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Evaluation of Anti-microbial Activity of Indigenous Medicinal plants Seed extracts of India

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ABSTRACT

In the present study, six medicinally important plants seed (Acacia catechu, Sida cordifolia, Momordica fotida, Albiziz procera, Mesua ferrea and Lantana camare) were screened for their phytochemical and screened for anti-microbial activity by using 4 different solvent, water, Acetone, Petroleum Ether and chloroform. All the plants which are selected for the study contains phytochemicals like alkaloids, flavonoids, tannins, steroids, terpinoids, phenols and saponins. All the extracts showed high to moderate amounts of anti-microbial activity except A. procera against selected gram positive and gram negative bacterial species. The extracts from plants; A.catecu, S, cordifolia, M. foetida and L. camare showed highest zones of inhibition on gram positive bacteria than gram negative species. The bacterial species (B.cereus, Streptococcus pyrogens and S.epidermidis) showed highest sensitivity towards these four extracts. The gram negative bacterium Pseudomonas aeruginosa showed highest sensitivity to these extracts and Shigella flexneri showed highest category of resistance to these extracts. A. catechu, S.cordifolia and L.camare aqueous, acetone and petroleum ether extracts showed the best inhibition zones against these gram negative bacterial species.

Key words: Anti-microbial activity, gram positive bacteria, plant extracts.

INTRODUCTION

A variety of plants species have been used in different parts of the Globe to treat human diseases and infections [1]. Plants are used in much therapeutics in different parts of countries and are a source of many potential and powerful drugs [2]. Folk medicine using plant extracts continues to provide health coverage for over 80% of the Global population, particularly in the developing countries [3]. India is the largest producer of medicinal herbs and appropriately called as Botanical garden of the world [4]. Drugs extracted from the plants species are easily available, safe, and competent and have diminished side effects. Products obtained from higher plants may present a new foundation for anti-microbial agents with possibly novel mechanisms of action [5]. In contrast to synthetic pharmaceuticals based upon single chemicals, many phytomedicines exert their beneficial effects through the additive or synergistic action, eliminating the side effects associated with the dominance of a single xenobiotic chemical in the body [6]. The valuable medicinal property of plant materials typically result from the blends of secondary metabolites present in the plant. The therapeutic activitys of plants are unique to particular plant species or groups are dependable with this concept as the combinations of secondary products in a particular plant [7]. Secondary metabolites are synthesized in a specialized cell types and at distinct developmental stages, making their extraction and purification difficult. As a result secondary metabolites that are used commercially as biologically active compounds are generally high value-low volume products (e.g. -Steroids, Quinines, Alkaloids, Terpenoids

and Flavonoids) which are used in drug manufacture by the pharmaceutical industries. So in the present investigation we have studied 6 plants species: (*Acacia catechu, Sida cordifolia, Momordica fotida, Albiziz procera, Mesua ferrea and Lantana camare*) for their phytochemicals and anti-microbial activity. This might be a hope that the presences of phytochemicals (secondary metabolites) are responsible for their anti-microbial effects.

MATERIALS AND METHODS

Collection of Plant material
The plants were collected from their natural habitat, form different parts of south and north India. The plant material was identified and authenticated.

Chemicals:
The entire chemicals used in the present study are of analytical grade.

Preparation of plant extract
The collected plant material (seeds) was carefully washed under running tap water followed by sterilized distilled water, and was air dried at room temperature in laboratory for 10-25 days. These dried plant materials were then homogenized to a fine coarse powder using an electric blender and then stored in air tight containers until further use. Various organic solvents viz. water **[AQ]**, Acetone **[AE]**, Petroleum ether **[PE]**, and Chloroform **[CF]** were used for extractions. 100 gm of homogenized coarse powders of seeds were soaked in different conical flasks containing 100 ml of water **[AQ]**, Acetone **[AE]**, Petroleum ether **[PE]**, and Chloroform **[CF]** each and were allowed to stand for 30 min on a water bath with occasional shaking, which were then kept on rotary shaker at 200rpm for 24h [8-10]. Finally each sample extract (water **[AQ]**, Acetone **[AE]**, Petroleum ether **[PE]**, and Chloroform **[CF]**) were prepared by using Soxhlet apparatus and was filtered through sterilized Whatman No 1 filter paper and concentrated to dryness under vacuum at 40^0C using rotaevaporater. Thus the obtained dried extracts were lyophilized, labelled and stored at 4^0C in sterile bottles [11-12]. The extracted powder was dissolved in 10 % dimethyl sulfoxide (DMSO) for the further use. To detect various biologically active phytochemical constituents present in various solvent extracts the standard methods were followed [13-15].

