

In Vitro Antioxidants, Antimicrobials and Biochemical Response of Methanol Leaf Extract of *Eucalyptus camaldulensis* following Sub-Acute Administration to Rats

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Abstract

Methanol leaf extract of *Eucalyptus camaldulensis* was investigated for phytochemical compositions, antioxidants, antimicrobial and safety profile. The antibacterial study was carried out using agar well diffusion method, while antioxidant activities were evaluated by 2, 2'-diphenyl-1-picrylhydrazyl (DPPH) and ferric reducing antioxidant power (FRAP) assays. A total of fifteen rats were divided into three groups (5 rats each) and were given 0, 250 and 500 mg/kg bwt of the extract orally for 28 days. Results revealed that tannins (24.72±0.36 g) is the most abundant phytochemicals followed by phenols (6.01±0.89 mg/g) while alkaloid (0.19±0.67 mg/g) was the least. Extract demonstrated antioxidant activities with IC₅₀ of the 244.98±5.24 µg/mL and 462.755 ± 6.98 µg/mL in DPPH and FRAP assays respectively. The extract inhibited the bacteria growth with minimum inhibitory concentrations (MIC) and minimal bactericidal concentrations (MBC) values ranged between 7.5-60 µg mL⁻¹ and 60-12 µg mL⁻¹ respectively. The concentrations of albumins, bilirubins sodium, potassium, creatinine, serum transaminases and alkaline phosphatase (ALP) activities were not significantly (p>0.05) altered by the extract. Urea concentration was significantly (p<0.05) higher while proteins were lower in rats treated with 500 mg/kg bw of the extract. Methanol extract of *E. camaldulensis* could be considered as a cheap source of effective and safe herbal remedy with potential candidate for the development of a new drug.

Keywords: Phytochemicals, *In vitro* antioxidant, *Eucalyptus camaldulensis*, DPPH, Antibacterial.

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INTRODUCTION

Plants are free gifts of nature available at human disposal for various pharmacological uses. It has been used as a primary source of oral remedies for various ailments and diseases from a time immemorial [1]. The recent growth in knowledge of free radicals and reactive oxygen species (ROS) in biological systems is producing a medical revolution that promises a new age of health [2]. These reactive oxygen species play an important role in degenerative or pathological processes, such as aging, cancer, coronary heart disease, Alzheimer's disease, atherosclerosis, cataracts, and inflammation [3].

A number of studies have linked the antioxidants in natural products particularly, plants with

reduction of chronic diseases and inhibition of pathogenic bacteria growth, which are often associated with the termination of free radical proliferation in biological systems [4-6]. Microbial infections have always been considered as a leading cause of morbidity and mortality in humans. The microbial resistance to the existence antibiotics is well known, so it is very important to search for alternative antimicrobial agents from natural source like plants or herbs to overcome this challenge [7].

Eucalyptus camaldulensis Dehnh, also called the river red gum, it is a large aromatic tree that is endemic in Australia [8]. It is the most important genera of Myrtaceae family commonly used in Traditional medicine for the treatment of sore throat, wounds, asthma, burns, fever, influenza, arthritis, malaria, and

pharyngitis [9, 10]. Pharmacologically, leaves of *E. camaldulensis* are well documented for cytotoxic [11], antimicrobial [12], insecticidal [13], antioxidant [8] and anti-dermatophytes [14] activities. Previous phytochemistry revealed that acylated pentacyclic triterpenoids [15], eucalyptanoic acid [16] and essential oils [17] are the major bioactive metabolite in *E. camaldulensis*. The aim of the current study was to evaluate the in vitro antimicrobial, antioxidants and safety evaluations of *E. camaldulensis* leaves' extract in rats

MATERIAL AND METHODS

Sample preparation and extraction

The fresh leaves of *Eucalyptus camaldulensis* were collected from Minna, Niger State. The leaf was destalked, washed with clean-water, dried at room temperature and finally grounded using a grinder mill and dried at room temperature. Extraction of plant materials was performed by weighing 50 g of the powdered plants and extracted by soxhlet extraction using 200 ml of methanol.

Phytochemical Analysis

Evaluation of the extract for qualitative phytochemical compositions including saponins, tannins, glycoside, alkaloids, steroids and flavonoids were determined by the methods described by Harborne [18] and Sofowora [19]. The quantitative concentration of alkaloids in the extract was determined by the method described by Harborne [18], saponins contents as described by Chang *et al.* [16] flavonoids and phenols by methods described by Oloyed [14] while tannins contents was determined by the methods of AOAC [20].

