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Full Length Research Paper

Evaluation of phytochemical and antimicrobial potentials of roots, stem-bark and leaves extracts of *Eucalyptus camaldulensis*

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The extracts for phytochemical screening and antimicrobial activity were carried out on dried powdered leaves, stem-bark and roots of *Eucalyptus camaldulensis* using ethanol and water as solvent. The results of the phytochemical screening showed that the plant parts contained saponins, tannins, phenols and glycosides. The disc diffusion method was adopted for the antimicrobial activity of the plant extracts. The antimicrobial activity test of the plant extracts on *Escherichia coli*, *Staphylococcus aureus*, *Salmonella typhi* and *Bacillus subtilis* showed that both water and ethanol extracts had inhibitory activity on all the tested organisms except the roots and stem-bark extracts of ethanol which showed no measurable zone of inhibition. The results obtained from this study revealed that extracts of *E. camaldulensis* possess antimicrobial activities against some microorganism that causes diseases.

Key words: *Eucalyptus camaldulensis*, phytochemical, medicinal, ethanol, diseases.

INTRODUCTION

Antimicrobial agents are substances that interfere with the growth and metabolism of microbes. In common usage, the term denotes inhibition of growth and with reference to specific groups of organisms, terms as antibacterial, antifungal, antiviral and antiprotozoa are frequently employed. Antimicrobial agents may either kill microorganisms or inhibit their growth. Those that inhibit growth are called bacteria static. These agents depend on the normal host defenses to kill or eliminate the pathogens after its growth has been inhibited. For example, sulfa drugs, which are frequently prescribed for urinary infections, inhibit the growth of bacteria in the bladder until they are eliminated during the normal process of urination. Antimicrobial agents that kill are bactericidal. These antimicrobial agents are particular useful in situations in which the normal host defenses cannot be relied on to remove or destroy pathogens. A given antimicrobial can be bactericidal in one situation, yet bacteria static in another, depending on the concentration of the drug and the growth stage of the microorganism (Nester et al., 2004).

Some antimicrobial agents are used to treat infection and they are called chemotherapeutic agents. Chemotherapeutic agents are chemicals substance used for the treatment of infectious diseases or disease caused by the proliferation of malignant cells. These substances are prepared in the chemical laboratory or obtained from microorganisms and some plants and animals in general, naturally occurring substances are distinguished from synthetic compounds by the name antibiotics. Some antibiotics are prepared synthetically, but most of them are prepared commercially by microbial biosynthesis.

Chemotherapeutic agents must have selective toxicity for the parasite, which means a low toxicity for host cells and high toxicity for the parasite (Pelezer et al., 1993).

In Nigeria, application of medicinal plants especially in traditional medicine is currently well acknowledged and established as a viable profession (Kafaru, 1994).

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Table 1. Results of phytochemical screening of *E. camaldulensis*

Bioactive compounds	Water extracts			Ethanol extracts		
	Roots	Stem-bark	Leaves	Roots	Stem-bark	Leaves
Tannins	+	+	-	+	+	+
Saponins	+	+	-	+	-	+
Phenols	+	+	+	+	-	+
Glycosides	+	+	+	+	+	+
Alkaloids	-	-	-	-	-	-

+ = Present, - = Absent.

Extraction of bioactive compounds from medicinal plants permits the demonstration of their physiological activity. It also facilitate pharmacology studies leading to synthesis of a more portend drugs with reduced toxicity (Ebana et al., 1991).

Presently, in the developing countries, synthetic drugs are not only expensive and inadequate for the treatment of diseases, but are also often with adulterations and side effects (Shariff, 2001) and the increasing resistance of most synthetically derived antimicrobial agents are of utmost concern (Adeniyi and Ayepola, 2008). Therefore, there is need to search for suitable plants of medicinal value to be effectives in the treatment of diseases, which must be harmless to human tissue.

Eucalyptus species have been planted on many parts of the world. It 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 (Adeniyi et al., 2006).

