J. Phytol. Res. **20**(2) : 161-170, 2007

ANTI-BACTERIAL RESPONSE OF TWELVE PLANTS OF FOLK-LORE MEDICINE USED AGAINST *DIABETIS MELLITUS*

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Herbal medicine is the root of all traditional medicinal systems practiced in India (Unani and Ayurveda) and elsewhere and WHO survey has estimated that 80% of population on earth relies on this system for primary health care needs. In current years, the trend has been towards the elucidation of these medicinal plants for their anti-microbial activity with the view of discovering new therapeutics against microbes. *In vitro* experiments were conducted to assess the anti-bacterial activity of hexane and methanol extracts of twelve important plants of folk–lore medicine against four gram–negative and three gram–positive bacteria. Moreover, plants of the present studies have been used for their hypoglycemic activity against diabetes. Therefore, it is proposed to test whether they exhibit any pattern of relationship in their action against the screened microbes. The extracts of all the eleven plants excepting *Momordica* exhibited activity against the screened microbes but to different levels. The efficacy of each extract varied against each of the strain. To our knowledge there are only one or two reports in *Mimosa pudica*. The present study has revealed that its extract was a potential agent against the gram – positive bacteria like *Enterococcus faecalis* and *Proteus vulgaris* involving both hot and cold methanolic extracts. Inspite of their significance in folk-lore medicine, the plants like *Curcuma longa, Withania, Trigonella* and *Ficus bengalensis* were found to be less efficient in their activity against most of the positive and negative bacteria. However, our results have clearly shown the potential efficacy of *Emblica officinalis* as a broad spectrum microbial agent followed by *Syzygium cumunii* and *Azadirachta indica* in that order. It was also found that hexane extracts were less effective as compared to cold and hot methanolic extracts. Again, the distribution of no active zones (complete absence of zones) were more in the cold extract than in the hot one, more so with the gram – positive bacteria. It is concluded that the kind of extract profoundly influences the nature of anti-microbial activity indicating the importance of aqueous extracts in such studies.

Keywords: Anti- bacterial activity; Hexane and methanolic extracts; Medicinal plants.

Introduction

The traditional medicine systems around the world including ancient Chinese, Indian (constituting the two major branches - Unani and Ayurveda) and Amazonian ethano medicine, rely heavily on herbs for health preservation and healing and it was China that has accumulated rich knowledge on the use of medicinal plants for treatment of various diseases and in the discovery and development of new drugs of plant origin[1]. The recent survey by World Health Organization (WHO) states that more than 80% of the world's population relies on traditional medicine for primary health care needs.

Herbal medicines have been described in traditional texts and are used for several purposes such as anti, stress, anti – inflammatory and antioxident, anticancer[2], antifungal and antimicrobial agents[3]. It is not surprising that the use of alternative supplements has become so popular with the cure/prevention of diverse kinds of human ailments[4]. However, world surveys have shown that relatively small percentage (5 – 10%) of the estimated 5,00,000 plant species on the Earth have only been used so far to the optium extent and requires further systematic survey and confirmation. Currently, about one – quarter to one–half of the investigations are intended for elucidating the use of many folk-lore medicinal plants as anti-microbials and people have shown keen interest on the topic of anti-microbial properties of plant extracts for two main reasons; the life span of any antibiotic is limited inspite of the fact that two or three new antibiotics derived from microorganisms are added each year[5] requiring additional exchequer[6]. Secondly, the public is becoming increasingly aware of the problems in the over prescription and misuse of traditional antibiotics[7].

The recent appearance of strains with reduced susceptibility to antibiotics[8] shows the urgency to the search for new infection fighting strategies. The development of drug resistance against commonly used antibiotics has also necessitated the need for new antimicrobial substances from other sources including plants[9]. Besides these reasons, antimicrobials of plant origin, contrary to the synthetic drugs, are not associated with many side effects and appear to have huge therapeutic potential against many infectious diseases [10]. The phytochemical research based on ethanopharmacological informations is generally considered an effective approach in the discovery of new anti-infective agents from higher plants[11].

