MedCrave Step into the World of Research
International Journal of Complementary & Alternative Medicine

Research Article

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Phytochemical and antimicrobial potentials leaves extract of eucalyptus globulus oil from maichew Tigray Ethiopia

Abstract

The extracts for phytochemical screening and antimicrobial activity were carried out on dried fresh leaves, Eucalyptus globulus using 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 water extracts had inhibitory activity on all the tested organisms. The results obtained from this study revealed that extracts of Eucalyptus globules possess antimicrobial activities against some microorganism that causes diseases.

Keywords: eucalyptus globulus phytochemical, medicinal, water, diseases

Volume 3 Issue 1 - 2016

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Received: September 24, 2015 | **Published:** January 13, 2016

Introduction

Since the first a agricultural settlement, mankind exploited plants such as forth grass, herbs and fruit yielding trees for their medicinal values. Nowadays, following the discovery of different type of medicinal plants and development of their therapeutic application, the practice of traditional medicine well acknowledge and established as a proffesion. Through a number of medical and clinical researches, researchers are able to demonstrate the specific physiological activity of a particular medicinal plant of the extraction of its bioactive compound. These also helps to perform a pharmacological study to synthesis drug from medicinal plants with a reduced toxicity and side effect.[1] Furthermore, the active components of herbal remedies have found combined with other inactive substance. However, these complementary components give the plant as a whole a safety and efficiency much superior to that of its isolated and pure active components.[2] Therefore, it is found to be very crucial to conduct more research on the therapeutic value of medicinal plants.

Presently in the developing countries, synthetic drugs are not only expensive and inadequate for the treatment of diseases but are also often with adulteration and side effects. Of the tropical and sub-tropical plants, Eucalyptus is one of the medicinal plants which belong to the order myrtles and Myrtaceae and a large genus of aromatic trees indigenous to Australia, Tasmania and the neighboring Island, and now extensively cultivated in many other countries including Ethiopia.[3] 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 ant protozoa 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.[4] Some antimicrobial agents are chemotherapeutic with a chemical 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.[5] In Ethiopia, application of medicinal plants especially in traditional medicine is currently well acknowledged. The increasing resistance of most synthetically derived antimicrobial agents is of utmost concern.[6] Therefore, suitable medicinal plants having effective remedy value which is less harmful to human tissue need to be explored. 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.[7] It may also provide antiseptic properties.[8] Some species of Eucalyptus such as globulus, maculate and Viminal is with inhibition effect on some Gram-positive bacteria have been reported.[9] Fungicide activity has also been reported.[10,11]

The purpose of this study was to investigate the phytochemical composition and antimicrobial activities of fresh leaves extracts of Eucalyptus globules against some pathogenic microorganisms.

Materials and methods

Plant material

Leaves of Eucalyptus globules were collected from matched Tigray Ethiopia. The plant was authenticated at the department of biology Mikelle University Ethiopia. The leaves were air-dried in a well ventilation place until the moisture content reduced to a minimum suitable for grinding.

Isolation of essential oil

The essential oil was extracted by subjecting the air-dried leaves of Eucalyptus globules to hydro distillation using a Clevenger-type apparatus. Fresh seed (100g) were chopped and mixed with distilled water in a 5 iter round bottom flask. The hydro distillation lasted for 3hours and the oil collected was dried with sodium sulphate and stored at 4°C in a refrigerator for further use.[12]

Phytochemical analysis

The preliminary phytochemical analysis of the extracts was carried out to determine the presence of tannins, saponins, alkaloids, phenols and glycosides as described.[13–16] Results are as shown in Table 1.

+ = Present

- = Absent

Table 1 Results of phytochemical screening of Eucalyptus Globulus

Bioactive Compounds	Water extract of leaves
Tannins	+
Saponins	+
Phenols	+
Glycosides	+
Alkaloids	-

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.

Table 2 Results of antimicrobial activity of leaves of Eucalyptus Globulus

Inhibition zone in (mm)	Amount added (20µL)	
	Water extract	Gentamicin (control)
E.coli	13	26
S. aureus	15	23
S.thyphimurium	15	28
B.subtilis	13	22

Test for saponins

5ml of the extract with 10ml of water in a test tube was shaken, and then a full mass of small bubbles formation was taken as an indication of the presence and absence of saponins.

Test for tannins

Extract plus 4ml of water and drops of ferric chloride were mixed, and then immediate green precipitate formation was taken as an indication for the presence of tannins.

