρ-cymen-7-ol (6.39 %). Globulol (2.52 %) and α-phellandrene (2.20 %) were also among the constituents identified. The antioxidant features of the essential oil was evaluated using inhibition of 2,2-diphenyl-1-picrylhydrazyl radical, a concentration dependent radical scavenging activity with IC_{50} value of 136.87 µl/ml was observed. Cytotoxic effect was assayed using the brine shrimp lethality test, Probit's analysis of the result revealed a LC_{50} value of 9.59 µl/ml. The absence of 1,8-cineole and presence of α-phellandrene coupled with low antioxidant activity and high cytotoxic effect of the Eucalyptus oil investigated in the study suggest it may not be suitable for medicinal purposes but can be used as insecticidal agents.

Key words: *Eucalyptus globulus*, leaf essential oil, antioxidant, cytotoxic, oxygenated monoterpenes.

INTRODUCTION

Eucalyptus (family, Myrtaceae) is one of the world's most widely planted genera (1). *Eucalyptus globulus* Labill., commonly referred to as Tasmanian Blue Gum, is a fast growing, evergreen tree, bearing pendant leaves, native to Tasmania and south-east Australia (2). Of all Australian

eucalypts, this specie is the most widely introduced overseas (3) and has been established in many countries including Nigeria. Apart from its extensive use in the pulp industry, it also produces *Oleum eucalypti* (*Eucalyptus* oil) that is extracted on commercial scale in many countries like China, India, South Africa, Portugal, Brazil and Tasmania (4) as raw materials in perfumery, cosmestics, food, beverages, aromatherapy and phytotherapy (5 6).

Essential oils are natural products composed mainly of terpenes and terpene-derivatives in addition to some other non-terpene components (7). The leaves of *E. globulus* contains up to 3.5 % w/w essential oil (6,8). 1,8-cineole (eucalyptol) is the principal constituent found in *Eucalyptus.* However, other chemotypes such as α-phellandrene, ρ-cymene, γ-terpinene, ethanone, spathulenol, among others have been documented (1,9,10). Composition pattern of essential oils reflects their nutritional, cosmetic, pharmaceutical or medicinal values.

Medicinal and pharmaceuticals properties of the plant extracts are also exploited in folk phytotherapy and aromatherapy. Aromatherapy is the therapeutic use of fragrances or mere volatile (essential) oils by inhalation to cure or prevent diseases and infections (11). Study on the biological activities of essential oils extracted from plants are generating great interest among researchers. Antidiabetic, antioxidants, antimicrobial, and insecticidal activities of essential oil extracts from leaf of *E. globulus* have been established (3,12.13.14).

Antioxidant agents are compounds that have the potentials to scavenge reactive oxygen species or free radicals.. These free radicals play important roles in energy production, synthesis of some biomolecules, phagocytosis, and cell growth in living systems (15). An imbalance in the rate of production of free radicals or removal by the antioxidant defense mechanisms leads to a phenomena, referred to as oxidative stress. In disease conditions such as diabetes, cancer and cardiovascular diseases, an aggravated imbalance could occur in deleterious oxidation of biomolecules resulting in cell or tissue damage (16,17,18).

Due to characteristics adverse effects of synthetic antioxidants (19), there is need to explore phytotherapies to develop viable alternatives. Antioxidant activities of *E. globulus* leaf essential oil as well as some of its main constituents have been reported (13). However, it has also been established that the composition pattern of essential oil is affected by factors such as geographical location (20,21), which consequently influence their biological activities (22). It is on this basis, we investigated the chemical composition, antioxidants and cytotoxic effects of essential oil extracted from the leaves of *Eucalyptus globulus* grown in Abuja, North Central Nigeria.

MATERIALS AND METHODS

Plants Material

Fresh leaves of *Eucalyptus globulus* were collected from Sheda Science and Technology Complex (SHESTCO), Sheda, Abuja. Identification of the leaf was carried out at Herbarium of Plant Biology Department, University of Ilorin, where voucher specimens were deposited.

