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Phytochemical Screening and Antibacterial Activity of the Ethanolic Stem Bark Extract of *Eucalyptus Camaldulensis*

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ABSTRACT

In this study, the stem bark of *Eucalyptus camaldulensis* was subjected to preliminary phytochemical and anti-bacterial analysis. The extraction of the stem bark was done with ethanol with a recovery of 12.1 %. The phytochemical evaluation as well as the antibacterial analysis of the ethanolic leaf extract was carried out. The phytochemical analysis showed the presence of carbohydrates, terpenol, cardiac glycoside, flavonoids, tannin and corderoites. The antibacterial analysis of the ethanolic extract showed activity on *Staphylococcus aureus*, *Streptococcus pyogenes*, *E-coli*, and *Pseudomonas aeruginosa* at different concentrations. *Eucalyptus camaldulensis* possesses medicinal value owing to the presence of important phytochemicals as well as inhibitory potential against some selected microbes. Also, *Eucalyptus camaldulensis* could be exploited in the treatment of diseases related to the investigated microbes.

1. Introduction

Plants have continued to be a major source of medicine, as they have always been throughout human history. Medicinal plants, due to their immense therapeutic value, have played an essential role in the healthcare system since ancient times, and they could be a key source of new antimicrobial medications to battle pan- and multi-drug resistant pathogens. These new antibacterials could be found in herbal extracts and essential oils. *Eucalyptus camaldulensis* is a major medicinal plant [1-3].

Herbal remedies are widely available for the treatment and prevention of various diseases and often contain highly active pharmacological compounds. Many plants synthesize substances that are useful for the maintenance of health in humans and other animals. These substances, most of which are phenols or their oxygen substituted derivatives such as tannins, secondary metabolites of plants which have been isolated are at least 1200. In many cases, these substances (particularly alkaloids) serve as plant mechanisms against predation by microorganisms, insects, and herbivores [4]. Plant-originated drugs present safer health benefits in comparison to chemical drugs, hence, more than 80 % of the population of the world opt for medicinal plants for the sustenance of their primary health care needs [5-6].

Phytochemicals are active secondary plant metabolites that are responsible for the claimed medicinal activities of plants. *Eucalyptus camaldulensis* is one of those plants that possess these phytochemical properties and is reported to possess medicinal activities on various ailments. the phytochemical constituents of various parts of this plant have been investigated using standard methods of phytochemical screening. The presence of these phytochemicals in *E. camaldulensis* could therefore justify the applications of the plant in the management and curing of various ailments as claimed traditionally. Most phytochemicals are more useful in extracted forms and the extraction is normally done by using suitable solvents because the required components may not involve the whole plant and in some cases, non–

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useful toxicants need to be eliminated [7]. Phytochemicals are most useful in several aspects of life including corrosion inhibition, pharmaceutical applications among others [8]. Studies have indicated that different solvents extract phytochemicals to the different extents [9].

The primary process of obtaining compounds in their crude form from plants is known as extraction [3]. The quantity extracted as well as the quality are a function of the type of processes, structures of the compound, and natural resources. The solvent extraction methods that have been used over the years for extraction include decoction, infusion, maceration, percolation, and digestion. Various solvents have also been used in this process. These include methanol, water, ethanol, dimethyl-sulfoxide, chloroform, among many others [10]. However, these techniques are demanding regarding the extraction process duration, organic solvent consumption, and lack of extraction automation.

The plant, *eucalyptus camaldulensis*, commonly known as the river red gum, belongs to the family of Myrtaceae [11]. It is a large evergreen, fast-growing plant (20 – 40 m high) and can withstand very high temperatures while tolerating long drought periods. It has a stout trunk, widely spreading, irregular smooth bark shedding at intervals throughout the year to show white, yellow, and grey, becoming rough at the base [12-13]. It is regarded as one of the most planted eucalypts especially in Nigeria, Argentina, Arizona, California, etc [14]. The reported properties of the extracts of this plant include but are not limited to antihyperglycemic, ulcer healing, antioxidant, cytotoxic effect, antibacterial activity, antimicrobial and anti-inflammatory activity [13,15-21]. Medically, *E. camaldulensis* has been used as an analgesic, anesthetic, astringent and antiseptic for the treatment of gastrointestinal symptoms – dysentery, colic, and diarrhea – arrests bleeding cuts and open wounds, decocts for the relief of pains, spasms and aches, and respiratory disease – laryngitis, trachalgia, colds, laryngalgia, coughs, sore throat and asthma [3,13,21].