Reports on a number of individual plants or their essential products are innumerable in the literature for certain of the plants[12-16] although there are very few instances of such anti-microbial studies atleast in some of the plants used in the present investigation[17-19]. A polyherbal formulation (Dianex) consisting the aqueous extracts of *Momordica charantia, Aegle marmelos, Cassia auriculata, Withania somnifera* and *Curcuma longa* was found to be hypoglycemic and antidiabetic reversing many other diabetic complications[20]. Further, the extracts of *Curcuma* and *Withania* also effect related enzyme systems as well[21]. Anti-microbial activity of either the crude extracts[22,23] or their component active compounds and neem oil[24-26] indicate that the results are inconsistent.

The information on *Curcuma longa* is mostly confined to isolating the active principles and their characterization[27,28] with a little information on anti-microbial activity[3,23,29]. The plant *Murraya koenigii* is used in the treatment of diarrhoea, insect bites and other ailments and many phytochemical studies are known in this plant[30-33] while the effects of alkaloids as anti-microbial agents, in the recent studies, are worth considering[34,35]. The activity studies on *Mimosa pudica, Aegle marmelos, Ficus bengalensis* and to a lesser extent on *Emblica officinalis* are meagre in the literature[2, 36-38] in spite of their significance in folk-lore medicine.

The literature survey mentioned here states that screening of plants for anti-microbial activities is important in the identification of new potential compounds of therapeutic use. In the present investigation we have choosen twelve plants having relatively much more prominence in folk-lore medicine. Moreover most of these plants are known for their hypoglycemic activity[20,22] against *Diabetes mellitus*; hence it is proposed to test the presumption that they may exhibit any relationship in their action against the screened microbial agents. The paper describes the results of anti-bacterial activities of extract of these twelve plants against seven bacterial strains using methanol and hexane as extraction solvents.

Materials and Methods

The leaves of *Aegle marmelos, Azadirachta indica, Gymnema sylvestre, Murraya koenigii,* and *Withania somnifera* were collected from A.P. Forest of Endada. The whole plant of *Momordica charantia* was collected from Srikakulam. The barks of *Ficus bengalensis, Syzygium cumunii* and the whole plant *Mimosa pudica* were collected from A.U. campus. The fruits of *Emblica officinalis* were obtained from Kambalakonda, Visakhapatnam. Corm of *Curcuma longa* were collected from Vizianagaram farms and seeds of *Trigonella foenum-graecum* were purchased from a local commodities store. The plant materials were dried under the shade and ground to a fine powder with the help of an electrical grinder while the bark material is powdered in a flour mill.

Extract preparation-But for minor deviations depending on nature of the individual plant material, the following protocol is used to obtain the extract. Dried powder is subjected to maceration using 400ml of hexane solvent in a round bottomed flask with constant stirring for 5 days, filtered and subjected to waterbath distillation, concentrated using chloroform to obtain final extract. A similar procedure is used to obtain cold methanol extract while waterbath heating is done using a reflex condenser in case of hot methanol extract. The hexane and methanolic extracts of these plants were screened against the following seven bacterial strains.

Gram negative	Source	Gram positive	Source
Escheriachia coli	ATCC 9637	*Bacillus subtilis*	MTCC 2274
Klebsiella pneumoniae	MTCC 2405	*Enterococcus faecalis*	MTCC 0439
Proteus vulgaris	MTCC 0426	*Streptococcus faecalis*	MTCC 0459
Micrococcus luteus	MTCC 1538		

Anti-Bacterial susceptibility test-From the extract of each sample obtained above 100mg of it is mixed with 1ml of dimethyl sulfoxide (DMSO) in an eppendorf tube. The crude extracts of different plant species were subjected to anti-microbial assay by the cup/well diffusion method[39] on nutrient agar medium. 25 μl of sterile nutrient agar was dispensed into sterile universal bottles. These were then inoculated with 2μl of overnight bacterial cultures of investigated bacteria, mixed gently and poured into sterile petridishes. After setting, wells of 6mm diameter were made in the center of each petridish, to which 50μl of the tested different plant extracts were loaded and were allowed to diffuse for 45 minutes. The plates were incubated at 37°C for 24hr and the diameter of the resulting zone of inhibition was determined with transparent ruler in millimeters for

Table 1. Inhibitory zones of Hexane extracts of the medicinal plants investigated (in mm) against gram negative and positive bacteria.

