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Phytochemical Screening and Antimicrobial Activity from Five Indian Medicinal Plants Against Human Pathogens

[1]*Periyasamy Ashokkumar,* [1]*Rajkumar and* [2]*Mahalingam Kanimozhi*

[1]Department of Biotechnology, M.G.R. College, Hosur, Tamil Nadu, India
[2]Department of Botany, Ethiraj College for Women, Chennai, Tamil Nadu, India

Abstract: Five different Indian medicinal plants, *Achyranthes aspera, Cassia senna, Wrightia tinctoria, Aristolochia bracteolate* and *Rauvolfia tetraphylla* were examined using agar disc diffusion method against *Escherichia coli, Klebsiella pneumoniae, Pseudomonas aeruginosa, Bacillus subtilis Micrococcus luteus* and *Staphylococcus aureus.* Plant leaves were extracted using different solvents such as methanol, ethyl acetate, aqueous and chloroform. Phytochemical screening of these plants was performed for constituents: alkaloids, flavonoids, tannins, anthraquinones, saponins, glycosides and volatile oils. Among the different extracts, methanol extracts showed more antibacterial activity and moderate activity recorded with aqueous, ethyl acetate and chloroform extracts. *Achyranthes aspera* showed maximum antibacterial activity against all the tested bacteria than the other plants. All the bacteria were more susceptible to methonolic extracts than the other organic extracts. In future these plants can be further subjected to isolation of the therapeutic antimicrobials and to further pharmacological evaluation.

Key words: Medicinal plants · Phytochemical · Antimicrobial activity · Clinical bacteria

INTRODUCTION

Medicinal plants represent a rich source form which antimicrobial agents may be obtained. Plants are used medicinally in different countries and are a source of many potent and powerful drugs [1]. The use of medicinal plants to treat human diseases has its roots in pre-historical times. Medicinal plants are used by 80% of the world population as the only available medicines especially in developing countries [2]. A wide range of medicinal plant parts is used for extract as raw drugs and they possess varied medicinal properties. The different parts used include leaves, root, stem, flower, fruit, twigs exudates and modified plant organs. While some of these raw drugs are collected in smaller quantities by the local communities and folk healers for local used, many other raw drugs are collected in larger quantities and traded in the market as the raw material for many herbal industries [3]. Plants used for traditional medicine contain a wide range of substances that can be used to treat chronic as well as infectious diseases. Clinical microbiologists have great interest in screening of medicinal plants for antimicrobial activities and phytochemicals as potential new therapeutics. The active principles of many drugs found in plants are secondary metabolites [4, 5]. The antimicrobial activities of plant extracts may reside in a variety of different components, including aldehyde and phenolic compounds [6]. The beneficial medicinal effects of plant materials typically result from the combinations of secondary products present in the plant. In plants, these compounds are mostly secondary metabolites such as alkaloids, steroids, tannins, and phenol compounds, flavonoids, steroids, resins, fatty acids gums which are capable of producing definite physiological action on body. The development of drug resistance in human pathogens against commonly used antibiotics has necessitated a search for new antimicrobial substances from other sources including plants [7]. Screening of medicinal plants for antimicrobial activities and phytochemicals is important for finding potential new compounds for therapeutic use.

In the present study, we selected 5 different medicinal plants, such as *Achyranthes aspera, Cassia senna, Wrightia tinctoria, Aristolochia bracteolate* and *Rauvolfia tetraphylla* were screened for phytochemical constituents and antimicrobial activity against *Escherichia coli, Klebsiella pneumoniae, Pseudomonas aeruginosa, Bacillus subtilis Micrococcus luteus* and *Staphylococcus aureus.*

Corresponding Author: Periyasamy Ashokkumar, Department of Biotechnology, M.G.R. College, Hosur, Tamil Nadu, India

MATERIALS AND METHODS

Collection of Plant material: Fresh leaves of *Achyranthes aspera, Cassia senna, Wrightia tinctoria, Aristolochia bracteolate* and *Rauvolfia tetraphylla* were collected from in and around Hosur, Krishinagiri Distict, TamilNadu, India, during 2009-2010. The plants were identified taxonomically and authenticated at the Herbarium, Department of Botany, University of Madras, Chennai, Tamilnadu, India.

