



Phytochemical Analysis and Antioxidants effects of Methanol Stem Bark Extract of Eucalyptus Camaldulensis in Wister Albino Rats

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Authors' contributions

This work was carried out in collaboration among all authors. Author JN designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Authors IBA and MSA managed the analyses of the study. Author MSA managed the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

Aim: The aim is to evaluate the antioxidant potentials of *Eucalyptus camaldulensis* methanol stem bark extract (ECMSBE) on Wister albino rats.

Methodology: The phytochemical screening of ECMSBE was conducted using standard methods. A total of 36 albino rats were used for the antioxidant studies. The rats were divided into six (6) groups of six rats. Control group received distilled water orally at 2ml/kg. Oxidative stress was induced in groups 2 to group 6 CCl₄ (1ml/kg, s.c) at every 72hrs for 10 days. Group 2 was untreated while groups 3 – 6 received doses of 50, 100, 150 and 200mg/kg of ECMSBE respectively. On the 11th day, the rats were sacrificed and the liver was removed and homogenised and oxidative stress parameters were determined.

Results: Phytochemical analysis of ECMSBE revealed the presence of saponins, flavonoids, tannins, phenols, glycosides, steroids, terpenoids and resins. There was no significant difference (P<.05) between the CCl₄ induced group and the group treated with ECMSBE (50mg/kg). However,

their concentrations were significantly different from the group treated with ECMSBE (100mg/kg – 200mg/kg) when compared to the group treated with CCl₄ Only. The CCl₄-induced group had its vitamin A, vitamin C and vitamin E concentrations significant different (P<.05) from the groups treated with ECMSBE (100, 150 and 200mg/kg body weight). There was no significant difference (P>.05) in the levels of SOD, CAT and GPx between the group induced with CCl₄ only and the group treated with ECMSBE (50mg/kg), however, it these concentrations were significantly higher (P<.05). The enzymatic antioxidants concentration in the normal control group was not significantly different (P>.05) when compared the group that was treated with ECMSBE (200mg/kg)

Conclusion: The result suggest that the extract of *E. camaldulensis* possessed antioxidant properties which can be used as effective protecting agents against oxidative stress and other diseases.

Keywords: Phytochemical; Antioxidant; *Eucalyptus camaldulensis*; Carbon tetrachloride.

1. INTRODUCTION

E. camaldulensis belongs to the order Myrtales and Myrtaceae. It is a large genus of aromatic trees indigenous to Australia, Tasmania and the neighboring Island, but today, it can be found growing in subtropical regions of the world. The genus consists of about 700 species of evergreen trees and shrubs [1]. The tree can grow to 375-480 feet (125-160 meters). Their name originates from the Greek word eucalyptol which means well-covered. *Eucalyptus* trees thrive in environments that maintain average temperatures of about 60°C. The plants have been considered as sources of medicinal agents for the treatment of many diseases [2]

Eucalyptus is a traditional remedy for a variety of common ailments, particularly of respiratory tract, and burns in China. Also, it is widely used as natural antioxidant food additives [1]. Experimentally, it has been demonstrated that this plant possess a broad therapeutic properties as antimicrobial and antidiabetic. *E. camaldulensis* has been designated and used as a medicinal plant in northern Nigeria, West Africa, and other part of the world. According to the traditional medicinal and indigenous knowledge, *E. camaldulensis* is used to treat illnesses like diarrhoea, leprosy, male sterility, diabetes, cough, cold, cancer, ulcer, inflammation, antimicrobial and some others [3].

Antioxidants are compounds capable of either delaying or inhibiting the oxidation processes which occur under the influence of atmospheric oxygen or reactive oxygen species. They are used for the stabilization of polymeric products, of petrochemicals, foodstuffs, cosmetics and pharmaceuticals. Antioxidants are part of the defence mechanisms of the organism against the pathologies associated to the attack of free

radicals [4]. Endogenous antioxidants are enzymes, like superoxide dismutase, catalase, glutathione peroxidase. When endogenous factors cannot ensure a rigorous control and a complete protection of the organism against the reactive oxygen species, the need for exogenous antioxidants arises, as nutritional supplements or pharmaceutical products, which contain as active principle an antioxidant compound. Amongst the most important exogenous antioxidants are vitamin A, vitamin E, vitamin C [5]. There is an increasing interest in antioxidants, particularly in those intended to prevent the presumed deleterious effects of free radicals in the human body, as well as the deterioration of fats and other constituents of foodstuffs [4]. Free radicals are known to be involved in the etiology of many degenerative diseases [5]. Natural products of plant origin have been found to exhibit strong antioxidant activity. This thus necessitates the assessment of *E. camaldulensis* for its antioxidant potential.

