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Screening Of Phytochemical And Antibacterial Activity Of Polygala javana Plant Leaf, Stem And Root Extract Against Human Pathogen

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Abstract: Microorganisms have potential to cause human disease. The screening of antibacterial activity of medicinal plants is very important since vast numbers of medicinal plants have been used for centuries as remedies for human diseases. The present investigation mainly focused to screen the phytochemical studies and antibacterial activity of petroleum ether, benzene, chloroform, methanol and distilled water extracts of leaf, stem and root of the plant *Polygala javana* against ten species human pathogens viz., *Escherichia coli, Pseudomonas aeruginosa, Staphylococcus aureus, Streptococcus pyogenes, Salmonella typhii, Serratia marcescens, Klebsiella pneumoniae, Proteus vulgaris, Enterobacter aerogene,* and *Bacillus subtilis* using disc diffusion method. Results showed that crude extract of petroleum ether leaf extract has maximum activity (9mm) against *Escherichia coli, the* crude methanol stem extract has maximum activity (10mm) against *Pseudomonas aeruginosa* and the crude methanol root extract has maximum activity (11mm) against *Proteus vulgaris.*

Keywords: Phytochemical studies, antibacterial activity, *Polygala javana,* petroleum ether, benzene, chloroform and methanol.

INTRODUCTION

Plants have been used for a long time for their medicinal properties. Plant derived products *viz*., gums, resins, oils and extracts have been used for therapeutic purpose since ages. Systematic screening of folk medicinal plants has resulted in the discovery of novel effective compounds against harmful organisms[1]. Aromatic and medicinal plants are a group of unique plants containing certain chemicals having antimicrobial properties. Plant protection measures have been developed by the use of safer biocides which are environmentally and ecologically safe and could be exploited for commercialization. During recent years, these crops have been reported to possess potent antifungal and antibacterial activity[2]. Among aromatic and medicinal crops *Pogostemon patchouli* (Patchouli), *Rosmarinus officinalis* (Rosemary), *Lantana camara* (Lantana) and *Chromolaena odorata* (Eupatorium) are important crops possessing antimicrobial compounds in them. So, the present study is aimed to study the phytochemical screening and antibacterial activity features of leaf, stem and root of *Polygala javana*.

MATERIAL AND METHODS

Material

The experimental material selected for the present study is *Polygala javana DC* Wild belongs to the family *Polygalaceae*. The identity of the plant species was confirmed with Voucher specimen No: 1157 available in the Survey of Medicinal Plant Unit (SMP), Govt. Siddha Medical College, Palayamkottai. The Plant material was collected from Dr.Zakir Husain Collage campus, Ilayangudi and it was subjected to the phytochemical screening and antibacterial activity studies. The taxonomic features collected from the species have been confirmed with the Flora of Presidency of Madras[3] and The Flora of Tamil Nadu Carnatic[4].

Phytochemical analysis

Mature and healthy plant was collected and dried at room temperature (25-30^{0}C), for about two weeks. About 30gms of plant powder of each plant species were taken in a digestion flask and fitted to the soxhlet apparatus and was separately extracted with petroleum ether, benzene, chloroform, and methanol. The aqueous extract was repared from directly boiling the powder with distilled H_2O. These extracts were concentrated and kept in brown bottles used for the phytochemical screening [5]. The extracts were tested for steroids, alkaloids, triterpene, sugar, phenolic groups, flavone, catechin, saponin, tannin, anthroquinone, amino acid and reducing sugars.

Biochemical analysis:

The protein, phenol and carbohydrates are found to be essential bio chemicals for any plants to regularize the activity of the plant. Hence, the present study was also carried out for the analysis of protein[6], phenol[7] and carbohydrate[8].

Microorganisms tested

Escherichia coli, Pseudomonas aeruginosa, Staphylococcus aureus, Streptococcus pyogenes, Salmonella typhii, Serratia marcescens, Klebsiella pneumoniae, Proteus vulgaris Enterobacter aerogenes and *Bacillus subtilis* were obtained from P.G.Department of Microbiology, Dr.Zakir Husain College, Ilayangudi, Sivagangai, Tamil Nadu. Nutrient broth was used as the media for the culturing of bacterial strains and a loop full of the strains of all the human pathogens were inoculated in the nutrient broth and incubated for 37^{o}C for 18 hours and was used for this present study.