Eucalyptus oil is readily steam distilled from the leaves and can be used for cleaning, deodorizing and in very small quantities in food supplements, especially sweets, cough drops and decongestants (Schulz et al., 1998). It may also provide antiseptic properties (Chao and Young, 1998). The leaf extract of some species (*Eucalyptus globulus, Eucalyptus maculate and Eucalyptus viminalis*) have been reported to inhibit some Gram-positive bacteria (Takahashi et al., 2004). Fungicide activity has also been reported (Essien and Akpan, 2004; Mehraban et al., 2005).

The aim of this study was to investigate the phytochemical composition and antimicrobial activities of the crude leaves, stem-bark and root extracts of *Eucalyptus camaldulensis* against some pathogenic microorganisms.

MATERIALS AND METHODS

Sampling and sample preparation

Fresh sample of roots, stem-bark and leaves of the *E. camaldulensis* were collected from the premises of Modibbo Adama University of Technology, Yola and was identified by Dr. Jatau, D.F. The identified plant parts were washed with tap water and air-dried. The dried parts were chopped into pieces, milled into fine powder by pounding manually with a clean and sterile pestle and mortar. The dried powdered samples were each collected into sterile cellophane bags and labelled to prevent mix up. The samples were kept in cool dry place till further use. The dried powdered samples were used for extraction purposes.

Extraction procedure

250 ml of water was added to 50 g each of the dried powdered samples in a flask. Each of the soaked sample was stirred, sealed with aluminium foil and allowed to stand for 72 h. The content was then filtered with Whatman No. 1 filter paper. The filtrates were concentrated using rotary evaporator at 40℃. The extracts were stored in a universal bottle and refrigerated at 4℃ prior to use (Mann et al., 2008).

The same procedure was followed with dried powdered samples as described earlier except that ethanol was used as solvent in place of distilled water.

Phytochemical analysis

The preliminary phytochemical analysis of the extracts were carried out to determined the presence of tannins, saponins, alkaloids, phenols and glycosides as described (Harborne, 1998; Evans, 1998; Abulude, 2007; Fadeyi, 1987). Results are as shown in Table 1.

Test for saponins

5 ml of the extract was vigorously shaken with 10 ml of water in a test tube. Frothing which persisted was taken as an evidence for the presence of saponins.

Test for tannins

Extract plus 4 ml of water and drops of ferric chloride were mixed. Immediate green precipitate was taken as evidence for the presence of tannins.

Test for alkaloids

2 ml of the extract plus picric acid were mixed; an orange coloration was taken as evidence for the presence of alkaloids.

Table 2. Results of antimicrobial activity of roots, stem-bark and leaves of *E. camaldulensis.*

Test organism	Water extracts			Ethanol extracts			Gentamicin (Control)
	Roots	Stem-bark	Leaves	Roots	Stem-bark	Leaves	
E. coli	17	12	13	-	-	18	26
S. aureus	18	13	15	19	20	22	23
S. typhi	16	13	15	16	14	13	28
B. subtilis	17	12	13	22	17	24	22

- = No measurable zone of inhibition.

Test for phenols

Equal volume of the extract was added to equal volume of ferric chloride, a deep bluish green solution was taken as a positive test for the presence of phenols.

Test for glycosides

5 ml of extract plus 25 ml of dilute sulphuric acid were poured into a test tube. The mixture was boiled for 15 min, cooled and neutralized with 10% sodium hydroxide and 5 ml of Fehling A and B was added. Brick red precipitate is a positive test for the presence of glycosides.

Test organisms

The organisms used in this study include, *Escherichia coli*, *Staphylococcus aureus, Salmonella typhi* and *Bacillus subtilis*.

Collection of test organisms

The organisms were collected from the microbiology laboratory of the Peace Hospital, Jimeta, Yola with the help of the laboratory staff. *E. coli* was collected in peptone water and labeled S_1. The same procedure was repeated for the collection of *S. aureus*, *S. typhi* and *B. subtilis*. These were labeled S_2, S_3 and S_4, respectively.

Preparation of nutrient agar/nutrient broth

This was carried out as described by Monica (2000). 28 g of the nutrient agar powder was dissolved in 1000 ml of distilled water in a conical flask and was autoclaved for l5 min at 121°C, and then, it was allowed to cool to 47°C and dispensed into plates or slants. The slants were used in culturing and sensitivity test of the organisms.