Bacteria and Growth Conditions: Six bacterial species were employed as test organisms which include *E. coli*, *K. pnemoniae*, *P. aeruginosa*, *B. subtilis, M. luteus* and *S. aureus*. The bacteria were maintained in Mueller-Hinton Agar (MH). Inocula were prepared by adding an overnight culture of the organism in MH broth to obtain an OD 600 in 0.1. The cells were allowed to grow until they obtain the McFarland standard 0.5 (approximately 108 CFU/ml).

Extraction and Preparation of Plant Materials: *Achyranthes aspera, Cassia senna, Wrightia tinctoria, Aristolochia bracteolate* and *Rauvolfia tetraphylla* were collected from in and around Hosur for the present study. Fresh leaves were washed thoroughly 2-3 times with running tap water and then with sterile water followed by shade-dried, powdered and used for extraction. 10 g of the powder is mixed with 40 ml of Methanol in a 250-ml conical flask and was kept at 25°C for 12 h. The suspension was filtered through a Whatman no. 4 filter paper and the filtrate was evaporated by vacuum dryer at 40°C overnight to get the Methanol extract. Similarly, Ethyl acetate, chloroform and aqueous extracts were prepared by applying the same procedure. Finally, the extracted powder was resuspended in the respective organic solvents at a concentration of 100 mg/ml before it was tested for the antibacterial activity.

Phytochemical Tests

Molisch's Test for Carbohydrates: Few drops of Molisch's reagent was added to each of the portion dissolved in distilled water, this was then followed by addition of 1 ml of conc. H_2SO_4 by the side of the test tube. The mixture was then allowed to stand for two minutes and then diluted with 5 ml of distilled water. Formation of a red or dull violet color at the interphase of the two layers was a positive test [8].

Barfoed's Test Monosaccharides: About 0.5 g each portion was dissolved in distilled water and filtered. 1 ml of the filtrate was then mixed with 1 ml of Barfoed's reagent in a test tube and then heated on a water bath for a period of 2 minutes. Reddish precipitate of cuprous oxide was considered as a positive test [8].

Fehling's Test for Free Reducing Sugar: About 0.5 g each portion was dissolved in distilled water and filtered. The filtrate was heated with 5 ml of equal volumes of Fehling's solution A and B. Formation of a red precipitate of cuprous oxide was an indication of the presence of reducing sugars [8].

Fehling's Test for Combined Reducing Sugars: About 0.5 g each portion was hydrolysed by boiling with 5 ml of dilute hydrochloric acid and the resulting solution neutralised with sodium hydroxide solution. To this, few drops of Fehling's solution was added and then heated on a water bath for 2 minutes. Appearance of a reddish-brown precipitate of cuprous oxide indicates the presence of combined reducing sugars [8].

Test for Tannins: About 0.5 g each portion was stirred with about 10 ml of distilled water and then filtered. Few drops of 1% ferric chloride solution were added to 2 ml of the filtrate occurrence of a blue-black, green or blue-green precipitate indicates the presence of tannins [9].

Borntrager's Test: About 0.2 g of each portion to be tested was shaken with 10 ml of benzene and then filtered. Five milliliters of the 10% ammonia solution was then added to the filtrate and thereafter the shaken. Appearance of a pink, red or violet color in the ammoniacal (lower) phase was taken as the presence of free anthraquinones [8].

Liebermann-burchard Test for Steroids: To 0.2 g of each portion, 2 ml of acetic acid was added, the solution was cooled well in ice followed by the addition of conc. H2SO4 carefully. Color development from violet to blue or bluish-green indicated the presence of a steroidal ring i.e. glycone portion of cardiac glycoside [8].

Test for Terpenoids: A little of each portion was dissolved in ethanol. To it 1 ml of acetic anhydride was added followed by the addition of conc. H_2SO_4. A change in colour from pink to violet showed the presence of terpenoids [8].

