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ANTIMICROBIAL ACTIVITY OF *BURSERA PENICILLATA* LEAF EXTRACTS ON HUMAN INFECTIOUS PATHOGENS

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ABSTRACT: The purpose of this study was to evaluate antimicrobial activity of *Bursera penicillata* leaf extract against human infected bacteria. Antimicrobial activity of Petrolium ether, Methanolic and Aqueous extracts from leaves of *Bursera penicillata* were tested against pathogenic bacteria *Staphylococcus aureus, Escherichia coli, Proteus vulgaris, Klebsiella pneumoniae, Staphylococcus saprophyticus, Enterococcus faecalis* and *Enterobacter cloacae* using Agar well diffusion method. Solvent extracts from the leaves of the plant shown a considerable antimicrobial activity against most tested microorganisms. The most active extract was Methanolic extract from the leaf against *Klebsiella pneumoniae* and significantly inhibited the bacterial activity. Minimum inhibitory concentration for aqueous and methanolic extracts of leaves ranged from 5.0–20.0 mg/ml and ethyl acetate and petroleum ether leaf extracts ranged from 25.0µg-55.0 µg and 45.0-85 µg/ml. It further reflects a hope for the development of many more novel chemotherapeutic agents or templates from such plants which in future may serve for the production of synthetically improved therapeutic agents.

INTRODUCTION: Medicinal plants are of great importance to the health of individuals and communities. The medicinal value of these plants lies in same chemical substances that produce a definite physiological action on the human body. The most important of these bioactive constituents of plants are alkaloids, tannins, flavonoids, terpernoids and phenolic compounds [1]. Plant derived substances have become of great interest owing to their versatile applications [2]. It has been estimated that 14-8% of higher plant species are used medicinally and that 74% of pharmacologically active plant derived components were discovered after following up on ethno medicinal use of the plants [3].

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In the last few decades, the search continues for safe and effective antimicrobial agents with which can be treated a wide variety of bacterial infections. This need has been heightened recently by the emergence of many antimicrobial-resistant organisms [4]. This worldwide interest in medicinal plants reflects recognition of the validity of many traditional claims regarding the value of natural products in health care [5]. Plants are rich in a wide variety of secondary metabolite such as tannins, terpenoids, alkaloids, and flavanoids which have been proved *in vitro* to have anti microbial properties. The use of plant extracts and phytochemicals, both with known antimicrobial properties, can be of great significance in therapeutic treatments. In the last few years, a number of studies have been conducted in different countries to prove such efficiency [6].

Bursera is an aromatic essential oil plant introduced into India by two private enterprising Scotsman - P. J. Anderson and G. N. Hamphries in

1912 at Thatgunni estate near Bangalore, Karnataka State. Forest department has started it's cultivation since 1958. It grows well on lateristic red soils and prefers arid tropical climate with temperature variation between 18°C and 35°C and rainfall between 450mm and 650mm annually. Being a deciduous species tree remains leafless from November to March and new flush starts during April-May with simultaneous flowering. Trees start bearing 3 to 4 years after planting.

The oil is distilled by usual steam distillation of air dried husks which yield 10 to 14% of oil. About 25kg of oil can be expected from one hectare plantation. Rosita Arvigo (*Bursera simaruba*) reports that the bark is a common topical remedy in Belize for skin affections like skin sores, measles, sunburn, insect bites and rashes. A bark decoction is also taken internally for urinary tract infections, pain, colds, flu, sun stroke, fevers and to purify the blood.

The present study was done to screen antimicrobial activity of Methanol, ethyl acetate and petroleum ether extract of leaves of *Bursera penicillata* against seven pathogenic bacteria which belongs to two group gram positive and gram negative. These are *Staphylococcus aureus, Staphylococcus saprophyticus, Escherichia coli, Enterococcus faecalis, Enterococcus cloacae, Proteus vulgaris,* and *Klebsiella pneumoniae.*

MATERIALS AND METHODS:

Preparation of Plant Extract:

Preparation of *Bursera penicillata* extracts: *Bursera penicillata* plant was identified and authenticated (Deposited Number: - Bot /184/OU/H.0121/HYD) by Prof. Ramchandra Reddy, a taxonomist at the Dept. of Botany, Osmania University Hyderabad. *Bursera penicillata* leaves were collected from Nallamalla forest, Mahebub Nagar, Telangana, India.

