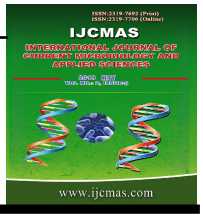
International Journal of Current Microbiology and Applied Sciences
ISSN: 2319-7706 **Volume 2 Number 5 (2013) pp. 211-221**
http://www.ijcmas.com



Original Research Article

Bioactive screening and antimicrobial activity of flowers from the selected three medicinal plants on chosen microbes

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Keywords

Antibacterial activity; phytochemical analysis; Medicinal plants; *Tribulus terrestris; Pavetta indica; Saraca asoca.*

A B S T R A C T

The aqueous, ethanol and benzene extracts of flower from three medicinal plants of *Tribulus terrestris, Pavetta indica* and *Saraca asoca* were examined for possible sources of antimicrobial activities and phytochemical constituents. Occurrence of the phytochemicals in all the three different plant flowers mainly depends upon the solvent efficiency. Moreover, presence of carbohydrates, alkaloids, tannins, saponins, flavanoids, anthraquinone, glycosides, steroids, terpenoids and sterols. Though, Flavanoids, Anthraquinone and glycosides absence in benzene flower extract of *S. asoca*. The preliminary evaluations of both the aqueous and solvent benzene ethanol extracts exhibited appreciable inhibitory activities on the tested pathogenic bacterial isolates at concentration of 30mg m/L. Results of inhibition zone in *S. asoca* showed more therapeutic activity where the benzene flower extract demonstrated significant ($P<0.05\%$) inhibitory activity 49±4.1mm in diameter on the tested *K. pneumonea* bacterial isolates. Similarly ethanol extract of *P. indica* flower showed of highest range of inhibition zone between (30±5.3 to 40±7.1 mm) observed on *S. aureus* and *S. typhi* respectively. From, this study explained the above said thee medicinal plant flower extracts were definitely used for the potential antibacterial agent.

Introduction

Plants have a limitless ability to synthesize aromatic substances mainly secondary metabolites, of which at least 12,000 have been isolated, a number estimated to be less than 10% of the total (Mallikharjuna *et al*., 2007). Traditional medicine has been practiced for many centuries by a plants as a source of pharmacologically active compounds has increased worldwide (Kostova and Dinchev, 2005). Plant extracts represent a continuous effort to find new compounds with the potential to act against multi-resistant bacteria. Approximately 20% of the plants found in the world have been submitted to

pharmacological or biological test, and a substantial number of new antibiotics introduced on the market are obtained from natural or semi-synthetic resources (Mothana and Lindequist, 2005).

Tribulus terrestris is a flowering plant of the Zygophyllaceae family, native to warm temperature and tropical regions it can thrive even in desert climates and poor soil (Abeywickrama and Bean, 1991). *T. terrestris* is used in folk medicine as tonic, aphrodisiac, analgesic, astringent, stomachic, anti-hypertensive, diuretic, lithon-triptic and urinary anti-infectives (Saad Aldein, 1986;Tsuchiya *et al*., 1996). Leaves stem, and flowers of *Saraca asoca*, an endangered medicinal plant in India, and young explants cultivated on Murashige and Skoog's medium containing 6-benzylaminopurine were analyzed for antibacterial potential depicted by Shahid *et al*., (2007).

Different parts of Turkish and Iranian *T. terrestris* have been reported to have antibacterial activity (Abbasoglu and Tosun, 1994; Kianbakht and Jahaniani, 2003). The treatment and control of diseases by the use of the available medicinal plants in a locality will continue to play significant roles in medical health care implementation in the developing countries of the world. Nearly, all cultures and civilizations from ancient times to the present day have depended fully or partially on herbal medicine because of their effectiveness, affordability, availability, low toxicity and acceptability (Bayoub *et al*., 2010). However, these defensive molecules give plants their medicinal value which is appreciated by human beings because of their great importance in health care of individuals and communities. Due to ineffectiveness of most drugs as a result of microbial resistance to available agents most especially in developing countries, more patients are seen in medical centers than ever. In view of this, it is therefore very important to search for effective but of low cost and reliable traditional therapeutic agents, hence also the abuse of drugs for ailment is in high increase which motivated drug resistant organisms. Though, the antimicrobial activity of *T. terrestris* flower part has not been studied till today. In the current study, we evaluated in vitro antimicrobial activity of flowers from three different plants of *T. terrestris*, *S. asoca* and *P. indica* using different extracts. This work is therefore aimed at studying three Indian medicinal plants used locally for treatment of some diseases for their phytochemical properties and antimicrobial activities in comparison to known antibiotics.