Figure 1: *Eucalyptus camaldulensis* Source: Behind Murtala Hall, University of Maiduguri

Pseudomonas aeruginosa is a gram negative bacterium with unipolar motility [22].

P. aeruginosa is an opportunistic pathogen of humans and plants. *Escherichia Coli* is a gram-negative bacterium that is commonly found in the lower intestine of warm-blooded animal animals.

Staphylococcus aureus is a gram-positive spherical bacterium that colonizes mainly the nasal passages. *S. aureus* causes a variety of supperative (pus-forming) infections and toxinoses in humans. It causes superficial skin lesions such as boils, styes, and furunculosis, more serious infections such as pneumonia, mastitis, phlebitis, meningitis, and unitary tract infections. *S-aureus* causes food poisoning by releasing enterotoxins into the food and toxic shock syndrome by the release of superantigens into the bloodstream. *Streptococcus* is a genus of gram-positive coccus or spherical bacteria that belongs to the family of *streptococcaceae*, within the order *Lactobacillales*, in the phylum *Firmicutes*. Cell division in streptococci occurs along a single axis, so as they grow, they tend to form pairs or chains that may appear bent or twisted. There are many different types of streptococci and infections vary in severity from mild throat infections to pneumonia.

This work aimed at screening phytochemicals and evaluating the antibacterial activity of ethanolic extract of the stem bark of *Eucalyptus camaldulensis*. To this end, the following objectives were achieved: extraction of the stem bark of *Eucalyptus camaldulensis*, phytochemical analysis of an ethanolic extract of stem bark of *Eucalyptus camaldulensis*, and study of antibacterial potential of the stem bark ethanolic extract against selected microbes (*Staphylococcus aureus, Streptococcus pyrogens, Escherichia coli, and Pseudomonas aeruginosa).*

2. Results and Discussion

The extraction of the stem bark of *Eucalyptus camaldulensis* using ethanol produced extracts with coffee brown colors which were powdery respectively. The yield was as high as 30.25 g as presented in the table below.

Ethanol has been reported as the best extractant for *E. camaldulensis* as it gives a percentage yield of 10 % and above. Al–Sanafi [21] and, Alghamdi and Ababutain [13] reported ethanol as best among water and methanol for the extraction of the bark and leaves of *E. camaldulensis.* In this work, however, the yield of 12.1 % is an improvement from the 11.6 % obtained by Alghamdi and Ababutain [13]. This variation may be due to the location and the accompanying soil compositional differences.

Table 1. The extraction profile of stem bark of E*ucalyptus camaldulens*

Extract	Yield (g)	Colour	Percentage(%)	Texture
Ethanol stem bark	30.25	brownish	12.1	powdery

Standard methods were used to study the phytochemical constituents and the result revealed that for stem bark extract of *eucalyptus camaldulensis,* saponins, flavonoids, tannins, terpineol, carbohydrates, cardiac glycosides, cardenolide, and carbohydrates were present as presented in table 2 below.

Phytochemical analysis of *Eucalyptus* ethanolic stem bark extract showed the presence of bioactive compounds including tannin, cardiac glycosides, saponins, flavonoids, and terpineol compounds (Table 2). Sani *et al* [27] had previously reported the presence of tannins, saponins, and steroids in ethanolic extract of *E. camaldulensis*. This agrees with previous research reported by Ishnava, *et al* [28] and Ishag *et al*, [29]. The result also showed that when it comes to secondary metabolism, *E. camaldulensis* has been reported as one of the richest plants due to the presence of the above bioactive compounds. This study is also in agreement with previous work done by Babayi *et al* [30], Ayepola and Adeniyi [31], and, Alghamdi and Ababutain [13].