Micro-organisms tested:
Bacterial strains: Total 6 strains including gram positive and gram negative bacteria were selected to assess the susceptibility test against the different solvent extract. These are, *Bacillus cereus*, *Streptococcus pyrogens*, *Streptococcus epidermidis*, *Protease vulgaris*, *Pseudomonas aeroginosa*, *Shigella flexneri*.

Assay of Antibacterial activity: Antibacterial activity of the crude extracts in different solvents of selected plants was tested by disc diffusion assay [16]. Mueller Hinton agar no. 2 (Hi Media, India) was used as the bacteriological medium. Medium was prepared and poured 20 ml each in sterilized Petri plates of 9 cm diameter and allowed to solidify. Bacterial cultures grown in nutrient broth and on agar slants were used. Bacterial suspension was prepared aseptically from 10 ml of saline (0.085 g NaCl in 10 ml Distilled water) under laminar. The plates, cultured with microbial suspension (100-150 µl) by spread plate technique. The zone of inhibition was measured after 24 hrs using disc diffusion assay. The concentration of extract was 10 mg/ml and 5 µl of each extract was used for antibacterial assay. For each bacterial strain controls were maintained where extract free pure solvents were used. The control zones were subtracted from the test zones and the resulting zone diameter [17] are reported.

RESULTS

Anti-bacterial Studies of six selected plants:
1)*Acacia catechu:*
All the seed extracts (AQ, AE, PE and CF) of *A.catechu* plant were tested for their anti-bacterial activity against selected three gram positive (*B.cereus, S.pyrogens* and *S.epidermidis*) and three gram negative (*P.vulgaris, Pseudomonas aeruginosa* and *Shigella flexeneri*) bacterial species. The AE extract showed highest anti-microbial activity against *Streptococcus epidermidis* (25 mm) and *B.cereus* (24 mm). When compared to the other extracts PE extract of *A.catechu* showed least anti-microbial activity against *Shigella flexeneri* (7 mm).The AQ and AE extracts reported the least activity with the *Shigella flexeneri* (10 and 8 mm) . All the four extracts (AQ, AE, PE and CF) showed marked antimicrobial activity on all the gram positive bacteria than compared with gram negative bacteria

(Fig 1). In conclusion the AE extract is more effective and showed highest anti-microbial action on the selected bacterial species.

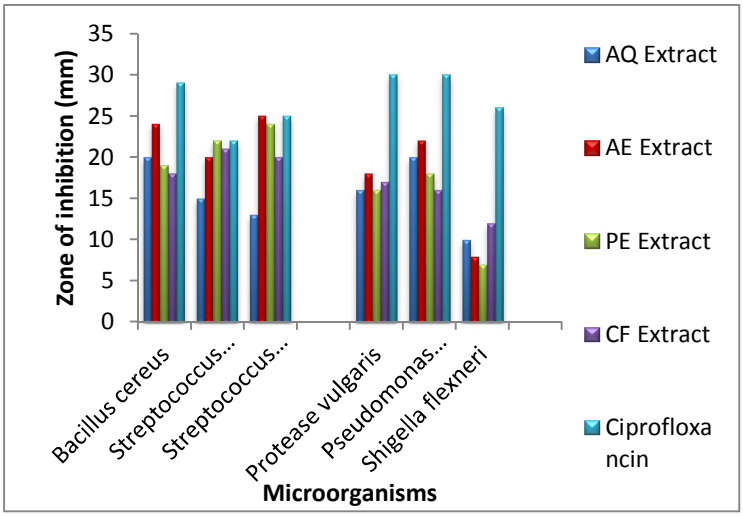

Fig 1: Anti-microbial activity of Different solvents (seed) extracts of *Acacia catechu*

2)Sida cordifolia:

Of all the four extracts made from *S.cordifolia* seeds, the PE extract showed maximum zones of inhibition against *Streptococcus pyrogens* (22 mm) and *Bacillus cereus* (20 mm). The AE and CF extract of the plant showed moderate inhibition action against all the bacterial species selected for the work (Fig 2). The AQ extract of *S.cordifolia* has shown least activity on *Shigella flexneri* at 10 mg/100 µl concentrations. But the other organic solvents extracts (AE, PE and CF) showed least anti-microbial activity on *Shigella flexneri* when compared with the other gram negative organisms.