Materials and Methods

Collection Plants Materials

Experimental healthy lowers were collected from Thiruvananthapuram and identified plant from Crop Protection Research Centre from St. Xavier's College (Autonomous), Palayamkottai. Fresh flowers were collected and properly washed in tap water (H_2O), and then rinsed in sterile distilled H_2O. The flowers were air dried and milled in a warring blender and sieved to obtain smooth powder. The smooth powder was stored in airtight glass containers protected from sunlight until required for further analysis.

Preparation of the extracts

Aqueous extracts

The air dried fine powdered plant flowers (150 gm) were infused in distilled water until complete exhaustion. The extract was

then filtered using Whatman No. 1 filter paper and the filtrate was evaporated in vacuo and dried using either a rotary evaporator at 60°C or a freeze drier (Kandil *et al*., 1994). The final dried samples were stored in labeled sterile bottles and kept at -20°C.

Ethanol extracts

Ethanol extracts were accomplished according to established protocols (le Grand *et al*., 1988). Each dried plant sample was ground and extracted in a percolator with 95% ethanol. About 10 ml of ethanol per gram of plant sample was used. The ethanol extract was dried under a reduced pressure at 40°C. The dried extract was stored in sterile bottles until further use.

Benzene extracts

Powdered samples (100gm) from each plant part were extracted with chloroform using a soxhlet extractor for continuously 10 h or until the used solvent turned pure and colorless (Chhabra *et al*., 1982). The solvent was removed using a rotary vacuum evaporator at 40°C to give a concentrated extract, which was then frozen and freeze-dried until use.

Microbial cultures

Ten strains of bacteria were used as tested microorganisms. The bacterial strains included Gram-positive *Staphylococcus aureus, Bacillus subtilis, Bacillus cereus* and *Corynebacterium diphtheriae*; Gram-negative *Escherichia coli, Proteus vulgaris, Serratia marcescens, Salmonella typhimurium, Klebsiella pneumoniae* and *Pseudomonas aeruginosa*. All microorganisms were clinical isolates, obtained from the Microbiology Laboratory at Nagercoil, Vivek medical Lab and very carefully identified using standard microbiological methods. Discrete colonies of fresh cultures of the different bacterial isolates were then picked and suspended in 5 ml Nutrient broth (NB, Oxoid), in well-labeled sterile Bijou bottles, and incubated for 24 h at 37ºC prior to antimicrobial susceptibility testing.

Extraction of plant material

Cold and hot extraction with H_2O, and soxhlet extractions with methanol (99%) and Benzene, as described by Junaid et al., (2006), was carried out. Twenty gram of each sample was weighed into 100 ml of the solvent (methanol and Benzene). For cold extraction the samples and solvent were stirred every 30 min for 3 h and allowed to stand for 24 h, while for hot extraction the samples and solvent were heated for 30 min and stirred every 30 min for 3 h and allowed to stand 24 h. After preparation of the crude extract as described, the organic extracts were diluted using 50% dimethylsulphoxide (DMSO), while the aqueous extracts were reconstituted using sterile distilled H_2O to make the final concentrations which kept in refrigerator till used.

