

Original Research Article

## Antibacterial activity of methanolic and acetone extract of some medicinal plants used in indian folklore

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**Abstract**

Antibacterial study of methanolic and acetone extract in crude and treated (with 50 % lead acetate) form of medicinal plants *Alstonia scholaris* Linn. R.Br. (Stem bark, Apocynaceae), *Achyranthus aspera* Linn. (Whole plant, acanthaceae), *Moringa oleifera* Lam. (Leaves, Morinaceae), *Tinospora cordifolia* (Stem, Menispermaceae), and *Enicostema hyssopifolium* (Willd) (Stem, Gentianaceae). Extractive values in methanol were found higher than the extractive value in acetone, for all plants. All extract of plants were tried at 40-mg/ml concentrations against eight strains of bacteria, by agarwell-difusion test. Acetone extract was found more active as compared to methanol extract. Phytochemical investigation revealed crude and treated extracts of all plants were containing more or less same type of chemical constituents (except protein and carbohydrate). Selected eight strains of bacteria were study for antibiotic susceptibility against standard antibiotics like Ampicillin (10 $\mu$ g), Tetracycline (25 $\mu$ g), Gentamicin (30 $\mu$ g), Co-Trimoxazole (25 $\mu$ g), Amikacin (10 $\mu$ g), by Octadisc. Treated extract of *M. oleifera* and *A. scholaris* were count as to new source of antimicrobial agent for the infectious diseases (Typhoid).

**Keywords:** Antibacterial activity, Medicinal Plants, infectious diseases.

### Introduction

Plants are invaluable sources of pharmaceutical products [1] and plants are recognized for their ability to produce a wealth of secondary metabolites and mankind has used many species for centuries to treat a variety of diseases [2]. Despite the wide availability of clinically useful antibiotics and semisynthetic analogues, a continuing search for new anti-infective agents remains indispensable because some of the major antibacterial agents have considerable drawbacks in terms of limited antimicrobial spectrum or serious side effects [3]. The negative health trends call for a renewed interest in infectious disease in the medical and public health communities and renewed strategies on treatment

and prevention. Proposed solutions are outlined as a multi-pronged approach that includes: prevention, (such as vaccination); improved monitoring; and the development of new treatments. It is this last solution that would encompass the development of new antimicrobials [4]. There is an urgent need to discover new antimicrobial agents for human and veterinary therapeutic uses, as resistance to current drugs increases in severity and extent [5, 6]. The identification of new natural products with antimicrobial activity, extraction methods, and hopefully new modes of action, is one of the ways of tackling this problem. Lack of scientific knowledge has often constituted a major constraint to consideration of the use of



traditional herbal remedies in conjunction with or as an affordable alternative to orthodox medical treatment. In present study methanolic and acetone extract of five plants (traditionally used in many diseases) are studied for their antimicrobial activity in crude form and after treated with 50 % lead acetate treatment.

## Materials and methods:

### Plant material

Authentic sample (powder) of *Alstonia scholaris* Linn. R.Br. (Stem bark, Apocynaceae), *Achyranthus aspera* Linn. (Whole plant, acanthaceae), *Moringa oleifera* Lam. (Leaves - Morinaceae), *Tinospora cordifolia* (Stem-Menispermaceae), and *Enicostema hyssopifolium* (Willd) (Gentianaceae), collected from Bapalal Botanical Vaidya Research Center Surat (Gujarat).

### Extraction

Coarsely powdered air-dried material 4 g was placed in a glass stoppered conical flask and macerated with 100 ml of analytic grade solvents (methanol, and acetone) shaking frequently, and then allowing it to stand for 18 hours. Filter it rapidly through Whatman No. 1 filter paper, taking care not to lose any solvent. Transfer 25 ml filtrate to flat-bottom dish and evaporate solvent on a water bath. Dry at 105 °C for 6 hours, cool in a desiccator for 30 minutes and weigh without delay, calculate the content of extractable matter in mg/g of air-dried material [7]. Crude extract (10 ml) treated with 200 µl of 50 % lead acetate (Hi-media, Mumbai), mix it properly and precipitated by centrifugation (REMI, India) at 10000 rpm for 15 min. Carefully remove the supernatant in watch glass, evaporate at room temperature, dry at 105 °C for 6 hours, cool in a desiccator for 30 minutes. Extracts were dissolved in 90 % DMSO to concentration 40 mg/ml were used for antimicrobial activity.