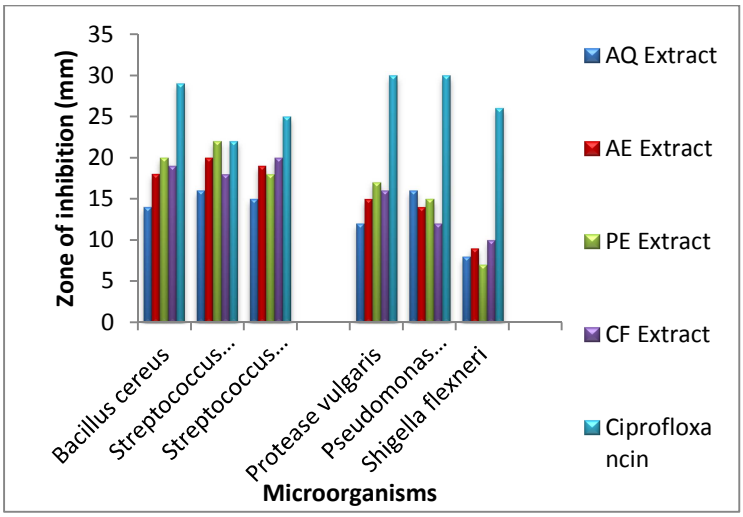

Fig 2: Anti-microbial activity of Different solvents seeds extracts of *Sida cordifolia*

***3)Momordica foetida*:**

The result of AQ and AE seed extract of *Momordica foetida* plant showed highest- moderate anti-bacterial activity on organisms tested but failed to inhibit the growth of *Shigella flexneri*. The least inhibition activity on the entire gram positive and gram negative bacterial species was seen in the result obtained with PE extract but PE extracts inhibited the *Shigella flexneri* (10 mm) (Fig 3). The least anti-microbial effect was recorded with PE and CF extract

of this plant when compared with the standard drug Ciprofloxin (1 gm/ml). In conclusion the AQ and AE extract recorded highest zones of inhibition on all the bacterial species (except *Shigella flexneri*) and it is more effective than PE and CF extracts against these microorganisms.

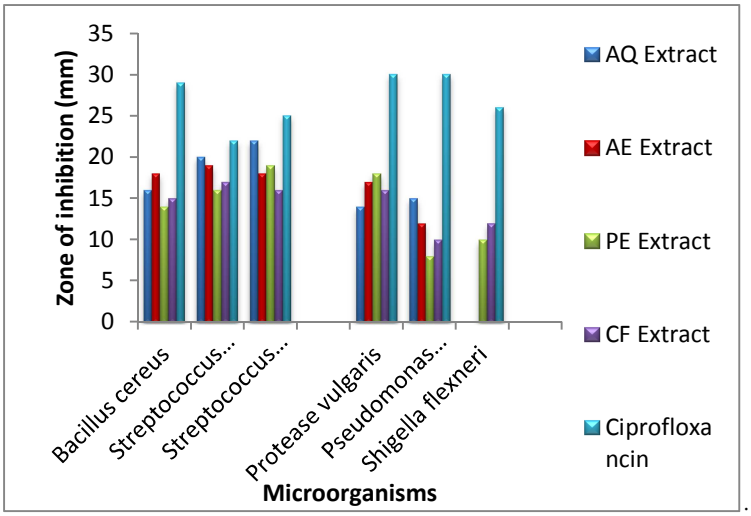

Fig 3: Anti-microbial activity of Different solvents seed extracts of *Momordica foetida*

4)Albizia procera:

The result obtained from anti-microbial activity study of four extracts (AQ,AE, PE and CF) of the plant *A.procera* showed no activity on gram positive bacteria and gram negative bacteria. All the extract failed to produce zones of inhibition against these microorganisms (Fig 4).