1.5kg of each powdered plant part was extracted at room temperature (25±2°C) with ethanol and water for 48 h. The resulting mixtures were filtered and evaporated in a shaker water bath sustained at 55-65°C. The obtained dried crude extracts were contained in plastic containers and labeled appropriately as AE (aqueous extract) and EA (ethanol extract)

Antibacterial Screening

The antibacterial activities of the crude

plant extracts were evaluated by agar well diffusion (Nair and Chando, 2005). Nutrient agar (Lab M and Nutrient broth (Lab M) were used for the sub-culturing the bacterial isolates. Mueller-Hinton agar (Hi-media) was used for the sensitivity screening. The crude extracts were prepared in 5% v/v aqueous dimethyl sulphoxide (DMSO) at concentration of 20mg/ml. The inocula of the tested bacterial strains were prepared from 24h broth culture. The absorbance was read at 530nm and adjusted with sterile distilled water. From the prepared bacterial solutions, other dilutions were prepared to give a final concentration of 106 Colony Forming Unit (Cfu) per milliliter. 0.2ml each of the bacterial suspension was obtained with sterile syringe and needle and spread plated on molten Mueller-Hinton agar. The plates were allowed to stand for 1.5 h for the test bacterial isolates to be fully embedded and well established in the seeded medium. With a sterile cork borer wells of equal depth were dug with a previously sterilized No 4 cork borer. The wells were aseptically filled up with the extracts avoiding splash and overfilling. The plates were incubated at 370Cfor 24-48 h. The sensitivity of the test organisms to each of the extracts were indicated by clear halo around the wells. The halo diameters were taken as an index of the degree of sensitivity. Sterile 5% aqueous DMSO was used as negative control while streptomycin and methicilin (2mg/ml) was used as the positive control. All experiments were carried out in triplicates.

Minimum Inhibitory Concentration (MIC)

The MIC was determined by the method of Sahm and Washington (1990). 1ml of the extract solution at concentration of 20mg/ml was added to 1mL of Muller-Hinton broth and subsequently transferred. 1mL from the first test tube to the next test tube and this continue up to the seventh test tube. Then 1ml of 24 h culture of the test bacteria organisms (1.0 x 106cell/ml) was inoculated into each test tube and mixed thoroughly. The test tubes were then incubated at 370C for 24 h. The tube with the lowest dilution with no detectable growth was considered as the MIC. For every experiment, a sterility check (5% v/v aqueous DMSO and medium) negative control (5% v/v aqueous DMSO, medium and inoculum) and positive control (5% aqueous DMSO, medium, inoculum and water soluble antibiotics) were included.

Preliminary Screening of secondary metabolites

Phytochemical Screening

Phytochemical tests were carried out on the flower *T. terrestris*, *P. indica* and *S. asoca* Aqueous, Benzene and Methanol extract of using the procedure outlined by Harborne, (1973); Jigna *et al*., (2006).

Statistical Analysis

One way ANOVA was used to determine the significance antimicrobial broad spectrum activity of six bacterial pathogens. Data's were analysed with one way of ANOVA test using pp version-4 Window. Results with $p<0.05$ were considered as a statistically significant.

Results and Discussion

Phytochemical screening of the flower extracts revealed the presence of carbohydrates, alkaloids, tannins, saponins, Flavanoids, anthraquinone, glycosides, steroids, terpenoids and sterols. Though, Flavanoids, Anthraquinone and glycosides absence in

benzene flower extract of *S. asoca* (Table 1). In addition Sterols, Methylated sterols were uniformly absent throughout the ethanolic extract of three experimental flowers.

From the present results revealed the presence of bioactive properties in the part flower at various degrees. Local users of these plants employ primarily water as solvent of extraction for the preparation of infusions and decoctions in different parts of India. Among the three plants *Saraca asoca* exhibited more antibacterial activity (49±4.1) in Benzene extract against the tested bacteria. Similarly, flower benzene extract of *T. terrestris* showed further maximum (45±9.3) mean inhibitory zone was formed against *K. pneumonea,* similar effect also been noticed on *L.acidophilus* organism. Meanwhile in the third plant of *P. indica* showed that the significant peak activity has been noticed in Ethanol extract against *S. typhi*. Eventhough, a similar inhibitory values were noticed in aqueous extract from *T.terrestris* flower on *S.aureus* and *K.pneumonea* (25±9.3) and *P. indica* (25±9.3) ethanolic extract against *E. coli* (25±5.5) (Tables 2). Furthermore, minimum inhibitory zone was formed in *L. acidophilus* aqueous extract of T. *terrestris*. Subsequently very low activity showed in 6 ±0.30 aqueous extract of *P. indica* it was insignificantly. In addition very poor activity denoted on benzene extract of *S. asoca* flower extract.