Name of the plant	*E. coli*	*Klebsiella pneumoniae*	*Proteus vulgaris*	*Micrococcus luteus*	*Bacillus substilis*	*Enterococcus faecalis*	*Streptococcus faecalis*
Aegle marmelos	13	17	11	14	NA	18	19
Azadirachta indica	13	11	28	19	NA	15	12
Momordica root	12	14	19	24	NA	26	23
Momordica stem	12	12	NA	11	NA	12	18
Momordica leaf	15	15	16	19	12	24	14
Momardica fruit	-	-	-	-	-	-	-
Ciprofloxacine	15	20	20	22	-	-	-
Penicillin (5mg/disc)	-	-	-	-	17	11	16

Table 2. Inhibitory zones of cold methanolic extracts of the medicinal plants investigated (in mm) against gram -negative and positive bacteria.

Name of the plant	*E. coli*	*Klebsiella pneumoniae*	*Proteus vulgaris*	*Micrococcus luteus*	*Bacillus substilis*	*Enterococcus faecalis*	*Streptococcus faecalis*
Aegle marmelos	18	12	18	12	NA	20	27
Azadirachta indica	18	14	33	21	10	23	20
Curuma longa	10	10	12	12	10	12	10
Emblica officinalis	28	29	22	23	22	34	24
Ficus bengalensis	11	NA	15	13	NA	13	NA
Gymnema sylvestre	14	10	13	14	NA	NA	NA
Mimosa pudica	17	14	22	10	18	17	12
Syzyzium cumunii	22	24	20	21	18	22	20
Trigonella foenum graceum	10	NA	14	NA	NA	21	NA
Withania somnifera	12	NA	13	15	NA	13	11
Murraya koenegii	10	18	16	11	NA	12	NA
Momordica leaf	15	15	16	19	-	-	-
Momordica stem	NA	16	18	18	NA	13	20
Momordica root	13	13	14	14	13	NA	NA
Momardica fruit	13	12	12	12	15	NA	NA
Ciprofloxacine	15	20	20	22	-	-	-
Penicillin	-	-	-	-	17	11	16