Flavonoids were reported as prostaglandin synthase inhibitors [30]. Prostaglandins are known to be involved in pain perception [31]. The presence of tannins and saponins possibly might have given rise to the observed anti-bacterial property and contributed to the antipyretic activity of the plant extract. Saponins possess a wide range of therapeutic actions in the body including an anti-inflammatory, anti-pyretic, expectorant, anti-malarial, and hemolytic effects on red blood cells, while tannins are compressed for cuts and wounds, hemorrhoids, varicose veins, and in medicine diarrhea, catarrh, heavy menstrual flows and inflammatory conditions of the digestive tract [23]. Cardiac glycosides incretion increase the force of myocardial contraction and reduce conductivity within the atrioventricular (AV) node. These compounds have been known to exert pharmacological and antagonistic effects. Phytochemicals such as alkaloids, flavonoids, glycosides, and saponins possess a wide range of therapeutic actions in the body including antipyretic.

Table 2. Phytochemical Constituents of ethanolic stem bark extract of *eucalyptus camaldulensis*

S/No		Test	Inference
1	Alkalide	Drangendroof,s reagent	-
		Mayer's reagent	-
2	Flavonoids	Shinoda's	+
		Ferric Chloride	+
		Lead Acetate	+
		NaOH	-
3	Cardiac glycoside	Salkoski's test	+
		Liberman-Burchorid	+
4	Corderoites	Killer-killiani	+
5	Glycosides	Free anthraquinone	-
		Combined anthraquinone	-
6	Tannin	Ferric chloride	+

		Lead Acetate	+
7	Terpenol	Terpenol	+
8	Carbohydrates	Molisch	+
		Monosaccharide	+
		Reducing sugar	+
		Combined reducing sugar	+
		Ketoses	
9	Saponin	Frothing's test	+
10	Phlobatannin		+

The antibacterial activity *of E. camaldulensis* stem bark extracts was assessed against four bacterial isolates (*Pseudomonas aeruginosa, Escherichia Coli, Staphylococcus aureus,* and *Streptococcus pyogenes).* The results showed that the *E. camaldulensis* extract has inhibitory activity against all tested bacteria. This agrees with earlier work done by Abubakar [32] who tested and got a positive results on the use of crude leaf extracts of *Eucalyptus camaldulensis* against clinical isolates of *Escherichia coli, Staphylococcus aureus, Salmonella typhi, Proteus mirabilis,* and *Klebsiella pneumoniae*. *E. camaldulensis* extracts may be used as a natural antibiotic for their bactericidal effect to treat serious infections caused by pathogenic bacteria. The antimicrobial activity of *E. camaldulensis* has also been reported by Nwabor *et al* [33] who researched on the antioxidant and antibacterial effect of ethanolic leaf extract of *E. camaldulensis* and reported from the findings that the plant's leaf extract can be a potential alternative source of bio preservative agent against *Lysteria monocytogenes*

Table 3: Susceptibility test for CEE on the test organism at different concentrations ml/disc and their zone of inhibition (mm). Concentration (mg/disc) of the CEE and standard drug diameter zone of inhibition (mm) as mean ± SEM

Test organisms	**Gentamicin**	**Zones of inhibition(mm)**		
		100	**150**	**200**
Staphylococcus aureus	10.25±0.74	0.00±0.00	0.00±0.00	4.25±0.56
Streptococcus pyogenes	0.00±0.00	0.00±0.00	8.25±0.56	25 .0±55.9
Escherichia coli	9.75.5±0.56	0.00±0.00	0.00±0.00	19.75±0.56
Pseudomonas aeruginosa	10.25±0.92	0.00±0.00	0.00±0.00	0.00±0.00

Key: < 08 = Inactive, 09 – 11 = Weakly active, 12 – 15 = Moderately active, 16 – 18 = Highly active, > 19 = Very highly active

3. Experimental

3.1. Sample collection and identification

The stem bark of Eucalyptus camaldulensis was collected at the University of Maiduguri Campus, Maiduguri Borno State, Nigeria and authenticated by a plant taxonomist at the Department of Biological Sciences, University of Maiduguri, Nigeria. The sample was air-dried at ambient temperature (28 – 31°C) and pulverized using a porcelain mortar and pestle. It was thereafter sieved using a 2.00 mm sieve, after which, 500 g was weighed, mixed thoroughly for experimental tests.

3.2. Plant Extraction

A quantity equivalent to 1 L of 95 % ethanol was poured into a round bottom flask. A 250 g of sample was placed in the thimble and the thimble was inserted into the center of the extractor. The extractor with its content was heated under reflux at 60 ^{o}C for three hours.