28 g of nutrients broth powder was dissolved in 1000 ml of distilled water in a conical flask and was autoclaved at 121°C for 15 min; it was then allowed to cool to 47°C and was dispensed into test tubes.

Antimicrobial investigation

The stocks were maintained on nutrient agar slant and sub-cultured in nutrient both for incubation at 37°C prior to each antimicrobial testing. Inoculation of the test organisms on nutrient agar prepared plates was achieved by flaming a wire loop on a spirit lamp, cooling the wire loop (air cooling) and fetching the test organisms. The discs were prepared using a Whatman filter paper, kept in vials-bottles and sterilized in an oven at 150°C for 15 min. Prepared discs containing the various extracts were carefully placed on the inoculated plates using a sterilized forceps in each case (Fatope, 1993). The plates were then turned upside-down and inoculate at 37°C for 24 h in an incubator. After incubation, the inoculated plates were observed for zones of inhibition (in mm diameter). The result was taken by considering the zone of growth and inhibition of the organisms by the test fractions (Mackie and McCartney, 1989). Activity and inactivity were observed in accordance with the standard and acceptable method. Results are as shown in Table 2.

RESULTS AND DISCUSSION

The phytochemical analysis (Table 1) revealed the presence of tannins, saponins, phenols and glycosides in the roots and stem-bark of water extracts. Only phenols and glycosides were present in the water extract of the leaves. In the ethanol extracts, all the bioactive compounds tested were present in the roots and leaves except alkaloids which are absent. Tannins and glycosides were present in the stem-bark of ethanol extract, but saponins, phenols and alkaloids were absent. It is interesting to note that in both the water and ethanol extracts of the roots, stem-bark and leaves of *E. camaldulensis,* alkaloids was completely absent. Phytochemical screening of the extracts varies from one plant part to another as revealed in the results. It could also vary from place to place due to geographical location, climatic conditions and soil condition of a particular area. This may explain why it could be possible to have differences in chemical composition of the same plant of study in other areas.

The crude extracts of medicinal plant studied were found to contain the following phytochemical compounds, saponins, tannins and phenols. Other investigators reported the presence of these components, cardiac glycoside and volatile oils (Ahmad et al., 1998).

The antimicrobial activities of the test organisms are as shown in Table 2. Antimicrobial susceptibility of the extracts against the test organisms showed that both extract has activities on the entire test organism except *E. coli* which shows no measurable zone of inhibition with ethanol extract from roots and stem-bark of the plant.

The inhibitory effects of these medicinal plants on the microorganisms may therefore, be due to the presence of the aforementioned phytochemical components. The

results of the present study showed that the crude extracts of *E. camaldulensis* inhibit the growth of *S. typhi, E. coli*, *B. subtilis* and *S. aureus*. Differences in polarity among various solvents have been reported to be accountable for the differences in solubility of plant active principles, hence, variation in degree of activities. Results also showed that activities of all the extracts were concentration dependent. Highest activity was demonstrated by the standard antibiotic gentamicin (control). This is because the antibiotic is in it pure state and has refined processes that have established it as a standard antibiotic (Prescott et al., 2002). Results of this study therefore have shown that *E. camaldulensis* is a potential source of antibiotic substances for drug development for use against this group of organisms that causes diseases. Thus, both extracts had antimicrobial activities and confirmed the historical use of *E. camaldulensis* oil as an antimicrobial agent (Kumar, 1988).

Conclusion

This study has shown that the extracts of *E. camaldulensis* possess antimicrobial potentials found to be effective against pathogenic microorganisms involved in wounds infections, urinary tract infections, gastrointestinal tract infections and typhoid fever. Therefore, the results of this study provide a rationale for the use of the plant parts in traditional medicine practice in Nigeria. The activities of *E. camaldulensis* should further be investigated against wide range of microorganisms. Also, purification and toxicological studies should be carried out with a view of sourcing antimicrobial agents for drug development.

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