Test for Saponins: One gram of each portion was boiled with 5 ml of distilled water, filtered. To the filtrate, about 3 ml of distilled water was further added and shaken vigorously for about 5 minutes. Frothing which persisted on warming was taken as an evidence for the presence of saponins [8].

Shinoda's Test for Flavonoids: About 0.5 of each portion was dissolved in ethanol, warmed and then filtered. Three pieces of magnesium chips was then added to the filtrate followed by few drops of conc. HCl. A pink, orange, or red to purple colouration indicates the presence of flavonoids [9].

Ferric Chloride Test for Flavonoids: About 0.5 of each portion was boiled with distilled water and then filtered. To 2 ml of the filtrate, few drops of 10% ferric chloride solution were then added. A green-blue or violet colouration indicated the presence of a phenolic hydroxyl group [9].

Lead Ethanoate Test for Flavonoids: Few quantity of the each portion was dissolved in water and filtered. To 5 ml of each of the filtrate, 3 ml of lead ethanoate solution was then added. Appearance of a buff-coloured precipitate indicates the presence of flavonoids [9].

Sodium Hydroxide Test for Flavonoids: Few quantity of the each portion was dissolved in water and filtered; to this 2 ml of the 10% aqueous sodium hydroxide was later added to produce a yellow colouration. A change in colour from yellow to colourless on addition of dilute hydrochloric acid was an indication for the presence of flavonoids [9].

Test for Alkaloids: Few quantity of the each portion was stirred with 5 ml of 1% aqueous HCl on water bath and then filtered. Of the filtrate, 1 ml was taken individually into 2 test tubes. To the first portion, few drops of Dragendorff's reagent were added; occurrence of orange-red precipitate was taken as positive. To the second 1 ml, Mayer's reagent was added and appearance of buff-coloured precipitate will be an indication for the presence of alkaloids [8].

Test for Soluble Starch: Few quantity of each portion was boiled with 1 ml of 5% KOH, cooled and acidified with H_2SO_4. A yellow colouration was taken as the presence of soluble starch [10].

Antimicrobial Susceptibility Test: The disc diffusion method was used to screen the antimicrobial activity [11]. *In vitro* antimicrobial activity was screened by using Mueller Hinton Agar (MHA) obtained from Himedia (Mumbai). The MHA plates were prepared by pouring 15 ml of molten media into sterile petriplates. The plates were allowed to solidify for 5 minutes and 0.1% inoculum suspension was swabbed uniformly and the inoculum was allowed to dry for 5 minutes. The different concentrations of extracts (50, 100 and 150µl/disc) were loaded on 6 mm sterile disc. The loaded disc was placed on the surface of medium and the compound was allowed to diffuse for 5 minutes and the plates were kept for incubation at 37°C for 24 hrs. At the end of incubation, inhibition zones formed around the disc were measured with transparent ruler in millimeter. These studies were performed in triplicate.

RESULTS AND DISCUSSION

The results of the phytochemical screening of leaves extract of *Achyranthes aspera, Cassia senna, Wrightia tinctoria, Aristolochia bracteolate* and *Rauvolfia tetraphylla* is as presented in Table1. These classes (such as alkaloids, saponins, tannins, anthraquinones and flavonoids) of compounds are known to have curative activity against several pathogens and therefore could suggest the use traditionally for the treatment of various illnesses [12, 13]. The broad antibacterial activities of this extracts could be as a result of the plant secondary metabolites (alkaloids, anthraquinones, flavonoids, tannins, saponins) present in the extracts; in line with these findings, Usman and Osuji (2007) [13], reported that tannins had been widely used topically to sprains, bruises and superficial wounds as such, it could be probable that tannins and other plant phenolics from this extract were responsible for these broad activities. The extract and its partitioned portions were further subjected to antimicrobial studies. The susceptibility pattern against the test organisms is shown in Table 2.

