

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

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**Abstract:** Five different Indian medicinal plants, *Achyranthes aspera*, *Cassia senna*, *Wrightia tinctoria*, *Aristolochia bracteolata* 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 methanolic extracts than the other organic extracts. In future these plants can be subjected to isolation of the major constituent's antimicrobials and to further pharmacological evaluation.

**Key words:** Medicinal plants • Phytochemical • Antimicrobial activity • Clinical bacteria

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### INTRODUCTION

Medicinal plants represent a rich source from 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 bracteolata* 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*.

## MATERIALS AND METHODS

**Collection of Plant Material:** Fresh leaves of *Achyranthes aspera*, *Cassia senna*, *Wrightia tinctoria*, *Aristolochia bracteolata* and *Rauwolfia 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. pneumoniae*, *P. aeruginosa*, *B.s 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 bracteolata* and *Rauwolfia 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 was 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 extract 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  $\alpha$ -naphthol 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. Formation of a red or dull violet color at the interphase of the two layers was a positive test [8].

**Test for Tannins:** About 0.5 g of each plant powder 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 plant powder 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 and Terpenoids:** To 0.2 g of each, 2 ml of acetic acid was added, the solution was cooled well in ice followed by the addition of conc.  $H_2SO_4$  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 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 may be the presence of saponins [8].

**Shinoda's Test for Flavonoids:** About 0.5g 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].

**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].

**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 1 ml, Mayer's reagent was added and appearance of buff-coloured precipitate will be an indication for the presence of alkaloids [8].

**Antimicrobial Susceptibility Test:** The disc diffusion method was used to screen the antimicrobial activity [10]. *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 bracteolata* and *Rauvolfia tetraphylla* is presented in Table 1. 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 [11, 12]. 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 [12], 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 bracteolata* 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).

The different organic extracts of *A. aspera* exhibit significant antimicrobial activity against *E. coli*, *K. pneumoniae*, *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. Edwin *et al.* [13] and Varuna *et al.* [14] 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 [15]. These differences might also be attributed to the changes in environmental conditions.

Table 1: Phytochemical screening of selected medicinal plants from Hosur, TamilNadu, India.

Phytochemicals	Inference				
	<i>Achyranthes aspera</i>	<i>Cassiasenna</i>	<i>Wrightia tinctoria</i>	<i>Aristolochia bracteolata</i>	<i>Rauvolfia tetraphylla</i>
Flavanoids	+	+	+	+	+
Alkaloids	+	+	+	+	+
Glycosides	+	+	+	+	+
Saponins	+	-	+	+	+
Phytosterols	+	+	+	+	+
Triterpenoids	+	+	+	+	+
Tannins	+	-	-	-	+
Carbohydrates	+	+	+	+	+
Proteins	+	+	+	+	+
Anthraquinones	-	-	-	-	-

Key = + present; - = absent

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

Methanol	Methanol			Ethyl acetate			Aqueous			Cholorofrom		
	50µl	100µl	150µl	50µl	100µl	150µl	50µl	100µl	150µl	50µl	100µl	150µl
<b><i>Achyranthes aspera</i></b>												
<i>P.aeruginosa</i>	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
<i>K.pneumoniæ</i>	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
<i>E.coli</i>	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
<i>M.luteus</i>	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
<i>B.subtilis</i>	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
<i>S.aureus</i>	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
<b><i>Cassia senna</i></b>												
<i>P.aeruginosa</i>	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±0.51
<i>K.pneumoniæ</i>	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±0.42
<i>E.coli</i>	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
<i>M.luteus</i>	10±0.40	14±0.50	16±0.57	6±0.24	7±0.25	12±0.43	8±0.32	10±0.36	14±0.50	5±0.20	7±0.25	8±0.28
<i>B.subtilis</i>	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
<i>S.aureus</i>	6±0.27	9±0.40	9±0.28	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
<b><i>Aristolochia bracteolata</i></b>												
<i>P.aeruginosa</i>	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
<i>K.pneumoniæ</i>	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
<i>E.coli</i>	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
<i>M.luteus</i>	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
<i>B.subtilis</i>	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
<i>S.aureus</i>	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
<b><i>Wrightia tinctoria</i></b>												
<i>P.aeruginosa</i>	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
<i>K.pneumoniæ</i>	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
<i>E.coli</i>	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
<i>M.luteus</i>	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
<i>B.subtilis</i>	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
<i>S.aureus</i>	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
<b><i>Rauvolfia tetraphylla</i></b>												
<i>P.aeruginosa</i>	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±0.37	9±0.57
<i>K.pneumoniæ</i>	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±0.03	8±0.48
<i>E.coli</i>	11±0.22	13±0.39	15±0.45	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
<i>M.luteus</i>	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
<i>B.subtilis</i>	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
<i>S.aureus</i>	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±0.32

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 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 extracts had significant inhibitory activity against *K. pneumoniae* and *M. luteus* and moderate activity against *E. coli*, *B. subtilis*, *P. aeruginosa* and *S. aureus*. Similarly, these results was supported by Kavitha and Nirmaladevi [16]. Extracts of *Wrightia tinctoria* are also showed similar pattern of *Aristolochia bracteolata*, 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 extract 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 [17].

The methanolic 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*, [18] 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 [19], phenolics and polyphenols [20], tannins [21], terpenoids [22], sesquiterpenes [23], 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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