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Antimicrobial Potency and Phytochemical Screening of Leaf and Bark Extracts of *Eucalyptus globulus*

Preeti Singh[1*], Ridhima Mathur[1] and Tarana Ara Khan[2]

[1]*Department of Bioscience and Biotechnology, Banasthali Vidyapith, Rajasthan, India*

[2]*Department of Lifesciences, Chatrapati Shahu Ji Maharaj University, Kanpur, India*

[*]**Corresponding author:** Preeti Singh, Department of Bioscience and Biotechnology, Banasthali Vidyapith, Rajasthan, India, Tel: +9119300495; E-mail: preetispb13@gmail.com

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Abstract

The objective of the present study was to determine the antibacterial and antifungal activity by *Eucalyptus globulus*. The methanolic, ethanolic, petroleum ether extracts of leaf and bark were observed against the microbes that are pathogenic to humans. It was observed that methanolic extract of bark showed the best result, the zone of inhibition was (18-23 mm) in diameter. The antibacterial and antifungal activity may be due to presence of phytoactive compounds like saponins, alkaloids, steroids, terpenoids. The results of the study indicate that methanolic bark and leaf extract might be exploited as natural medicines and drugs for treatment of various infections caused by microorganism.

Keywords: *Eucalyptus globulus*; Antimicrobial; Phytoactive; Phytochemical; Zone of inhibition

Introduction

Mankind has been benefited by the nature in various ways. In recent time, the use synthetic drugs lead to number of side effects and this converges an alternate way to use natural products. As plant based medicines have least or no side effects. Eucalyptus is an important medicinal pant belongs to the family Myrtaceae. It is tall evergreen tree and is native to Australia, widely used as in treatment of skin disorders, inflammations, chest problems. Almost every part of this plant has medicinal properties [1].

"Leaf extract of Eucalyptus" has been approved as a natural food additive and it is included among the antioxidants in the "List of Existing Food Additives in Japan" [2]. The leaf, bark extracts of *Eucalyptus* have shown various bio potent activity such as antioxidants, antibacterial, antifungal, or anti-hyperglycaemic [3]. Oil of this plant is beneficial for curing lung infections and fungal infections [4].

In past three decades, a large number of antibiotics have been produced, but it has been clinically threatened by exposure and emergence of various multidrug resistant pathogens [5]. World Health Organization (WHO) reported that medicinal plants would be the ideal source to obtain diverse drugs [6]. The main objective of this study is to focus on antimicrobial activity and phytochemical screening of Eucalyptus leaf and bark.

Materials and Methods

Fresh mature leaves and bark were collected from Banasthali Vidyapith Campus, Rajasthan, India. The leaves and bark were washed thoroughly with normal tap water and once washed with sterile distilled water. The samples of leaves and bark were air dried under shade for one week at room temperature. Electrical grinder was used to make the leaves and bark in powdered form. The powder was stored in dark air tight container separately.

Preparation of extracts

Methanolic, Ethanolic and Petroleum ether solvents were used for preparation of extracts. 20 gm of leaf powder and 20 gm of bark powder were weighed and homogenised with 100 ml of these three solvents. Leaf and Bark extract were prepared separately. The crude was left over night for extraction. The crude was filtered after 24 hours. The extracts were obtained and stored at 4°C.

Antimicrobial screening

In order to determination of antimicrobial activity by *E. globulus*, the strains of bacteria used for this study were *Pseudomonas aeruginosa*, *E. coli*, *Klebsiella pneumoniae*. The fungal strains used were *Aspergillus niger* and *Penicillum* species.

Bacterial cultures were grown in Nutrient Broth (Himedia, Mumbai, India) at 37°C ± 0.5°C while fungi were cultured in Potato Dextrose Broth (Himedia, Mumbai, India).

Agar well diffusion method was employed for screening of antimicrobial activity. Bacterial cultures were spread over the nutrient agar with the help of sterile spreader. Four Wells of 6 mm were made on each plate, to one well 30 µl of leaf extracts was filled and in second well 30 µl of bark extract was filled. In third well 30 µl was filled with-Ciproflaxin (Positive control) and fourth well was filled with DMSO-Dimethyl Sulphoxide (Negative control) and diameter of zone of inhibition was calculated after incubation period of 24 hours at 37°C. The same procedure was done for fungi and diameter of zone of inhibition was calculated after 5 days of incubation at 37°C, positive control was FLU–Flucanozole.