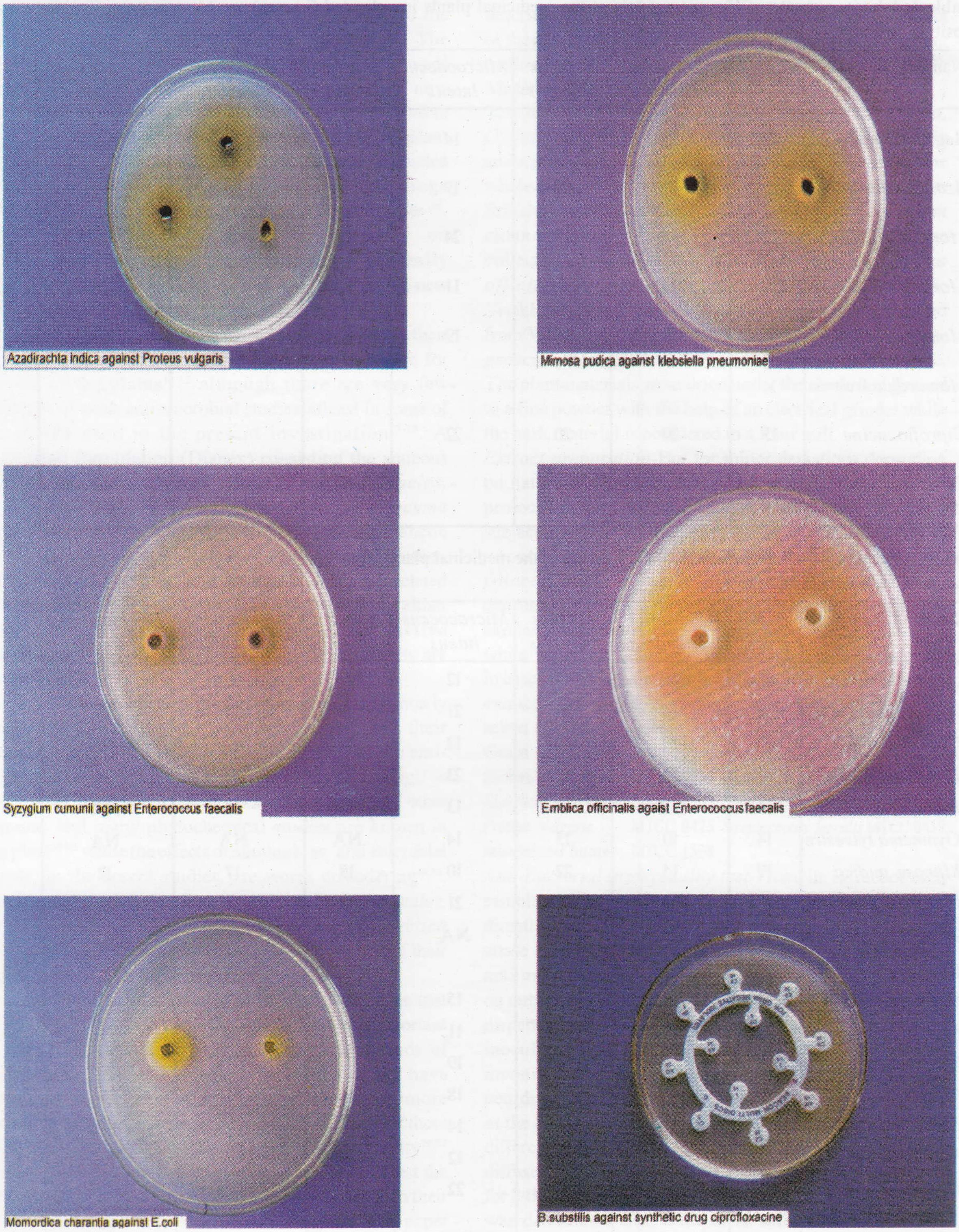

Fig.1. Nutrient - agar media showing inhibition zones in response to representative plant extracts.

Table 3. Inhibitory zones of hot methanolic extracts of the medicinal plants investigated (in mm) against gram -negative and positive bacteria.

Name of the plant	*E. coli*	*Klebsiella pneumoniae*	*Proteus vulgaris*	*Micrococcus luteus*	*Bacillus substilis*	*Enterococcus faecalis*	*Streptococcus faecalis*
Aegle marmelos	19	14	20	16	NA	20	27
Azadirachta indica	22	28	17	31	10	23	20
Curuma longa	12	12	17	14	10	12	10
Emblica officinalis	30	33	22	24	22	34	24
Ficus bengalensis	21	28	22	19	NA	13	NA
Gymnema sylvestre	19	17	17	17	NA	NA	NA
Mimosa pudica	19	20	31	14	18	17	12
Syzyzium cumunii	23	28	22	23	18	22	20
Trigonella foenum graceum	10	10	16	10	NA	21	NA
Withania somnifera	15	10	17	18	NA	13	11
Murraya koenegii	12	27	17	11	NA	12	NA
Momordica leaf	15	15	16	19	-	-	-
Momordica stem	17	- 16	18	15	NA	13	20
Momordica root	15	16	17	16	13	NA	NQ
Momardica fruit	14	13	13	14	15	NA	NA
Ciprofloxacine	15	20	20	22	-	-	-
Penicillin	-	-	-	-	17	11	16

each treatment; duplicates were maintained for each experiment. Penicillin was taken as the standard for gram - positive bacteria while Beacon multidisc ciprofloxacine was used as a standard for gram - negative bacteria (Fig. 1).