The plant leaves were air dried for 8 days, and then grounded using a mechanical grinder at room temperature and made into a coarse powder. The dried powder (200g) of *Bursera penicillata* was extracted sequentially using different organic solvents in increasing order of polarity (Petroleum ether, Ethyl acetate and Methanol) as per standard protocols (6, 7). Extraction mixtures were kept in a dark room for 72hrs at room temperature in sterilized bottles wrapped with aluminum foil to avoid evaporation. After 72hrs, mixtures were filtered through whattman no.1 filter paper.

This step was continued three to four times to remove any undissolved plant material which was subsequently extracted with another solvent. These filtered extracts were concentrated using a vacuum rotary evaporator (Superfit TM) and kept at 37ºC to remove any traces of solvent. Concentrated preparations of *Bursera penicillata* were stored at -20ºC until further use. The yield for 200g of dried powder was 20 g, 11 g and 32 g of Petroleum ether, Ethyl acetate and methanol extracts respectively.

Bacterial culture:

Plant extracts have been assayed for their antimicrobial activity against different species of bacteria was used as test microorganisms. Seven bacterial strains *Escherichia coli* ATCC 25922, *Enterococcus faecalis* ATCC 10741, *Enterobacter cloacae* ATCC 10699, *Proteus vulgaris* ATCC 12454, *Klebsiella pneumoniae* ATCC 15380, Staphylococcus aureus ATCC 25923 and Staphylococcus saprophyticus ATCC 35552 were used in our study. Microbial cultures were preserved at -20°C in microcentrifuge tube having 40% sterile glycerol.

Antimicrobial Activity:

Extract were tested against the strains for their inhibitory activity by 'Disc diffusion method'. Nutrient broth for inoculum preparation and Muller-Hinton agar media have been used for screening the antimicrobial activity. The wells were created onto the surface of a solid agar medium like Mueller Hinton with well puncture [10], Trypton soy agar [11] or Nutrient agar [12]. The media has been pre-inoculated with test organisms.

The standard inoculum size is of 1-2 x 108 CFU/ml of bacteria for inoculating diffusion plates [13] which is equal to McFarland 0.5 turbidity standard. After that test compound was inoculated into the well and plate incubated at 37°C for 24 hrs. Each test was carried out in triplicate with controls. Microbial growth was determined by measuring the diameter zone of inhibition with the help of scale.

Minimum Inhibitory Concentration (MIC):

The minimum inhibitory concentration was determined using the tube dilution techniques. Varying concentrations each extract have been prepared using single dilution method. A 9 ml of the nutrient broth was pipetted into the various test tubes and sterilized at 121°C for 15 minutes. Each tube was inoculated with an overnight standard inoculum size is of 1-2 x 108 CFU/ml of *Proteus vulgaris, Staphylococcus aureus, Klebsiella pneumoniae, Escherichia coli, Staphylococcus saprophyticus, Enterococcus faecalis* and *Enterobacter cloacae* which is equal to McFarland 0.5 turbidity standard and then transferred into the test tube containing the extract. The test tubes were incubated at 37°C for 24 hours.

The least concentration of the plant extract that does not permit any visible growth or turbidity of the inoculated test organisms in broth culture were taken as the minimum inhibitory concentration in each case. Control experiment with plant extracts and another tube with no plant extract were also performed [14].

RESULTS AND DISCUSSION:

Antibacterial Activity of *Bursera penicillata* Extracts:

The extracts of leaves of *Bursera penicillata was* showed antibacterial activity with diameters of zone of inhibition ranging from 12-25 mm. Ampicilin, streptomycin and chloramphenicol were used as positive control. Methanol, ethyl acetate and petroleum ether were used as negative controls not produced zones of inhibition. The antibacterial activity of leaves extracts of *Bursera penicillata is* shown in **Table 1**.

