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Evaluation of Anti-bacterial Activity of Indigenous Medicinal plants Species of India

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ABSTRACT

In the present study 10 medicinally important plant species were screened for their phytochemicals by using 4 different solvents, aqueous, Acetone, Petroleum Ether and Chloroform and subjected to anti-bacterial activity. All the extracts showed high to moderate amounts of anti-microbial activity against selected gram positive and gram negative bacterial species. The aqueous and organic solvents extracts from 10 selected plants showed highest zones of inhibition on gram positive bacteria than gram negative species. The gram bacterial species B.cereus, S.aureus and S.epidermidis showed highest sensitivity towards these four extracts. The gram negative bacterium P. aeruginosa showed highest sensitivity to these extracts and Shigella flexeneri showed highest category of resistance to these extracts. The Woodfordia fruticosa stem aqueous, acetone and extracts showed the best inhibition zones against these gram negative bacterial species. The S.saponaria, fruit pericarpic extracts showed the best zones of inhibition against all the bacterial species tested. All the A.squamosa seeds cotyledon extracts showed unsatisfactory zones of inhibition when compared to the other plants extracts against these gram positive and gram negative bacteria.

Key words: Alkaloids, anti-bacterial, extracts, saponins, species.

INTRODUCTION

Plants have an almost limitless ability to synthesize aromatic substances, most of which are phenols or their oxygen-substituted derivatives [1]. Most are secondary metabolites, of which at least 12,000 have been isolated, a number estimated to be less than 10% of the total [2]. In many cases, these substances serve as plant defense mechanisms against predation by microorganisms, insects, and herbivores. Some, such as terpenoids, give plants their odours; others (quinones and tannins) are responsible for plant pigment. Many compounds are responsible for plant flavor (e.g., the terpenoid capsaicin from chilli peppers), and some of the same herbs and spices used by humans to season food yield useful medicinal compounds. Phytochemical studies regarding acetone, petroleum Ether and chloroform extracts in indigenous medicinal plants of *Garcinia indica*, *Jatropha curcas*, *Nigella sativa*, *Levisticum officinales*, *Dracacetonena loureiri*, *Woodfordia fruticosa*, *Vaccinium macrocarpon*, *Foeniculum vulgare* (stem), *Sapindus saponaria* (pericarp), *Annona squamosa* (seeds) are scanty. So, the study was taken up for the evaluation of plant secondary metabolites in these plants and screened for their anti-bacterial activity.

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 in the Department of Botany, P. B. Siddhartha College, Vijayawada, Andhra Pradesh, India.

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

Preparation of plant extract
The collected plant material was carefully washed under running tap water followed by sterilized distilled water, and was air dried at room temperature in laboratory for 30-45 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 , Acetone, Petroleum ether, and Chloroform were used for extractions. 10 gm of homogenized coarse powders of leaf, stem, pericarp and seed were soaked in different conical flasks containing 100 ml of water, Acetone, Petroleum ether, and Chloroform 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 [3-5]. Finally each sample extract (water, Acetone, Petroleum ether and Chloroform) 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 rotacetonevaporater. Thus the obtained dried extracts were stored at 4^0C in labeled and stored in sterile bottles [6-7]. 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 [8-10] (Results not shown here).

Micro-organisms tested: Total 8 strains including gram positive and gram negative bacteria were selected to assess the susceptibility test against the different solvent extract. These are, *Bacillus cereus*, *Streptrococcus pyrogens*, *Streptococcus epidermidis*, *Staphyloccus aureus*, *Protease vulgaris*, *Pseudomonas acetoneroginosa*, *Salmonella typhi*, *Shigella flexneri*.

Antibacterial Assay: Antibacterial activity of the crude extracts in different solvents of selected plants was tested by disc diffusion assay [11]. 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/100 µl and 4 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 [12] are reported.

RESULTS

All the results obtained for the anti-microbial activity of all the extracts are given in Fig: 1-10

1)*Garcinia indica :*
The petroleum ether extract showed highest anti-microbial activity against *S. aureus*(30 mm) and *B.cereus*(29 mm). The aqueous extract of *G.indica* showed least anti-microbial activity against *Shigella flexeneri* (10 mm). The acetone and chloroform extracts reported the least activity with the *Shigella flexeneri*. All the four extracts showed marked antimicrobial activity on all the gram positive bacteria than compared with gram negative bacteria (Fig 1).

