ANTIMICROBIAL ACTIVITY AND PHYTOCHEMICAL ANALYSIS OF *EUCALYPTUS TERETICORNIS* BARK AND LEAF METHANOLIC EXTRACTS

Pranay Jain[1], Shekhar Nimbrana[2] and Gaurav Kalia[2]
[1]Assistant Professor, University Institute of Engineering and Technology, Kurukshetra University, Kurukshetra, India.
[2]B. Tech. Biotechnology, University Institute of Engineering and Technology, Kurukshetra University, Kurukshetra, India.
***Email: drpranayjain@gmail.com**

ABSTRACT

In the present investigation, the methanolic bark and leaf extracts of *Eucalyptus tereticornis* were evaluated for antimicrobial activity against common human pathogens and subsequently phytochemical analysis of the crude extracts was carried out to determine the active phytochemical constituents responsible for antimicrobial activity. It was observed that methanolic bark extract of *E. tereticornis* was more effective in inhibiting all the four test pathogens with zone of inhibition ranging between 17mm and 27mm as compared to methanolic leaf extract (18 to 24mm). Phytochemical screening of crude bark and leaf extracts revealed that both the extract contain saponins, tannins, steroids and flavonoids, whereas only the bark extract consisted of cardiac glycosides. The results indicate that the methanolic bark and leaf extracts might be exploited as natural drug for the treatment of several infectious diseases caused by these organisms.

Keywords: *Eucalyptus tereticornis*, Methanolic Extracts, Antimicrobial activity.

INTRODUCTION

Nature has been a source of medicinal agents for thousands of years and since the beginning of mankind. The application of medicinal plants especially in traditional medicine is currently well acknowledged and established as a viable profession[1].

Extraction of bioactive compounds from medicinal plants permits the demonstration of their physiological activity. It also facilitates pharmacology studies leading to synthesis of a more potent drug with reduced toxicity[2]. Furthermore, the active components of herbal remedies have the advantages of being combined with many other substances that appear to be inactive. 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[3].

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[3]. There is therefore the need to search for plants of medicinal value. Eucalyptus is a tall, evergreen tree, native to Australia and Tasmania, successfully introduced worldwide, now extensively cultivated in many other countries including India[4].

In the present investigation, the methanolic bark and leaf extracts of *Eucalyptus tereticornis* were evaluated for antimicrobial activity against common human pathogens and subsequently phytochemical analysis of the crude extracts was carried out to determine the active phytochemical constituents responsible for antimicrobial activity.

MATERIALS AND METHODS

Collection of plant material

The plant materials were collected from different localities in Kurukshetra district. The samples were washed thoroughly to remove dirt particles present on the surface. The samples were then dried in oven. The samples were crushed into powdered form by mortar and pestle.

Preparation of methanol extract

Twenty five grams of the material was soaked in 100 ml of methanol and allowed to stand for 24 hrs followed by boiling until the volume was reduced to one-third. The crude extracts were obtained by filtration and stored in a refrigerator at 4ºC [5].

Procurement and maintenance of test pathogens

The various human pathogenic microorganisms were procured from Microbial Type Culture Collection (MTCC), Institute of Microbial Technology (IMTECH), Chandigarh, which included Gram positive bacteria, *Streptococcus mutans* (MTCC 497), *Staphylococcus aureus* (MTCC 7443) and Gram-negative bacterium *Escherichia coli* (MTCC 5704) and a yeast *Candida albicans* (MTCC 3017). The slants of brain heart infusion agar were made to preserve the cultures. All the slants were kept at 4^0C in the refrigerator for further studies.

Standardization of inoculum

The microbial inoculum was standardized at 0.5 McFarland. In microbiology, McFarland standards are used as a reference to adjust the turbidity of bacterial suspensions so that the number of bacteria will be within a given range. Original McFarland standards were made by mixing specified amounts of barium chloride and

sulfuric acid together. Mixing the two compounds forms a barium sulfate precipitate, which causes turbidity in the solution. A 0.5 McFarland standard is prepared by mixing 0.05 ml of 1.175% barium chloride dihydrate ($BaCl_2 \cdot 2H_2O$), with 9.95 ml of 1% sulfuric acid (H_2SO_4). The standard could be compared visually to a suspension of bacteria in sterile saline or nutrient broth[6].