Phytochemical analysis

Test	Leaf			Bark		
	A	B	C	A	B	C
Steroid	++	+	+	++	+	+
Saponins	++	+	+	++	+	++
Alkaloid	+	+	+	+	+	+
Flavonoid	++	+	+	+	+	+
Terpenoid	+	+	+	+	+	+
Glycosides	_	_	_	_	_	_

Table 1: Phytoconstituents present in the Extracts of Methanol(A), Ethanol(B), Petroleum Ether (C).

The bioactive compounds or the phytoconstituents found in the leaf and bark (Methanol, Ethanol, Petroleum Ether extracts) were steroids, saponins, flavonoids and alkaloids. The test were done as per the standard methods (Table 1) [7,8].

Results and Discussion

It was observed that methanolic extracts had shown greater zone of inhibition for both bacterial and Fungal cultures whereas ethanolic extract had shown lesser zone of inhibition and the Petroleum ether had minimum zone of inhibition in leaf and bark both.

We can compare the antimicrobial activity by the tables given below (Tables 2-5).

Leaf Extarcts	**Zone of inhibition in diameter (mm)**		
	Escherichia coli	*Klebsiella pneumoniae*	*Pseudomonas aeruginosa*
Methanolic	21	19	17
Ethanolic	19	15	14
Petroleum ether	17	13	11
−Ciproflaxin (Positive control)	26	24	25
DMSO–Dimethyl Sulphoxide (Negative control)	__	__	__

Table 2: Antibacterial activity by Leaf extract.

Bark Extarcts	**Zone of inhibition in diameter (mm)**		
	Escherichia coli	*Klebsiella pneumoniae*	*Pseudomonas aeruginosa*
Methanolic	23	20	19
Ethanolic	21	18	16
Petroleum ether	18	15	12
−Ciproflaxin (Positive control)	28	26	25
DMSO–Dimethyl Sulphoxide (Negative control)	__	__	__

Table 3: Antibacterial activity by Bark extract.

Leaf extract	**Zone of inhibition in diameter(mm)**	
	Aspergillus niger	*Penicillum species*
Methanolic extract	17	14
Ethanolic extract	16	11
Petroleum ether extract	11	9
FLU–Flucanozole (Positive control)	24	19
DMSO–Dimethyl Sulphoxide (Negative control)	__	__

Table 4: Antifungal activity by Leaf extracts.

Bark extract	Zone of inhibition in diameter (mm)	
	Aspergillus niger	*Penicillum* species
Methanolic extract	20	17
Ethanolic extract	18	12
Petroleum ether extract	13	10
FLU–Flucanozole (Positive control)	25	21
DMSO–Dimethyl Sulphoxide (Negative control)	—	—

Table 5: Antifungal activity by bark extracts.

Phytochemical screening of the crude of the Leaf and Bark extracts were screened using the methods of Trease and Evans [9]. Presence of the secondary metabolites in Eucalyptus can be useful in preparing drugs and medicines against bacteria and fungi [10]. The presence of bioactive agents such as flavonoids, alkaloids, saponins, steroids served as defence mechanism against microbes and they can disrupt the cell membranes and form the complex proteins, hence inhibits the growth of microorganisms [11-14]. These bioactive compounds (secondary metabolites) would be helpful in curing various kinds of infections or diseases such as influenza, gastrointestinal infections, lung infections, skin rashes. It is also used as antiseptic and ant diabetic agent. Now a days Eucalyptus oil is used in soaps, shampoos and ointment [15].

In present investigation, the antimicrobial potency of the methanolic, ethanolic and petroleum ether extracts of leaf and bark of *Eucalyptus globulus* have shown their activity against microbes, in medical concern multidrug resistant would be active in search for antimicrobial and bio potent compounds present in nature [16]. From (Tables 2 and 3) we can compare the leaf and bark extracts activity, methanolic bark activity is higher i.e 23 mm in diameter against *E.coli* whereas Petroleum ether has shown least activity against *Pseudomonas aeruginosa* i.e 11 mm in case of antibacterial activity (Table 2).

In case of Fungi, Methanolic Bark extract has shown the maximum activity against *Pseudomonas aeruginosa* while Petroleum ether extract has shown least activity against *Penicillum* species i.e. 9 mm (Table 4).

The inhibitory effect may be due to the bioactive compounds present in Eucalyptus and may be exploited as natural drug for treatment of antibacterial and antifungal drug [17].

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