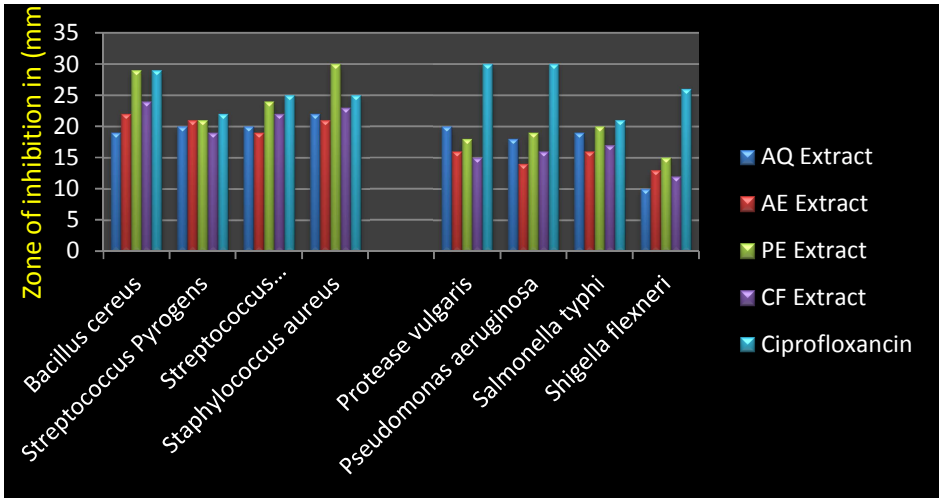

Fig 1: Anti-microbial activity of Different solvents extracts of *Garcinia indica* (leaf)

2) *Jatropha curcas:*

Of all the four extracts made from *J.curcas*, the aqueous extract showed maximum zones of inhibition against *P.acetoneruginosa* (31 mm) and *S.epidermidis* (26 mm). The chloroform extract of the plant leaf showed least inhibition action against all the bacterial species selected for the work (fig 2). The aqueous extract of *J.curcas* has shown no activity on *P.vulgaris* at 10 mg/100 µl concentrations. But the other organic solvents extracts showed least anti-microbial activity on *P.vulgaris* when compared with the other gram negative organisms.

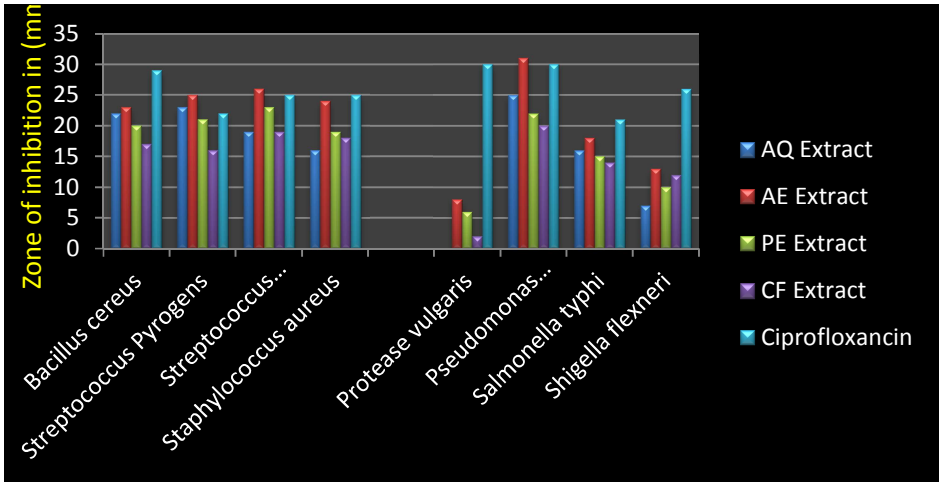

Fig 2: Anti-microbial activity of Different solvents extracts of *Jatropha curcas* (leaf)

3) *Nigella sativa*:

The result of petroleum ether extract of *Nigella sativa* plant showed highest antibacterial activity on *B.cereus* and *S.epidermidis* with zone of inhibition of 30mm and 30mm respectively and moderate inhibition activity on the entire gram positive and gram negative bacterial species (Fig 3). The least anti-microbial effect was recorded with chloroform extract of this plant when compared with the standard drug Ciprofloxin (1 gm/ml). The least zone of inhibition was observed for *Shigella flexeneri* with 8 mm which was a gram negative bacterium. The aqueous extract showed moderate activity on all the gram positive and negative bacteria tested. In conclusion the petroleum ether extract recorded highest zones of inhibition on all the bacterial species and it is more effective than chloroform and acetone extracts against these microorganisms.