2. MATERIALS AND METHODS

2.1 Plant Collection and Identification

E. camaldulensis stem bark was collected from Kalgo Town, Kalgo Local Government, Kebbi State, Nigeria. The plant was identified by a Taxonomist from the Department of Plant Science and Biotechnology Department, Kebbi State University of Science and Technology Aliero and a Voucher specimen was deposited at the Herbarium of the same Department for future reference.

2.2 Experimental Animals

The Wistar albino rats were purchased from Animal House, Usmanu Danfodiyo University

Sokoto. Thirty six (36) healthy Wister albino rats of both sexes weighing 150 – 200 g were used for this study. All protocols were carried out in compliance with NIH guidelines for care and use of Laboratory Animals (Pub. No. 85-23, Revised 1985).

2.3 Qualitative Phytochemical Screening

Alkaloids, tannins and saponins were estimated by the method of Sofowora [6]. Glycosides were estimated by the method of Trease and Evans [7]. Flavonoids were estimated by the method of Edeoga et al. [8], whilst anthraquinones, steroids, terpenoids and phenols were estimated by the method of Harborne, [9].

2.4 Experimental Design for Antioxidants Study

Induction of oxidative stress was done with slight modification according to the method of Bell et al. [10]. Thirty six (36) adult albino rats weighting 150-200 were divided in to six groups, of six rats each.

Group I served as normal control, received distilled water (2ml/kg body weight) for ten days

Group II received 30% carbon tetrachloride (1ml/kg body weight) in liquid paraffin for every 72 hrs for fourteen days.

Group III, IV, V and VI: received the methanol stem bark extract of *E. camaldulensis* dose of 50, 100, 150 and 200mg/kg respectively; once a day for ten days followed by CCl₄ induction for every seventy two (72) hours.

At the end of the experiments, the animals in various groups were sacrificed after 48 hours of 30% CCl₄ induction under chloroform anaesthesia, liver samples were collected.

2.5 Preparation of Liver Tissue Homogenate

Liver tissue was carefully removed from the sacrificed animals using a pair of gloves, prior to the dissection the liver was weighed and perfused with phosphate buffer saline to remove any red blood cells and clots. Some part of the liver was perfused with cold 0.86% KCl. One ml of homogenization buffer (0.1 M phosphate buffer, pH 7.4 containing 1mM EDTA) per 100mg of tissue added. Then liver tissue sample was homogenized. After homogenization, the sample was centrifuge at 1500 rpm for 5 minute at 4°C to obtain post mitochondrial supernatant, which was then transferred to a clean tube for the estimation of enzymatic and non-enzymatic antioxidants.

2.6 Biochemical Analysis

Superoxide dismutase (SOD) activity was determined by the method of Marklund [11]. Catalase (CAT) by the method of Johansson and Borg [12]. Glutathione peroxidase (GPx) activity was assayed by the method of Paglia and Valentine [13]. Vitamin A was estimated using the method of Rutkowski et al. [14]. Vitamin C was assayed by the method of Rutkowski *et al.*, [15] while Vitamin E was assayed by the method of Hashim and Schuttringer [16]. The concentration of MDA was determined by the method of Shah and Walker, [17]. Reduced Glutathione (GSH) by the method of Baker and Cerniglia, [18].

2.7 Statistical Analysis

All data were reported as Means ± Standard Error of Mean (SEM). The values were analysed using Statistical Package for Social Sciences (SPSS) 20.0, Duncan Post Hoc. Comparison test were used to check the differences between the individual groups. Test of significance between means were carried out using one-way analysis of variance (ANOVA). Differences in mean was considered significant if P<.05.

3. RESULTS

3.1 Phytochemical Screening

The phytochemical analysis of *Eucalyptus camaldulensis* stem bark revealed the presence of the saponins, flavonoids, steroids, phenols, tannins, terpenoids, glycosides and resin (Table 1).