### Phytochemical Screening

Crude extract in methanol (M1), and acetone (A1) along with after treatment of 50 % lead acetate (M2) and (A2) respectively, of all plants were subjected to qualitative phytochemical screening for protein, carbohydrate, saponin, tannin, glycoside, alkaloids, flavanoids, terpenoids, steroids and fixed oil according to the method [8].

### Procured Bacterial Strain

Test organisms used in this study were collected from the Microbiology Department of Sardar Patel University, V.V.Nagar, Gujarat. The Gram-positive bacteria are *Staphylococcus aureus* (ATCC9144) (SA), *Micrococcus luteus* (ATCC4698) (ML), *Klebsiella pneumoniae* (ATCC15380) (KP), *Bacillus subtilis* (ATCC 6051) (BC), and Gram-negative bacteria are *Pseudomonas aeruginosa* (ATCC25668) (PA), *Enterobacter aerogens* (ATCC13048) (EA), *Salmonella typhi* (NCTC 8394) (ST) and *Salmonella paratyphi- A* (SPA). Strains were maintained on nutrient agar.

### Agar diffusion Assay

Antimicrobial screening was done using agar well diffusion methods [9]. For this 25 ml of sterile Mueller –Hinton Agar No.2 (Hi-media), was poured in sterile autoclaved petri plates, before pouring 100 µl of activated culture of microorganism was added, and then allowed to stand for solidify completely. The wells were prepared with the help of sterile 10 mm diameter cork-borer. Then 100 µl of prepared plant extract (40 mg/ml) solution were poured into the wells. Then the plates were sealed with plasticine and transferred to the refrigerator to diffuse out for 30 min. The plates were then incubated in the incubator at 37 °C for 24 hrs. Triplicate plates were prepared for each treatment and the average zone of inhibition excluding well, were recorded. 90% DMSO was used as negative control, and 0.01mg/ml tetracycline was used as positive control. Inoculum turbidity was maintained constant throughout the experiment to 0.8 OD at

660 nm. Level of turbidity is equivalent to approximately  $1 \times 10^8$  CFU/ml.

### Antibiotic susceptibility of selected bacterial strains

Susceptibility of selected bacterial strain was done against the standard antibiotics viz. Ampicillin(10 $\mu$ g), Tetracycline(25 $\mu$ g), Gentamicin(30 $\mu$ g), Co-Trimoxazole(25 $\mu$ g), Amikacin(10 $\mu$ g) by octadisc (Hi-media, Mumbai). Twenty ml of sterilized nutrient agar seeded with activated bacterial culture was poured in petri dish, allowed to solidify and octadisc was placed gently on surface by pointed forceps. Seal the plates with plasticine and incubate in the incubators at 37 °C for 48 hrs.

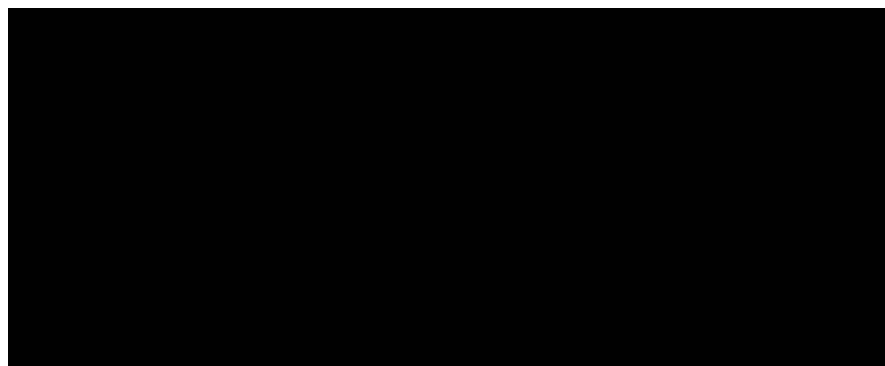
## Results

### Extractive value

The results of extractive value in acetone and methanol are shown in graph 1. All plants showed higher percentage of extraction in methanol then the acetone. The yield for selected plants in acetone was found in order to E.hyssofolia (7.11 %)> A.scholaris (2.50 %)> T.cordifolia (1.82%)> M. oleifera (1.22 %) > A.aspera (0.46 %), while in methanol yield was E.hyssofolia (26.5 %)> A.scholaris (18.5 %)> M.oleifera (6.36%)> T.cordifolia (3.47 %) > A.aspera (3.05 %).

