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The Antibacterial Activity of Leaf Extracts of *Eucalyptus camaldulensis* (Myrtaceae)

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Abstract: The antibacterial activity of the leaf extracts of *Eucalyptus camaldulensis* was studied against *Klebsiella spp, Salmonella typhi, Yersinia enterocolitica, Pseudomonas aeruginosa* (Gram-negative), *Staphylococcus aureus* and *Bacillus subtilis* by the agar diffusion method. The methanol extract, dichloromethane fraction and methanol residue at 10mg mL^{-1} displayed broad spectrum activity against all the test organisms but the petroleum ether fraction showed no activity. The antibacterial activity of the extracts was compared to the drug gentamycin. The minimum inhibitory concentrations of the methanol extract and dichloromethane fraction determined by the agar dilution method ranged between 0.04 and 10mg mL^{-1} with that of *Bacillus subtilis* being the least. Phytochemical screening of the plant revealed the presence of tannins, saponins and cardiac glycosides. The results of this study support the traditional use of *Eucalyptus camaldulensis* leaves as an antibacterial agent.

Key words: *Eucalyptus camaldulensis*, antibacterial activity, minimum inhibitory concentration, methanol extract, dichloromethane fraction.

INTRODUCTION

Plants have been used for the treatment of diseases all over the world before the advent of modern clinical drugs and are known to contain substances that can be used for therapeutic purposes or as precursors for the synthesis of useful drugs [14]. Thus over 50% of these modern drugs are of natural products origin and as such these natural products play an important role in drug development in the pharmaceutical industry [7].

Eucalyptus camaldulensis is an important ethnomedicinal plant belonging to the family, Myrtaceae. It is used as a remedy for sore throat and other bacterial infection of the respiratory and urinary tracts. Essential oils of the leaves are used in the treatment of lung diseases while the volatile oils are used as expectorants [2]. Topical ointments containing eucalyptus oil have also been used in traditional Aboriginal medicines to heal wounds and fungal infections. Eucalyptus oil obtained by steam distillation and rectification of the fresh leaves has Eucalyptol (1,8-cineole) as its active ingredient and this is responsible for its various pharmacological actions [16]. The antimicrobial activities of the methanolic extracts of E. camaldulensis have also been reported [3,10].

The emergence of bacterial resistance to the currently available antimicrobial drugs necessitates further research in the discovery of new safe and effective antibacterial agents[9]. The investigation of certain indigenous plants for their antimicrobial activity is therefore of utmost importance. This study is aimed at investigating the antimicrobial activity of *Eucalyptus camaldulensis* against Gram-positive and Gram-negative bacteria thereby establishing it as a potential antimicrobial agent.

MATERIALS AND METHODS

Plant Material: *Eucalyptus camaldulensis* leaves were collected around the Department of Forestry, University of Ibadan and authenticated at the Department of Botany and Microbiology, University of Ibadan. A voucher specimen was deposited at the Herbarium for reference purposes. The leaves were air-dried and then ground before use for this study.

Extraction Procedure: The dried and powdered leaves (400g) were subjected to soxhlet extraction with methanol as the extraction solvent. A part of the methanol extract was then partitioned into petroleum ether and dichloromethane. The extracts were filtered and allowed to evaporate to dryness. Each extract was transferred into clean and dried airtight vials until ready for use.

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Phytochemical Screening: Samples of *Eucalyptus camaldulensis* leaves were screened phytochemically for the presence of secondary metabolites using the standard methods of Harbone,[6] and Trease and Evans [15]. The secondary metabolites screened for are saponins, alkaloids, anthraquinones, tannins and cardiac glycosides.

Bacterial Strains: The organisms used in this study were four Gram-negative bacteria and two Gram positive bacteria namely, *klebsiella spp, Salmonella typhi, Yersinia enterocolitica, Pseudomonas aeruginosa* (Gram-negative), *Staphylococcus aureus* and *Bacillus subtilis* (Gram-positive). The organisms were obtained from the Department of Pharmaceutical Microbiology, Faculty of Pharmacy, University of Ibadan.

Determination of antibacterial activity: The antibacterial activity of the extracts was determined using the agar cup diffusion as described by Adeniyi *et al* [1]. A 1 mL of an overnight culture of each bacterial isolate (equivalent to 10^7-10^8cfu mL^{-1}) was used to seed sensitivity test agar plates maintained at 45°C. The seeded plates were allowed to set and a sterile cork borer of 8mm diameter was used to cut equidistant wells on the surface of the agar. The wells were filled with 0.1mL solution of each extract reconstituted with methanol at a concentration of 10mg mL^{-1}. Gentamycin at 5µg mL^{-1} was included as positive control. The plates were incubated at 37°C for 24h after which the diameter of zones of inhibition were measured.

Determination of Minimum Inhibitory Concentration (MIC): The determination of the minimum inhibitory concentration of the methanol extract and the dichloromethane fraction was carried out using the agar dilution method described by Lajubutu *et al* [8]. Different concentrations of the extracts were prepared to give a final concentration in the range of 10 to 0.039 mg mL^{-1}. 2ml of each dilution was mixed with 18ml of Mueller-Hinton agar, poured into Petri dishes and allowed to set. The agar was streaked with an overnight broth culture of the test organisms and incubated overnight. The lowest concentration inhibiting growth was regarded as the minimum inhibitory concentration of the extracts.