Assay for antibacterial activities

Antibacterial activity of the extract was carried out using agar-well diffusion method as described by Gaherwal *et al.* [21]. Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) were determined by tube dilution method for each of the test organism as described by Tsado *et al.* [22] in triplicates.

Assay for Antioxidant activities

DPPH radical scavenging activity of the plant extract at varying concentrations (2.5-100 µg/mL) was measured in vitro via the 2, 2'-diphenyl-1-picrylhydrazyl (DPPH) assay as described by Tsado *et al.* [23], while Fe³⁺ ion reducing power of the sample was evaluated using varying extract concentrations (2.5 - 100 µg/mL) according to the methods described previously [53,54]. The extract concentration providing 50% inhibition (IC₅₀) was calculated from the plot of inhibition (%) against extract concentration. Ascorbic acid at the same concentrations was used as the reference antioxidants.

Toxicological Study

Acute toxicity was carried out as described previously [24]. In the subacute toxicity animals (5 each) were dosed 0 (control), 250 and 500 mg/kg bwt of the extract orally for 28 days. Procedures described by Shittu *et al.* [15] and Yusuf *et al.* [26] were followed during blood sample collection and serum preparation for biochemical analysis. Serum activities of alkaline phosphatase (ALP), Aspartate transaminase (AST) and alanine transaminase (ALT) were determined as described previously [27]. The concentrations of serum total proteins [28], bilirubins, albumins [29], urea creatinine [30], sodium, potassium, and chloride [31] were determined using standard methods.

STATISTICAL ANALYSIS

Values were analyzed using statistical package for social science (SPSS) version 16 and presented as means ± SE of the mean. Comparisons between different groups were carried out by one-way analysis of variance (ANOVA) followed by Duncan's Multiple Range Test (DMRT). The level of significance was set at $P < 0.05$ [32].

RESULTS

Phytochemical composition

The qualitative analysis of phytochemicals revealed the presence of phenols, tannins, alkaloids, saponins, glycoside, terpenes, anthraquinone and flavonoids. Phlobatannins was absence (Table 1). Quantitatively, tannins (24.72±0.36 g) is the most abundant phytochemicals in methanol leaf extract of *Eucalyptus camaldulensis*, followed by phenols (6.01±0.89 mg/g) while alkaloid (0.19±0.67 mg/g) was the least abundant phytochemicals.

Table-1: Phytochemical composition of methanol leaf extract of *Eucalyptus camaldulensis*:

Phytochemicals	Inferences	Quantity (mg/g)
Phenol	+	6.01±0.89 ^d
Flavonoids	+	0.71±0.14 ^b
Tannins	+	24.72±0.36 ^e
Saponins	+	3.13±0.45 ^c
Alkaloids	+	0.19±0.67 ^a
Cardiac glycosides	+	
Anthraquinone	+	
Steroids	+	
Terpenes	+	
Phlobatannins	-	

Data are Mean ± SEM of triplicate determination

Antioxidants activities

Methanol extract of *Eucalyptus camaldulensis* promoted an inhibition of DPPH radical with increasing concentrations (Fig. 1). The IC₅₀ of the of the extract against DPPH radicals is 244.98±5.24 µg/mL as compared with 79.64 ±3.78 µg/mL recorded for the reference drug (Ascorbic Acid). The extract also exhibited FRAPS activities that increase with increase extract concentration (figure 2). The IC₅₀ recorded for

the extract in FRAP assay was $462.755 \pm 6.98 \mu\text{g/mL}$ as compared with $204.50 \pm 1.78 \mu\text{g/mL}$ recorded for

reference drug (ascorbic Acid).

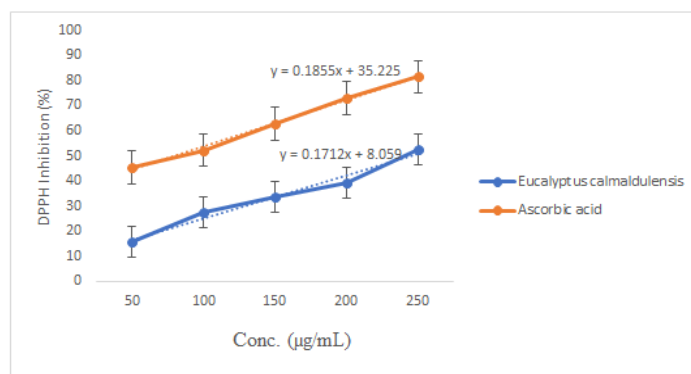


Fig-1: DPPH radical scavenging activities of methanol extract of *Eucalyptus camaldulensis*. Values are mean \pm SEM of 3 determinations. $\text{IC}_{50} = 244.98 \pm 5.24 \mu\text{g/mL}$ (*Eucalyptus camaldulensis*), $79.64 \pm 3.78 \mu\text{g/mL}$ (Ascorbic Acid).