Table 1. Qualitative Phytochemical Components of *Eucalyptus camaldulensis* stem bark

Tests	Inference
Saponins	+
Flavonoids	+
Steroids	+
Phenols	+
Tannins	+
Terpenoids	+
Glycosides	+
Alkaloid	-
Resin	+

Key
 - = Not detected
 + = Present

3.2 Antioxidant Activity

3.2.1 Effect of *E. camaldulensis* methanol stem bark extract on oxidative stress markers

The effect of *E. camaldulensis* on GSH and MDA showed that there was no significant difference ($P < .05$) between the CCl_4 induced group and the group treated with ECMSBE (50mg/kg). However, their concentrations were significantly different from the group treated with ECMSBE (100mg/kg – 200mg/kg) when compared to the group treated with CCl_4 Only. The normal control group was significantly different ($P < .05$) from all the treated groups (Table 2).

3.2.2 Effects of administration of ECMSBE on Antioxidants vitamins

The result of the effect of administration of ECMSBE in liver homogenate on antioxidants vitamins in liver homogenate of rats with CCl_4 -induced oxidative stress is presented in Table 3. The CCl_4 -induced group had its vitamin A, vitamin C and vitamin E concentrations significant different ($P < .05$) from the groups treated with ECMSBE (100, 150 and 200mg/kg body weight). The group treated with distilled water (2mg/kg) had its antioxidant vitamins significantly different ($P < .05$) from all the other groups.

3.2.3 Effects of administration of ECMSBE enzymatic antioxidants

Table 4 shows the effect of administration of ECMSBE on enzymatic antioxidants in rats with CCl_4 – induced oxidative stress. There was no significant difference ($P > .05$) in the levels of SOD, CAT and GPx between the group induced with CCl_4 only and the group treated with ECMSBE (50mg/kg), however, it these concentrations were significantly higher ($P < .05$). The enzymatic antioxidants concentration in the normal control group was not significantly different ($P > .05$) when compared the group that was treated with ECMSBE (200mg/kg).

4. DISCUSSION

The antioxidants effects of several medicinal plants has been attributed to the presence of phytochemical constituents like saponins, flavonoids, terpenoids, phenols, tannins, steroids, resins and glycosides [19]. Flavonoids are polyphenolic compounds that are ubiquitous

in nature [20]. Studies have shown that flavonoids and other polyphenolic compounds play a role in scavenging free radicals and in the inhibition of lipid peroxidation. Plant phenolics are one of the major groups of compounds acting as primary antioxidant free radical terminators [21]. Saponins are glycosides that consist of polycyclic aglycones attached to one or more sugar side chains. The non-sugar part of saponins has a direct antioxidant activity which may results in benefits such as reduced risk of cancer and liver damage [20]. Tannins are water-soluble phenolic compounds that have the ability to chelate metal ions such as Fe (II) and interfere with one of the reaction steps in Fenton reaction and thereby retard oxidation. The inhibition of lipid peroxidation by tannin constituent can act via the inhibition of cyclooxygenase [4].

Oxidative stress is associated with the diminished capacity of a biological system to counteract an overproduction or invasion of reactive oxygen species and other radicals. It has been reported that acute administration of CCl_4 induced oxidative stress in rats [22]. Free radicals can be scavenged through chemoprevention by utilizing natural antioxidant compounds present in foods and medicinal plants. Almost all organisms are well protected against free radical damage by enzymes such as superoxide dismutase and catalase, or compounds such as ascorbic acid, tocopherols and glutathione. Some medicinal plants have been shown to have both chemopreventive and/or therapeutic effects on human diseases [2]. Lipid peroxidation is one of the prime factors involved in cellular damage caused by free radicals. Malondialdehyde (MDA) is one of the final products of polyunsaturated fatty acids peroxidation in the cells and are thus commonly termed as a marker of oxidative stress [23].