The results of the antimicrobial determinations for all the organic extracts of the leaves of *Achyranthes aspera, Cassia senna, Wrightia tinctoria, Aristolochia bracteolate* and *Rauvolfia tetraphylla* against the six bacterial species were investigated by disc-diffusion assay. The disc diffusion method for antibacterial activity showed significant reduction in bacterial growth in terms of zone of inhibition. The zone of inhibition increased on increasing the concentration of extract. This showed the concentration dependent activity (Table 2).

Table 1: Properties and phytochemical screening of selected medicinal plants from Hosur, TamilNadu, India

	Inference				
Phytochemicals	*Achyranthes aspera*	*Cassia senna*	*Wrightia tinctoria*	*Aristolochia bracteolata*	*Rauvolfia tetraphylla*
Flavanoids	+	+	+	+	+
Alkaloids	+	+	+	+	+
Glycosides	+	+	+	+	+
Saponins	+	-	+	+	+
Phytosterols	+	+	+	+	+
Steroids	-	-	+	+	-
Triterpenoids	+	+	+	+	+
Tannins	+	-	-	-	+
Carbohydrates	+	+	+	+	+
Proteins	+	+	+	+	+
Anthraquinones	-	-	-	-	-

Key = + present; - = absent

Table 2: Antibacterial activity of the different extracts of selected medicinal plants