Three different extracts from flowers of *T. terrestris*, *P. indica* and *S. asoca* were tested at various concentrations (0.10~25.00 mg/ml), and the evaluated MIC values are reported in Table. 3. All the plant parts showed antibacterial activity against most tested bacteria. Aqueous extract from *T. terrestris* fruits showed good activity against the tested bacteria and the strongest activity was seen against *S. typhi* (*MIC*=0.62 mg/ml), which was similar to what was achieved by the standard drug chloramphenicol. Mean while, *M. variance* was inhibited using the maximum extract concentration (*MIC*=6.47 mg/ml). In addition, *L. acidophilus* and *P. aeruginosa* resisted all aqueous extracts of various concentrations. Ethanol and Benzene extracts of *T. terrestris* flower extract demonstrated very close activities against all reference bacteria. Very strong activity was seen against *S. aureus*, *B. subtilis*, *B.cereus*, *S. typhi*, *E. coli* and *P. vulgaris* using both extracts. The highest antibacterial activity was seen against *B.subtilis*, *B. cereus*, *S. typhi* and *P.vulgaris* in the ethanol extract (*MIC*=0.15 mg/ml), while *B. subtilis*, *B. cereus* and *C. diphtheriae* were the most sensitive bacteria to the benzene extract (*MIC*=0.31 mg/ml). *S.typhimurium*, *K.pneumoniae*, *P.aeruginosa* and *M. variance* were inhibited by highest concentrations of ethanol and benzene extracts ranging from (*MIC*=3.15and 6.57 mg/ml). Similarly other two flower extracts were clearly showed that peak activity noticed on gram positive bacteria of *S. typhi* ethanol extract. In addition *S. asoca* showed maximum activity against organisms used.

The reference antibiotics(Chloramphenicol and methicilin) as the positive control in concentration of 2mg/ml as used in this study though higher in inhibitory affinity (39-45mm) Chloramphenicol and lower in inhibitory affinity (12-20mm) methicilin were not active (0mm) on *E.coli*, *S. typhi*, *C. jejumum* and *K. pneumoniae.* The reference antibiotics (streptomycin and methicilin) as the positive control in concentration of 2mg/ml as used in this study though higher in inhibitory affinity (39-45mm) [streptomycin] and lower in

Table. 1 Phytochemical screening of flowers from three medicinal plants of *T. terrestris, P. indica* and *S. asoca*

Phytochemical constituents	Name of the plants					
	Benzene			Ethanol		
	T. terrestris	*P. andrographis*	*S. asoca*	*T. terrestris*	*P. indica*	*S. asoca*
Alkaloids	+	++	++	+	+	+
Carbohydrates	+	++	+	+	-	+
Tannins	+	-	++	+	+	+
Saponins	+	+++	+	+	+	+
Flavanoids	+	+	-	+	+	-
Anthraquinone	+	+	-	+	-	-
Glycosides	+	+	-	+	+	+
Steroids	+	+	+++	+	+	+
Terpenoids	+	++	+	+	-	+
Sterols	+	+	++	-	-	-
Methylated sterols	-	-	+	-	-	-

inhibitory affinity (12-20mm) methicillin were not active (0mm) on *E.coli, S. typhi, L. acidophilus* and *K. pneumoniae*. Despite this position, these organisms were quite susceptible to the plant extracts though at higher concentrations than the reference drugs. The MIC of the extracts against the tested bacterial organisms is shown in table 3.

The activity of the plant against both Gram-positive and Gram-negative bacteria may be indicative to the presence of broad spectrum antibiotic compounds or simply general metabolic toxins in the plant, in addition to the plant (fruits, leaves and root) content of pharmacological active metabolites like furostanol and spirostanol saponins (Kostova and Dinchev, 2005), phytosterols and some amides (Xu *et al*., 2000). All parts (fruits, stems plus leaves and roots) of Turkish and Iranian *T. terrestris* showed antibacterial activity against *Enterococcus faecalis*, *S. aureus*, *E. coli* and *P. aeruginosa* (Kianbakht and Jahaniani, 2003). In addition previously postulated contrast to aerial parts of Yemeni *T. terrestris* which had no detectable antibacterial activity against these bacteria (Awadh-Ali *et al.*, 2001), and only fruits and leaves of Indian *T. terrestris* were active exclusively against *E. coli* and *S. aureus* (Williamson, 2002).