### Phytochemical screening

Results of phytochemical screening for methanolic extract of all plants in crude (M1) form and after treated with 50 % lead acetate (M2) are shown in Table 1. And results for crude acetone extract (A1), and after treated with 50 % lead acetate (A2) are shown in Table 2. Alkaloids were present in M1 extract of E.hyssofolia, A.scholaris, T.cordifolia, M. oleifera, A.aspera (Table 1), while absent in A1 extracts of E.hyssofolia, A.scholaris, T.cordifolia (Table 2). Primary metabolites like carbohydrate and protein were detected in both M1 and A1 extract of all selected plants, and absent in M2 and A2 extract. Steroids were present only in both crude extract (M1 and A1) of A.scholaris (Table 1 and 2). M1 extract of A.scholaris, M. oleifera and E.hyssofolia were showed positive result for glycoside (Table 1), while glycoside was present in all A1 extract of all plants (Table 2). Both M1 and A1 extract of all five plants were showed presence of flavanoids (Table 1 & 2). But M2 and A2 extracts of M. oleifera and E.hyssopifolium were only showed presence of flavanoids (Table 1 & 2). Saponin was detected in M1, M2, A1, A2 extracts of all plants (Table 1 & 2). Terpenoids was absent in M1 extract of A.aspera (Table 1), while positive for the A1 extract (Table 2). Other all plants were positive for the presence of terpenoids in crude (M1, A1) as well as treated (M2, A2) extracts (Table 1 & 2). Crude extract (A1) of A.scholaris and M. oleifera were only positive for the tannin (Table 2). Fixed oil was detected in any types of extracts in all selected plants.



Graph 1. Extractive value of plants.

**Table 1: Phytochemical screening of Methanol extract.**

Phytoconstituents	A.scholaris		A.aspera		M.oleifera		T.cordifolia		E. hyssofolium	
	M1	M2	M1	M2	M1	M2	M1	M2	M1	M2
1. Alkaloids	+	+	+	+	+	+	+	+	+	+
2. Carbohydrates	+	-	+	-	+	-	+	-	+	-
3. Protein	+	-	+	-	+	-	+	-	+	-
4. Steroids	+	+	-	-	-	-	-	-	-	-
5. Glycosides	+	+	-	-	+	+	-	-	+	+
6. Saponin	+	+	+	+	+	+	+	+	+	+
7. Flavanoids	+	-	+	-	+	+	+	-	+	+
8. Tannins	+	+	-	-	+	+	+	+	+	+
9. Triterpenoids	+	+	-	-	+	+	+	+	+	+
10.Fixed oils	-	-	-	-	-	-	-	-	-	-

+ = Present, - = absent.

**Table 2. Phytochemical screening of Acetone extract.**

Phytoconstituents	A.scholaris		A.aspera		M.oleifera		T.cordifolia		E. hyssofolium	
	A1	A2	A1	A2	A1	A2	A1	A2	A1	A2
1. Alkaloids	-	-	+	+	+	+	-	-	-	-
2. Carbohydrates	+	-	+	-	+	-	+	-	+	-
3. Protein	+	-	+	-	+	-	+	-	+	-
4. Steroids	+	+	-	-	-	-	-	-	-	-
5. Glycosides	+	+	+	+	+	+	+	+	+	+
6. Saponin	+	+	+	+	+	+	+	+	+	+
7. Flavanoids	+	-	+	-	+	+	+	-	+	+
8. Tannins	+	+	-	-	+	+	-	-	-	-
9. Triterpenoids	+	+	+	+	+	+	+	+	+	+
10.Fixed oils	-	-	-	-	-	-	-	-	-	-

+ = Present, - = absent.