	(Zone of mm)											
	Methanol			Ethyl aceteate			Aqueous			Cholorofrom		
Achyranthes aspera	50µl	100µl	150µl	50µl	100µl	150µl	50µl	100µl	150µl	50µl	100µl	150µl
P.aeruginosa	12±0.77	12±0.63	14±0.90	6±0.38	8±0.42	10±0.64	8±0.51	10±0.52	12±0.77	8±0.51	10±0.52	12±0.77
K.pneumoniae	11±0.41	13±0.07	15±0.91	6±0.22	8±0.046	10±0.60	8±0.30	11±0.06	13±0.79	8±0.30	11±0.06	13±0.7
E.coli	12±0.36	14±0.42	16±0.48	8±0.24	10±0.30	12±0.36	10±0.30	12±0.36	14±0.42	10±0.30	12±0.36	14±0.42
M.luteus	12±0.48	14±0.50	17±0.61	8±0.32	10±0.36	12±0.43	10±0.40	12±0.43	14±0.50	10±0.40	12±0.43	14±0.50
B.subtillus	13±0.68	15±0.48	16±0.51	7±0.37	9±0.28	11±0.35	9±0.47	14±0.45	11±0.35	9±0.47	14±0.45	11±0.35
S.aureus	12±0.54	13±0.58	15±0.48	6±0.27	10±0.45	12±0.38	8±0.36	13±0.58	12±0.38	8±0.36	13±0.58	12±0.38
Cassia senna												
	50µl	100µl	150µl	50µl	100µl	150µl	50µl	100µl	150µl	50µl	100µl	150µl
P.aeruginosa	8±0.51	12±0.63	14±0.9	6±0.38	8±0.42	10±0.64	6±0.38	10±0.52	12±0.77	5±0.32	6±0.31	8±.51
K.pneumoniae	8±0.30	10±0.05	12±0.72	5±0.18	6±0.03	8±0.48	6±0.22	8±0.04	10±0.60	4±0.15	5±0.02	7±.42
E.coli	8±0.24	10±0.30	14±0.42	5±0.15	6±0.18	8±0.24	6±0.18	8±0.24	12±0.36	4±0.12	5±0.15	6±0.18
M.luteus	10±0.40	14±0.50	16±0.57	6±0.24	7±0.25	12±0.43	8±0.32	10±.36	14±0.50	5±0.20	7±0.25	8±0.28
B.subtillus	7±0.37	9±0.28	10±0.32	4±0.21	6±0.19	9±0.28	6±0.31	9±0.28	10±0.32	4±0.21	6±0.19	5±0.16
S.aureus	6±0.27	9±0.40	9±.28	5±0.22	5±0.22	8±0.25	6±0.27	7±0.31	9±0.28	4±0.18	6±0.27	6±0.19
Aristolochia bracteolate												
	50µl	100µl	150µl	50µl	100µl	150µl	50µl	100µl	150µl	50µl	100µl	150µl
P.aeruginosa	15±0.96	17±0.89	20±1.28	6±0.38	8±0.42	10±0.64	8±0.51	10±0.52	12±0.77	5±0.32	7±0.37	8±0.51
K.pneumoniae	13±0.49	14±0.08	15±0.91	8±0.30	10±0.05	12±0.7	10±0.37	12±0.06	14±0.85	6±0.22	8±0.04	10±0.60
E.coli	12±0.36	14±0.42	17±0.5	6±0.18	8±0.24	10±0.30	8±0.24	10±0.30	12±0.36	5±0.15	6±0.18	8±0.24
M.luteus	17±0.68	19±0.68	21±0.75	6±0.24	10±0.36	12±0.43	8±0.32	12±0.43	14±0.50	6±0.24	8±0.28	10±0.36
B.subtillus	15±0.79	16±0.51	17±0.54	7±0.37	8±0.257	10±0.32	7±0.37	10±0.32	11±0.35	5±0.26	6±0.19	8±0.25
S.aureus	10±0.45	12±0.54	13±0.41	6±0.27	7±0.31	9±0.28	8±0.36	9±0.40	11±0.35	6±0.27	8±0.36	9±0.28
Wrightia tinctoria												
	50µl	100µl	150µl	50µl	100µl	150µl	50µl	100µl	150µl	50µl	100µl	150µl
P.aeruginosa	9±0.57	11±0.58	15±0.96	6±0.38	8±0.42	10±0.64	8±0.51	10±0.52	12±0.77	5±0.32	6±0.31	8±0.51
K.pneumoniae	10±0.26	12±0.06	14±0.85	6±0.15	8±0.04	10±0.60	8±0.21	10±0.05	12±0.72	5±0.13	6±0.034	8±0.48
E.coli	9±0.18	12±0.36	15±0.45	6±0.12	8±0.24	11±0.33	7±0.14	10±0.30	13±0.39	5±0.1	6±0.18	9±0.27
M.luteus	12±0.30	15±0.54	17±0.61	8±0.20	10±0.36	12±0.43	10±0.25	12±0.43	14±0.50	6±0.15	8±0.28	10±0.36
B.subtillus	10±0.25	10±0.32	12±0.38	7±0.17	8±0.25	10±0.32	9±0.22	9±0.28	11±0.35	5±0.12	7±0.22	10±0.32
S.aureus	11±0.49	9±0.40	10±0.32	7±0.31	8±0.36	10±0.32	9±0.40	9±0.40	10±0.32	5±0.22	7±0.31	10±0.32
Rauvolfia tetraphylla												
	50µl	100µl	150µl	50µl	100µl	150µl	50µl	100µl	150µl	50µl	100µl	
P.aeruginosa	10±0.64	13±0.68	15±0.96	6±0.38	9±0.47	11±0.70	8±0.51	11±0.58	13±0.83	6±0.38	7±.37	9±0.57
K.pneumoniae	10±0.26	12±0.06	14±0.85	6±0.15	8±0.04	10±0.60	8±0.21	10±0.05	12±0.72	5±0.13	6±03	8±0.48
E.coli	11±0.22	13±0.39	15±.45	7±0.14	9±0.27	11±0.33	9±0.18	11±0.33	13±0.39	6±0.12	7±0.21	9±0.27
M.luteus	12±0.30	14±0.50	16±0.57	8±0.20	10±0.36	12±0.43	10±0.25	12±0.43	14±0.50	6±0.151	8±0.28	10±0.36
B.subtillus	9±0.2	11±0.35	13±0.41	5±0.12	6±0.19	9±0.28	10±0.25	12±0.38	13±0.4	7±0.17	9±0.28	10±0.32
S.aureus	8±0.36	10±0.45	12±0.38	4±0.18	7±0.31	8±0.25	8±0.36	10±0.45	10±0.32	6±0.27	9±0.40	10±.032

