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Review Article

ANTIMICROBIAL ACTIVITY AND PHYTOCHEMICAL SCREENING OF *PTEROSPERMUM RETICULATUM* WIGHT & ARN.

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ABSTRACT

The *in vitro* antimicrobial activity of leaf and stem extracts (ethanolic and water) of *Pterospermum reticulatum* was carried out against 4 bacterial (*Staphylococcus aureus*, *Bacillus subtilis, Escherichia coli, Pseudomonas aeruginosa*) and 3 fungal strains (*Aspergillus niger, Candida albicans*, *Trichoderma viridae*) following disc diffusion method. Minimum inhibitory activity (MIC) was carried out by broth dilution method. The ethanolic extract of leaf and stem exhibited the maximum antibacterial activity against all tested bacterial strains. The highest bactericidal property observed against *B. subtillis* and *S. aeureus* with MIC values of 648 µg/ml and 620µg/ml respectively. Among the extracts tested for antifungal activity, water extract of leaf exhibited good activity against *C. albicans*. There was no antifungal activity observed against *A. niger and T. viridae.* The preliminary phytochemical analysis of the extracts revealed the presence of phenols, glycosides, tannins, saponins and terpenoids.

Keywords: Antimicrobial, *Pterospermum reticulatum*, Minimum inhibitory activity, Phytochemicals.

INTRODUCTION

The use of plants as source of remedies for the treatment of many diseases dated back to prehistory and people of all continents have this old tradition. Despite the remarkable progress in synthetic organic chemistry of the twentieth century, over 25% of prescribed medicines in industrialized countries derived directly or indirectly from plants[1]. Herbs and spices are known for their antimicrobial and antioxidative properties. Due to an increasing demand for natural food additives, herbs and spices have emerged as popular ingredients and have a tendency of replacing synthetic antimicrobial and antioxidant agents.

Within the recent years, infections have increased to a greater extent; simultaneously the resistance to antibiotics has become an ever-increasing therapeutic problem [2]. Natural products of higher plants may possess a new source of antimicrobial agents with possibly novel mechanism of action [3, 4]. They are effective in the treatment of infectious diseases, while simultaneously mitigating many of the side effects that are often associated with synthetic antimicrobials [5]. Therefore, it is a new challenge to seek for the *in vitro* antimicrobial activity of natural compounds such as polyphenols from ethnomedicinal plants on pathogenic bacteria.

Polyphenols are a group of highly hydroxylated phenolic compounds present in the extractive fraction of several plant materials. Polyphenols in plants include hydroxycoumarins, hydroxycinnamate derivatives, flavanols, flavanones, anthocyanins, proanthocyanidins (tannins), hydroxystilbenes, aurones, etc. Polyphenols are well documented to have microbicide activities against a huge number of pathogenic bacteria[6, 7].

Pterospermum reticulatum wight & Arn. (Sterculiaceae) a is tree growing up to 25 m tall distributed in the evergreen forests of Western Ghats at low altitudes. Some of the plants belonging to family Sterculiaceae have been reported to be medicinally important. Hidayathulla reported antimicrobial activity in stem and leaves of *P.divesifolium*[8] and Jaiganesh reported antimicrobial activity in *P. Canescens*[9]. *P. acerifolium* reported to have antimicrobial and antihelmintic activity[10], antioxidant and anti-inflammatory[11], hepatoprotective effect[12]. There is no information about antimicrobial activity of *P. reticulatum.* In this context the present study was designed to evaluate phytochemicals and antimicrobial activity of stem and leaf extracts of *P. reticulatum*.

MATERIAL AND METHODS

Collection of plant material

The stem and leaves of *Pterospermum reticulatum* were collected from the arboretum of Mangalore University, Mangalore, and Karnataka, India. The leaves were shade dried, grinded into fine powder and stored in air tight polythene bags until use.

Preparation of extract

Fifty grams of dried and powdered sample (stem and leaves separately) was soxhleted with ethanol for 8 hours and the extracts were concentrated using rotary evaporator (Superfit, Model-Supervac) and the residue obtained was stored at 4°C. For water extract, 50 grams of dried powdered sample (bark and leaves separately) was kept in water bath for 8 hours at 40°C 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 $FeCl_3$ tests), tannins (gelatin tests), saponins (foam tests), sterols (Liberman-Burchard and Salkowski tests) and Glycosides (Molishs test, Benedicts) following Harborne method [13].

Antibacterial activity

Four bacterial cultures viz. two Gram-positive (*Staphylococcus aureus* ATCC 6538, *Bacillus subtilis* ATCC 6633) and Gram-negative (*Escherichia coli* ATCC 8739 and *Pseudomonas aeruginosa* ATCC 27853) used in this study were obtained from National chemical laboratory, Pune, India. The bacterial strains were maintained on nutrient agar slants. 200 µl of overnight 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.

Two of the fungal strains viz. *Aspergillus niger* MTCC No. 1344, *Candida albicans* MTCC No. 227 were obtained from IMTECH, Chandigarh, India and *Trichoderma viridae* was obtained from Plant pathology laboratory CPCRI, Kasaragod, India.