### Antimicrobial activity

Results of comparative antimicrobial activity of M1 extract and M2 extracts of all plants are recorded in Table 3, and results of A1 and A2 are shown in Table 4. M1 and M2 extracts of all plants were completely inactive against BS (Table 3). M1, M2 extracts of *M. oleifera* were found most active extracts as only BS and KP only were not inhibited by extract (Table 3). In methanolic extract highest inhibition (10mm) was found by *A.scholaris* against ML (Table 3). Highest zone of inhibition (22mm) was observed by A2 extracts of *A.scholaris* against EA. KP was completely resistant towards acetone extracts

(A1, A2) of *E.hyssofolia*, *A.scholaris*, *T.cordifolia*, *M. oleifera*, *A.aspera* (Table 4). BS, EA, and SA were found sensitive to A1 and A2 extracts of all plant (Table 4). Moderate kind of sensitivity was observed in ML and SPA, and least activity found in PA and ST, against A1 and A2 extract (Table 4). One strong observation was observed in present study was that in all plants A2 and M2 extract showed higher antibacterial activity than the A1 and M1 at same concentration (40mg/ml) respectively. All strain of bacteria was susceptible to positive control, and DMSO 90 % as negative control was not inhibiting any bacterial strain.

**Table 3. Antimicrobial activity of Methanol at 40 mg/ml**

Plants		BS	EA	KP	ML	PA	SA	ST	SPA
A.scholaris	M1	-	-	2	6	2	-	-	-
	M2	-	-	4	10	5	-	-	-
A. aspera	M1	-	-	-	2	-	4	2	-
	M2	-	-	-	5	-	8	6	-
M.oleifera	M1	-	4	-	2	4	2	2	2
	M2	-	8	-	5	9	7	6	6
T. cordifolia	M1	-	2	-	-	-	-	-	-
	M2	-	7	-	-	-	-	-	-
E. hyssofolium	M1	-	-	-	-	-	-	2	-
	M2	-	-	-	-	-	-	6	-

A-Methanol, B- Methanol fraction after treatment of 50 % lead acetate, - : no inhibition zone.

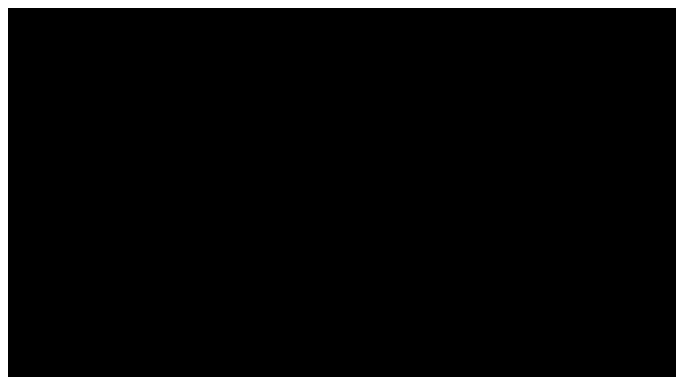
**Table 4. Antimicrobial activity of acetone fraction at 40 mg/ml.**

Plants		BS	EA	KP	ML	PA	SA	ST	SPA
A. scholaris	A1	6	20	-	10	-	4	-	-
	A2	8	22	-	14	-	7	-	-
A. aspera	A1	4	4	-	2	-	3	-	-
	A2	7	8	-	4	-	4	-	-
M.oleifera	A1	7	8	-	10	6	10	6	2
	A2	9	12	-	13	9	13	10	5
T. cordifolia	A1	2	5	-	-	-	5	-	8
	A2	6	9	-	-	-	10	-	12
E. hyssofolium	A1	4	6	-	-	-	7	-	-
	A2	8	9	-	-	-	10	-	-

A-Acetone, B- Acetone fraction after treatment of 50 % lead acetate, - : no inhibition zone

### Antibiotic susceptibility of bacterial strains

Results of antibiotic susceptibility of the selected bacterial strains are shown in (Graph.2). ML was more susceptible to all antibiotics. EA, PA, and SA were completely resistant to co-trimoxazole (25 µg/disc). STB was also showed negligible inhibition (4mm) against co-trimoxazole (Graph 2). Highest susceptibility (22mm) was found in KP against tetracycline (25 µg/disc). Ampicillin at 10µg/disc was showed negligible activity against EA (2mm), STB (4mm), PA (2mm), and SA (2mm).