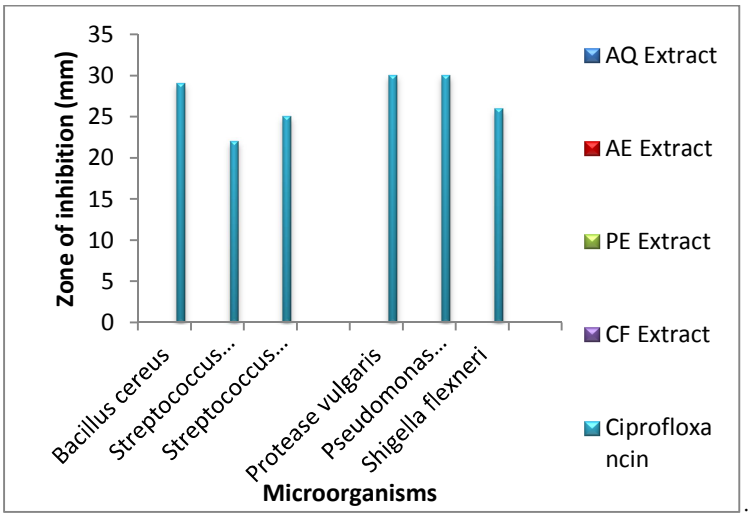

Fig 4: Anti-microbial activity of Different solvents seed extracts of *Albizia procera*

***5)Mesua ferrea*:**

All the seed extracts (AQ, AE, PE and CF) of *Mesua ferrea* plant were tested for their anti-bacterial activity against selected three gram positive (*B.cereus, S.pyrogens* and *S.epidermidis*) and three gram negative (*P.vulgaris, Pseudomonas aeruginosa* and *Shigella flexeneri*) bacterial species. The results for all the four extracts has shown some variations in the inhibitory activity for the gram positive and gram negative bacterial species. High anti-microbial effect (26 mm zone of inhibition) was recorded against *Streptococcus epidermidis* with the AE extract and a low level of inhibition (5 mm) was recorded against *Shigella flexneri* (Fig 5). All the four extracts showed high to moderate anti-microbial activity against gram positive bacterial species. The extracts failed to show anti-microbial

effect on the gram negative bacterial species. Weak zones of inhibition was observed against *Shigella flexneri* for all the three organic extracts (5, 6 and 3 mm).The AE and PE extracts from the *Mesua ferrea* has showed moderate activity against gram positive bacteria,when compared with the standard antibiotic Ciprofloxacin (1mg/ml), which is acting as a positive control. The AQ extract has reported mild anti-microbial activity on these organisms.

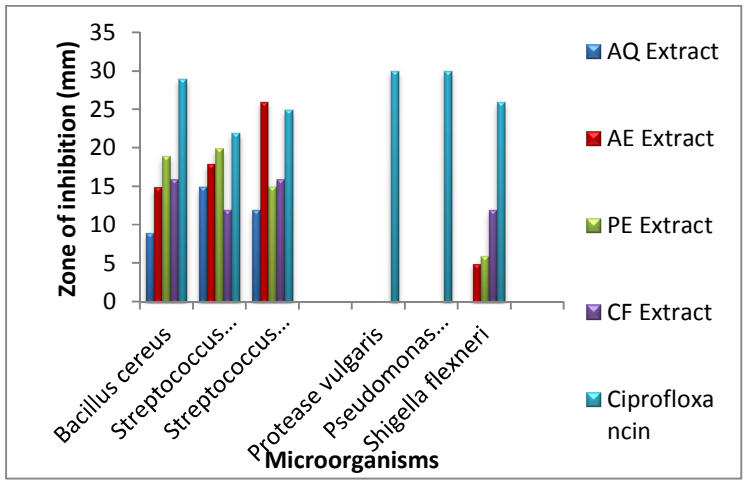

Fig 5: Anti-microbial activity of Different solvents seed extracts of *Mesua ferrea*

6)Lantana camare:

In the present experiment to analyze the anti-microbial activity of plant *L.camare* seeds was selected and different extracts were made with the help of solvents (AQ. AE, PE and CF). Of all the four extracts PE extract showed the highest anti-microbial activity on gram positive bacteria and moderate activity on gram negative bacteria. The AE extract and CF extract have shown moderate activity on the selected microbes. The highest zone of inhibition was recorded against *Streptococcus pyrogens* with zone diameter of 20 mm in PE extract and least was recorded against *Protease vulgaris* and *S.flexneri* with 9mm in AE and PE extracts (Fig 6). The AQ extract showed no inhibition activity against *Bacillus cereus*, *Protease vulgaris* and *Shigella flexneri* .In conclusion the *L.camare* seed PE extract are very effective against gram positive bacteria than compared with the gram negative bacterial species.