Previously, similar results been noted by Firas and Hassan, 2008 in the on aqueous extract from *T. terrestris* leaves revealed the close results to the fruit extract, except it was active against *P. vulgaris* with an MIC value of 2.50 mg/ml and resisted by *S. typhimurium*, *S. marcescens* and *P. aeruginosa*. Ethanol and chloroform extracts from leaves also possessed promising results against the tested bacteria with less action compared with fruit ethanol and chloroform extracts. The best MIC values were calculated against *B. subtilis*, *P. vulgaris* and *K. pneumoniae* (MIC=0.31 mg/ml) using the ethanol extract and *B. subtilis* (MIC=0.31 mg/ml) using chloroform extract. *S. marcescens* resisted the chloroform extract and was inhibited by the highest ethanol extract concentration this views also agreed by Zhang *et al*., (2006); Seethram *et al*., (2003).

Table.2 Mean inhibitory (mm) as expressed by the crude aqueous and ethanol extracts of the three medicinal plants at 30mg/ml.

Bacterial organism	***T. terrestris***			***P. indica***			***S. asoca***		
	AQ	**B**	**E**	**AQ**	**B**	**E**	**AQ**	**B**	**E**
B. cereus	23±0.5	14±0.61	24±.19	14±5.31**	31±1.4	32±1.0	17±3.46	10±2.3	31±1.6
S. aureus	13±0.52	25±0.7	16±1.3	18±1.6	12±1.6	30±5.3*	12±0.87	5±0.41	20±1.0
L. acidophilus	10±1.6^{is}	11±1.42	21±1.5	11±0.7^{is}	36±3.6	19±2.8	9±1.24	11±1.0	23±2.9
K.pneumoniae	16±1.6	45±9.3**	25±9.3	12±2.67*	13±2.2	18±4.0	15±1.71	49±4.1**	22±0.5
E. coli	20±0.5	17±4.71	14±2.3	15±0.5	22±.5.3	25±5.5	15±2.14	23±4.5	19±1.6
S. typhi	13±1.2	15±1.11	26±5.7	6 ±0.30	20±3.1	40±7.1*	18±2.35	20±2.9	21±3.5

* - Statistically significant at 5% level, ^{is}- Statistically insignificant at 5% level. The results are the mean values of triplicate tests measured in two directions after 24-48h incubation at 37°C.

Table.3 In vitro antimicrobial activity of ethanolic and benzene flowers extracts from three medicinal plant on Gram-positive and Gram-negative organisms

Microorganisms	*MIC* (mg/ml)										
	T. terrestris			***P. indica***			***S. asoca***			**Control**	
	A	E	B	A	E	B	A	E	B	Ch	Methicillin
Gram-positive											
S. aureus	2.50	0.6	0.6	2.50	1.3	3.0	5.00	1.02	0.41	0.1	NT
B. subtilis	1.25	0.15	0.31	1.25	0.31	0.31	2.50	0.62	0.31	0.01	NT
B. cereus	1.3	0.2	0.4	3.20	0.62	0.65	5.00	1.25	0.62	0.39	NT
S. typhi	0.62	0.15	0.31	1.25	0.72	1.05	2.50	1.25	0.71	0.72	NT
Gram-negative											
P. vulgaris	5.00	0.15	1.25	2.50	0.31	1.25	5.00	2.50	1.25	0.45	NT
E. coli	1.25	0.62	0.62	2.50	0.62	1.25	4.95	2.50	1.25	0.45	NT
L. acidophilus	5.00	2.50	2.50	5.10	5.15	5.05	5.00	5.00	5.00	2.50	NT
M. variance	6.57	1.85	2.50	5.00	1.25	5.00	5.00	6.47	2.50	2.50	NT
K. pneumoniae	3.15	1.80	1.25	2.50	0.31	2.50	5.00	2.50	1.25	1.25	NT
P. aeruginosa	4.00	1.75	2.50	5.00	1.25	2.50	5.00	2.50	1.25	2.50	NT

A: Aqueous extract; E: Ethanol extract; Benzene extract; Ch: Chloramphenicol; Methycilin at concentration of 2mg/ml were used as the positive reference drugs.