The present study showed a significant increase in the level of MDA in liver homogenate of CCl_4 -induced non-treated group when compared with the normal non treated groups. This increase in MDA levels suggests enhanced lipid peroxidation leading to tissue damage and failure of antioxidant defense mechanism to prevent formation of excessive free radicals [22]. This in turn alters the ratio of polyunsaturated to other fatty acids, thus, leading to a decrease in the membrane fluidity which may be sufficient to cause cell death [20]. However, the administration of *E. camaldulensis* stem bark extract to test groups revealed a significant

Table 2. Effect of *E. camaldulensis* methanol stem bark extract on oxidative stress markers

Dose Administration	GSH (mg/dl)	MDA ($\mu\text{mol/g}$)
Distilled water (2mg/kg)	31.17 \pm 0.69 ^c	28.10 \pm 1.3 ^a
CCl ₄ Only	14.33 \pm 0.33 ^a	65.17 \pm 0.52 ^c
ECMSBE (50mg/kg) + CCl ₄	16.83 \pm 0.60 ^a	61.69 \pm 1.94 ^c
ECMSBE (100mg/kg) + CCl ₄	29.93 \pm 0.42 ^b	53.33 \pm 2.71 ^b
ECMSBE (150mg/kg) + CCl ₄	18.17 \pm 0.31 ^{ab}	53.16 \pm 1.12 ^b
ECMSBE (200mg/kg) + CCl ₄	28.67 \pm 0.42 ^b	41.54 \pm 0.22 ^b

Values are expressed as mean \pm standard error of mean, n = 5. Mean values having the different superscript letters in a column are significantly different (P<.05) (one-way ANOVA followed by Duncan's multiple range test). GSH = Reduced Glutathione, MDA = Malondialdehyde, ECMSBE = *Eucalyptus camaldulensis* Methanol Stem Bark Extract

Table 3. Effects of administration of ECMSBE on antioxidants vitamins

Dose Administration	Vitamin A ($\mu\text{mol/l}$)	Vitamin C ($\mu\text{mol/l}$)	Vitamin E (mg/dl)
Distilled water (2mg/kg)	10.20 \pm 1.10 ^c	122.12 \pm 0.93 ^c	19.97 \pm 0.65 ^c
CCl ₄ Only	4.89 \pm 0.23 ^a	81.87 \pm 0.39 ^a	11.76 \pm 0.66 ^a
ECMSBE (50mg/kg) + CCl ₄	5.31 \pm 0.23 ^a	97.73 \pm 3.94 ^a	14.31 \pm 0.67 ^a
ECMSBE (100mg/kg) + CCl ₄	7.34 \pm 1.27 ^{ab}	116.54 \pm 1.22 ^b	17.34 \pm 0.62 ^{ab}
ECMSBE (150mg/kg) + CCl ₄	6.68 \pm 0.32 ^{ab}	117.42 \pm 1.71 ^b	16.34 \pm 0.52 ^{ab}
ECMSBE (200mg/kg) + CCl ₄	9.76 \pm 0.33 ^c	111.75 \pm 2.19 ^b	18.14 \pm 0.55 ^b

Values are expressed as mean \pm standard error of mean, n = 5. Mean values having the different superscript letters in a column are significantly different (P<.05) (one-way ANOVA followed by Duncan's multiple range test). ECMSBE = *Eucalyptus camaldulensis* Methanol Stem Bark Extract

Table 4. Effects of administration of ECMSBE on enzymatic antioxidants

Dose Administration	SOD (U/mg)	CAT(U/ml)	GPX (nmol/min/ml)
Distilled water (2mg/kg)	84.33 \pm 2.07 ^b	123.21 \pm 3.98 ^c	65.25 \pm 4.70 ^b
CCl ₄ Only	49.05 \pm 5.78 ^a	79.04 \pm 6.42 ^a	41.17 \pm 3.65 ^a
ECMSBE (50mg/kg) + CCl ₄	53.05 \pm 2.93 ^a	83.89 \pm 2.32 ^a	55.84 \pm 1.81 ^a
ECMSBE (100mg/kg) + CCl ₄	85.01 \pm 3.42 ^b	102.10 \pm 5.15 ^b	63.90 \pm 1.93 ^b
ECMSBE (150mg/kg) + CCl ₄	64.59 \pm 2.59 ^{ab}	97.28 \pm 4.26 ^{ab}	64.58 \pm 2.68 ^b
ECMSBE (200mg/kg) + CCl ₄	75.71 \pm 2.04 ^b	114.47 \pm 3.46 ^c	76.09 \pm 0.91 ^b

Values are expressed as mean \pm standard error of mean, n = 5. Mean values having the different superscript letters in a column are significantly different (P<.05) (one-way ANOVA followed by Duncan's multiple range test). SOD = Superoxide Dismutase, CAT = Catalase, GPX = Glutathione Peroxidase, ECMSBE = *Eucalyptus camaldulensis* Methanol Stem Bark Extract

decrease in MDA levels. This observed decrease might be due to inhibitory effect of the plant extract on lipid peroxidation and also the ability to antagonize the formation of free radical damage caused by CCl₄ [22].