Assay for antibacterial activity

Antibacterial and antifungal assay was carried out by disc diffusion method [14].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. Sterile paper discs (6 mm in diameter) impregnated with 25 µl of the extract concentrations (1.5mg/disc) 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 antifungal activity was assayed by inoculating the fungal spores on the potato dextrose agar (PDA) medium pre-impregnated with discs containing plant extracts. Nystatin was used as positive reference standard against fungal strains.

The Minimum Inhibitory Concentration (MIC) of ethanol extracts of leaf and bark was determined by broth dilution method of NCCLS[15]. 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 phenols, glycosides, tannins, saponins and terpenoids in ethanolic extract of stem and leaf (Table1). Tannins, phenols, glycosides, saponins were detected in water extracts of stem and leaf. Alkaloids, flavonoids, steroids, resins were not detected in any of the extracts. The ethanolic extract of leaf and stem exhibited the maximum antibacterial activity against all tested bacterial strains (Table 2).The highest bactericidal property observed against *B. subtillis* and *S. aeureus* (Gram + ve) compared to *E.coli* and *P.aerugenosa* (Gram – ve). MIC values of ethanolic stem extracts found to be the least (620µg/ml) against *S. aeureus* (Table3) whereas bark ethanolic extract exhibited highest MIC value (1410µg/ml). Ethanolic extracts of both leaf and stem showed lower MIC values against *B. subtillis* (651;648µg/ml).

Among the extracts tested for antifungal activity, leaf water extract exhibited good activity against *C. albicans* (Table4). There was no antifungal activity observed against *A.niger, T.harzianum*.

Table 1: Analysis of phytochemicals in leaf and stem extracts of *Pterospermum reticulatum*

Materials	**Leaf**		**Stem**	
Solvents	**Ethanol**	**Water**	**Ethanol**	**Water**
Phytochemicals				
Alkaloid	-	-	-	-
Flavonoids	-	-	-	-
Glycosides	+	+	+	+
Phenols	+	+	+	+
Tannins	+	+	+	+
Saponins	+	+	+	+
Steroids	-	-	-	-
Resins	-	-	-	-
Terpenoids	+	-	+	-

+ = Presence of phytochemicals - = Absence of phytochemicals

Table 2: Antibacterial activity of the *Pterospermum reticulatum* leaf and stem extracts (n=3; Mean±SD)

Materials	**Solvents**	**Zone of Inhibition(mm)**			
		E.coli	***P.aerugenosa***	***B. subtillis***	***S. aeureus***
Leaf	Ethanol	3.5±0.6	3.1±0.5	5.4±0.5	4.6±1.0
	Water	1.1±1.9	1±1.2	5.0±0.9	3.8±1.4
Stem	Ethanol	4.5±1.5	2.3±2.6	5.3±0.5	5.6±1.0
	Water	2.7±0.9	1.3±0.7	5.1±0.3	4.5±0.4
Streptomycin(10µl/ml)		19.5±2.1	16.1±1.5	21.9±3.2	23.2±2.3

Table 3: MIC of ethanol extracts by Broth dilution method

Materials	**Solvents**	**Minimum inhibitory concentration (µg/ ml)**			
		E. coli	***P. aerugenosa***	***B. subtillis***	***S. aeureus***
Leaf	Ethanol	1210	1231	651	841
Stem		821	1410	648	620

Table 4: Antifungal activity of the *Pterospermum reticulatum* leaf and stem extracts (n=3; Mean±SD)

Material	**Solvent**	**Zone of Inhibition(mm)**		
		A. niger	***T.viridae***	***C. albicans***
Leaf	Ethanol	--	--	3.0±0.5
	Water	--	--	5±0.17
Stem	Ethanol	--	--	2.3±0.5
	Water	--	--	2.3±0.5
Nystatin (10µg/ml)		19±0.9	18±0.23	20±1.2

-- = No activity

DISCUSSION

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.

Phytochemical constituents such as alkaloids, flavonoids, tannins, phenols, saponins, and several other aromatic compounds are secondary metabolites of plants that serve a defense mechanism

against any microorganisms, insects and other herbivores[17]. The present study carried out on the plant samples revealed the presence of medicinally active constituents. The presence of phenols, tannins, saponins and steroids in the extracts could be responsible for the observed antimicrobial property.

These bioactive compounds are known to act by different mechanisms. Tannins bind to proline rich proteins and interfere with the protein synthesis[18]. 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 membrane[19], damage membrane protein, interfere with membrane integrated enzymes[20],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.. Antimicrobial property of saponin is due to its ability to cause leakage of proteins and certain enzymes from the cell [21] .Steroids have been reported to have antibacterial properties, the correlation between membrane lipids and sensitivity for steroidal compound indicates the mechanism in which steroids specifically associate with membrane lipid and exerts its action by causing leakages from liposomes action [22]

In the present study, *S. aureus* and *B.Subtilis* was found to be most sensitive to all extracts. The highest sensitivity of these strains may be due to their cell wall structure and outer membrane [23]. The present results suggest that gram-positive bacteria are generally more sensitive to the herb extracts. This was consistent with the previous studies on other spices and herbs [24, 25]

The antibacterial and antifungal activity of *P. reticulatum* is being reported for the first time. The crude extracts obtained from the leaf and stem of *P. reticulatum* may be used to treat diseases caused by bacteria and fungi after evaluating their toxicity and further clinical trails .

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