The investigation was made on methanolic extract of leaf have highest activity against *Klebsiella pneumoniae* (25.0 mm), *Staphylococcus aureus* (17.0 mm) and *E. coli* (14.0 mm) and least inhibition zone of aqueous extract of leaves was observed against *E. faecalis* (11.0 mm) and *E. cloacae* (9.7 mm) and ethyl acetate and petroleum ether shown considerable zone of inhibition but lesser than the methanol extract.

TABLE 1: ANTIBACTERIAL ACTIVITY OF LEAF EXTRACTS OF *BURSERA PENICILLATA* BY WELL DIFFUSION TEST

Test Organism	Zone of Inhibiton (mm)						
				Bursera penicillata leaf extracts			
	Amp	Strp	Chl	Methanol	Ethyl acetate	Pet. ether	Control
S. aureus	+	+	+	17.0±0.21	8.9±0.77	10.4±0.01	0
S. saprophyticus	+	+	+	12.5±0.04	8.3±0.64	10.87±0.8	0
Proteus vulgaris	+	+	+	14.5±0.16	12.8±0.92	9.15±0.29	0
Enterococcus faecalis	+	-	+	11.0±0.55	9.5±0.42	11.05±0.5	0
Klebsiella pneumoniae	+	+	+	25.0±0.08	11.35±0.66	13.5±0.33	0
Enterobacter cloacae	-	-	-	9.7±0.11	6.4±0.22	7.55±0.07	0
Escherichia coli	-	+	+	14.0±0.73	7.5±0.33	6.3±0.22	0

Minimum Inhibitory Concentration:

The extracts of leaves were shown considerably good antibacterial activity for each test organisms were selected to determine. Values of MICs were dependent on the bacterial species. Minimum inhibitory concentration for methanolic extracts of leaves ranged from 5.0-20.0µg/ml and ethyl acetate and petroleum ether leaf extracts ranged from 25.0-55.0 µg and 45.0-85 µg/ml (**Table 2**).

TABLE 2: MINIMUM INHIBITORY CONCENTRATION OF EXTRACTS OF *BURSERA PENICILLATA* BY BROTH DILUTION METHOD

Test organism	MIC values of *Bursera penicillata* leaf extracts (µg/ml)		
	Methanol	Ethyl acetate	Pet. ether
S. aureus	12.5 ± 0.12	25.0±0.02	66.5±0.12
S. saprophyticus	8.5±0.04	32.5±0.043	50.0±0.04
Proteus vulgaris	7.0±0.15	28.0±0.11	45.0±1.05
Enterococcus faecalis	20.0±0.02	43.5±0.21	67.5±0.09
Klebsiella pneumoniae	5.0±0.05	36.5±0.34	73.5±0.02
Enterobacter cloacae	11.25±0.65	42.0±0.55	81.5±1.76
Escherichia coli	14.5±0.33	55.0±0.22	85.0±1.02

The present study was indicated the activity of plant against gram positive and gram negative bacteria. The leaves of plants were shown antimicrobial activity against all reference bacteria. The highest activity was shown in methanolic extract of leaf against *Klebsiella pneumoniae* which is a gram negative bacterium that is known to cause destructive changes to human lungs via inflammation and hemorrhage with cell death (necrosis) that sometimes produces a thick, bloody, mucoid sputum (currant jelly sputum).

CONCLUSION: In conclusion, fruits and leaves from *Bursera penicillata* possessed inhibitory activity against the tested bacteria. Methanolic extracts of leaf showed almost comparable antibacterial activity, which support their traditional use against infectious diseases. Since, Indian *Bursera penicillata* showed activity against all tested bacteria but the highest was against *Klebsiella pneumoniae,* the use of plant as an anti infective to destructive changes to human lungs via inflammation and hemorrhage with cell death (necrosis).

Furthermore, it may help to discover new chemical classes of antibiotics that could serve as selective agents for the maintenance of animal or human health and provide biochemical tool for the study of infectious diseases. It further reflects a hope for the development of many more novel chemotherapeutic agents or templates from such plants which in future may serve for the production of synthetically improved therapeutic agents.

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