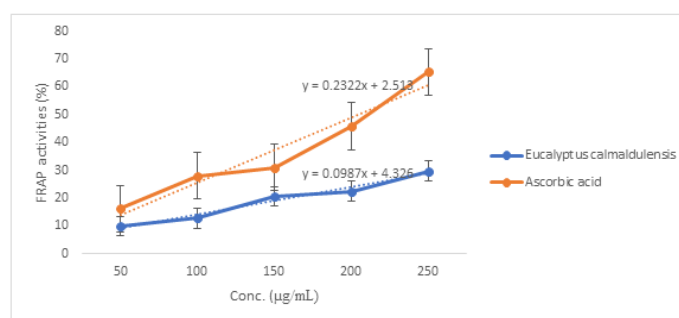


Fig-2: FRAP activity of leaf extracts of methanol extract of *Eucalyptus camaldulensis*. Values are mean \pm SEM of 3 determinations. $\text{IC}_{50} = 462.755 \pm 6.98 \mu\text{g/mL}$ (*Eucalyptus camaldulensis*), $204.50 \pm 1.78 \mu\text{g/mL}$ (Ascorbic Acid)

Antimicrobial activities

Methanol leaf extract of *Eucalyptus camaldulensis* inhibited the growth of *Streptococcus pyogenes*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Staphylococcus aureus* and *Bacillus subtilis* in a dose dependent manner. The extract at 20 and 40 $\mu\text{g/mL}$ were not active against *Streptococcus pyogenes*. The highest zone of inhibition (33.00 ± 1.90

$\mu\text{g/mL}$) was recorded against *Pseudomonas aeruginosa* while the least ($22.00 \pm 2.03 \mu\text{g/mL}$) was recorded against *Streptococcus pyogenes* (Table 2). Overall results showed that the extract of *E. camaldulensis* leaves had MIC values ranged between $7.5\text{--}60 \mu\text{g mL}^{-1}$ and MBC values ranged between $60\text{--}120 \mu\text{g mL}^{-1}$ (Table 3).

Table-2: Zone of Inhibition of the organism caused by methanol leaf extract of *Eucalyptus camaldulensis*

Bacteria (Test organism)	Conc ($\mu\text{g/mL}$)				
	20	40	60	80	100
<i>Streptococcus pyogenes</i>	Nil	Nil	13.00 ± 0.59	19.00 ± 1.98	22.00 ± 2.03
<i>Klebsiella pneumoniae</i>	18.00 ± 1.93	14.00 ± 1.90	17.00 ± 1.09	22.00 ± 2.03	30.00 ± 2.89
<i>Pseudomonas aeruginosa</i>	13.00 ± 0.78	14.00 ± 0.97	24.00 ± 2.08	28.00 ± 2.89	33.00 ± 1.90
<i>Staphylococcus aureus</i>	Nil	21.00 ± 2.89	23.00 ± 1.09	24.00 ± 1.98	25.80 ± 2.32
<i>Bacillus subtilis</i>	9.70 ± 0.67	12.20 ± 0.98	19.00 ± 2.06	21.00 ± 1.90	24.00 ± 1.90

Data are Mean \pm SEM of triplicate determination

Table-3: Minimum inhibitory and minimum bactericidal concentrations of methanol leaf extract of *Eucalyptus camaldulensis*

Bacteria (Test organism)	MIC ($\mu\text{g/mL}$)	MBC ($\mu\text{g/mL}$)
<i>Streptococcus pyogenes</i>	60	60
<i>Klebsiella pneumoniae</i>	7.5	30
<i>Pseudomonas aeruginosa</i>	30	60
<i>Staphylococcus aureus</i>	60	120
<i>Bacillus subtilis</i>	60	60

Biochemical Parameters

The concentrations of albumins, bilirubins sodium, potassium, creatinine, serum aspartate

transaminase, alanine transaminase and alkaline phosphatase (ALP) activities were not significantly ($p < 0.05$) altered by treatment with 250 and 500 mg/kg

bw *E. camadulensis*. However, serum concentration of urea were significantly ($p < 0.05$) higher while proteins were lower in rats treated with 500 mg/kg bw *E. camadulensis* when compared with the normal control.