The different organic extracts of *A. aspera* exhibit significant antimicrobial activity against *E. coli*, *K. pnemoniae*, *P. aeruginosa*, *B. subtilis, M. luteus* and *S. aureus*. Methanolic extracts from leaf part of *A. aspera,* produced consistent level of inhibition of bacterial growth and followed by aqueous, chloroform and ethyl acetate. The ethanol and aqueous extracts of leaves of *Achyranthes aspera* were prepared and its wound healing and antioxidant activity were evaluated. [14] and Varuna *et al*. 2010 [15] reported *Achyranthes aspera* ethanolic extract of root, leaves, stem gives different biological activity and also isolate chemical constituents like Betaine and Achyranthine. These chemical constituents are used in treatment of gallbladder stone, asthma, high and low Blood Pressure. Whereas the leaves of *A. aspera* different extracts showed low activity against all the bacteria tested [16]. These differences might also be attributed to the changes in environmental conditions.

Four organic different leaves extracts of *C. senna* showed antibacterial activity against all the tested organisms. The present study was supported by the Hatil Hashim *et al.* [2] reported ethonolic extracts of *C. occidentalis*, *C. tora*, *C. senna*, *R. minima* var. *memnonia*, *A. maritime* and *C. nervatus* showed equal or nearly equal antibacterial activity both against Gram-positive and Gram-negative bacteria.

In vitro antibacterial studies of the four different leaf extracts of *Aristolochia bracteolata* revealed that the methanol extract had significant activity against most of the organism and followed by aqueous extract, while the ethyl acetate extract possessed moderate activity (Table 2). Methanol extract exhibited the maximum inhibitory effect against *M. luteus*, *B. subtilis* and *P. aeruginosa* and considerable inhibitory activity against *E. coli, K. pneumoniae* and *S. aureus*. Aqueous ethyl acetate and chloroform extract had significant inhibitory activity against *K. pneumoniae,* and *M. luteus* and moderate activity against *E. coli, B. subtilis, P. aeruginosa* and *S. aureus*. Similarly, this result was supported by Kavitha and Nirmaladevi [17]. Extracts of *Wrightia tinctoria* are also showed similar pattern of *Aristolochia bracteolate*, this plant extracts inhibit all the test bacteria at different patterns (Table 2). Similarly, *Wrightia tinctoria* leaves extracts possessed potent antimicrobial properties against bacteria and dermatophytic fungi. In particular, methanol and ethanol extracts were active against bacteria and hexane extract was active against dermatophytic fungi, suggesting that the active principles may be useful in the topical treatment of superficial skin infections [18].

The methonolic extracts of *R. tetraphylla* showed maximum activity against *E. coli*, *K. pneumoniae*, *P. aeruginosa* and *M. luteus* then followed by *Bacillus subtilis* and *S. aureus*. These results revealed that extracts of *R. tetraphylla* exhibited significant antibacterial activity. In testing, inhibition zone increased with increase in drug concentrations and thus exhibiting concentration dependent activity. Suresh *et al,* [19] reported the better antimicrobial activity of ethanol extract obtained from *Rauvolfia tetraphylla* showed maximum activity against *E.coli, Enterobacter aerogenes, Alcaligenes faecalis* and different fungi tested *Aspergillus niger* and *Penicillium spp* were found to be more sensitive to crude extract when compared to others. The plants are the vital source of innumerable number of antimicrobial compounds. Several phytoconstituents like flavanoids [20], phenolics and polyphenols [21], tannins [22], terpenoids [23], sesquiterpenes [24], are effective antimicrobial substances against a wide range of microorganisms.

The discovery of a potent remedy from plant origin will be a great advancement in bacterial infection therapies. The results of present investigation clearly indicate that the antibacterial activity vary with the species of the plants and plant material used. These results suggest that the plant extracts possess compounds with antimicrobial properties that can be further explored for antimicrobial activity. This antibacterial study of the plant extracts demonstrated that folk medicine can be as effective as modern medicine to combat pathogenic microorganisms. The millenarian use of these plants in folk medicine suggests that they represent an economic and safe alternative to treat infectious diseases. These plants could serve as useful sources for new antimicrobial agents. Further work is needed to isolate the active principle from the plant extracts and to carry out pharmaceutical studies.

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