3.3. Phytochemical Screening of Eucalyptus Camaldulensis

Preliminary phytochemical screening was carried out on stem back ethanolic extracts of the Eucalyptus camaldulensis to check for the presence of carbohydrates, flavonoids, cardiac glycoside, alkaloids, anthraquinone, tannin, terpineol, and saponin.

Molish's Test: This test was carried out on the extract using distilled water, molish's reagent, and H_2SO_4 according to a method by Trease and Evans [23].

Test for Monosaccharide*:* The test was done on 0.5 g of the extract, distilled water, Whatman number 4 filter paper, and Barfoed's reagent in accordance with a method by Trease and Evans [23].

Test for Free Reducing Sugar: Fehling's test was used to check for reducing sugar [23].

Test for Combined Sugar: For the combined sugar, 0.2 g of the extract, HCl, NaOH, and Fehling's solution were used for this test [23].

Test for Alkaloids: this test was carried out on 0.5 g of the extract using 1 % HCl, Drangendroff's reagent, Mayer's reagent, and Wagner's reagent [23].

Test for Flavanoids

Ferric Chloride Test: distilled water and 10 % ferric chloride was used to test for the presence of phenolic hydroxyl group [23].

Shinoda's Test: Ethanol, magnesium chips, and HCl were used to test for the presence of flavonoids on 0.5 g of the extract [24].

Lead Ethanoate Test: This test was done using lead ethanoate solution [23].

Sodium Hydroxide: 10 % $NaOH_{(aq)}$ and $HCl_{(dil)}$ were used in this test [23].

Test for Cardiac Glycosides

Test for steroidal Nucleus: Salkowski's, Liebermann-Burchard's, and Killer-Killiani's tests were used to achieve this test [25].

Test for Anthraquinone and Combined Anthraquinone: These tests were carried out using Borntrager's test [23].

Test for Tannins: Ferric Chloride and Lead Acetate tests were used to carry out this test [23].

Test for Terpenoids: This was done using ethanol, acetic anhydride, and H_2SO_4 [23].

Test for Ketones was achieved using Salivanoff's test [26].

Test for Saponins: The froth test and emulsion test as described by Harborne [26] was used to determine the presence of saponins.

Test for Phlobatannins was carried out on the extract using distilled water and 1% $HCl_{(aq)}$ [23].

3.4. Antimicrobial study

Test for organisms: The organisms tested for include *Staphylococcus aureus*, *Streptococcus pyrogens, Escherichia coli, and pseudomonas aeruginosa*. These were clinical laboratory isolates obtained from the department of medical microbiology university of Maiduguri, Borno state.

Preparation of the media**:** Mueller Hinton agar was weighed and prepared in an autoclave at 121 ^oC for 15 minutes. It was dispensed into a sterile petri dish and allowed to solidify.

Inoculation of Organism on Culture Media**:** Gram-positive (*Staphylococcus aureus*, *Streptococcus pneumoniae*) and gram-negative (*Escherichia coli, and Pseudomonas aeruginosa*) were used for this work. Respective organisms/isolates were dispersed inside peptone water and allowed to reach a Mcfarland standard. It was poured unto the dried media. The media was freely allowed to dry of its content. Four different wells were made on the media containing the organisms. The wells were labeled according to the concentration of the extract (CD, DD) and antibiotic control (gentamicin). A 25 µL of the concentrated extract was placed on the culture plate and incubated at 37 ^oC for 18 hrs. The zones of inhabitation were measured to determine the activity of the extract against respective organisms.

Preparation of Extract: A 10 mL sterile distilled water was placed into a sterile universal container, then 1 g of the plant extract was added making a concentrated solution of the extract. A 5 mL of sterile distilled water was placed into two different containers. A 5 mL from the concentrated extract was transferred into a universal container with 5 mL of sterile distilled water making it a diluted solution of the extract from the diluted solution of the extract was transferred into the last container containing 5ml of water and mixed making the volume 10 mL and concentration doubled. Generally, we have three solutions containing concentration (100 mL), dilute (150 mL), and double dilute solution (200 mL).

4. Conclusion

The results of this investigation confirm the presence of bioactive phytochemicals in *Eucalyptus camaldulensis* ethanolic extract. It further reveals that the stem bark of plants contains some major bioactive compound that inhibits the growth of microorganisms thereby proving to be very effective as an alternative source of antibiotics.

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