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Research Article

PHYTOCHEMICAL AND ANTIBACTERIAL SCREENING OF SEVEN APOCYNACEAE SPECIES AGAINST HUMAN PATHOGENS

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ABSTRACT

The antibacterial activity of the methanol extracts of seven selected plant species belonging to the family Apocynaceae, collected from different localities of Tirunelveli district of Tamilnadu were screened against ten infectious pathogens. Antibacterial activities of the extracts were determined by the agar disc diffusion method. MIC of the plant extracts that showed some efficacy against pathogens at the minimum concentration was noted. Ciprofloxacin (5 mcg/disc) was used as positive control for comparison of the inhibition zones. The methanolic extracts of *A. cathartica* and *P. alba* showed active inhibition against most of the tested human pathogens.

Keywords: Apocynaceae, Antibacterial activity, Human pathogens, Minimum Inhibitory Concentration.

INTRODUCTION

The steadily increasing bacterial resistance to existing drugs is a serious problem in antibacterial therapy and necessitates continuing research into new classes of antibacterials [1, 2]. One way to prevent antibiotic resistance of pathogenic species is to use new compounds that are not based on existing synthetic antimicrobial agents [3]. It is anticipated that phytochemicals with adequate antibacterial efficacy could be used for the treatment of bacterial infections [4].

A special feature of higher angiospermic plants is their capacity to produce a large number of organic chemicals of high structural diversity. The so-called secondary metabolites [5], which are grouped into different categories based on their mechanism of function like chemotherapeutic, bacteriostatic, bactericidal and antimicrobial [6]. Screening of medicinal plants for antibacterial activities and phytochemicals is important to find out the potential new compounds for therapeutic use. Among many plants used as antibacterials, the members belonging to Apocynaceae are reported to have better effect against pathogens and these plants are being locally available [7, 8, 9, 10].

The family Apocynaceae consists of about 250 genera and 2000 species of tropical trees, shrubs and vines [11]. A characteristic feature of this family is that almost all species produce milky sap. In traditional medicine, Apocynaceae species are used to treat gastrointestinal ailments, fever, malaria, pain and diabetes [12]. The roots, leaves and latex of Apocynaceae are used to treat skin and liver diseases, leprosy, dysentery, worms, ulcers, tumours and ear aches [13].

In the present study, seven medicinal plants belonging to Apocynaceae are selected and screened for phytochemical constituents and antibacterial activity against ten human pathogens

MATERIALS AND METHODS

Plant materials:

The leaves of *Catharanthus roseus* (A_1), *Rauvolfia tetraphylla* (A_2), *Nerium Oleander*(A_3), *Tabernaemontana divaricata* (A_4), *Allamanda cathartica* (A_5), *Thevetia peruviana* (A_6) and *Plumeria alba* (A_7) belonging to Apocynaceae were collected from different locations of Tirunelveli District of Tamilnadu, India. All the species were identified and checked with herbarium specimens in Xavier's College Herbarium (XCH). India.

Crude Methanolic Extract

The dried powdered leaf material was macerated with 95% methanol (100 g dried powder sample/500 ml of 95% methanol) for 7 days at room temperature. The filtrated solvent of each species was removed under vacuum at 40°C by using a rotary evaporator. The obtained crude extract was stored at 4°C.

Preliminary screening

The condensed extracts were used for preliminary screening of phytochemicals such as alkaloids, steroids, reducing sugars, catechins, anthroquinones, flavonoids, terpenoids, sugars, phenols, saponins, tannins and aminoacids. The presence of phytochemicals from methanol extract of all the samples was qualitatively determined[14].

Determination of antibacterial activity

a) Microorganisms used

The pathogens *Salmonella typhi, salmonella paratyphi, Pseudomonas vulgaris, Streptococcus aureus, Staphylococcus aureus, Klebsiella vulgaris, Shigella dysenteriae, Shigella boydii,* Methicillin Resistant *Staphylococcus aureus* (MRSA) and *Escherichia coli* were obtained from Microbial Type Culture Collection (MTCC), Chandigarh. The bacterial strain was cultured in nutrient broth at 37°C and maintained on nutrient agar (HiMedia) slant at 4°C.

