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Research Article

PRELIMINARY PHYTOCHEMICAL SCREENING AND ANTIMICROBIAL POTENTIAL OF *PTEROSPERMUM CANESCENS* ROXB, (STERCULIACEAE)

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ABSTRACT

In the present investigation, preliminary phytochemical screening and antimicrobial potential of the petroleum ether, chloroform and ethanolic leaf extracts (100, 50, 25 mg/ml) of *Pterospermum canescens* Roxb., (Sterculiaceae) were carried out against certain gram-positive (*Bacillus subtilis, Staphylococcus aureus, Streptococcus pneumoniae*) and gram-negative (*Proteus vulgaris, Escherichia coli, Vibrio vulnificus*) bacteria and fungi (*Aspergillus niger, Candida albicans*) by detecting the zone of inhibition using agar well-diffusion method. Preliminary phytochemical screening showed the presence of alkaloids, flavonoids, phenolic compounds and steroids. Petroleum ether, chloroform and ethanolic leaf extracts (100, 50, 25 mg/ml) exhibited significant antimicrobial activity against the various bacteria and fungi using the respective standard drugs (10 µg/ml).

Keywords: *Pterospermum canescens*, Phytochemistry, Antimicrobial activity

INTRODUCTION

Medicinal plants have been used for centuries as remedy for human diseases because they contain the compounds of therapeutic values[1]. Infectious diseases are the leading cause of death worldwide. Many infectious diseases have been known to be treated with herbal remedies throughout the history of mankind. Natural products, as a pure compound or as a standardized plant extracts provide unlimited opportunities for new drug lead because of the unmatched availability of chemical diversity. An increasing failure of chemotherapeutics and antibiotic resistance exhibited by pathogenic and infectious agents has led to the screening of medicinal plants for their antimicrobial potential. In recent years, secondary metabolites (phytochemicals) previously with unknown pharmacological activities have been extensively investigated as a source of medicinal plants. Thus, it is anticipated that the phytochemicals with adequate anti-infective efficacy will be used for the treatment of various infections caused by pathogens[2]. Therefore, there is a need to develop the efficient, safe and inexpensive drugs from plant source are of great importance.

The genus *Pterospermum* Schreb., (Sterculiaceae) represents of about 40 species in the world, of which 12 species were reported from India[3] and 8 species has been reported from Tamil Nadu state[4]. An ethnomedicinal plant species *Pterospermum canescens* Roxb., (Syn. *Pterospermum suberifolium* Lam.) locally known as *Sempulavu* was distributed in all districts of Tamil Nadu. Ethnomedicinally, the leaves are used for headache[5], treatment of fractured bones[6] and small pox[7]. The plant has been reported to contain β-amyrin, betulin, kaempferol, lupeol, quercetin, scopoletin and β – sitosterol[8]. After the scrutiny of literatures, it was confirmed that so far no other work has been carried out on this plant. Hence, the present study aims to develop an antimicrobial lead of therapeutic interest from this selected ethnomedicinal plant.

MATERIALS AND METHODS

Collection of plant material

The leaves of *Pterospermum canescens* Roxb., were collected from the Kalapet vicinity of Pondicherry and the collected plant material was botanically identified and confirmed by the Plant Taxonomist Mr. A.C.Tangavelou and the herbarium specimen (KPJ 42) was prepared and deposited at Bio-Science Research Foundation, Pondicherry for future reference.

Preparation of extracts

The collected leaves were chopped into small pieces, shade dried and coarsely powdered by using a pulverizor.Then the leaf powder was subjected to successive solvent extraction with organic solvents of increasing polarity such as petroleum ether, chloroform and ethanol by continuous hot percolation method using soxhlet apparatus[9]. The extracts were collected and distilled off on a water bath at atmospheric pressure and the last trace of the solvent was removed *in vacuo*. The resulted extracts were used for preliminary phytochemical screening and antimicrobial activities.

Preliminary phytochemical screening

All the extracts were subjected to preliminary phytochemical screening followed by the standard methods [9, 10].

Antimicrobial activity

Petroleum ether, chloroform and ethanolic leaf extracts of the selected plant were used to prepare various concentrations such as 100, 50 and 25 mg/ml respectively. These were used for the screening of antimicrobial potential compared with respective standard antibiotics.

Test microorganisms

The following bacterial and fungal strains were used for the screening of antimicrobial activity. All the microbial strains of human pathogens used were procured from IMTECH, Chandigarh and the procured microbes are the Gram-negative bacteria, such as *Escherichia coli* (MTCC 724), *Proteus vulgaris* (MTCC 426), *Vibrio vulnificus* (MTCC 1145) and the Gram-positive bacteria such as *Bacillus subtilis* (MTCC 441) and *Staphylococcus aureus* (MTCC 96), *Streptococcus pnemoniae* (MTCC 655) and fungi such as *Aspergillus niger* (MTCC 1344) and *Candida albicans* (MTCC 227) respectively.

Determination of antimicrobial activity

Agar well-diffusion method[11] was followed to determine the antimicrobial activity. Nutrient agar (NA) and Potato Dextrose Agar (PDA) plates were swabbed (sterile cotton swabs) with 8 hours old - broth culture of respective bacteria and fungi. Two wells (10mm diameter) were made in each of these plates using sterile cork borer. About 0.3 ml of different concentrations (100, 50, 25 mg/ml) of plant extracts were added using sterilized dropping pipettes into the wells and allowed to diffuse at room temperature for 2 hours. The plates were incubated at 37°C for 18-24 hours for bacterial pathogens and 28°C for fungal pathogens. Respective solvent control for leaf extracts was also maintained and the diameter of zone of inhibition was recorded in mm and compared with standard values. Triplicates were maintained and the experiment was repeated thrice and the average values were recorded for antimicrobial activity.