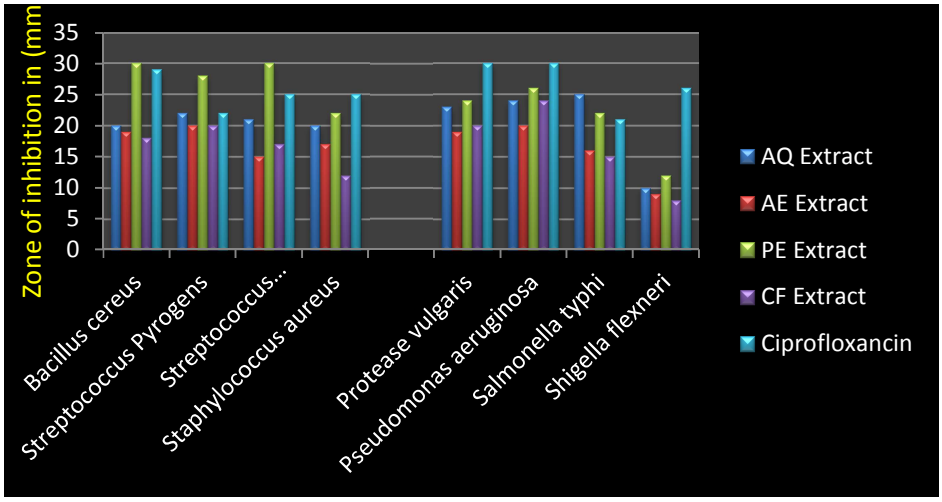

Fig 3: Anti-microbial activity of Different solvents extracts of *Nigella sativa* (leaf)

4) *Levisticum officinale*:

The result obtained from *L.officinale* showed that the acetone and chloroform extracts had highest zones of inhibition on gram positive bacteria and moderate inhibition towards gram negative bacteria. The petroleum ether extracts has reported least anti-microbial activity against these microorganisms. The aqueous extract when compared with the standard antibiotic showed moderate levels of inhibition against these organisms. The least zone of inhibition was reported against *S.typhi* with zone diameter of 13 mm in petroleum ether extract and a highest value of 28mm was recorded for *S.aureus* in acetone extract (fig 4).

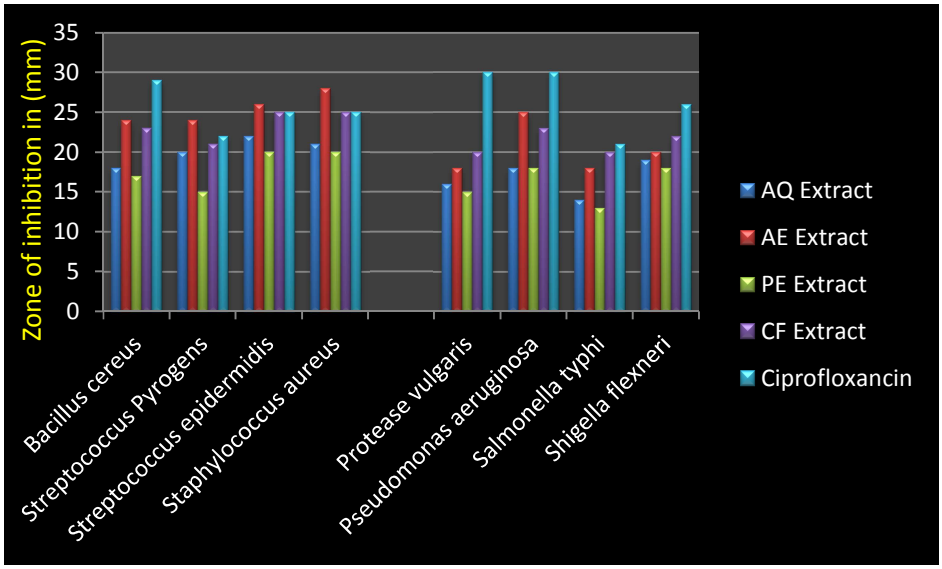

Fig 4: Anti-microbial activity of Different solvents extracts of *Levisticum officinale* (leaf)

5) *Dracacetonena loureiri*:

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 (30mm zone of inhibition) was recorded against *S.aureus* with the petroleum ether extract and a low level of inhibition (12 mm) was recorded against *P.vulgaris* with aqueous extract (fig 5). The petroleum ether extract showed high inhibitory anti-microbial activity against both the gram positive and gram negative bacterial species. The acetone and chloroform extracts from the *D.loureiri* has showed

moderate activity when compared with the standard antibiotic Ciprofloxacin (1mg/ml), which is acting as a positive control. The aqueous extract has reported least anti-microbial activity on these organisms.