Based on the present study, previously depicted the plants understudied of the *T. terrestris P. indica S. asoca* leaves; stem bark and roots to be good sources of antimicrobial property. This results obtained conforms to the findings of Verma *et al*., (2010). The bioactive compounds on the medicinal plants employed contain various secondary metabolites such as phenols, tannins, alkaloids, flavonoids, steroids and glycosides in appreciable quantities (Sing and Jain, 2011). The effective inhibitory potency observed with the plants parts; proof it that the inhibitory compounds were extractable by the employed solvents against the tested pathogenic bacterial isolates. This observation as reported correlates with Obadoni and Ochuko, (2001) who emphasized that these compounds are known to show medicinal activity as well as exhibiting physiological activity.

This concentration was visibly active on the tested bacterial isolates due to the combinative therapeutic actions of the various secondary metabolites contained in the plants. Some of the tested bacterial isolates such as *S. dysentariae, P. aeruginosa, E. coli* and *K. pneumoniae* reported to be associated with nosocomial and community acquired infections (Indrayan *et al*., 2002; De and James, 2002) were found susceptible to the plants crude extracts used in this study. This proof emphasized that some nosocomial and community acquired infections could be prevented or alleviated with the use of most especially, the ethanol extracts of the studied plants. These plants, most especially their leaves and stem bark in dry powder form could be used for direct consumption as various kinds of beverages, decoctions and infusions. Healthy fresh leaves of these plants could as well be prepared as soup.

The percentage MIC values of the plants were based where both the ethanol and aqueous extracts have the same MIC values on the tested bacterial isolates. This perhaps helps to interpret that differences in inhibitory diameters (mm) could result in the same therapeutic potency when varied in concentrations, depending on the organism's susceptibility to the antibacterial components present in the extracts. The presence and the phytochemical components of the studied plants, the inhibitory zones and the MIC concentrations at which values were effective on the tested organisms, highlights that there were variations in the antibacterial potency of the plants extracts Prajapati *et al.,* (2003). The variations in the sensitivity could also be attributed to the differences in growth rate of the tested organisms, nutritional requirements, temperature and inoculum size (Gaill and Jon, 1995).

It has been reported that antibiotics are not the only antibacterial agents and this study observed the effective potency of the studied plants extracts on the selected pathogenic bacterial isolate than some highly rated antibiotics (reference drug) in disease cure and prevention. One problem in the use of medicinal plants is the quantity desired to effect cure hence most times, medication is basically on unspecified quality of decoctions and infusions. Irrespective of the plants parts in this study and methods of extraction (ethanol and water), a dosage of between 1.2-10mg exhibited appreciable inhibitory values on the tested bacterial species. Furthermore, Pal *et al*., (2007) also observed the somewhat similar result such as methanolic extract was active against *Bacillus subtilis*. Moreover, we found that the methanolic extract was more effective in cases of *Staphylococcus aureus* and

Escherichia coli. Although our extracts were inferior to the positive control as far as zones of inhibition were concerned, the differences between the zones of inhibition produced by the positive control and the extracts against *E. coli* and *B. subtilis* were not remarkable. Even at low concentration (100μg/ml), both the extracts showed antibacterial activity. Those extracts at 200μg/ml produced good antibacterial activity against *E. coli* and *S. aureus*, respectively. From the above mentioned results, it may be concluded that both the extracts contains antibacterial activity. But in the current study proved that the low concentration standardized amount is 30mg/ml of flower extracts (Aq, B and E) were necessary for formation of the zone of inhibition against the six bacterial pathogens. The intractable problem of antimicrobial resistance has led to the resurgence of interest in herbal products as sources of novel compounds to suppress or possibly eradicate the ever increasing problems of emergence of newer diseases thought to be brought under control (Akharaiyi and Boboye, 2010).