Results and Discussion

The table 1 shows the data of inhibitory zones (in millimeters) for a concentration of 5mg/well in three of the medicinal plants involving hexane solvent as extracting system. Appearance of inhibition zones in most cases indicate the active constituents in each of the extract to a lesser or greater extent. It is the strain *Bacillus* that is almost insensitive to these extracts excepting in the leaf sample of *Momordica*. However, compared to the standard penicillin zones at similar concentrations, anti-bacterial activity is limited to only a few cases. Hexane extracts of all the investigated plants are effective in the control of *Enterococcus faecalis* whereas none of the extracts can inhibit the growth to the levels of standards antibiotics in case of the bacterial strains *E.coli* and *Klebsiella*. The root extracts of *Momordica charantia* are very effective against *Enterococcus, Micrococcus* and *Streptococcus* but to a lesser extent with *Proteus vulgaris*, whereas the leaf extract exhibited inhibitory zones against *Enterococcus* while that from the stem is less effective against *Streptococcus faecalis*.

The table 2 represents the cold methanolic extracts of as many as twelve plants including the four different plant parts of *Momordica charantia*. The extracts of all the plants have exhibited inhibitory zones against the strain *E.coli* (Fig. 1) although the more efficacy is seen with *Emblica officinalis* and *Syzygium cumunii* and to a considerable extent by the extracts of *Aegle marmelos* and neem plant; extracts from any of the parts of *Momordica* is efficient in the control of this strain. The same kind of situation was visualized for the second gram-negative bacterium *Klebsiella pneumoniae* with the extracts of *Emblica officinalis* and *Syzygium* being highly potential anti-microbial agent against this strain also. However, unlike in *E.coli* the strains are almost insensitive to the extracts of *Ficus bengalensis, Trigonella foenum - graecum* and *Withania somnifera* inhibition zone formation.

As regards to the gram-positive bacterium, *Bacillus subtilis*, the cold methanol extracts of most of the studied plants are ineffective exhibiting lack of inhibitory zones in most cases. The extracts of *Mimosa pudica* and *Syzygium cumunii* produced some satisfactory results while those from the two important medicinal plants like neem and turmeric are less active; however, the extract from *Emblica officinalis* is a potent agent. *Enterococcus faecalis* is another gram-positive bacterium that has been screened here. But for the absence of inhibitory zone with *Gymnema sylvestre*, the extracts of other medicinal plants are effective to different extents compared to the standard values. Reasonable inhibition is observed with extracts of *Murraya, Withania, Curcuma* and *Mimosa pudica*. However, *Syzygium cumunii, Trigonella and Azadirachta* and more so *Emblica officinalis* extracts are far efficient in controlling the activity of this strain (Fig.1).

The extract of *Mimosa pudica* appears to be a highly efficient anti-bacterial agent against the only strain *Proteus vulgaris* and to a satisfactory level against *Enterococcus*. The extracts of neem plant followed by that of *Emblica officinalis* are even greater efficient in the control of *Proteus* with the maximum inhibitory zone observed with the extract from neem tree. But for the absence of inhibitory zone with *Trigonella* extract, appearance of zones of different diameters are evident against *Micrococcus luteus* with as much as 23mm in the case of *Emblica officinalis* . However, none of these plant extracts are considered equivalent to the standard antibiotic at similar concentrations in controlling this bacterial strain.