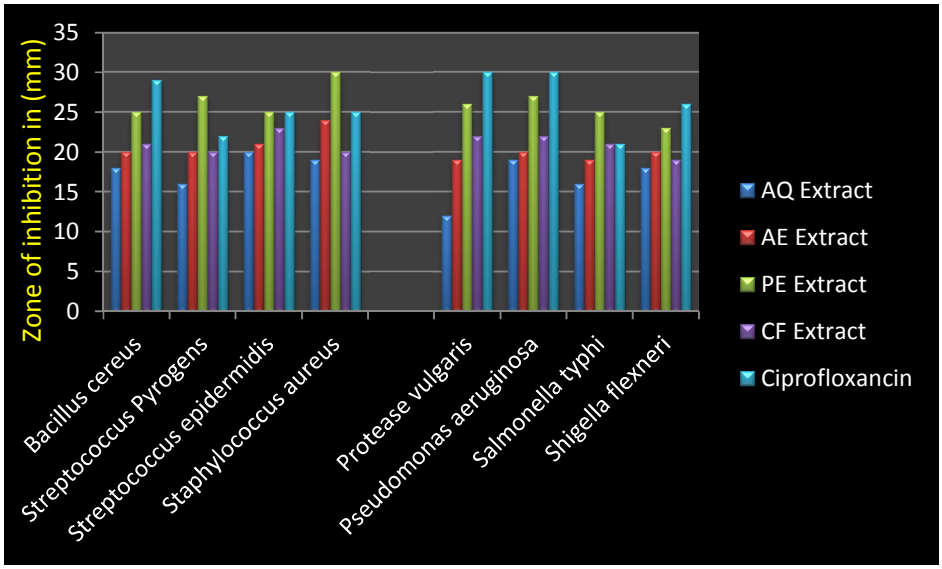

Fig 5: Anti-microbial activity of Different solvents extracts of *Dracacetonena loureiri* (leaf)

6) *Woodfordia fruticosa*:

In the present experiment to analyze the anti-microbial activity of plant *W.fruticosa* stem part was selected and different extracts were made with the help of solvents (aqueous, acetone, petroleum ether and chloroform). Of all the four extracts aqueous extract showed the highest anti-microbial activity on gram negative bacteria and moderate activity on gram positive bacteria. The petroleum ether extract and acetone extract have shown least activity on the selected microbes. The highest zone of inhibition was recorded against *Salmonella typhi* with zone diameter of 26mm and least was recorded against *B.cereus* and *S.aureus* with 8mm (fig 6).

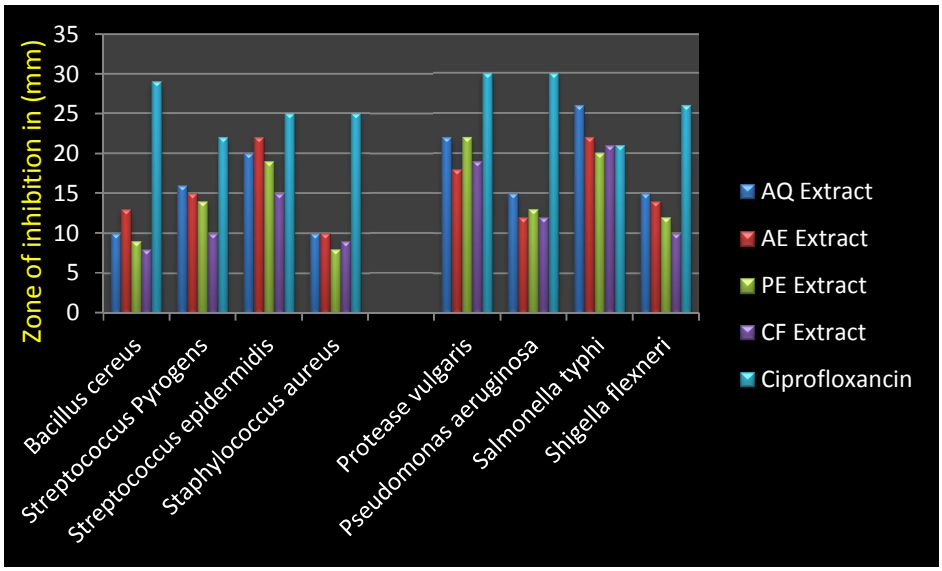

Fig 6: Anti-microbial activity of Different solvents extracts of *Woodfordia fruticosa(Stem)*