Table 1: Preliminary phytochemical screening of various leaf extracts of *Pterospermum canescens* Roxb.

Phytoconstituents	Petroleum ether extract	Chloroform extract	Ethanolic extract
Alkaloids	-	-	+
Amino acids & Proteins	-	-	-
Anthraquinones	-	-	-
Carbohydrates	-	+	+
Catechins	-	-	-
Flavonoids	+	+	+
Glycosides	-	-	-
Gums & Mucilages	+	-	-
Phenolic compounds	+	+	+
Saponins	-	-	-
Steroids	+	+	+
Tannins	-	+	+

(+) = Presence of phytoconstituents; (-) = Absence of phytoconstituents

Table 2: Antimicrobial activity of various leaf extracts of *Pterospermum canescens* Roxb., against different human pathogens

Test Microorganism	Zone of inhibition in mm									
	Petroleum ether extract (mg/ml)			Chloroform extract (mg/ml)			Ethanol extract (mg/ml)			Standard
	100	50	25	100	50	25	100	50	25	(10µg/ml)
Gram-negative bacteria										
Proteus vulgaris	15	15	16	21	22	23	12	17	14	34 (Cl)
Escherichia coli	18	18	14	16	15	28	21	15	13	35 (A)
Vibrio vulnificus	16	19	14	17	26	17	17	14	13	36 (K)
Gram-positive bacteria										
Bacillus subtilis	14	14	16	13	14	15	12	23	17	34 (A)
Staphylococcus aureus	21	24	13	13	16	16	15	13	12	35 (A)
Streptococcus pneumoniae	17	21	23	17	19	15	18	27	18	32 (C)
Fungi										
Aspergillus niger	27	27	16	22	17	21	20	28	19	33 (P)
Candida albicans	21	18	14	18	20	14	21	19	14	32 (P)

(A) = Ampicillin; (C) = Ciprofloxacin; (Cl) = Clotrimaxazole ; (K) = Kanamycin ; (P) = Penicillin

RESULTS

Preliminary phytochemical screening

The results of Preliminary phytochemical screening were given in the Table 1. showed the presence of flavonoids, phenolic compounds and steroids in petroleum ether, chloroform and ethanolic leaf extracts of *Pterospermum canscens* Roxb. Tannins were present in both the chloroform and ethanolic extracts but not in petroleum ether extract. Alkaloids were present only in ethanolic extract whereas absent in petroleum ether and chloroform extracts. Amino acids, anthraquinones, catechins, glycosides and saponins were completely absent in all the three extracts.

Antimicrobial activity

The results of antimicrobial activity of petroleum ether, chloroform and ethanolic leaf extracts of *Pterospermum canescens* Roxb., against the various pathogens were tabulated in the Table 2. It was found that petroleum ether leaf extract exhibited significant activity against *Aspergillus niger*, *Candida albicans* and *Staphylococcus aureus* have moderate activity against *Proteus vulgaris*, *Escherichia coli*, *Vibrio vulnificus*, *Bacillus subtilis* and *Streptococcus pneumoniae* (100, 50, 25 mg/ml). Chloroform leaf extract showed significant activity against *Proteus vulgaris*, *Vibrio vulnificus*, *Aspergillus niger* and *Candida albicans* and at the same time, produced moderate activity against *Escherchia coli*, *Bacillus subtilis*, *Staphylococcus aureus*, *Streptococcus pneumonia*e (100, 50, 25 mg/ml). Ethanolic leaf extract (100, 50, 25 mg/ml) exhibited significant results against *Escherchia coli*, *Bacillus subtilis*, *Streptococcus pneunomiae*, *Aspergillus niger*, and *Candida albicans* but exerted moderate activity against *Proteus vulgaris*, *Vibrio vulnificus* and *Staphylococcus aureus*. Antimicrobial potential of petroleum ether, chloroform and ethanolic leaf extracts were compared with the standard drugs such as ampicillin, ciprofloxacin, clotrimaxazole, kanamycin and penicillin (10µg/ml) against the respective human pathogens.

DISCUSSION

Secondary metabolites in plant products are responsible for several biological activities in man and animals[12]. The active components usually interfere with growth and metabolism of microorganisms in a negative manner[13]. Antimicrobial properties of several plant extracts have been attributed due to the secondary metabolites [14, 15, 16]. The reason for the difference sensitivity between the gram-positive and gram-negative bacteria could be ascribed to the morphological differences between these microorganisms, gram-negative pathogens having an outer phospholipidic membrane carrying the structural lipopolysaccharide components. This makes the cell wall impermeable to lipophilic solutes, while porins constitute a selective barrier to hydrophilic solutes with an exclusion limit of about 600 Da. The gram positive bacteria should be more susceptible having only an outer peptido glycone layer which is not an effective permeability barrier[17]. Phenolic content of plant extracts possess antimicrobial activity[18] and highly oxidized phenols are more inhibitory because of phenolic toxicity to microorganisms[19]. In addition, leaf extracts also possess antimicrobial potential against all pathogens which may be due to the presence of steroids[20]. It is not surprising that the differences in antimicrobial effect of plant species due to the variation in the phytochemical property between the plant species. Further explorations on plant derived antimicrobials are needed, to determine the identity of antimicrobial compounds within this plant and also to determine their full spectrum of efficacy.

In conclusion, *Pterospermum canescens* leaf extracts possess a broad-spectrum of antimicrobial activity against pathogens responsible for various infectious diseases.

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