Test for alkaloids

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

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

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

Test organisms

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

Collection of test organisms

The microorganisms were collected from the microbiology laboratory of the Ayderreferral Hospital with the help of the laboratory staff. *E. coli* was collected in peptone water and labeled S1. The same procedure was repeated for the collection of S. aureus, S. typhi and B. subtilis. These were labeled S2, S3and S4, respectively.

Preparation of nutrient agar/nutrient broth

This nutrient media prepared using a standard protocol, Monica (2000). 28g of the nutrient agar powder was dissolved in 1000ml of distilled water in a conical flask and was autoclaved for 15min 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 28g of nutrients broth powder was dissolved in 1000 ml of distilled water in a conical flask and was autoclaved at 121°C for 15min; it was then allowed to cool at 47°C and was dispensed into test tubes.

Antimicrobial investigation

The stocks on the nutrient agar slant sub-cultured into nutrient broth and incubated at 37°C.Then the test organism inoculated on the nutrient plate agar and the discs were prepared using a Whatman filter paper; kept invials-bottles and sterilized in an oven at 150°C for 15min. prepared discs containing the various extracts were carefully placed on the inoculated plates using a sterilized forceps in each case.[17] The plates were then turned upside-down and inoculate at 37°C for 24h 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.[18]

Results and discussion

The Phytochemical analysis (Table 1) revealed the presence of phenols; glycosides tannin and saponins were present in the water extract of the leaves.

Phytochemical screening of all the tested aqueous leaves extract of eucalyptus revealed the presence of tannins, saponins phenols, and glycosides. Regardless of the known antimicrobial activities of these phytochemical compounds, their presence in the extract is perhaps the reason for the antimicrobial effects exhibited by these plants on S. *aureus* and S. *typhi.* These substances are known to have antimicrobial effects. Tannins, for example are known to be made up of phenolic compounds and phenols, and phenolic compounds have been used extensively as disinfectants. Action of tannin may be due to protein denaturation and is found to be non-specific.[19] Tannins possess astringent and homeostatic properties and are therefore widely used as topical application on sprains, bruises, and superficial wounds and infections.

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 antimicrobial activities of the test organisms as shown in Table 2, the results of the present study showed that the crude extracts of Eucalyptus globulus inhibit the growth of S. typhimurium, *E. coli*, B. subtilis and S. aureus even though highest activity was demonstrated by the standard antibiotic Gentamicin (control), this may be because the antibiotic is in it pure state and has refined processes that have established it as a standard antibiotic[20] phytochemical components. Results also showed the microorganisms have different resistance properties toward the extract of this plant. E. globulus showed highest inhibiting activity on S. typhimurium, and S. aureus while it was found to have a low activity on *E. coli*, B. subtilis which might be attributed to the genetic factor of the strain of the bacteria. The result also showed that the sensitivity of S. *aureus* and *E. coli* toward the extract of Eucalyptus globulus revealing (13mm) and (15mm) zone of inhibition in diameter, respectively. This result demonstrated the highest antibacterial activity of this plant species collected from this region compared to those found by (Bachir Raho Ghalem and Benali mohamed 2008) in which the recorded result was (4mm) and (2.4mm) zone of inhibition using the same concentration of extract of Eucalyptus globulus on S. aureus and *E. coli* respectively. Which was collected from Mascara willaya city North West of Algeria. As the result showed this Eculaptus globulus has high efficacy to be serving as a potential source of antibiotic substances for drug development against these two types of bacteria that causes diseases and confirmed the historical use of Eucalyptus globulus oil as an antimicrobial agent.[21]

Conclusion

The result of phytochemical analysis of this plant showed that all the tested extracts have bioactive substances which display antimicrobial activity. For instance, tannins are known to be made up of phenolic compounds, and phenols and phenolic compounds that have been used as disinfectant. Due to differences in geographical location of these plants, the phytochemical constituent can be greatly affected.[22–25] These influences can also contribute to differences in the antimicrobial activities of plant extracts on the tested microorganisms as shown in Table 2. Generally, this study has shown that the extracts of Eucalyptus globules 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 Ethiopia the activities of Eucalyptus globules 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.[26–31]

Moreover, in order to use wide verities of plants which may have medicinal and pharamacognaostic value and therapeutic significance, a number of researches investigating the true antimicrobial nature of the phytochemical constituents of the interest plant has to be done.

Acknowledgments

None.

Conflicts of interest

Author declares there are no conflicts of interest.

Funding

None.

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Citation: Amabye TG, Bezabh AM, Mekonen F. Phytochemical and antimicrobial potentials leaves extract of eucalyptus globulus oil from maichew Tigray Ethiopia. *Int J Complement Alt Med.* 2016;3(1):1–4. DOI: 10.15406/ijcam.2016.03.00056