The cold extracts of *Ficus, Gymnema, Trigonella* and *Murraya* are not active against *Streptococcus faecalis*, while inhibitory zones lesser than the standard, are recorded for *Curcuma, Mimosa* and *Withania*. The growth of the seven bacterial strains screened is highly inhibited by extracts from neem tree, *Emblica* and *Syzygium*. The efficacy of *Aegle marmelos* cold methanol extract is maximum with *Streptococcus faecalis*, with the exception of the effect of stem extract of *Momordica* on *Proteus vulgaris*; the extracts of all these three parts are ineffective as anti-bacterial agents on any of the screened bacteria.

The table 3 shows the anti-microbial survey of hot methanolic extracts of eleven medicinal plants and in the four plant parts of *Momordica charantia*. Appearance of inhibitory zones for the two gram-negative bacteria, *E.coli* and *Klebsiella*, is seen with all the plant extracts unlike cold extracts. The plant constituents of *Murraya, Curcuma, Withania* and *Trigonella* are less effective on the strain *E.coli* as evident from weak inhibitory zones. The results of *Aegle marmelos* is just at par with the standards, the maximum zone of inhibition was recorded for *Emblica officinalis, Syzygium Cumunii* and *Azadirachta indica* in that order. The extract of *Aegle* is not so effective on the other gram-negative bacterium *Klebsiella*. The plants like neem, *Syzygium* and to a higher extent, *Emblica officinalis* could serve as anti-microbial agents against this strain as well.

In addition, extract of *Murraya koenigii* has recorded very large zones against *Klebsiella*. However, the data in table 3 shows that it was not effective for the rest of the strains studied while activity was absent with *Bacillus subtilis* and *Streptococcus faecalis*. Similarly, the other spicy plant *Trigonella* also shows no activity against *Streptococcus faecalis* and is limited in its activity over five of the strains studied, while its efficacy is evident only against *Enterococcus faecalis* and to a lesser extent against *Bacillus subtilis*. Inspite of the high potential of *Curcuma longa* as a medicinal plant, it is far less efficient in the control of any of the seven bacterial strains investigated.

The susceptibility of *Bacillus subtilis* to hot methanolic extract of *Aegle marmelos, Gymnema sylvestre* and *Murraya koenigi* is negligible. Inspite of the formation of inhibitory zones with the extracts from other plants, the efficacy in the control of this bacterial strain is attributable to only those of *Emblica officinalis, Enterococcus faecalis* is another member of the gram-positive bacteria. Excepting *Momordica charantia*, the extracts from all other medicinal plants are effective against this strain. However, they could be grouped based on efficacy as satisfactory *(Murraya koenigii, Withania somnifera, Gymnema sylvestre* and *Curcuma longa)*, intermediate (*Aegle marmelos, Mimosa pudica* and *Ficus bengalensis)* and highly potent ones (*Azadirachta indica* and *Emblica officinalis)*.

The hot extracts of plants of *Emblica officinalis, Ficus bengalensis* and *Syzygium cumunii* are with equal efficacy against *Proteus vulgaris* (Fig. 1) and only a little above the standards, while the strain is less susceptible to the most important plants like neem, turmeric and *Withania* in addition to *Murraya koenigii*. Although larger inhibition zones are recorded against *Micrococcus luteus*, it is only the extract from *Azadirachta indica* that is at par with the standard in its efficacy against this strain. Surprisingly, the extract from *Emblica officinalis*, which has exhibited very large inhibitory zones with all the other strains and in both cold and hot extracts, its efficacy is less compared to the standard antibiotic. To a limited extent this is true with *Syzygium cumunii* also. The inhibition by extracts of other plants is far lesser against *Micrococcus luteus.*

