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RESEARCH ARTICLE

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IDENTIFICATION AND PRELIMINARY SCREENING OF PHYTOCONSTITUENTS IN *EUCALYPTUS GLOBULUS* ROOT WITH A TLC-BASED STUDY

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ABSTRACT: The current work seeks to analyze TLC profile, phytochemical constituents, and antimicrobial activities of *Eucalyptus globulus* root extract. Eucalyptus globulus including other medicinal plants are sources of bioactive compounds; however, the root extracts of this species are still unknown. In order to close this gap, a methanolic extract of the roots was prepared by the use of soxhlet extraction technique giving a percentage yield of 7.23% w/w which could be considered high since it suggests good recovery of phytoconstituents have been made. Phytochemical analysis of the extract call for presence of several bio active compounds among them are flavonoids, terpenes, phenol, tannin, saponin and glycoside that possess various pharmacological activities. TLC profiling for flavonoids had an R_f value of 0.87 while terpenes had an R_f value of 0.31, phenols had an R_f of 0.25 and tannins had R_f of 0.19. These findings agreed with the chemical variation and abundance of the extract. Apart from the phytochemical characterization, the ant-bacterial efficiency of the extract was also assessed against different bacterial and fungal isolates. The extract showed moderate antibacterial efficacy against the examined bacteria especially gram-positive bacteria *S. aureus* and *K. pneumoniae* with MIC values comparable to the standard Gentamycin. Still, the activity documented against the gram-negative bacteria and the fungal strains was relatively low. However, the inhibition zones noticed during the antifungal assays indicate that extract has the ability to cure fungal infections. The outcome of this research disproves the use of Eucalyptus globulus root to give phytochemicals used in antimicrobial products. On one hand, its moderate antifungal potency, however, demonstrate its SOME limitation, but on the other, the extract exhibit activity against gram-positive bacteria making it a potential natural source for manufacture of efficient antimicrobial compounds. Subsequent research works on the identification and profiling of these molecules may significantly improve the therapeutic value of the plant in creating new forms of herbal medicine and antimicrobial pharmaceuticals.

Key Words: *Eucalyptus globulus*, Phytochemical Screening, Thin-Layer Chromatography (TLC), Antimicrobial Activity, Gram-Positive Bacteria, Natural Antimicrobial Agents

INTRODUCTION

The rise of antimicrobial resistance in the world has shifted focus to develop new therapies which have prompted a lot of interest in natural product. Current abuse and misuse of these drugs have led to appearance of multi-drug resistant strains of bacteria and fungi, which are a major threat to human life [1]. In this regard, medicinal plants hold a lot of future in view of being potential source of new bioactive molecules having therapeutic prospects. Among of them *Eucalyptus globulus* or Tasmania blue gum has been widely used in folk medicine all over the world [2].

It is incorporated into the Myrtaceae family and is commonly referred to as the globe silver tree and grows in Australia. But it has been widely grown all over the world because it is fairly resilient and has so many uses [3]. The plant is used mainly for the aromatic leaves as primary source of essential oil that have various pharmacological actions including antimicrobial, anti-inflammatory and analgesic properties [4]. These essential oils, containing eucalyptol (1,8 cineole), are explored and incorporated significantly into medicines, cosmetics, and effective natural insecticides. Apart from leaves the bark, and the wood itself is also of commercial importance being utilized in areas such as timber and pulp for paper. In its raw form and in its constituent oils, *Eucalyptus globulus* has been the subject of numerous investigations; however, its roots are comparatively understudied [5].

Surprisingly, roots of the plants are also that part which is under investigation for it contains profile of secondary metabolites that is different from rest parts of the same plant. These metabolites can be alkaloids, flavonoids, tannins, phenolics and glycosides, many of which possess bioactivities [6].

In vitro investigations concerning the plant roots have shown their efficacy against microbial infections, controlling oxidative stress, and even exhibiting certain forms of cell killing effects on particular cancer cells. Because of the recent popularity in the search for eco-friendly and naturally-derived remedies, the present study aims to examine the root system of Eucalyptus globulus [7].