Chloride concentration was higher in rat's dosed 250 and 500 mg/kg bw *E. camadulensis* when compared with the control.

Table-4: Effect of methanol extract of *E. camadulensis* on biochemical parameters in rats

	Control	250 mg/kg bw	500 mg/kg bw
AST (U/L)	56.23±2.09 ^a	59.89±4.32 ^a	55.08±3.23 ^a
ALT (U/L)	72.93±3.21 ^a	74.09±5.89 ^a	79.89±3.94 ^a
ALP (U/L)	156.34±5.43 ^a	162.93±4.32 ^a	158.34±7.93 ^a
Total proteins (mg/dL)	52.88±1.23 ^b	54.21±2.34 ^a	31.90±2.90 ^a
Albumin (mg/dL)	2.09±0.03 ^a	2.12±0.27 ^a	2.11±0.63 ^a
Bilirubin (mg/dL)	1.69±0.22 ^a	1.59±0.21 ^a	1.55±0.14 ^a
Urea (mg/dL)	123.23±3.24 ^a	126.09±3.21 ^a	171.89±4.34 ^b
Creatinine (mg/dL)	3.78±0.78 ^a	3.89±0.24 ^a	3.18±0.13 ^a
Sodium (Meq/L)	45.32±2.34 ^a	43.90±4.32 ^a	46.32±3.89 ^a
Potassium (Mmol/L)	7.09±0.78 ^a	7.12±0.53 ^a	7.05±0.14 ^a
Chloride (Mmol/L)	132.90±3.45 ^a	159.23±3.97 ^b	165.98±5.43 ^b

Data are Mean ± SEM of triplicate determination. Value followed by different superscript alphabet along the column were significantly different ($p < 0.05$).

DISCUSSION

The present study revealed the presence of various important medicinal phytochemicals in methanol leaves extract of *eucalyptus calmaldulensis*. In line with the present study, Azzah and Ibtisam [10] also reported the presence of saponins, tannins, flavonoids, carbohydrate and protein compounds in leaves and bark extracts of *E. camaldulensis* [10].

Quantitatively, tannins (24.72±0.36 g) is the most abundant phytochemicals in methanol leaf extract of *Eucalyptus camaldulensis*, followed by phenols (6.01±0.89 mg/g) while alkaloid (0.19±0.67 mg/g) was the least abundant phytochemicals. Previous studies stated that phenolic compounds are responsible for antioxidants and antimicrobial activity of the plant extracts [33, 34, 35, 35]. Flavonoids are most diversified groups of phenolic compounds found in plant, its biological activity include, antioxidants antibacterial, anti-inflammatory, anti-allergic, protect against ulcers, vireses and antitumor effect [37]. Saponin has been reported to have anti-inflammatory, cardiac depressant and hyper-cholesterolemic effect [38]. Alkaloids have been found to have microbiocidal effect, antihypertensive antifungal, anti-inflammatory and antifibrogenic effect [39]. Previous researchers have also demonstrated the antimicrobial and antioxidant effect of glycosides [40]. Thus, the phytochemical constituents indicate that the methanol extract of *eucalyptus calmaldulensis* could yield a drug of pharmaceutical significance. However, the absence of phlobatannins agrees with the earlier study which reported that not all phytochemicals are present in medicinal plant and those present differs with the solvent used in the extraction process [14]

The antimicrobial activities of methanol leaf extract of *Eucalyptus calmaldulensis* showed that the extracts had good antimicrobial activity against the test organism. This consistent with the prior studies [42, 34,

43, 44], that proved *E. camaldulensis* extracts possess the ability to inhibit the growth of Gram-positive and Gram-negative bacteria. The ability of the methanol leaf extract of *Eucalyptus calmaldulensis* to inhibit the growth of Gram-positive and Gram-negative bacteria explains why it is used in folk medicine for the treatment of sore throat, wounds, asthma, burns, fever, influenza, arthritis, malaria, and pharyngitis [9, 10]. Increase in the concentration of the extract results in corresponding increase in the zones of inhibitions. This linear relationship between extract concentrations and zones of inhibition could be that the extract was able to diffuse into the inoculated nutrient agar [23, 45].