Extracts of the plants of *Emblica officinalis* and *Syzygium cumunii* are also potent against *Streptococcus faecalis.* In addition *Aegle marmelos* plant exhibited such higher inhibitory zones against this strain. While its susceptibility is negligible with those of *Trigonella, Withania* and *Murraya* and far less than standard with neem, turmeric, *Ficus* and *Gymnema.* But the extract of *Mimosa pudica* produced zones at levels equivalent to the standard. Considering the plant *Momordica charantia,* the cold methanolic extract from all the four plant parts had its efficacy to a limited extent on the bacterial strain *Bacillus subtilis.* Excepting this, the data shows that the extracts from all these parts are ineffective against other strains studied. While no activity is exhibited by extracts from all the four parts against *Streptococcus faecalis,* the activity is nil with that of root and fruit with the other strain *Enterococcus faecalis.*

Literature survey indicates that in country like India, about 70-80% of the population live in rural remote areas and depend on products of plant origin for many purposes. About ten plants have been suggested by world surveyors to meet, in a sustainable manner, the nutrient and health needs of the poor in these areas. Incidentally these include the following five plants *Azardirachta indica, Curcuma longa, Aegle marmelos, Trigonella foenum-graecum* and *Mormordica charantia,* used in the present investigation, suggesting the significance of anti-microbial studies in these plants. Moreover, in addition to *Eugenia jambolana* and *Cassia auriculata,* plants like *Gymnema sylvestre, Momordica charantia, Azadirachta indica, Aegle marmelos, Withania somnifera and Curcuma longa* are all used for their hypoglycemic activity against diabetes mellitus[20]. It is likely that the anti-microbial studies of these plants may share some commonality in their action against the microbial strains in the present investigation. A perusal of the data indicates that it is variable among the twelve plants investigated. Yet the activity is limited or even absent in most cases of these plants, excepting the case of *Azadirachta indica* and *Gymnema sylvestre.* The vital plants like *Curcuma* and *Withania* exhibited very little anti-microbial activity on one or two strains as of *Momordica.* It is believed that the same active principle may interact differently in their action against the microbes or that diverse unknown related substances may also influence their action on these microbes. It is also likely that the plant microbe interaction is more complex than is inferred from such studies and needs further thorough investigation.

The major constituent of turmeric is curcumin (diferuloylmethane) which constitute upto 90% of the total cucuminoid content with dimethoxy curcumin and bis-dimethoxycurcumin comprising the remainder[4]. Ethylacetate, methanol and water extracts from this plant has been studied against the most important hospital and community pathogen called methicillin- resistant *Staphylococcus aureus*[29] with some positive findings. The results of the present study indicate that the concentrations of the methanolic extracts used are not effective against the microbes tested. An anonymous monograph on turmeric, however, depicts its use as anti-inflammatory, anti-carcinogenic and anti-microbial, and suggests the need for further experimentation using this plant. The anti-microbial activity of *Momordica charantia* leaf extracts with two other plants using cold 95% ethanol on *E.coli, Salmonella paratyphi* and *Shigella dysenterae* are very promising; it is claimed that the leaf extracts have compounds that exhibit anti-microbial properties against test organisms. However, the anti-bacterial activity results from the extracts of four parts of *Momordica charantia* in the present studies are not very encouraging with a slight efficacy of leaf extract recorded against *E.coli* (Fig. 1) and *Proteus vulgaris.* Similar negative effects were noticed, although the isolated MAP 30 protein from this plant was found to be antiviral, antitumor and antimicrobial in its action.

To our knowledge as on today, there are only one or two reports on the anti-microbial studies on *Mimosa pudica.* Its anti-microbial activity along with six others on *Vibreo cholerae*[37] suggests some positive response, although more pronounced activity is associated with *Terminalia avicenniodes.* The present investigation has recorded very high efficacy of hot as well as cold methanolic extract of *Mimosa* against gram-negative *Proteus vulgaris.* However, the efficacy of hot methanolic extract is lesser against the other two gram-negative bacteria *E.coli* and *Klebsiella.* Infact its promising influence on *Enterococcus faecalis* clearly suggests the use of *Mimosa pudica* as a potent anti-microbial agent. Anti-microbial activity of the extracts of *Azadirachta indica* and *Curcuma longa*[23] were effective on *E.coli, Staphylococcus faecalis, Bacillus cereus* etc. and needs further confirmation. Earlier studies on *Azadirachta*[24] claim that a spermicidal fraction of neem oil (NIM-76) is more effective as an anti-microbial agent as compared to the neem oil itself, especially its effect is less on *E.coli* and *Klebsiella.* The present report also shows very high efficacy of the methanolic extract of neem against *Enterococcus faecalis* and *Proteus vulgaris* (Fig. 1) and to a lesser extent against *Streptococcus faecalis* with cold extract. The hot extract is effective on *Enterococcus* in addition to *Micrococcus* but not on *Proteus*. Almas[40] also reported anti-microbial activity of neem oil on *E.coli* and *Klebsiella* with hot methanolic extract but not with cold extracts and hexane extracts, as is influenced by the pH of the final extract.