This research therefore seeks to fill this gap through an emphasis on the phytochemical content and antimicrobial activity of methanol extract of *Eucalyptus globulus* root. TLC analysis was used to determine the extract potency and composition to help in the identification of the produced phytoconstituents, and broth microdilution methods were used to measure extract effectiveness against various bacterial and fungal isolates. Consequently, this study aims at exploring the bioactive features of *Eucalyptus globulus* roots so as to shed light on other unknown therapeutic uses of the plant besides its use in producing natural antimicrobials.

MATERIALS AND METHODS

The whole plant of *Eucalyptus globulus* was identified and the roots were collected from Jhansi city of India in November 2022. The rooted part was dried under the shade and then reduce to powdered form. Soxhlet extraction in methanol gave a crude extractive yield of 7.23% w/w. The obtained extract also went through a preliminary experiment in studying the phytochemical content that includes carbohydrate, flavonoid, tannin, Saponin, glycosidal and phenolic contents [8].

TLC analysis was used to identify the various chemicals in the extract. A mobile phase consisting of methanol, ethyl acetate, water, and formic acid. Their R_f values were then determined using the developing solvent system in the ratio 3:5:2 and iodine vapour as the detecting agent. R_f values were used to determine the individual components in order to compare the results [9].

The study also examined the efficiency of the extract against several bacterial and fungal species. MICs were obtained through a broth dilution method while the effectiveness of antifungal agents was evaluated by the cup plate method. A number of drugs were used to establish a comparison with the efficacy of the extract by measuring zones of inhibition [10].

RESULTS AND DISCUSSION

Phytochemical Composition

The qualitative test for phytochemical screening of the methanolic extract of the root part of Eucalyptus globulus indicated that the plant contained flavonoids, tannins, phenolics, glycosides and saponin constituents. These phytochemicals are especially effective as antimicrobial, antioxidants, and anti-inflammatory agents. The lack of alkaloids, gums and mucilage confirms the selectivity of the secondary metabolites of the roots which is typical in plants adaptation for therapeutic usage (Table 1).

Table 1: Preliminary Phytochemical Screening of *Eucalyptus globulus* Root Extract

Phytochemical Test	*Result*
Alkaloids	Absent
Carbohydrates	Present
Resins	Present
Glycosides	Present
Saponins Glycosides	Present
Phenolic Compounds and Tannins	Present
Proteins and Amino Acids	Present
Gums and Mucilages	Absent
Flavonoids	Present

Thin Layer Chromatography (TLC) Analysis

The major phytoconstituents in the methanolic extract of the root bark of Eucalyptus globulus were profiled using TLC profiling. The extract, developed using Methanol: The phytochemicals present in the plant were analyzed using the solvent system: Ethyl acetate: Water: Forming acid (10:3:5:2) as the mobile phase, which of which under iodine vapour detection, showed four bands or spots (Table 2 and Fig. 1).

Fig. 1: Chromatogram of TLC of methanolic extract of roots of *E. globulus*

Table 2: TLC Study of *Eucalyptus globulus* Root Extract

Extract	*Mobile Phase*	*R_f Value*	*Identified Compound*
80% Methanol	Methanol: Ethyl Acetate: Water: Formic Acid (10:3:5:2)	0.87	Flavonoids
80% Methanol	Methanol: Ethyl Acetate: Water: Formic Acid (10:3:5:2)	0.31	Terpenes
80% Methanol	Methanol: Ethyl Acetate: Water: Formic Acid (10:3:5:2)	0.25	Phenols
80% Methanol	Methanol: Ethyl Acetate: Water: Formic Acid (10:3:5:2)	0.19	Tannins

The obtained R_f values are a measure of the mobility of the separated compounds; the flavonoids having the highest mobility (R_f = 0.87) while the tannins have the least mobility (R_f = 0.19). The results of this study are in support of the chemical heterogeneity of the extract and form a basis for future pharmacological analyses of the individual compounds.

