



PHYTOCHEMICAL EVALUATION AND ANTIBACTERIAL ACTIVITY OF *PTEROSPERMUM DIVERSIFOLIUM* BLUME

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ABSTRACT

Phytochemicals from the leaves of *Pterospermum diversifolium* were extracted using different solvents of various polarity such as Hexane, Ethyl acetate, Methanol and Water. The antibacterial activity was carried out against *Escherichia coli*, *Staphylococcus aureus*, *Bacillus subtilis* and *Pseudomonas aeruginosa* by Disc diffusion method and Minimum Inhibitory Concentration (MIC) by broth dilution method. The water extract was able to inhibit *Staphylococcus aureus* and *Bacillus subtilis*. Among the solvent extracts methanol extract was most effective against the tested micro organisms. Phytochemical analysis revealed the presence of phenols, flavonoids, tannins, glycosides and terpenes.

Keywords: *Pterospermum diversifolium*, Phytochemicals, Antibacterial activity, Disc diffusion.

INTRODUCTION

Over the past few decades, there has been much interest in natural materials as source of new anti bacterial agents. Different extracts from traditional medicinal plants have been tested. Many reports show the effectiveness of traditional herbs against micro organisms as a result plants have become one of the bases of modern medicine.¹ Plants have given the western pharmacopeia and number of top selling drugs of modern times such as Quinine, Artemisinin, Shikonin and Camptothecin². Plants have been used for the treatment of disease all over the world before the advent of modern clinical drugs. Natural phytochemicals are known to contain substance that can be used for therapeutic purposes or as precursor for the synthesis of novel useful drugs. Total of 50% modern drugs are of natural products origin and as such these natural products play an important role in drug development in pharmaceutical industry. Use of plant as a source of medicine has been inherited and is an important component of the health care system³.

Phytochemical analysis and antibacterial activities of wild plants was carried out by Jain⁴, Antibacterial activity of *Andrographis paniculata* by Vinoth Kumar⁵, *Coleus aromaticus* by Subhash chandrappa⁶ *Cinnamomum species* by Sandigawad and Patil⁷, *Acacia nilotica* by Mahesh and Satish⁸, *Samanea Saman* by Raghavendra⁹ and *Eucalyptus camaldulensis* by Ayepola and Adeniyi¹⁰ were carried out in the recent years. Natural product of higher plants may give a new source of antibacterial agents with possibly a novel mechanism of action. The selection of crude plant extract for screening the crude antibacterial activity has the potential of being more successful in the initial steps than screening of pure compounds⁶. Some organisms have developed resistance to the existing antibiotics, therefore the development of bacterial resistance to the currently available antibiotics has necessitated the research for new antibacterial agents⁴.

Pterospermum diversifolium a rusty pubescent tree with white flowers belonging to family Sterculiaceae, is distributed in the Western ghats, Maharashtra and Indomalaysia. The leaves and bark rich in tannin, are used in traditional medicine e.g. as poultice against itching, and to treat wounds, and taken internally to treat dysentery. Leaves of *Pterospermum diversifolium* are given to cattle suffering from stomach disorder.¹¹

MATERIAL AND METHODS

Collection of plant material

The fresh leaves of *Pterospermum diversifolium* were collected from the Charmady region of the Western Ghats. The leaves were shade

dried, grinded into fine powder and stored in air tight polythene bags until use.

Preparation of leaf extract

Fifty grams of dried and powdered sample was soxhleted using methanol, ethyl acetate and hexane as solvents. The samples were concentrated using rotary evaporator.

For water extract, 50 grams of dried powdered sample was boiled in water for 4 hours in a water bath and the extract was filtered through six layers of muslin cloth and centrifuged at 5000g for 15 minutes. The supernatant was collected and concentrated using rotary evaporator. All the extracts were stored at 4°C until use.