**Graph 2. Antibiotic susceptibility of bacterial strain to antibiotics.**

A- Ampicillin, T- Tetracycline, G- Gentamicin, Co- Co-Trimoxazole, AK- Amikacin

## Discussion

The higher yield of the methanol extracts compared with the acetone extracts suggested that secondary metabolite of plants were more soluble in methanol than the acetone. In present study cold maceration was used to extract secondary metabolite, are the probably reason for low yield of extracts, as maceration [10] and cold extraction [11, 12] generally been reported to give lower yield of plant extracts compared to hot and soxhlet extraction.

Mostly both types of extraction (acetone and methanolic) extract same type of phytochemical like alkaloids, saponin, glycoside, flavanoids, tannins, and terpenoids as particular to different plants. And these compounds were well defined as antimicrobial agents, like tannin [13, 14], glycosides [15], Saponins [16], terpenoids and flavanoids, [17] and Alkaloids [18] in plants. A1 extracts found more active than M1 extract, which indicate that, active component of plants extract in acetone or may extract in very low concentration in methanol, as solubility of compound depend on polarity of extraction solvent [19] and [20]. High sensitivity of Gram-positive bacteria (except KP) than the Gram-negative bacteria, for selected plant extracts (M1, M2, A1, A2) were found in present study. The high resistance of gram-negative bacteria could be because of the phospholipid membrane in addition to the inner peptidoglycan layer, which makes the cell more impermeable for exogenous molecules [21].

Methanolic extract of *A.aspera* was inactive against BS, EA, KP, PA, and SPA, while acetone extract is active against BS and KP, these findings are corroborate with findings of [22]. Acetone extract of *A.scholaris* was more active than the methanol extract, results indicate active components in *A.scholaris* were more soluble in relatively non-polar solvent, findings are agree with [23]), as they reported butanol fraction has broad spectrum of antibacterial activity. Goyal et al. [24] reported alkaloids, sterols alkenes, are key antimicrobial agent in *A.scholaris*, result

matching with our finding. *M. oleifera* was most active plant among other selected plants. Activity of plant is attribute to the presence of saponin, tannin alkaloids and phenols [25]. Acetone extracts of *M. oleifera* only show the antimicrobial activity against the ST and STA, same results observed by Doughari et al. [26] for *Salmonella typhi*. Both bacteria are causative agent for the Typhoid fever, and recent year there has been a rapid rise in multidrug resistance by ST all over the world [27, 28, 29]. A2 extract of *M. oleifera* can be used to developed antityphoid agent. Activity of methanolic and acetone extract of *E.hyssofolium* was attributed to presence of flavanoids a major constitute in *E.hyssofolium* [30]. Singh et al. [31] reported presence of terpenoids and glycoside in *T.cordifolia*. These components are the responsible for the antibacterial activity of these plant [32].

M2 and A2 extracts of all plants were more active than their crude extract (M1, A1), phytochemical screening revealed that, carbohydrate and protein were absent in M2 and A2, while these present in higher concentration in crude extract, which may be interfered in diffusion of active component. Moreover the differences of antimicrobial activity of extracts were difficult to speculate; however, many antibacterial agents may exhibit their action through inhibition of nucleic acid, protein and membrane phospholipids biosynthesis [33]. Extract with analytical grade acetone gave a relatively wide spectrum of antimicrobial activity (37.5% – 87.5%) against the test bacterial strains compared to methanol extract (12.5 % - 75 %). The relatively wider spectrum of activity of the acetone extracts over the methanol extracts is difficult to explain since all the extracts contained the metabolites, though not in the same proportions. Perhaps, the active principles were more soluble in analytical acetone than the methanol solvents.

Antibacterial alternative for selected bacterial strains were always in focused because of its infectious nature and bacteria has ability to develop the genetic ability to transmit and acquire

resistance to drugs, which are utilized as therapeutic agents [34]. Several studies indicate the usefulness of plant for control of resistant strain of bacterial like *S.aureus* [35] and *P.aeruginosa* [36, 37, 38]. Among selected plants acetone extract of *A.scholaris* and *M. oleifera* can be used for the new antibacterial agent, for EA, SA and ML.

## Conclusion

Acetone extract after treatment of 50 % lead acetate was found to be best as antimicrobial agent. It is suggested if large amount of extract needed, cold maceration should be avoided. *M.oleifera* and *A.scholaris* can be further investigation to developed new weapon to combat infectious diseases.

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