Oil Isolation

Pulverised leaves of *E. globulus* was subjected to steam-distillation for 3 hr in a Clevenger type apparatus according to the British Pharmacopoea Specification. The resulting oil was collected, preserved in a sealed sample tube and stored under refrigeration until required for analysis.

Gas Chromatography / Mass Spectrometry

A Hewlett-Packard ITP5890A GC, interfaced with a VG analytical 70-250S double focusing mass spectrometer was used. Helium was the carrier gas at 1.2 ml/min. The MS operating conditions were: ionization voltage 70 eV, ion source 2300C. The GC was fitted with a 25 m×0.25 mm, fused silica capillary column coated with CP-sil 5. The film thickness was 0.15 μm. The GC operating conditions were identical with those of GC analysis. The MS data were acquired and processed by on-line desktop computer equipped with disk memory. The percentage compositions of the constituents of the oil were computed in each case from GC peak areas. The identification of the components was based on the comparison of retention indices (determined relative to the retention time of series of n-alkanes) and mass spectral with those of authentic samples (NIST 05.L database/chemstation data system) and with data from literature (23,24,25).

Antioxidant Assay

The DPPH assay was measured by following the bleaching of a purple methanol solution of DPPH (26,27). 0.5 ml of 1 mM solution of DPPH was added to 3ml of various concentrations of the standard (ascorbic acid) and essential oil samples in methanol. After 30 min incubation at room temperature, the absorbance was read against a blank at 517 nm. The actual decrease in absorption was measured against that of the control. All experiments were carried out in triplicates and percentage inhibition (% I) values were calculated as given below:

$$\% I = (A_0 – A_T / A_0) \times 100$$

where A_0 is the absorbance of the control (containing all reagents except the test compound) and A_T is the absorbance of the test samples.

Cytoxicity Assay

The brine shrimp lethality test (BST) was used to predict the toxicity of the oil. The modified method of McLaughlin *et al*. (28) was employed in this study. Different concentrations (1000, 100, 10 ppm) of the leaf essential oil of *E. globulus* were prepared using dimethylsulfoxide (DMSO). After 48 h, a drop of DMSO and 4 ml of sea water were added to each of the sample bottles containing the oil sample. Ten brine shrimp larvae were carefully counted into each of the sample bottles and the volume of the sea water was made up to 5 ml. Tests for each concentration was done in triplicate. A control experiment containing 5 ml of sea water, a drop of DMSO and ten brine shrimp larvae was set along side. The experiment was maintained at room temperature for 24hrs, the number of surviving larvae were counted and recorded, the data obtained were subjected to Finney's Probit analysis to determine the LC_{50} of the oil.

RESULTS AND DISCUSSION

Pulverized leaves of *Eucalyptus globulus* on steam-distillation afforded oil of 0.89 g/cm^3 and a yield of 0.96 % (v/w) on fresh weight basis. This compared favorably with the yield obtained from the plant leaves grown in other geographical regions (10,29). The total ion chromatogram of the essential oil is shown on Figure 1. The essential oil were quantified by the peak area normalization method. Table I depicts the retention indices, relative percentages, mass spectra data and identities of oil constituents. a total of 16 compounds were identified from the retention indices and mass spectra data. Oxygenated and hydrocarbon monoterpenes constituted 46.5 and 23.37 % of the oil respectively, 17.44 % were aromatic compounds, while hydrocarbon and oxygenated sesquiterpenes were 11.46 and 1.23 % respectively.