SOD is considered a first-line defence against ROS. This enzyme is present in nearly all cells, and converts $\cdot\text{O}_2$ to H₂O₂. Mitochondrial and bacterial SOD contain Mn, while cytosolic SOD is a dimer containing Cu and Zn. As the H₂O₂ may still react with other ROS, it needs to be degraded by either one of the other two antioxidant enzymes, GSH-Px or catalase [20]. GSH peroxidase is located in the mitochondria. It

catalyzes degradation of H₂O₂ by reduction, where two glutathione (GSH) molecules are oxidized to glutathione disulfide (GSSG). Regeneration of GSH by GSH-reductase, requires NADPH, which is oxidized to NADP⁺. A decline in cellular level of GSH has been considered to be indicative of oxidative stress [23]. Catalase, on the other hand, is localized primarily in peroxisomes, and so it detoxifies the H₂O₂ that diffuses from the mitochondria to the cytosol, converting it into water and molecular oxygen [24].

ROS detoxification agents also includes non enzymatic antioxidants such as vitamin E (α -

tocopherol), vitamin A (retinol), and vitamin C (ascorbic acid) they act in concert to protect against the free radical-induced damage [24]. The present study showed decreased of non-enzymatic antioxidants: Vitamin E, C and A in liver homogenate of CCl₄-treated rats when compared with the normal control group. Ascorbic acid has been proposed to have roles on regulation of photosynthesis and help regenerate GSSG back into GSH [20]. Ascorbic acid is readily oxidised to monodehydro ascorbic acid as part of its antioxidant function [21]. Vitamin E (α -tocopherol) is probably the most important lipid-soluble antioxidant protecting membranes, lipids and lipoproteins [25]. Vitamin E is one of the few nutrients for which supplementation with higher than recommended levels have been shown to enhance immune response and resistance to diseases [22].

Many studies have suggested that high intake of Vitamin E may slow down the development and progression of atherosclerosis. Some clinical trials also reported beneficial effects of Vitamin E supplementation in the secondary prevention of cardiovascular events [23]. The changes in activities of antioxidant enzymes might offer some important clues to explain pathologic mechanism of abnormal free radical metabolism. Increase in activities of antioxidants such as SOD, CAT, and GSH could be an adaptive response of these enzymes to increased production of reactive oxygen species (ROS) [24].

The impairment of the antioxidant defense system is considered as a critical event in CCl₄-induced hepatotoxicity. In this study, the marked inhibition of enzymatic antioxidants including GPx, SOD, and CAT activities accompanied by depletion in non-enzymatic antioxidants including GSH, Vitamin C and E levels of CCl₄ - induced indicates its ability to induce oxidative stress. However, administration of methanol stem bark extract of *E. camaldulensis* significantly reduced CCl₄ induced MDA elevation and increased SOD, GPx, CAT, activities, GSH, Vitamin C, A and E levels indicating the potential of the plant extract as an antioxidant. Phytochemicals such as phenols, saponins and flavonoids are known to possess antioxidant activities and provoke free radical scavenging enzyme system, thus may protect against oxidative stress caused by hepatotoxic agents [24]. The reduction in MDA level, elevation of GSH, Vitamin C and E, enhanced activities of SOD, GPx, and CAT in the liver homogenate of CCl₄ induced treated groups

might be to the inhibitory effect of the plant extract on lipid peroxidation and ability to antagonize the formation of free radical damage caused by CCl₄ [26].

5. CONCLUSION

The results from this study indicate that the methanol stem bark extract of *E. camaldulensis* possesses antioxidant properties. The antioxidant effect of *E. camaldulensis* may be attributed to the presence of phytoconstituents detected in the plant. Further study is recommended to investigate the active principles of *E. camaldulensis* and its mechanism of action.

CONSENT

It is not applicable.

ETHICAL APPROVAL

All authors hereby declare that "Principles of laboratory animal care" (NIH publication No. 85-23, revised 1985) were followed, as well as specific national laws where applicable. All experiments have been examined and approved by the appropriate ethics committee

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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