The plants parts antibacterial effectiveness on the tested bacterial isolates resulted within 24h of incubation in both the crude extract screening and MIC values. The aqueous extracts of the plants displayed extensively a competitive inhibitory potency with the more effective ethanol extracts of the plants parts on the tested isolates. The plant parts though effective on all the bacterial isolates, there were variations in inhibitory potency resulting from variations in the secondary metabolites concentrations in the plant parts. The result of this study also supports the traditional application of the plant and suggests that the plant extracts possess compounds with antibacterial properties that can be used as antibacterial agents in novel drugs for the treatment of various diseases. Further pharmacological evaluations, toxicological studies and possible isolation of the therapeutic antibacterial from this plant are the future challenges.

Acknowledgement

We would like to sincere thanks Rev. Fr. Premkumar (M.S.W) Secretary and Correspondent in Malankara Catholic College, Mariagiri for valuable suggestions, insightful advices and a boost of in my scientific endeavors.

References

Abbasoglu, U., and Tosun, F. 1994. Antimicrobial activity of *Tribulus terrestris* L. growing in Turkey. Hacettepe Universitesi Eczacilik Fakultesi Dergisi. 14:81–85.

Abeywickrama, K., and Bean, G. A. 1991. Toxigenic *Aspergillus flavus* and aflatoxins in Sri Lankan medicinal plant material. Mycopathol.113:187–190.

Akharaiyi, F. C., and Boboye, B. 2010. Antibacterial and Phytochemical Evaluation of Three Medicinal Plants. J. Natur. Product. 3:27-34.

Awadh-Ali, N.A. , W.D. Jülich, C. Kusnick and Lindequist, U. 2001. Screening of Yemeni medicinal plants for antibacterial and cytotoxic activities. J Ethnopharmacol. 74(2):173–179.

Bayoub, K., T. Baibai, D. Mountassif, A. Retmane and Soukri, A. 2010. Antibacterial activities of the crude ethanol extracts of medicinal plants against *Listeria monocytogens* and some other pathogenic strains. African J.Biotechnol. 9(27):4251-4258.

Chhabra, S.C., J.F. Shao and Mshiu, E.N.1982. Antifungal activity among traditionally used herbs in Tanzania. The Dar Medi. J.. 9:68–73.

De, N., and James, N.E., 2002. Antibacterial spectrum of ectracts of *Ocimum gratissimum* L (Basil) ND *Xylopia aetiopica* A. Rich (Dunal). Nig. J. Appl. Sci. 11: 165-175.

Firas, A., Al-Bayati and Hassan, F. Al-Mola. 2008. Antibacterial and antifungal activities of different parts of *Tribulus terrestris* L. growing in Iraq. J. Zhejiang. Univ .Sci. B. 9(2): 154–159.

Gaill, W., and Jon, A.W., 1995. Antimicrobial susceptibility test; dilution and disc diffusion methods. Manual of Clinical Microbiology, 6th Edn., pp.1327-1332.

Harborne, J.B., 1973. Phytochemical methods, London. Chapman and Hall, Ltd. pp. 49-188.

Ikan, R., 1981. Natural products: A laboratory guide, Academic Press, London.

Indrayan, A.K., A. Shama, B.S. Gideria and Gupta, C.P. 2002. Antibacterial activity of dye from *Ensalpina sappan* (Patang/Brazilworld). In. J. Microbiol. 42: 359-360.

Jigna, P., K. Nehal and Sumitra, C. 2006. Evaluation of antibacterial and phytochemical analysis of *Bauhinia variegates* Evaluations of antibacterial and phytochemical analyses of *Bauhinia variegate* L. bark. Afr. J. Biomed. Res. 9(1): 53-56.

Kandil, O., N.M. Radwan, A.B. Hassan, A.M. Amer and El-Banna, H.A.1994. Extracts and fractions of *Thymus capitatus* exhibit antimicrobial activities. J Ethnopharmacol. 44(1):19–24.

Kianbakht, S., and Jahaniani, F. 2003. Evaluation of antibacterial activity of *Tribulus terrestris* L. growing in Iran. Iranian J. Pharmacol. 2:22–24.