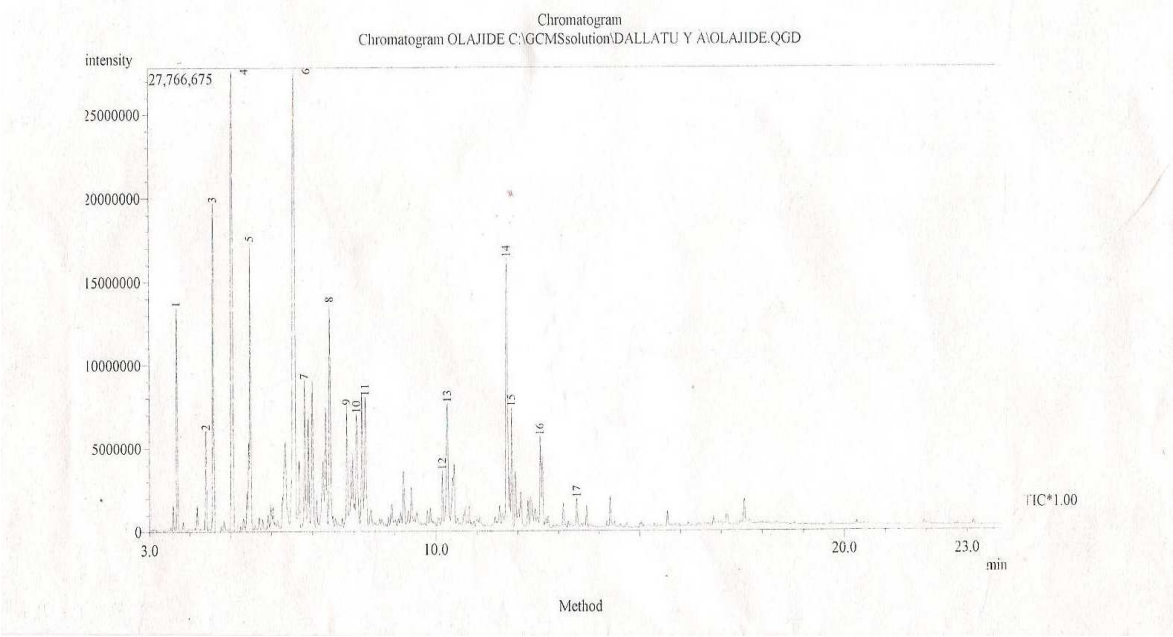

Figure 1. Total ion chromatogram of leaf essential oil of *Eucalyptus globulus* grown in Abuja, North Central, Nigeria

The bulk of the oil was characterized by oxygenated monoterpenes with Tepinen-4-ol (23.46 %) as the most abundant compound, other monoterpenoids identified include piperitone (5.90 %), apple oil (5.55 %), methane-1,2,3-triol (4.41 %), pinanediol (4.07 %) and cis-sabinol (3.11 %). γ-terpinene (17.01) was found as the predominant hydrocarbon monoterpene, α-pinene (4.16 %) and α-phellandrene (2.20 %) were also found in appreciable amount. Notable aromatic compounds identified were ρ-cymene (8.10 %) and its hydroxyl derivative ρ-cymen-7-ol, others include eudesmeneol (2.38 %) and α-selinene (0.57 %). Moreso, two oxygenated sesquiterpenes spathulenol (8.94 %) and globulol (2.52 %) as well as Cycloprop(e)azulene (1.23 %), a hydrocarbon sesquiterpene were also quantified.

The leaf oil composition pattern of the Nigerian grown *E. globulus* differs from those of other locations, the predominant compounds in the oil were terpinen-4-ol and its hydrocarabon precursor γ-terpinene. γ-terpinene (28-49 %) was found in Paskitani grown *E. globulus* leaf oils (10). Also, terpinene derivatives such as terpinen-4-ol found in appreciable quantity in the leaf

oil of this study, have also been reported, though in minor quantities (less than 1%), in *E. globulus* leaf oil grown in Yunnan province of China (29). Glubulol, a peculiar compound of *E. globulus* plant leaf essential oil found in appreciable quantity in the Chinese grown oil was also detected in the Nigerian grown oil.