7) *Vaccinium marocarpon*:
Of all the four extracts made from *V.marcarpon* plant leaf, the petroleum ether extract showed maximum zones of inhibition against gram positive bacteria and gram negative bacterial species. The acetone and chloroform extracts showed moderate zones of inhibition activity against these organisms .The highest zone of inhibition (18mm) (fig 7) was observed against *S.epidermidis* in petroleum ether extract and least zones of inhibition was recorded against *S.epidermidis* (6mm) and *S.flexeneri* (6mm) with aqueous extracts. The low zones of inhibition were observed in aqueous extracts against these organisms tested.

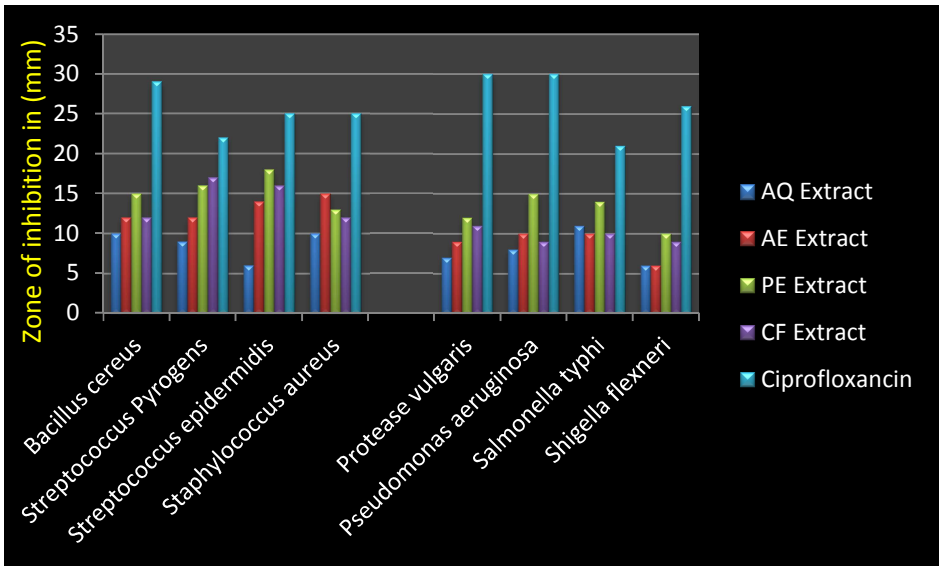

Fig 7: Anti-microbial activity of Different solvents extracts of *Vaccinium marocarpon*(leaf)

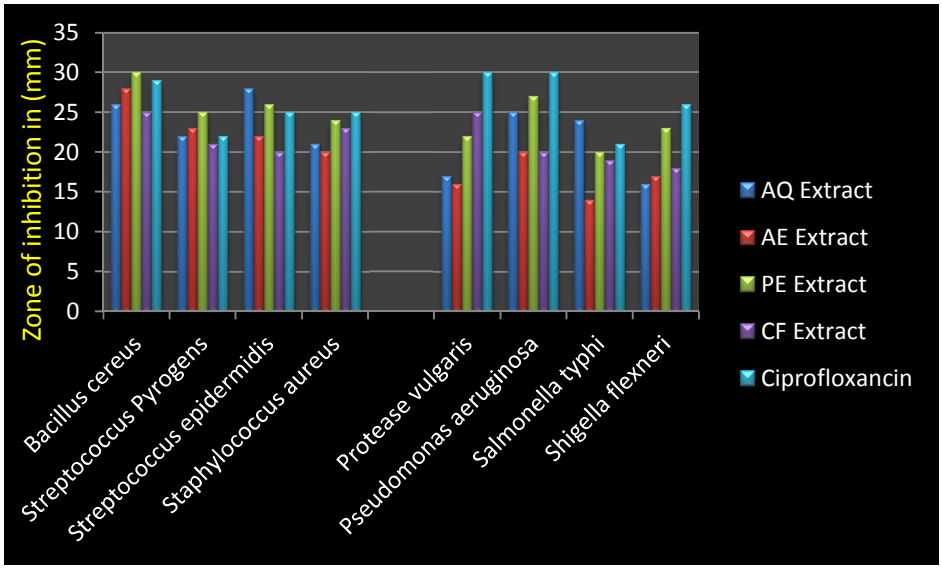

Fig 8: Anti-microbial activity of Different solvents extracts of *Foniculum vulgare*