b) Antibacterial activity

Antibacterial assay was carried out by disc diffusion method [15] using microorganisms cell suspension whose concentration was equilibrated to 0.5 McFarland standards. For this, 0.1ml (10^{-5} CFU /ml) of 24 hrs old bacterial culture was placed on Mueller Hinton agar medium and spread throughout the plate by spread plate technique. The sterile filter paper disc of 6mm diameter soaked with 3.12, 6.25, 12.5, 25, 50 mg/ml of plant extract dissolved in DMSO 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. Ciprofloxacin (5 mcg / disc) was used as positive control, negative controls were done using paper disc loaded with 25 µl of DMSO. The entire test was performed in triplicate. The Minimum Inhibitory Concentration (MIC) of methanol extract was determined as the lowest concentration of the plant extract inhibiting the visible growth of organism.

c) Determination of % of Relative Inhibition Zone Diameter

The antibacterial activity was calculated by applying the expression: % RIZD = (IZD sample - IZD negative control) / IZD antibiotic standard × 100, where RIZD is the relative inhibition zone diameter and IZD is the inhibition zone diameter (mm) [16].

f) Statistical analysis

All values are expressed as mean ± standard deviation. The Inhibition Zone Diameter data of each concentration was analyzed using one way analysis of variance (ANOVA). P value < 0.05 was considered as significant. The software OriginPro 7.5 was employed for the statistical analysis.

RESULTS AND DISCUSSION

Phytochemical screening

The medicinal uses of the selected species of Apocynaceae are listed in Table 1.

The phytochemical screening of the selected species showed the presence of reducing sugars and Phenolic compounds in all the seven plants, Triterpenoids, sugars and anthroquinones were present only in *C. roseus.* Catechin and tannins were present in all the plants except *N. oleander* and *T. peruviana* respectively. Steroids were present in *C. roseus*, *R. tetraphylla*, *T. peruviana* and *P. alba*. Flavonoids were present in *A. cathartica* and *P. alba* whereas aminoacids were absent in both the plants. Saponins were present in *C. roseus* and *N. oleander*. Alkaloids were present in *C. roseus*, *R. tetraphylla*, *N. oleander* and *P. alba* (Table 2).

Phytochemical constituents such as tannins, flavonoids, alkaloids and several other aromatic compounds or secondary metabolites of plants serve as defense mechanism against predation by many microorganism, insects and herbivores. The curative properties of medicinal plants are perhaps due to the presence of various secondary metabolites such as alkaloids, flavonoids, glycosides, phenols, saponins, steroids, etc., [17].

Antibacterial activity

Almost all the selected species of Apocynaceae showed antibacterial activity against the investigated ten human pathogens, the presence and absence of antibacterial activity is summarized in the Table 3.

The zone of inhibition ranged from 7 mm to 13 mm. The highest inhibition zone 13 mm (52 % of RIZD) was formed by the extract of *P. alba* against *Salmonella typhi* at the highest concentration, followed by 11 mm of inhibition zone against *Streptococcus aureus. Klebsiella vulgaris, Shigella dysenteriae* and *Shigella boydii*. Same inhibition zone was observed by *A. cathartica* against *Shigella dysenteriae* and *Escherichia coli* (Fig. 1).

The methanol extracts that showed antibacterial activity against the pathogens was active in 50 and 25 mg/ml concentration, the lower concentrations 6.25 & 3.12 mg/ml showed no activity, whereas, 12.5 mg/ml concentration was found to be the minimum inhibitory concentration against most of the pathogens. The zone of inhibition (mm) and % of Relative Inhibition Zone Diameter are given in the Table 4. None of the investigated plants showed activity against Methicillin Resistant *Staphylococcus aureus*. *T. peruviana* showed no activity against the pathogens except *Salmonella typhi* and *Salmonella paratyphi.* Similarly the methanol extract of *T. divaricata* showed its activity only against *Streptococcus aureus* with minimum inhibition zone of 7 mm. Among all the seven species investigated *P. alba* and *A. cathartica* was found to be efficient against most of the investigated pathogens.

Using Originpro software the values of the inhibition zone was statistically analysed through one way analysis of variance (ANOVA) followed by Tukey's test. Values of $P<0.05$ were considered statistically significant and the data of the effective antibacterial activity are presented in the Table 5.