Antimicrobial Activity

The qualitive MIC values and zones of inhibition were employed to determine the antimicrobial activity of the methanolic extract. Table 3 and 4 enshrine the results respectively and Fig. 2 and 3 graphically illustrate it.

Table 3: MIC Values of *Eucalyptus globulus* Extract vs. Gentamycin

Organism	*MIC of Extract (µg/ml)*	*MIC of Gentamycin (µg/ml)*
S. typi	500	3.625
S. enteritidis	500	3.625
E. coli	500	3.625
S. aureus	500	1.8625
K. pneumoniae	250	1.8625

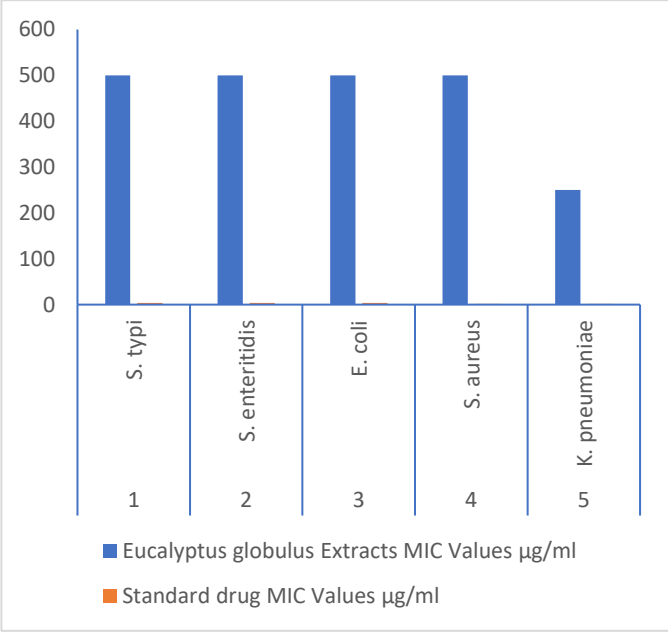

Fig. 2: Effect of roots of *Eucalyptus globulus* on selected bacterial strains

As shown by this bar chart, the MIC values of the methanolic extract are lower than Gentamycin. This extract demonstrated relatively good level of inhibitory effect, or in specific case of K. pneumoniae with IC 250 µg/ml, moderate effect, however, all other tested strains with their MIC 500 µg/ml demonstrated lesser effect than Gentamycin.

Zone of Inhibition

The antimicrobial efficacy was also ascertained by using the cup plate method. Thus, the extract produced inhibitory zones around all the tested bacteria; with larger zones resulting from higher concentrations (2000µg/ml).

Table 4: Zone of Inhibition of *Eucalyptus globulus* Extract

Organism	*Extract (1000 µg/ml)*	*Extract (2000 µg/ml)*	*Standard Drug (Well 1)*	*Standard Drug (Well 2)*
S. typi	1.2 cm	2.1 cm	1.4 cm	2.4 cm
S. enteritidis	1.3 cm	2.3 cm	1.4 cm	2.4 cm
E. coli	1.2 cm	2.7 cm	1.1 cm	2.1 cm
S. aureus	1.5 cm	2.7 cm	1.0 cm	2.3 cm
K. pneumoniae	1.1 cm	2.5 cm	1.5 cm	2.7 cm

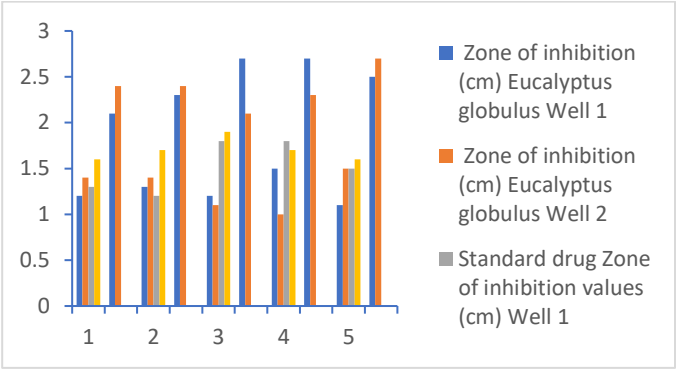

Fig. 3: Effect of *Eucalyptus globulus* on selected fungal strains

The bar chart shows the inhibition zones of the extract and the standard drug. However, at the extract concentration of 2000 µg/ml, the inhibition was significantly close to that of the standard drug for some of the strains. Of all the bacteria under test, *K. pneumoniae* and *E. coli* turned out to be most sensitive to the extract.