Antibacterial activity

Antibacterial activity was assayed by filter paper disc diffusion method. What man No. 1 filter papers of 5 mm diameter were used. These discs were sterilized before use. The extracts of the medicinal plant parts were added to the sterile discs. Each sterile disc was incorporated individually with 200 – 500 µ of extract of various medicinal plant parts using micropipette. Precautions were taken to prevent the flow of the solvent extract from the outer surface of the discs. The condensed extracts were applied in small quantities and the discs were allowed for air – drying. Then another dose of the extract was applied. Already prepared Muller – Hinton Agar medium sterilized well at 120^o C/15 lbs for 20 minutes. After the temperature reduced up to the level of handling the media poured in already sterilized Petri plates inside the laminar airflow hood. After solidification it used to streak the microbes. Muller – Hinton Agar broth was prepared in ten test tubes. These were cotton plugged and autoclaved. The test tubes were labeled according to the type of the bacterial cultures to be inoculated. The nutrient broth was incubated at 37 ± 1.5^o for 18 hrs. After incubation period, the microbial strains were smeared on sterile Muller – Hinton agar plates. Streptomycin presoaked and dried discs of 5 mm diameter of Whatman filter paper No. 1 were used as positive control. The plates were incubated at 37^o C for 18 hrs. After the incubation period the inhibition zones around the discs were measured and recorded. Three replicates for each concentration were carried out.

RESULT AND DISCUSIION

Phytochemical analysis

Flavonoid group of compounds from chloroform extract of *Lantana camara* has highly inhibited the plant pathogens. The results were previously reported with the extracts of *Croton bonplandianum* made from chloroform solvent were found to be effective against most of the organisms tested as reported[9]. The phytochemical characteristics of *Polygala javana DC* plants tested were summarized (**table-1).** The results revealed the presence of medically active Petroleum ether, benzene, chloroform and methanol extract in the *Polygala javana DC* plants studied. From the table, it could be seen that, Reducing Sugar proteins, carbohydrates, phenols, alkaloids and tannins, steroids, flavonoids and saponins were present in all the extract from *Polygala javana DC*.

Table.1: Preliminary phytochemical analysis of powder extracts of *Polygala javanan* DC

Polygala javana plant extracts	Samples	Reducing sugar	Protein	Phenol	Alkaloid	Steroid	Tri terpenoid	Flavonoid	Catachin	Saponin	Tannin	Anthro quinone
Petroleum ether	Stem	+	+	-	-	+	-	+	+	-	+	-
	Leaf	+	+	+	-	+	-	+	+	-	+	-
	Root	+	+	-	-	+	+	-	-	-	-	-
Benzene	Stem	-	+	-	+	+	-	-	-	-	+	-
	Leaf	-	+	+	+	+	-	-	-	-	+	-
	Root	+	+	-	+	+	+	-	-	-	+	-
Chloroform	Stem	+	+	+	+	+	-	-	-	-	+	-
	Leaf	+	+	+	+	+	-	-	-	-	+	-
	Root	+	+	+	+	+	+	-	-	-	+	-
Methanol	Stem	+	+	+	+	+	+	+	+	-	+	-
	Leaf	+	+	+	+	+	+	+	+	-	+	-
	Root	+	+	+	+	+	+	+	+	-	+	-
Distilled water	Stem	-	+	+	-	+	+	-	-	-	+	-
	Leaf	+	+	+	+	+	-	+	+	-	+	-
	Root	-	+	+	+	+	-	-	-	-	+	-

Biochemical analysis

Biochemical studies reveal that, the extract has carbohydrate, protein and phenol. It was found that the leaf contains maximum carbohydrates (8.5mg/g/fw), protein (7.4mg/g/fw) and phenol (17.2mg/g/fw).