RESULTS AND DISCUSSION

Qualitative phytochemical screening of the extracts of *E. camaldulensis* (Table 1) demonstrated the presence of tannins, saponins and cardiac glycosides while anthraquinones and alkaloids were absent.

The results of the antibacterial screening of the plant leaves (Table 2) revealed that the methanol extract, dichloromethane fraction and the methanol residue represent a broad spectrum of activity. All extracts showed varying degrees of inhibition on the tested microorganisms at a concentration of 10 mg mL^{-1}. The methanol extracts showed greater activity against *Salmonella typhi, Staphylococcus aureus* and *Bacillus subtilis* (15 -16mm) than *Klebsiella spp, Yersinia enterocolitica* and *Pseudomonas aeruginosa* (14mm). The dichloromethane fraction exhibited higher activity against *Klebsiella spp, Salmonella typhi, Yersinia enterocolitica* and *Bacillus subtilis* (15–16mm) than *Staphylococcus aureus* and *Pseudomonas aeruginosa* (13-14mm). The methanol residue had a lower activity against all the test organisms except *Klebsiella spp* and *Salmonella typhi.* The result also showed that *Klebsiella spp* and *Yersinia enterocolitica* which were not inhibited by gentamycin used as a positive control in this study were inhibited by the extracts. However the petroleum ether fraction showed no activity on all test organisms.

The minimum inhibitory concentration of the methanol extract and dichloromethane fraction of the leaves ranged between 0.04 and 10mg mL^{-1} (Table 3). The lowest MICs for both extracts were those for *Bacillus subtilis* (0.04 - 0.079 mg mL^{-1}).

Table 1: Phytochemical components of the leaf extracts of *E. camaldulensis*

Phytochemical components	*E. camaldulensis*
Alkaloids	-
Tannins	+
Saponins	+
Anthraquinones	-
Cardiac Glycosides	+

Note: + = Present- = Absent

Discussion: The antibacterial activity of the leaf extracts of *Eucalyptus camaldulensis* can be attributed to the action of the phytochemical compounds it contains. Babayi *et al*[4]. reported the presence of saponins, saponin glycosides, steroid, cardiac glycosides, tannins, volatile oils, phenols and balsam (gum) in the plant. The presence of these compounds in the family Myrtaceae to which *E. camaldulensis* belong has been reported by Pamplona-Roger[11].

Polyphenolic compounds and /or volatile oils are known to inhibit a wide range of organisms[5]. The antimicrobial activity of the extracts could be explained by the presence of tannins. The mechanism of action of tannins is based on their ability to bind proteins thereby inhibiting cell protein synthesis[13]. There was no significant difference in the antimicrobial activity of the extracts on Gram-negative and Gram positive bacteria despite the differences in their cell wall components. Akin-Osanaiye *et al*[3]. reported the antibacterial activity of *E. camaldulensis* extracts against *Staphylococcus aureus* while Sherry *et al* [12]

Table 2: Antibacterial activities of the leaf extracts of *E. camaldulensis* at 10mg mL-1

Organisms	MeOH	Pet. Ether	Dichl.	MeOH-residue	Gentamycin
Klebsiella spp	14	-	15	15	-
Salmonella typhi	16	-	15	15	17
Yersinia enterocolitica	14	-	16	13	-
Pseudomonas aeruginosa	15	-	14	13	26
Staphylococcus aureus	15	-	13	14	28
Bacillus subtilis	16	-	16	14	28

Resistance= no zone of inhibition, Positive control-Gentamycin (5µg mL^{-1}), MeOH- Methanol, Pet. Ether- Petroleum ether fraction, Dichl- Dichloromethane fraction.

Table 3: Minimum Inhibitory Concentration (MIC) of the leaf extracts of *E. camaldulensis*

Organisms	ME (mg mL^{-1})	DF (mg mL^{-1})
Klebsiella spp	10	10
Salmonella typhi	10	10
Yersinia enterocolitica	0.157	0.625
Pseudomonas aeruginosa	10	10
Staphylococcus aureus	1.25	0.625
Bacillus subtilis	0.04	0.079

ME= Methanol Extract, DF= Dichloromethane Fraction

revealed that topical applications of eucalyptus oil clears methicillin resistant *Staphylococcus aureus* infections. The methanol extract of *E. camaldulensis* has been found to be effective against *staphylococcus aureus* and Bacillus subtilis[4]. The results of the tests for minimum inhibitory concentration (MIC) revealed that the MIC for *Yersinia enterocolitica*, *Staphylococcus aureus* and *Bacillus subtilis* (between 0.04mg mL^{-1} and 0.625mg mL^{-1}) were lower than that of *Klebsiella spp*, *Salmonella typhi* and *Pseudomonas aeruginosa* (10mg mL^{-1}). This means that higher doses of the antimicrobial agent will be needed in the treatment of infections caused by *Klebsiella spp*, *Salmonella typhi* and *Pseudomonas aeruginosa* provided they are not toxic to the tissues.

Conclusion: The results of this study have shown that the leaf extracts of *E. camaldulensis* have great potential as antimicrobial agents in the treatment of infectious organisms. Further detailed investigation of the active components of the plant for the exact mechanism of action will contribute greatly to the development new pharmaceuticals.

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