Table I: Chemical composition of leaf essential oil of *Eucalyptus globules*

Compound[a]	RI[b]	% Composition	Mass Spectra
α – pinene	948	4.16	136,121,105,**93**,77,67
α- phellandrene	969	2.20	136,121,105,**93**,77,65
γ- terpinene	998	17.01	136,121,**90**,77,65,43
ρ- cymene	1042	8.10	134,119,**91**,77,65,51
Apple oil	1054	5.55	173,129,103,105,**70,**57
Cis- sabinol	1085	3.11	152,137,110,**82,**54,41
Terpinen-4-ol	1137	23.46	154,136,111,93,**71,**67
Piperitone	1158	5.90	152,137,110,**82,**54,41
Pinanediol	1276	4.07	170,152,111,**43**,41,30
ρ- cymen-7-ol	1284	6.39	150,**135**,91,77,65,37
Cycloprop(e)azulene	1386	1.23	204,161,119,105,91,**41**
α- Selinene	1474	0.57	218,189,175,105,91,**41**,39
Menthane-1,2,3-triol	1503	4.41	202,145,127,109,**43**,41
Spathulenol	1536	8.94	220,187,119,91,**43,**41
Globulol	1530	2.52	222,204,189,109,**43**,41
Eudesmeneol	1593	2.38	222,204,149,108,**59**,41

[a]Compounds are listed in order of elution from Silica Capillary Column coated on CP-Sil 5; [b]Retention indices on fused Silica Capillary Column coated with CP-Sil 5.

Most essential oil extracted from *E. globulus* contains at least 4 % to as high as 90 % 1,8-cineole, a characteristic constituent found in most *Oleum Eucalypti* (29,30). However, eucalyptol (1,8-cineole) was not identified in *E. globulus* leaf essential oil investigated in this study. Geographical and climatic conditions have been implicated as factors responsible for this variations (21). Other factors may include age of plant, time of harvest and method of extraction. Although steam and hydro-distillations are the most prominent technique for extraction. Demerit of this technique includes modification of components by auto-oxidation during distillation (20). Identification of ρ-cymene and terpinene as well as their respective hydroxyl derivatives ρ-cymen-7-ol and terpinen-4-ol may be attributed to oxidation of hydrocarbon terpenes to oxygenated derivatives during extraction.

The composition pattern of the *Oleum Eucalypti* reflects the uses of such oil. British pharmacopeia required *Oleum Eucalypti* to contain not less than 70 % 1,8-cineole and be free of phellandrene to be of high medicinal value (10). To further establish this requirement, some bioactivities of the oil were carried out. The antioxidant activity was evaluated by ability of the essential oil to scavenge DPPH radical in methanol. DPPH-radical is converted to DPPH-H, in the presence of antioxidants and the characteristics purple colour lightens. The result (Figure I) showed that the oil exerted a concentration dependent radical scavenging activity and at lower concentrations the activity of the oil was significantly lower ($p < 0.05$) when compared with the standard drug ascorbic acid (Table II) and this reflects in the high IC_{50} values (136.87 µl/ml; $y = 0.2913x + 10.13$) recorded. IC_{50} value is defined as the amount of antioxidant agents required to decrease the initial DPPH concentration by 50 % (31).