DISCUSSION

Efficacy Against Gram-Positive and Gram-Negative Bacteria

The MIC results corroborate that the evaluated extract provides reasonable antibacterial activity against gram-negative bacteria such as *S. typi* and *E. coli*. But it possessed significant activity against gram-positive bacteria especially *S. aureus* and *K. pneumoniae* equally to Gentamycin at 250-500 µg/ml conc. Such a finding only points towards a selective antibacterial character of the meat, most probably due to action of phenolic compounds and tannins that target at bacterial cell membrane.

Comparison with Standard Drugs

Although Gentamycin gave lower MIC values inferring more inhibitory activity the extract's potential for growth inhibition and formation of zones of inhibition proves it to be a potent source of natural antimicrobial substance. Even greater zones of inhibition were obtained with higher extract concentration (2000µg/ml) implying that its action is concentration dependent.

Phytochemical Influence

This probably explains the noted antimicrobial activity based on flavonoids and phenolic compounds. Information regarding these compounds indicate that they impede function of microbial cell membrane and restrict important enzymes. The TLC developed was further in concordance with the antimicrobial results confirming the presence of these bioactive constituents.

Implications for Future Research

Nevertheless, the level of effectiveness shown in the extract was moderate, but further isolation and purification of the desirable specific active compounds may significantly improve the

extract's antimicrobial action. Moreover, in vivo experiments and interaction with other known antimicrobials could expand the potential usage.

CONCLUSION

The phytochemical analysis of methanolic extract of Eucalyptus globulus roots represents diverse profile of bioactive compounds including flavonoids, tannins, phenol, glycoside and saponin. These compounds responsible for antimicrobial, antioxidant and ant-inflammatory activity are also observed for pharmacological activity of the extract. Further preliminary phytochemical screening and thin layer chromatography have shown that the root extract is chemically diverse, and therefore promising for containing a therapeutic constituent or constituents.

Endophytic bacteria from different families were sensitive to the extract with moderate zone of inhibition for bacterial strains, especially the gram-positive bacteria S. aureus and K. pneumoniae. It had equal efficacy inhibitory against gram-positive pathogens as the standard antibiotic Gentamycin and that it could be useful if used as natural solutions to tackle certain bacterial infections. Even though it was less active against the gram-negative bacteria, the observed antimicrobial property of the extract is a result of being able to produce measurable zone of inhibition. The TLC profiling further supported the identification of signature phytoconstituents where Rf values signified flavonoids, terpenes, phenols and tannins The prior literature exposed that phytochemicals causing disruption in microbial cell walls and depletion of vital enzymes. The present investigation forms a basis for the subsequent steps of the detailed bioassay-directed fractionation which is crucial in identifying and elucidating the constituents accountable for the extract's antimicrobial activity.

Nevertheless, due to its promising activity, the extract was less potent compared to synthetic antibiotics. However, to enhance the therapeutic efficacy of the plant, subsequent researches are required to purify the active ingredients. Furthermore, the further *in vivo* tests of the highly active and selective compound and relevant toxicity studies are required to confirm its effectiveness and safety. Its antimicrobial synergy with other standard antibiotics should also be studied to potentially increase the extract's potency and to expand the spectrum of susceptibility. Therefore, the results presented in this paper demonstrate that the root extract from Eucalyptus globulus has the possibility of being used as a natural antimicrobial. Despite moderate activity against pathogens, the chemical composition of the extract highlights its value as an alternative source of treatment to address AMR. If this line of research is expanded, the extract could help in the discovery and invention of other natural remedies that could be of great importance in the control of bacteria related infections hence meeting the ever increasing market for natural products.

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CONFLICT OF INTEREST: NIL

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