Emblica officinalis was known for its greater anti-proliferative activity[2] compared to many other plants including *Aegle marmelos*. Anti-microbial studies on 82 plants with a number of other bacterial and fungal strains[36] have shown that only 56 plants were effective which includes *Emblica officinalis* indicating its ability as an anti-microbial agent against many microbes. It was also reported that on the whole, alcoholic extracts showed greater activity than corresponding aqueous and hexane extracts. The present report recorded very high efficacy of *Emblica officinalis* against six of the seven tested bacteria with cold methanol while the high activity was evident on all bacterial strains with hot methanolic extract. These results clearly support the usefulness of *Emblica officinalis* as a broad-spectrum anti-microbial agent against a wide range of microbes.

Another significant observation was that hexane extracts were less effective in their anti-microbial activity as evident from the data of three plants (Table 1). It is also clearly evident that cases of complete inactivity (NA = inhibition zones absent altogether) are more with cold extractions as compared to hot methanolic extractions. Further, the hot extract from all the eleven plants excepting *Momordica charantia* can inhibit the growth of *Enterococcus faecalis*; these results prove the greater efficiency of hot methanolic extracts in their anti-microbial activity. It may be mentioned that the nature of the extract and the solvent system profoundly influence the efficacy in its anti-bacterial activity and hence the conditions optimizing the activity are to be clearly ascertained to each of the investigative plant system for a proper usage thus points of NA (inhibition zones altogether absent) were more with cold extract as against hot extract. Further, the gram-positive bacteria were more resistant to the plant constituents of both cold and hot methanol extracts and possess more efficient means of withstanding the inhibitory action of the phytochemicals of most plant systems than the gram-negative bacteria.

Medicinal plants, although have huge potentials in therapeutic use, can sometimes be poisonous or toxic if wrong plant parts are used or if the concentrations and formulations are not appropriate[41,42] and would cause adverse effects also. As such formulations and concentrations have to be evaluated thoroughly in their successful use as anti-microbial agents. Though, not directly used as anti-microbial agents at present by the public, many of these plant constituents are consumed as unregulated botanical preparations; it is essential to evaluate the consequence of such self-medication. Moreover, tribals mostly use them as water extracts as compared to the processed hexane and methanolic extracts like in the present studies. Given that the methanolic extracts were more effective than hexane extracts in many of the other investigations as in the case with the present study also, it is also likely that simply water extracts will also be as effective or even more; this needs to be assessed with further minimizing of the processing events. The out come of such findings authentify the traditional knowledge of those public increasingly depending on these products of plant origin.

In conclusion, the present study has shown that the activity was variable among the tested plants and that hexane extracts were less effective as compared to cold and hot methanolic extracts. Again, the absence of inhibitory zones (NA) recorded were more with cold extracts rather than hot methanol with a further differential action against the gram-positive and gram-negative bacteria. The hot methanolic extracts were thus more in their efficacy; the gram-positive bacteria seem to possess more efficient means of withstanding the phytochemical action of most of the plants of present investigation although, *Emblica officinalis*, followed by *Syzygium* and *Azadirachta* in that order exhibited efficacy against positive and negative strains at equal levels.

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