8)*Foeniculum vulgare*:
All the extracts which were prepared from *F.vulgare* plant leaf, are subjected to test the anti-microbial activity. The petroleum ether extracts showed highest zones of inhibition activity against *B.cereus* (30mm) and least zone of inhibition was observed in *S.typhi* (20mm) microorganisms (fig 8) .The acetone and chloroform extracts showed moderate effect on all the selected species of bacteria compared with the standard drug Ciprofloxacin (1mg/ml) and

petroleum ether extract. The aqueous extract showed least to moderate effect when compared to standard. But highest zone of inhibition was observed against *S.epidermidis* (28mm).

9) *Sapindus saponaria*:

All the extracts which were prepared from *Sapindus saponari* fruit pericarp, are subjected to test the anti-microbial activity. The petroleum ether extracts showed highest zones of inhibition activity against *S.epidermidis* (29mm) and *S.flexeneri* (28mm) in petroleum ether and aqueous extracts (fig 9). The acetone and chloroform extracts showed moderate effect on all the selected species of bacteria. But least zone of inhibition was observed against *P.aeruginosa* (17mm) in acetone extract.

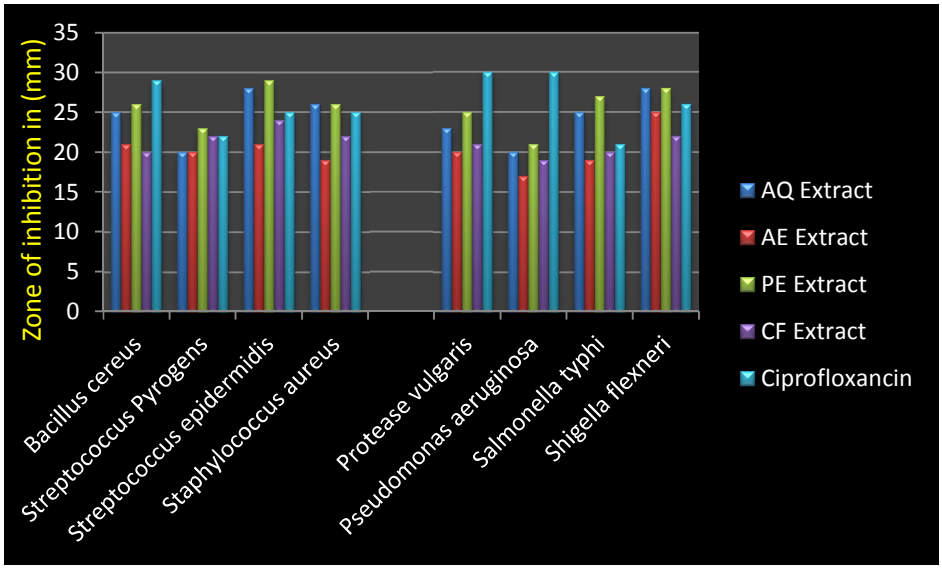

Fig 9: Anti-microbial activity of Different solvents extracts of *Sapindus saponari (Pericarp)*