Kostova, I., and Dinchev, D.2005. Saponins in *Tribulus terrestris* chemistry and bioactivity. Phytochem Rev. 4(2-3):111–137.

le Grand, A., P.A. Wondergem, R. Verpoorte and Pousset, J.L. 1988. Anti-infectious phytotherapies of the tree-savannah of Senegal (West-Africa) II. Antimicrobial activity of 33 species. J. Ethnopharmacol.22(1):25–31.

Mallikharjuna, P.B., L.N. Rajanna, Y.N. Seetharam and Sharanabasappa, G.K. 2007. Phytochemical studies of *Strychnos potatorum* L.f. A medicinal plant. E-jour. Chem. 4 (4): 510-518.

Mothana, R.A., and Lindequist, U. 2005. Antimicrobial activity of some medicinal plants of the island Soqotra. J Ethnopharmacol. 96(1-2):177–181.

Obadoni, B.O., and Ochuko, P.O., 2001. Phytochemical studies and comparative efficacy of the crude extracts of some homeostatic plants in Edo and Delta States of Nigeria. Glob.J.Pure Appl. Sci. 86: 2003-2008.

Pal, S. C., A.P. Maiti, B.P. Chaterjee and Nandy. 2007. Antibacterial activity of flower and buds of International Journal of Pharmaceutical Research andDevelopment *Saraca indica* Linn, Int. J. Med. Res. 82: 188-189.

Pradhan, P., L. Joseph, V. Gupta, R. Chulet, H. Arya, R. Verma and Bajpai, A. 2009. *Saraca asoca* (Ashoka):A review. J. Chem. Pharm.Res.19 (1): 62-71.

Saad Aldein, S.M.,1986. Medicinal Herbs. 1st Ed. Baghdad: Dar Al-Shoun Al-Thaqafia Al-Aama for Publishing. pp.70.

Seethram, N., H. Sujeetha, G. Jyothiswaran, A. Barad, G. Sharanbasappa and Praveen, S. 2003.

Antibacterial activity of *Saraca Asoka* Bark, Ind. J. Pharm.Sci. 65(6): 658-659.

Shahid, M., A. Shahzad, A. Malik and Anis, M. 2007. Antibacterial activity of aerial parts as well as in vitro raised calli of the medicinal plant Saraca asoca (Roxb.) de Wilde. Can. J .Microbiol. 53 (1):75-81.

Sing, R.P., and Jain, D.A. 2011. Evaluation of Antibacterial activity of alcoholic and aqueous extracts of five plants used in Traditional Medicine in North India. Int. J. Pharm. Tech. Res. 3 (1):4-9.

Srivastava, G. N., G.D. Bagchi and Srivastva, A. K.1988. Pharmacognosy of Ashoka stem bark and its adulterants, Int. J. Crude drug Res, 26 (2):65

Tsuchiya, H., M. Sato, T. Miyazaki, S. Fujiwara, S. Tanigaki, M. Ohyama, T. Tanaka and Iinuma, M. 1996. Comparative study on the antibacterial activity of phytochemical flavanones against methicillin-resistant *Staphylococcus aureus*. J. Ethnopharmacol.50 (1):27–34.

Verma, A., G.K. Jana, S. Sen, R. Chakraborty, S. Sachan and Mishra, A. 2010. Pharmacological Evaluation of *Saraca indica* Leaves for Central Nervous System depressant activity of mice. J. Pharm. Sci. Res.2(6): 338-343.

Xu, Y.X., H.S. Chen, L.Q. Liang, Z.B. Gu, W.Y. Lui, W.N. Leung and Li, T.J.2000. Three new saponins from *Tribulus terrestris*. Planta Med. 66(6):545–550.

Zhang, J.D., Z. Xu, Y.B. Cao, H.S. Chen, L.Yan, M.M. An, P.H. Gao, Y. Wang, X.M. Jia, Y.X. Jiang. 2006. Antifungal activities and action mechanisms of compounds from *Tribulus terrestris* L. J. Ethnopharmacol. 103 (1):76–84.