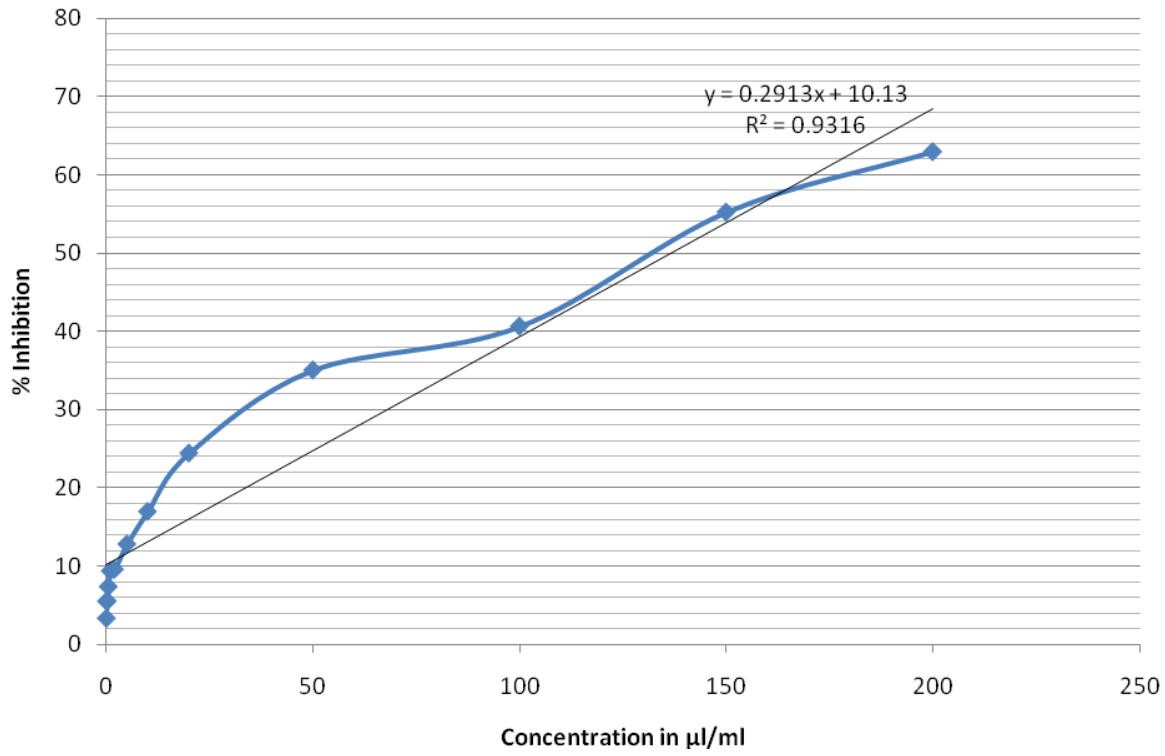

Figure II. Percentage Inhibition of DPPH radical by leaf essential oil of *Eucalyptus globules*

Essential oils are complex mixtures comprising many single compound, each of the constituents contributes to the biological effects of these oils (5). Moderate *in-vitro* antioxidant activities of terpinene and its derivatives such as terpinen-4-ol have been documented (32). Whereas, pinene and cymene possess relatively no significant or appreciable antioxidant activities (13,33). Strong antioxidant capacity of essential oils has been attributed to their phenolics constituents such as thymol and cavacrol, and probably 1,8-cineole with moderate DPPH radical scavenging activity reported by (7). Thus, the low antioxidant capacity of the oil may be attributed to absence of such compounds in the Nigerian grown *E. globulus* leaf oil. Moreso, possible antagonistic effect of various constituents of the oil might be responsible.

Table II. Percentage Inhibition of DPPH radical by leaf essential oil of *Eucalyptus globulus* and Ascorbic acid

E. globulus leaf essential oil		Ascorbic Acid	
µl/ml (0.89 mg/ml)	% Inhibition	mg/ml	% Inhibition
0.05	3.3	0.05	34.58
0.1	5.45	0.1	87.81
0.2	5.52	0.2	92.03

Basak and Candan (13) reported that principal constituents of *Eucalyptus camadulensis* which includes 1,8-cineole, cymene and pinene effected very low activities when compared to the high activity displayed by the leaf essential oil. The antioxidant capacity of the oil were attributed to probably minor constituents or synergistic effect of various components of the oil.

Cytotoxic effect was assayed using the brine shrimp lethality test, the assay principally assess the prospects of chemicals or substances as antitumor or cytotoxic agents (34). The result of the brine shrimp lethality assay of the *E. globulus* is shown in Table III. Finney's Probit analysis of the result revealed a LC_{50} values of 9.59 µl/ml indicating cytotoxic potentials. The results ascertained documented use of the plant extracts as repellant and insecticidal agents (14).

Table III. Brine shrimp lethality assay test of leaf essential oil of *Eucalyptus globules*

Conc (µl/ml)	Leaf essential oil of *E. globulus*	
	Survivor	Dead
1000	0	10
100	0	10
10	0	10
Control	10	2

LC_{50} = 9.59 µl/ml

CONCLUSION

The results of the present study revealed that the bulk of leaf essential oil of *Eucalyptus globulus* was constituted by oxygenated monoterpenes (46.5 %) with terpinen-4-ol (23.46 %) as the principal constituents. The absence of 1,8-cineole and presence of α-phellandrene coupled with low antioxidant activity and high cytotoxic effect of the *Eucalyptus* oil investigated in the study suggest it may not be suitable for medicinal purposes but can be used as repellant or antifeedant in insecticidal formulations.

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