ANTIBACTERIAL ACTIVITY OF METHANOLIC AND ACETONE EXTRACT OF SOME MEDICINAL PLANTS USED IN INDIAN FOLKLORE MEDICINE

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ABSTRACT

Antibacterial study of methanolic and acetone extracts of crude and treated (with 50 % lead acetate) extracts of medicinal plants viz, 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 (Wild) (Stem, Gentianaceae) was carried out. Extractive values in methanol were found to be higher than the extractive value in acetone, for all plants. All the extracts of the plants selected for the present study were tested for their antimicrobial activity at 40-mg/ml concentrations against eight strains of bacteria, by agarwell-diffusion test. Acetone extract was found to be more active as compared to that of methanol extract. The phytochemical analysis of crude and treated extracts of all the currently studied plants revealed that to contain more or less similar type of chemical constituents (except protein and carbohydrate). The eight strains of bacteria were selected for antibiotic susceptibility against standard antibiotics like Ampicillin (10µg), Tetracycline (25µg), Gentamicin (30µg), Co-Trimoxazole (25µg), Amikacin (10µg), by Octadisc.

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

MATERIALS AND METHODS

Plant material

Authentic (powder) samples of Alstonia scholaris Linn. R.Br. (Apocynaceae) Stem bark; Achyranthus aspera Linn. (Acanthaceae) whole plant; Moringa oleifera Lam. (Morinaceae) Leaves -. Tinospora cordifolia (Menispermaceae) Stem-, and Enicostema hyssopifolium (Wild) (Gentianaceae) whole plant, were collected from Bapalal Botanical Vaidya Research Center, Surat (Gujarat).

Procured Bacterial Strain

Test organisms used in this study were collected from the Department of Biosciences, Sardar Patel University, Vallabh Vidyanagar, Gujarat. The Gram-positive bacteria are Staphylococcus aureus (ATCC9144) (SA), Micrococcus luteus

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**Antibacterial activity of methanolic extracts.**

**RESULTS**

**Extractive value**

The results of extractive value in acetone and methanol are shown in Fig. 1. All the plants tested under study showed higher percentage of extraction in methanol than that of acetone. The yield for selected plants in acetone was found in the order of A. aspera (18.5 %) > T. cordifolia (1.82 %) > A. scholaris (2.50 %) > T. cordifolia (1.42 %) > M. oleifera (1.22 %) > A. aspera (0.46 %), while in methanol yield was in the order of E. hyssofolia (26.5 %) > A. scholaris (8.5 %) > M. oleifera (6.36 %) > T. cordifolia (3.47 %) > A. aspera (3.05 %).

**Phytochemical screening**

Results of phytochemical screening of methanolic extract of all plants in crude (M1) form and after treatment with 50 % lead acetate (M2) are shown in Table 1. Results for crude acetone extract (A1), and after treatment 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, 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. hyssofolium were only showed presence of flavanoids (Table 1 & 2). Sapin was detected in M1, M2, A1, A2 extracts of all plants (Table 1, 2). Terpenoids were 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.

**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 shown 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, 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.

**DISCUSSION**

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

Mostly both (acetone and methanolic) types of extractions yield same type of phytochemicals like alkaloids, saponins, glycosides, flavanoids, tannins, and terpenoids as particular to different plants. And the compounds such as tannin [13, 14], glycosides [15], Saponins [16], trepenoids and flavanoids, [17], and Alkaloids [18] were well defined as antimicrobial agents in plants.
Table - 1  Phytochemical screening of Methanol extract.

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<th>Phytoconstituents</th>
<th>Authentic powdered samples of Medicinal plants presently analyzed</th>
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<td></td>
<td>A. scholaris</td>
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<td>1. Alkaloids</td>
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<td>2. Carbohydrates</td>
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<td>3. Protein</td>
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<td>4. Steroids</td>
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<td>5. Glycosides</td>
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<td>6. Saponins</td>
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<td>7. Flavanoids</td>
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<td>8. Tannins</td>
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<td>9. Triterpenoids</td>
<td>+</td>
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<td>10. Fixed oils</td>
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Table - 2  Phytochemical screening of Acetone extract.

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Table - 3  Antimicrobial activity of Methanol fraction at 40 mg/ml (inhibition zone in mm)
A1 extracts were found more active than M1 extract, which indicates that active component of plants extracted more may get extracted in very low concentration in methanol, as solubility of compound depends upon the 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) was found in the 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 in accordance with the findings of Jigna et al. [22]. Acetone extract of A. scholaris was more active than that of methanol extract, results thus indicate active components in A. scholaris were more soluble in relatively non-polar solvent, as reported by Khan et al. [23], that butanol fraction has broad spectrum of antibacterial activity. Goyal et al. [24] reported that alkaloids, sterols alkenes, are key antimicrobial agents in A. scholaris and their results matching with our findings. M. oleifera was most active plant among the plants selected for the present study. Activity of plant is attributed to the presence of saponins, tannins, alkaloids and phenols [25]. Acetone extracts of M. oleifera only show the antimicrobial activity against the ST and STA. Similar results were observed by Doughari et al. [26] for Salmonella typhi. Both bacteria are causative agents of the Typhoid fever and recent years 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, for EA, SA and ML. Among the plants selected for the present study the acetone extract of A. scholaris and M. oleifera can be used for the new antibacterial agent, for EA, SA and ML.

**REFERENCES**


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