Phytochemical analysis

The extracts were used for preliminary screening of phytochemicals such as alkaloids (Wagner and Dragendorff's tests), flavonoids (Shinda and Lead acetate tests), Phenols (ellagic acid and FeCl₃ tests), tannins (gelatin tests), saponins (foam tests), sterols (Lieberman-Burchard and Salkowski tests) carbohydrates (Molish test, Benedicts) by Dey and Harborne method¹²

Antibacterial activity

In vitro antibacterial activity of different solvent extracts of *Pterospermum diversifolium* leaves were tested against Gram +ve *Staphylococcus aureus* ATCC 6538, *Bacillus subtilis* ATCC 6633, Gram -ve *Escherichia coli* ATCC 8739, and *Pseudomonas aeruginosa* ATCC 27853 procured from National Chemical Laboratory, Pune, India. The strains were maintained on nutrient agar slants. Two hundred micro liter of over night grown culture of each organism was dispensed into 20 ml of sterile nutrient broth and incubated for 4-5 hrs at 37°C to standardize the culture to 10⁻⁵ CFU/ml

Sterile empty discs (6mm diameter) were purchased from Himedia company, Mumbai, fifty mg of dried crude extract was dissolved in 1ml of 20% DMSO (Dimethyl sulphoxide) from the stock solution and 10µl of respective solvent extracts were added to the disc individually and aseptically. After drying they were used for screening the antibacterial activity.

Assay for antibacterial activity

Antibacterial assay was carried out by disc diffusion method¹³. For this, 0.1ml (10⁻⁵ CFU/ml) of 24 hrs old bacterial culture was placed on Muller Hinton agar medium and spread throughout the plate by spread plate technique. The sterile filter paper disc of 6mm diameter soaked with plant extract was placed on the surface of the medium and incubated at 37°C for 24hrs. Antibacterial activity was recorded by measuring the diameter of zone of inhibition. Streptomycin was

used as positive reference standard. The entire test was performed in triplicate.

The Minimum Inhibitory Concentration (MIC) of methanol extract was determined by broth dilution method of ¹⁴. The lowest concentration of the plant extract inhibiting the visible growth of organism was considered as MIC.

RESULTS

The preliminary phytochemical analysis of the extracts revealed the presence of saponins, glycosides, phenols and alkaloids in water extract (Table.1). Terpenes, flavonoids, tannins, phenols and saponins were detected in methanol extract. Phenols and saponins were positive in ethyl acetate extract. Terpenes and saponins were present in hexane extract.

The water and methanol extracts showed the maximum antibacterial activity against all the four bacterial strains tested (Table. 2). Antibacterial activity was higher against Gram +ve bacteria compared to the Gram -ve bacteria. The highest antibacterial activity was observed for the *Bacillus subtilis* followed by *Staphylococcus aureus* There was no inhibition by ethyl acetate and hexane extract against *E.coli* and *Pseudomonas aeruginosa*. The Minimum Inhibitory Concentration for methanol extract against bacterial strains showed variation (Table.3.). The higher MIC was observed against Gram negative organism, *Escherichia coli* (642 ±42µg/ml) followed by *Pseudomonas aeruginosa* (648 ±26µg/ml). The Minimum inhibitory concentration value for Gram positive strains was minimal compared to Gram negative strains. The Minimum Inhibitory Concentration of *Bacillus subtilis* was 320±26 µg/ml whereas *Staphylococcus aureus* it was 326±µg/ml.

Table 1: Analysis of phytochemicals in leaf extracts of *Pterospermum diversifolium*

Phytochemicals	Water extract	Methanol extract	Hexane extract	Ethyl acetate extract
Phenols	+	+	-	+
Flavonoids	-	+	-	-
Tannins	-	+	-	-
Terpenes	-	+	+	-
Saponins	+	+	+	+
Alkaloids	+	-	-	-
Glycosides	+	+	-	-

Table 2: Antibacterial activity of the *Pterospermum diversifolium* leaf extracts

Extracts/Antibiotic	Antibacterial activity (DIZ) in mm			
	<i>S. aureus</i>	<i>B. subtilis</i>	<i>P. aeruginosa</i>	<i>E. coli</i>
Water	8.3±0.5	17.9±2.1	5.9 ±1.2	5.2 ±1.3
Methanol	16.7±1.2	15.6 ±1.4	6.8 ±0.7	6.0 ±1.8
Ethyl acetate	10.4±0.8	6.8±0.9	-	-
Hexane	2.6±0.5	12.3±1.5	-	-
Streptomycin (10µl/ml)	23.2±2.3	21.9±3.2	19.2 ±3.1	20.2 ±1.9