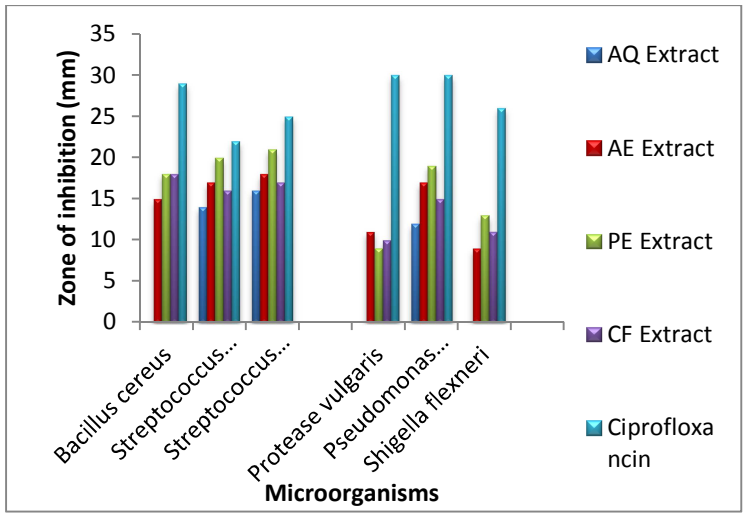

Fig 6: Anti-microbial activity of Different solvents seed extracts of *Lantana camare*

DISCUSSION

The plants evaluated in this work has different varieties of phytochemicals (Results not shown here) that could be considered as responsible for anti-microbial activities. The alkaloids are been detected in all most all the plant species except *Albizia procera* and the concentrations of alkaloids differed from plant to plant and extract to extract. Tannins have been diagnosed in all most all the solvents extracts extracted from these six selected medicinal plants [18]. They show antimicrobial activity by combining with proteins for example adhesins, substrates as well as cellular membrane proteins, therefore inactivating microbial adhesion and also causing membrane disruption [19-21]. Steriods have been reported to have antibacterial properties and they are very important compounds due to their relationship with compounds such as sex hormones [22].Terpenoids which belongs to one class of steroids are reported in most of the extracts of these selected plants seeds. The above said plant extracts which does not contains terpenoids showed moderate anti-microbial activity when compared with the other extracts because they show their roles in anti-bacteria [23]. Phenolics have been additionally detected in the plant they function by chealting metal ions like cobalt, manganese etc which are necessary as co-factors for microbial enzymes [21,24]. Saponins have been detected in all the extracts and the mode involving steps for the anti-bacterial properties may perhaps entail due to membranolytic activity of the saponins along with reducing of the surface area tension of the extracellular medium [25]. The *A. catechu, S.cordifolia* and *L.camare* AQ, AE and PE extracts showed the best inhibition zones against these gram negative bacterial species. This particular activity could be on account of high concentrations of phytochemicals which have been connected with antimicrobial properties [24,26]. The plant extracts had broad spectrum activity in that they inhibited growth of both Gram positive and Gram negative bacteria. The inhibition zones increased on increasing the concentration of the extract in the discs showing a concentration dependent activity and also varied with the species of bacteria tested. This could be due to the fact that the active components in the extract. Therefore, the concentration of the active components in the extract could be much lower than the standard antibiotic used. It is important to note that, if the active components were isolated and purified, they would probably show higher antibacterial activity than those observed in this study.

CONCLUSION

The plant based bio-active compounds have the effective dosage response with minimal side effects, when compared to the synthetic compounds. The studies conducted on these 6 selected plants species: (*Acacia catechu, Sida cordifolia, Momordica fotida, Albiziz procera, Mesua ferrea and Lantana camare*) showed the presences of phytochemicals and anti-microbial activity. The presences of phytochemicals (secondary metabolites) are responsible for their anti-microbial effects. All the extracts showed high to moderate amounts of anti-microbial activity against selected gram positive and gram negative bacterial species. The PE and AE solvents from 6 selected plants seed extracts showed highest zones of inhibition on gram positive bacteria than gram negative species. It further reflects a hope for the development of many more novel therapeutic agents or templates from such plants which in future may serve for the production of synthetically improved therapeutic agents.

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