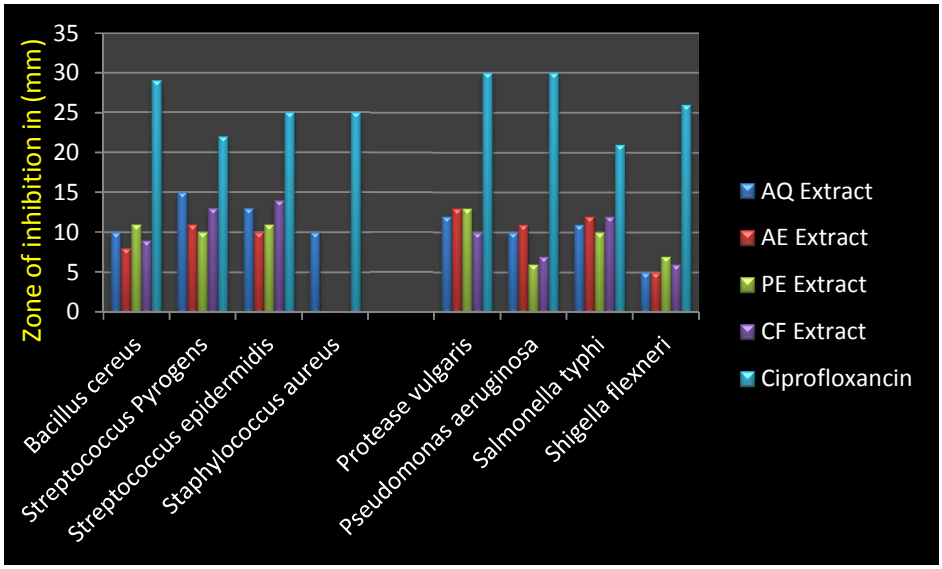

Fig10: Anti-microbial activity of Different solvents extracts of *Annona squamosa*(seed cotyledons)

10) *Annona squamosa:*

The results obtained from the anti-microbial activity study of four extracts extracted from the seed cotyledons' of *Annona squamosa* against the gram positive and gram negative bacterial species showed very minute effect on all

the microorganisms tested. All the extracts which were prepared showed moderate to low effect. The highest anti-microbial effect (14mm zone of inhibition) was recorded against *S.epidermidis* with the chloroform extract and a low level of inhibition (5 mm) was recorded against *Shigella flexeneri* with aqueous and acetone extract (fig 10).

DISCUSSION

Alkaloids have been detected in aqueous and petroleum ether extracts of *J.curcas*; all the extracts of *L.officinale*; aqueous, acetone and chloroform extracts of *D.loureiri*; all the extracts of *F.vulgare*(stem); *S.saponaria* extracts and aqueous, petroleum ether and chloroform extracts of *A.squamosa* of 10 plants selected for the work (Result not shown here). The plant alkaloids had extensive array task in inhibiting the progress of Gram positive as well as Gram negative bacteria. The inhibition areas and specific zones increases in increasing the concentration of the extracts and also different with the species involved bacteria screened. When they are compared to the standard Ciprofloxacin. Flavonoids are also observed in all most all the extracts except petroleum ether extracts of *S.saponaria* [1]. Tannins have been diagnosed in all most all the solvents extracts extracted from these 10 selected medicinal plants except in chloroform extract of *N.sativa* plants. 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 [13-15].Steroids have been reported to have antibacterial properties and they are very important compounds due to their relationship with compounds such as sex hormones [16]. Terpenoids which belongs to one class of steroids are reported in most of the extracts of the 10 selected plants. The absence of terpenoids was noticed in aqueous, acetone extracts of *J.curcas*; acetone extract of *N.sativa*; aqueous, petroleum ether and chloroform extracts of *D.loureiri* and they are completely absent in all the extracts of *F.vulgare*. 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[17].Phenolics have been additionally detected in the plant *J.curcas* and *S.saponaria* they function by chealting metal ions like cobalt, manganese etc which are necessary as co-factors for microbial enzymes [9,13]. 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 [18]. The aqueous and chloroform extracts when compared to the petroleum ether solvents extract showed less inhibition activity on tested bacteria. This particular lower activity could be on account of low concentrations of phytochemicals which have been connected with antimicrobial properties [18-20]. The results are in agreement with the other worker [21-25].

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 10 selected plants species: 1*)Garcinia indica* (leaves) 2) *Jatropha curcas* (leaves) 3) *Nigella sativa* (leaves) 4)*Levisticum officinale* (leaves) 5) *Dracacetonena loureiri* (leaves) 6) *Woodfordia fruticosa* (stem)7)*Vaccinium macrocarpon*(leaves)8) *Foeniculum vulgare* (stem); 9)*Sapindus saponaria* 10)*Annona squamosa* (seeds) showed the presences of phytochemicals and anti-bacterial activity. The presences of phytochemicals (secondary metabolites) are responsible for their anti-bacterial effects. All the extracts showed high to moderate amounts of anti-microbial activity against selected gram positive and gram negative bacterial species. The aqueous and organic solvents extracts from 10 selected plants 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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