Table 1: Medicinal uses of the seven selected species of Apocynaceae

Botanical name	Common name	Parts used	Uses
C. roseus	Nithyakalyani	Whole plant	Childhood leukemia, Hodgkin's disease, testicular cancer, diuretic, antidysenteric, hemorrhagic, antiseptic, anti-diabetic, stomachic, bleeding, sore throat, mouth ulcers.
R. tetraphylla	Pambukala	Leaves, fruits, seeds	Cholera, fever, eye disease, diarrhea, antihypertensive, dysentery, intestinal disorders
N. oleander	Arali	Flowers, Leaves, bark, root, latex	Warts, corns, cancerous ulcers, carcinoma, ulcerating or hard tumors
T. divaricata	Nanthiyarvattam	Leaves, stem, root	Inflammation, fever, pain, dysentery
A. cathartica	Manjal patti	Leaves, roots	Jaundice, complications with malaria
T. peruviana	Malaiaruli	Whole plant, seeds	Heart diseases,
P. alba	Champa	Flower, fruit, seed, bark, leaves, latex	Ulcers, herpes, scabies, haemostatic, tumours, purgative, cardiotonic, diuretic, hypotensive

Table 2: Results of the preliminary phytochemical analysis

S. No	Phytochemicals	A_1	A_2	A_3	A_4	A_5	A_6	A_7
1.	Steroids	+	+	-	-	-	+	+
2.	Triterpenoids	+	-	-	-	-	-	-
3.	Reducing sugar	+	+	+	+	+	+	+
4.	Sugars	+	-	-	-	-	-	-
5.	Alkaloids	+	+	+	-	-	-	+
6.	Phenolic compounds	+	+	+	+	+	+	+
7.	Flavonoids	-	-	-	-	+	-	+
8.	Catechin	+	+	-	+	+	+	+
9.	Saponins	+	-	+	-	-	-	-
10.	Tannins	+	+	+	+	+	-	+
11.	Anthroquinones	+	-	-	-	-	-	-
12.	Amino acids	+	+	+	+	-	+	-

Table 3: Results of the antimicrobial activity of the seven species of Apocynaceae

Bacteria	A_1	A_2	A_3	A_4	A_5	A_6	A_7
Salmonella typhi	-	-	-	-	+	+	+
Salmonella paratyphi	-	-	-	-	+	+	+
Pseudomonas vulgaris	-	-	+	-	-	-	+
Streptococcus aureus	+	-	+	+	+	-	+
Staphylococcus aureus	+	+	+	-	-	-	-
Klebsiella vulgaris	-	-	-	-	+	-	+
Shigella dysenteriae	+	+	-	-	+	-	+
Shigella boydii	+	+	+	-	+	-	+
MRSA	-	-	-	-	-	-	-
Escherichia coli	+	+	+	-	+	-	-

Table 4: Zone of Inhibition (mm) and % of RIZD of seven species of Apocynaceae

Bacteria	Conc. (mg/ml)	A_1	A_2	A_3	A_4	A_5	A_6	A_7
		Zone of Inhibition (mm) / % of RIZD						
Salmonella typhi	50	-	-	-	-	9±0.4/36	10±0.1/40	13±0.1/52
	25	-	-	-	-	8±0.1/32	9±0.1/36	11±0.1/44
	12.5	-	-	-	-	-	-	8±0.1/32
Salmonella paratyphi	50	-	-	-	-	10±0.1/42	7±0.2/29	10±0.2/42
	25	-	-	-	-	8±0.7/33	-	7±0.1/29
Pseudomonas vulgaris	50	-	-	10±0.2/50	-	-	-	10±0.2/50
	25	-	-	9±0.2/45	-	-	-	9±0.1/45
Streptococcus aureus	50	8±0.1/73	-	10±0.1/91	07±0.1/64	10±0.2/91	-	11±0.2/100
	25	7±0.2/64	-	8±0.1/73	-	8±0.1/73	-	9±0.1/ 82
	12.5	-	-	7±0.2/64	-	7±0.1/64	-	-
Staphylococcus aureus	50	7±0.1/39	7±0.1/39	9±0.2/50	-	-	-	9±0.2 / 50
	25	-	-	8±0.1/44	-	-	-	-
Klebsiella vulgaris	50	-	-	-	-	8±0.2/36	-	11±0.2/50
	25	-	-	-	-	7±0.1/32	-	10±0.1/45
	12.5	-	-	-	-	7±0.2 / 32	-	-
Shigella dysenteriae	50	7±0.1/54	9±0.1/69	-	-	11±0.3/85	-	11±0.2/85
	25	-	8±0.1/62	-	-	8±0.1/62	-	9±0.2/69
	12.5	-	7±0.1/54	-	-	7±0.1/54	-	-
Shigella boydii	50	8±0.1/35	9±0.2/39	9±0.2/39	-	9±0.1/39	-	11±0.3/48
	25	7±0.2/30	7±0.1/30	8±0.2/35	-	7±0.1/30	-	9±0.1/39
	12.5	-	-	7±0.1/30	-	-	-	8±0.1/35
Escherichia coli	50	9±0.1/36	9±0.1/36	11±0.1/44	-	11±0.2/44	-	-
	25	7±0.1/28	7±0.1/28	8±0.1/32	-	9±0.1/36	-	-