Values presented are means of six replicates, ± Standard error

Table 3: MIC of methanol extract against four Microorganisms by Broth dilution method

Microorganisms	Minimum inhibitory concentration (µg/ ml)
<i>S. aureus</i>	325±32
<i>B. subtilis</i>	320 ±26
<i>P. aeruginosa</i>	648 ±26.
<i>E. coli</i>	642 ±42

DISCUSSION

The increase of antibiotic resistance of microorganism to conventional drugs has necessitated the search for new efficient and cost effective ways for the control of infectious diseases, the result of different studies provide evidence that some medicinal plants might indeed be potential source of new antibacterial agents¹⁵ Plants are important source of potentially useful structures for the development of new chemotherapeutic agents, the first step towards this goal is *in vitro* antibacterial activity. The extracts of higher plant can be very good source of antibiotics against various bacterial pathogen ¹⁶. Plant based antimicrobial compounds have enormous therapeutics potential as they can serve the purpose without any side effects that are often associated with synthetic antibacterial compounds.

Analysis of four different solvent extracts of the plant leaf demonstrated the presence of phytochemicals like terpenes, flavonoids, Phenolics, Saponins, Alkaloids and glycosides. The plants are rich in a wide variety of secondary metabolites which were found to have *in vitro* antimicrobial properties ¹⁷. The antimicrobial activity found in the plant extracts have been attributed to some of the secondary metabolites^{18,19}. The presence of phenolic compounds in the extract may attribute antibacterial activity. Phenolic

compounds are thought to be toxic to micro organisms, inhibiting the enzymes which are essential for the growth of microorganism.

The antimicrobial activities of phenolic compounds may involve multiple modes of action for eg, oils degrade the cell wall, interact with the composition and disrupt cytoplasmic membrane²⁰, damage membrane protein, interfere with membrane integrated enzymes²¹, cause leakage of cellular components, coagulate cytoplasm, deplete the proton motive force, change fatty acid and phospholipid constituents, impair enzymatic mechanism for energy production and metabolism, alter nutrient uptake and electron transport.

In the disc diffusion antibacterial assay, methanolic and water extracts of *Pterospermum diversifolium* leaves were most effective against Gram +ve strains (*Staphylococcus aureus* and *Bacillus subtilis*) compared to Gram -ve strains (*Escherichia coli* and *Pseudomonas aeruginosa*). The MIC values were higher for Gram -ve strains compared to Gram +ve strains. These results are in agreement with earlier studies with different plants as reported by previous workers ^{23,24,25,27}. A possible explanation for these observations may be attributed to the significant differences in the outer layers of Gram negative and Gram positive bacteria. Gram negative bacteria possess an outer membrane and a unique periplasmic space not found in

Gram positive bacteria^{27,28}. The resistance of Gram negative bacteria towards antibacterial substances is related to the hydrophilic surface of their outer membrane which is rich in lipopolysaccharide molecules, presenting a barrier to the penetration of numerous antibiotic molecules. The membrane is also associated with the enzymes in the periplasmic space which are capable of breaking down the molecules introduced from outside²⁹. However, the Gram positive bacteria do not possess such outer membrane and Cell wall structures³⁰

The tested bacterial strains showed different pattern of inhibition. The alcoholic extract exhibited greater activity than the ethyl acetate and hexane extract against bacteria. The two possibilities that may account for the higher antibacterial activity of alcoholic extract are the nature of biologically active compounds (alkaloids, flavonoids, tannins, terpenoids which may be enhanced in the presence of the extract) and stronger extraction capacity of alcohol that may yield a greater number of active constituents responsible for the antibacterial activity³¹

The observation indicates the higher degree of solubility of the active principle in the polar solvents such as water and methanol as higher antibacterial activity was recorded in the polar solvent extracts compared to the non polar solvent extracts.

The antibacterial activity of *Pterospermum diversifolium* and nature of active principles present in the extracts of this plant is demonstrated for the first time against the pathogenic bacteria. These results suggest the possible exploitation of this plant in the management of the infectious diseases. Further purification of the extract may yield a novel antibacterial drug. Considering the rich diversity of plants, it is expected that screening and scientific evaluation of plant extract for their antimicrobial activity may provide some more new antimicrobial substances.

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