Minimum inhibitory concentration (MIC) is the lowest concentration of an extract that inhibit the visible growth of the test organism after 24hrs incubation [22]. In the present study MIC was measured based on turbidity or visible growth show by the organism. That the extract of *E. camaldulensis* leaves had MIC values ranged between 7.5-60 µg mL⁻¹ and MBC values ranged between 60-12 µg mL⁻¹ (Table 3). This value is however, higher than MIC values ranged between 0.391-25 µg mL⁻¹ reported for leaves and bark extract of *E. camaldulensis*. This broad-spectrum antibacterial activities of *E. camaldulensis* may be attributed to the presence of several secondary metabolite like saponins, tannins and flavonoids. Which, were mentioned in several studies to possess antibacterial properties [46, 47]. Many other members of the genus *Eucalyptus* including *E. viminalis*, *E. tereticornis*, *E. maculata* and *E. globulus* have also been reported for antimicrobial properties [47, 48, 49].

There are many reports that support the use of antioxidant supplement in reducing the level of oxidative stress and in preventing the development of complication associated with disease [36, 22]. In this study the methanol leaves extract of *Eucalyptus calmaldulensis* exhibited antioxidant property as confirmed by DPPH antioxidant assay. The DPPH

radicals were widely used to investigate the scavenging activity of some natural compounds. The extract was also found to have a high antioxidant property as confirmed by Fe^{3+} reducing power, The extract exhibited FRAP activities that increase with increase extract concentrations (figure 2). In DPPH scavenging assay The IC_{50} of the of the extract against DPPH radicals is $244.98 \pm 5.24 \mu\text{g/mL}$ as compared with $79.64 \pm 3.78 \mu\text{g/mL}$ recorded for the reference drug (Ascorbic Acid). The IC_{50} reported in this study was lower than IC_{50} value of 1562.52 and 155.17 reported for *C. adansonii* and *N. laevis* [23], $52.45 \pm 3.05 \mu\text{g/mL}$ reported for *Xylopi aethiopica* extract [26].

Serum biochemical parameters have been widely used as an indicator of pathological condition, toxicology or safety of a test substance, treatment outcome and general health status of animals [26, 22, 50]. Among these biochemical parameters, transaminases, alkaline phosphatases, proteins, albumin, bilirubins, urea creatinine and electrolyte are the most widely employed in assessing the integrity of liver and kidney following plant extract administration to animals [51]. Alterations in the normal activities or concentrations of these biochemical parameters are conventional indicators of any of the following conditions; renal or nephrotic impairments, hepatocellular injury, cellular leakage, loss of functional integrity of cell membrane, biliary cirrhosis or liver hepatitis [52]. Consequently, the concentrations of albumins, bilirubins sodium, potassium, creatinine, serum aspartate transaminase, alanine transaminase and alkaline phosphatase (ALP) activities were not significantly ($p < 0.05$) altered by treatment with 250 and 500 mg/kg bw *E. camadulensis*. This is an indication that the functional integrity of liver is well preserved and that the extract does not induced any form of pathological conditions to the liver.

The increases in chloride concentration in rats dosed 250 and 500 mg/kg bw *E. camadulensis* is an indication that the integrity of the kidney as regards to this metabolite has been compromised leading to increases production or decrease tubular excretion. Similarly, the observed increase in urea concentrations could be attributed to increase in protein catabolism which resulted in the decrease serum total proteins (Table 4). The extract might have interfered with the equilibrium in protein metabolism infavor of catabolism. Such drastic decrease in protein levels could, negatively affect cellular homeostasis and consequently affect the health of the animals.

CONCLUSION

This study has shown that the methanol leaf extract of *Eucalyptus calamdulensis* contains some useful potential bioactive principles that are inhibitory to some pathogenic organism as well as possess significant antioxidants properties. Thus, it may be

considered as a natural source of antimicrobials and antioxidants for therapeutic purposes.

Ethical approval

The principles governing the use of laboratory animals as laid out by the Federal University of Technology, Minna Committee on Ethics for Medical and Scientific Research and also existing internationally accepted principles for laboratory animal use and care as contained in the Canadian Council on Animal Care Guidelines and Protocol Review were duly observed.

Author's contributions

This work is a collaboration of all the authors. All authors read and approved the final manuscript

Consent for publication

Not applicable

Competing interests

The authors declare that they have no competing interests.

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