Agar well diffusion method

Antibacterial activity of methanolic bark and leaf extracts were tested using agar well diffusion method. 200µl of bacteria were aseptically introduced and spread using cotton swabs on surface of gelled sterile Muller Hilton agar plates. A well of about 6.0mm diameter with sterile cock borer was aseptically punched on each agar plate. 50µl of the methanolic bark and leaves of eucalyptus were introduced into the wells in the plates. A negative control well was too made with 50µl of the extracting solvent (DMSO). A positive control was made by placing antibiotic disc (Ciprofloxacin for bacteria and Ketoconazole for yeast) on agar plate. Plates were kept in laminar flow for 30 minutes for pre diffusion of extract to occur and then incubated at 37ºC for 24 hours. Resulting zone of inhibition was measured using a Hi media zone scale[7].

Phytochemical screening of crude extracts

The phytochemical screening of crude eucalyptus bark and leaf extracts were screened using the methods of Trease and Evans[8]. The components analyzed for are saponins, tannins, steroids, flavonoids and cardiac glycosides.

RESULTS AND DISCUSSION

Since multidrug resistance of microorganisms is a major medical concern, screening of natural products in a search for new antimicrobial agents that would be active against these microorganisms is the need of the hour[9]. In the present investigation, the antimicrobial activity of methanolic bark and leaf extracts of eucalyptus tree was evaluated against gram-positive and negative bacteria *S. mutans, S. aureus*, *E. coli* and a yeast *C. albicans*. Data presented in Table 1 revealed that methanolic bark extract of eucalyptus was more effective in inhibiting all the four test pathogens with zone of inhibition ranging between 17mm and 27mm as compared to methanolic leaf extract (18 to 24mm).

Table 1: Antimicrobial activity of methanolic leaf and bark extracts of *Eucalyptus tereticornis*.

Extracts	Zone of Inhibition (mm)			
	Staphylococcus aureus	*Streptococcus mutans*	*Escherichia coli*	*Candida albicans*
Eucalyptus Leaves	19	24	18	17
Eucalyptus Bark	17	27	26	21
Ciprofloxacin	40	31	37	N.A.
Fluconazole	N.D	N.D.	N.D.	N.A.

N.A.- No Activity; N.D.-Not Determined

Table 2: Phytochemical analysis of methanolic leaf and bark extracts of *Eucalyptus tereticornis*.

Phytoconstituents	Methanolic Leaf Extract	Methanolic Bark Extract
Saponins	+	+
Tannins	-	-
Steroids	+	+
Flavonoids	+	+
Cardiac glycosides	-	+

\+ Positive; - Negative

Our findings are similar to those reported by Farah *et al*. [10], Babayi *et al*. [11], Gamal and Sabrin[12] and Nair *et al*,[4] who also reported the inhibitory activity of eucalyptus essential oil against *S. aureus* and *E. coli*. Ciprofloxacin was used as positive control as an antibacterial antibiotic which produced the inhibition zones ranging between 31 and 40mm whereas *C. albicans* was found to be resistant to Ketoconazole which was used as positive antifungal antibiotic. The results are in accordance with those obtained by Pelletier *et al.* [13] who reported that the widespread use of azoles has led to the appearance of resistant *Candida* isolates.

Phytochemical screening of crude bark and leaf extracts revealed that both the extract contain saponins, tannins, steroids and flavonoids, whereas only the bark extract consisted of cardiac glycosides (Table 2). Our findings are in accordance with other investigators[3,11,14]. Who have also reported these components in members of the family Combretaceae. However, in the present study, tannins were found to be absent in both the extracts

which is in contrast with earlier studies carried out by Babayi *et al*. [11] who have reported the presence of tannins in *Eucalyptus camaldulensis*. The inhibitory effects of these extracts on the microorganisms may therefore be due to the presence of the above phytochemical components.

The present study revealed the antimicrobial potential of methanolic bark and leaf extracts of eucalyptus against gram positive and negative bacteria and yeast for which the problem of multidrug resistance has emerged thereby making them difficult to treat. The encouraging results indicate that the methanolic bark and leaf extracts might be exploited as natural drug for the treatment of several infectious diseases caused by these organisms and could be useful in understanding the relations between traditional cures and current medications.

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