Table 5: One Way ANOVA for the antimicrobial activity

Species	Bacteria	Conc. (mg/ml)	MS	F value	P value
A_1	*Strep. aureus*	50	0.13	5.40	0.0808
	S. boydii	50	3.37	112.50	0.0045**
	E. coli	50	2.94	98.00	0.0005**
	S. dysenteriae	50	0.24	9.60	0.0362**
A_2		25	0.37	15.00	0.3552
	S. boydii	50	2.94	58.80	0.0001**
	E. coli	50	3.84	384.00	0.0004**
	S. vulgaris	50	7.26	132.00	0.0003**
A_3		25	7.93	226.71	0.0001**
	Staph. aureus	50	0.01	0.60	0.4818
		25	0.13	3.85	0.1210
	Staph. aureus	50	0.06	1.50	0.2878**
	Strep. aureus	25	3.08	264.14	0.0008**
	S. boydii	50	2.94	84.00	0.0007**
		25	3.22	77.44	0.0009**
	E. coli	50	2.94	294.00	0.0007**
		25	4.33	216.75	0.0001**
	S. typhi	50	4.00	150.06	0.0002**
A_5		25	4.16	156.25	0.0002**
	S. paratyphi	50	2.94	117.60	0.0004**
		25	3.84	109.71	0.0004**
	K. vulgaris	50	3.01	262.13	0.0007**
	S. dysenteriae	50	0.06	0.92	0.3910
		25	0.37	10.71	0.0306**
	S. boydii	50	2.94	147.00	0.0007**
	E. coli	50	2.94	117.60	0.0004**
		25	3.84	384.00	0.0004**
	S. typhi	50	2.40	76.00	0.0009**
A_7		25	2.94	117.60	0.0004**
	S. paratyphi	50	2.94	45.23	0.0025**
	P. vulgaris	50	7.26	181.50	0.0001**
		25	7.93	226.71	0.0001**
	Strep. aureus	50	1.47	0.00	1.0000
		25	0.06	1.50	0.2878
	K. vulgaris	50	1.81	72.60	0.0010**
		25	2.16	108.00	0.0004**
	S. dysenteriae	50	0.60	1.09	0.3552
		25	0.24	6.00	0.0704
	S. boydii	50	2.16	36.00	0.0038
		25	3.37	112.50	0.0045**

P<0.05**- Significant; MS- Mean square; P- propability

The in vitro antibacterial activity of *P. alba* against Gram positive *Staphylococcus aureus, Bacillus subtilis,* Gram negative *Escherichia coli, Pseudomonas aeruginosa, Salmonella typhi* was carried out and efficient result was observed against *Staphylococcus aureus* and *Bacillus subtilis* [18]. Similar study was conducted using solvent extract of *A. cathartica* against both Gram positive and Gram negative bacteria and three fungal species, chloroform solvent extract showed significant antimicrobial activity against *Shigella dysenteriae* [19].

The plants are the vital source of innumerable number of antimicrobial compounds. Several phytoconstituents like flavonoids [20], phenolics and polyphenols [21], tannins [22], terpenoids [23], sesquiterpenes [24], etc., are effective antimicrobial substances against a wide range of microorganisms.

It can be seen from the above results that the leaf extract contains alkaloids, and some phenolic compounds. The phenolic compounds are known for their antimicrobial properties. The significance of these compounds is that these could be used as substitutes for synthetic antibiotics for the treatment of chronic kidney infection, bacterial endocarditis, carrier conditions of typhoid (where the organisms reside in gall bladder).

Further investigation is required for the isolation of the active principle which could serve as a broad-spectrum antibacterial agent for treating bacterial infections.

CONCLUSION

The phytochemicals that have efficient antibacterial activity could be screened, isolated and used as substitute for antibiotics. Among the seven Apocynaceae species screened, almost all the plants showed antibacterial activity but *P. alba and A. cathartica* were found to be more effective against most of the investigated pathogens. Investigation on the effect of active compounds would throw more light on the biological activity of the medicinal plants.

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