Antibacterial activity of leaf extract

The antibacterial activity of secondary metabolites from *Polygala javana* plant petroleum ether, benzene, chloroform, methanol and distilled water extract against 10 human bacterial pathogens were carried out by the present study and represented in table 2. It reveals that, the *Polygala javana* plant petroleum ether leaf extract showed maximum sensitivity (9mm and 7mm) against *Escheriachia coli* and *Staphylococcus aureus,* methanol leaf extract showed maximum sensitivity (8mm and 7mm) against *Streptococcus pyogenes* and *Klebsiella pneumoniae,* distilled water leaf extract showed maximum sensitivity (8mm) against *Streptococcus pyogenes.*

Antibacterial activity of stem extract

The antibacterial activity of secondary metabolites from *Polygala javana* plant petroleum ether, benzene, chloroform, methanol and distilled water extract against 10 human bacterial pathogens were carried out by the present study and represented in **table 2. It** reveals that, the *Polygala javana* plant benzene stem extract showed maximum sensitivity (9mm) against *Staphylococcus aureus,* methanol leaf extract showed maximum sensitivity (10mm) against *Pseudomonas aeruginosa,* distilled water leaf extract showed maximum sensitivity (9mm) against *Proteus vulgaris.*

Antibacterial activity of root extract

The antibacterial activity of secondary metabolites from *Polygala javana* plant petroleum ether, benzene, chloroform, methanol and distilled water extract against 10 human bacterial pathogens were carried out by the present study and represented in **table 2. I**t reveals that, the *Polygala javana* plant benzene and chloroform root extract showed maximum sensitivity (9mm) against *Proteus vulgaris,* methanol leaf extract showed maximum sensitivity (11mm) against *Proteus vulgaris,* distilled water leaf extract showed maximum sensitivity (9mm) against *Klebsiella pneumoniae.*

Table.2: Antibacterial activity of *Polygala javana* DC in leaf extract

Microorganisms	Leaf extract					
	Petroleum ether	Benzene	Chloroform	Methanol	Distilled water	Control
Diameter of inhibition zone (mm)						
Eseheriachia coli	9	-	-	-	6	18
Pseudomonas aeruginosa	6	-	6	8	-	8
Staphylococcus aureus	7	-	7	-	6	20
Streptococcus pyogenes	-	-	-	6	8	28
Salmonella typhii	6	-	-	-	-	28
Serratia marcescens	-	-	-	-	6	8
Klebsiella pneumoniae	-	-	-	-	7	20
Entrobactor areogenes	-	-	-	-	-	25
Proteus vulgaris	6	7	6	7	-	25
Bacillus subtillus	-	6	6	6	6	25

Table.3: Antibacterial activity of *Polygala javana* DC in stem extract

Microorganisms	Stem extract					
	Petroleum ether	Benzene	Chloroform	Methanol	Distilled water	Control
Diameter of inhibition zone (mm)						
Eseheriachia coli	-	-	-	-	6	18
Pseudomonas aeruginosa	-	9	6	10	6	21
Staphylococcus aureus	-	6	7	6	6	28
Streptococcus pyogenes	-	6	-	-	-	21
Salmonella typhii	-	6	6	-	6	18
Serratia marcescens	-	-	-	6	6	30
Klebsiella pneumoniae	-	-	-	-	-	22
Entrobactor areogenes	-	-	-	-	-	25
Proteus vulgaris	6	6	7	6	-	20
Bacillus subtillus	-	6	-	7	9	27

Table.3: Antibacterial activity of *Polygala javana* DC in root extract

Microorganisms	Root extract					
	Petroleum ether	Benzene	Chloroform	Methanol	Distilled water	Control
Diameter of inhibition zone (mm)						
Eseheriachia coli	-	-	-	-	7	35
Pseudomonas aeruginosa	6	-	6	6	-	25
Staphylococcus aureus	-	6	6	-	7	35
Streptococcus pyogenes	-	-	-	-	-	25
Salmonella typhii		7	8	6	8	19
Serratia marcescens	-	-	-	6	-	20
Klebsiella pneumoniae	-	-	-	-	9	20
Entrobactor areogenes	-	-	-	-	-	15
Proteus vulgaris	6	9	9	11	8	20
Bacillus subtillus	-	-	-	-	-	25

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