International Journal of Pharmacy and Biological Sciences-IJPBS™ (2019) 9 (2): 338-349
Online ISSN: 2230-7605, Print ISSN: 2321-3272
Research Article | Biological Sciences | Open Access | MCI Approved
UGC Approved Journal

Comparative Antimicrobial Activity of Ethanolic and Aqueous Extracts of *Asparagus resmosus*

Krishnadutt Pratihast[1], Rajneesh Kumar[2,*] and Sudhanshu Kumar Bharti[1,*]

[1]Department of Biochemistry, Patna University, Patna-800023, Bihar, India
[2]Department of Chemistry, Patna University, Patna-800023, Bihar, India

Received: 17 Jan 2019 / Accepted: 14 Mar 2019 / Published online: 1 Apr 2019
Corresponding Author Email: sudhanshu_bharti@rediffmail.com

Abstract

The present study was designed with the aim to evaluate and compare the effects of ethanolic (EtOH) and aqueous (H_2O) extracts of *Asparagus resmosus* (satavar; Family: *Asparagaceae*) plant extract according to phytochemical aspects for antibacterial efficacies. Phytochemical screening of the plant extract showed the presence of different percentage of active phytochemicals such as alkaloid, flavonoid, phenolic, saponin, tannin etc. The phenolic content of ethanolic extract was found to be 9.4 mg GAL/g while 6.8 mg GAL/g per gram dry weight basis for aqueous extract. The flavonoid content of ethanolic extract in terms of quercetin equivalent was found to be 0. 38 mg/g while for aqueous extract it was found to be 0.46 mg/g. The IC_{50} value of *A. racemosus* in ethanol was found to be 0.2016 µg/ml while that in water was found to be 0.2248 µg/ml. The ethanolic extract of *A. racemosus* showed the maximum zone of inhibition (7.50 mm) against *E. coli* followed by *Salmonella typhi* (7.00 mm) and *Bacillus cereus* (7.00 mm). The extract of *Asparagus racemosus* showed the minimum zone of inhibition (6.0 mm) against *Bacillus subtilis.* The aqueous extract of *Asparagus racemosus* showed the maximum zone of inhibition (7.50 mm) against *E. coli* followed by *Salmonella typhi* (7.00 mm) and *Bacillus subtilis* (5.00 mm). The extract of *Asparagus racemosus* showed the minimum zone of inhibition (4.0 mm) against *Bacillus cereus*.The minimum inhibitory as well as bactericidal concentration of investigated plant extract against bacterial strains of *E. coli* has been found to be 250 µg/ml.

Keywords

Asparagus resmosus, minimum inhibitory concentration, minimum bactericidal concentration, Gram positive bacteria, Gram negative bacteria, antimicrobial.

*****

1. INTRODUCTION

Microbial infections have caused a big burden of diseases and bacteria are listed in the first position among common micro-organisms responsible for opportunistic diseases (Rathee et al., 2012). In developing countries, bacterial infections are

prevalent due to factors such as poor hygiene, sanitation and overcrowding in the living conditions. On the other hand, amplified antibiotic resistance has become a global concern, in addition to the problem of microbial persistence, thus highlighting the need to develop novel microbial drugs that are not only active against drug resistant microbes, but more importantly, kill persistent micro-organisms and shorten the length of treatment. Despite toxicity, extended therapy also creates poor patient compliance (Mariita et al., 2010). In addition, antibiotics are associated with adverse effects on host, which include depletion of beneficial gut and mucosal microorganisms, immuno-suppression, hypersensitivity and allergic reactions (Kalayou et al., 2012). Also, there is not only the loss of antibiotics effectiveness against multidrug resistant microorganisms, but also global problem for the increased budget for disease treatment.

Bacterial infections are caused by a wide range of organisms resulting in mild infections to life threatening diseases. For instance, *Bacillus subtilis* is an aerobic, rod-shaped, motile, and endospore-forming Gram-positive bacterium. It is a soil inhabiting saprophytic organism (Sleigh and Timburg, 1998). Although harmless, it occasionally causes some opportunistic infections such as conjunctivitis. *Staphylococcus aureus,* a Gram-positive coccus bacterium, is part of the normal flora of human skin and mucous membranes (Murray et al., 1998). This organism is responsible for human staphylococcal skin infections (wounds and impetigo), soft tissues (septic arthritis) and pneumonia (Sleigh and Timburg, 1998). *Escherichia coli,* a Gram-negative rod-shaped bacterium is mostly found inhabiting the human gastrointestinal tract and commonly causes urinary tract infections (Najar et al., 2009). *Klebsiella pneumoniae* is a non-motile, rod-shaped Gram-negative bacterium. The organism is easily observed in culture as it forms large colonies. It is also part of the human intestinal and colon flora, having a prominent polysaccharide capsule that provides resistance against host defence mechanisms (Hugo, 1992). Common human *Klebsiella* infections include community acquired pneumonia, urinary tract infection, lower and upper respiratory tract infections (Einstein, 2000).

All through the ages, man has used plants to alleviate common infectious ailments and a number of these conventional medicines are still included as part of the consistent treatment of various diseases. The great ancient Indian civilizations provided written evidence of man's utilization of plants for the treatment of a wide range of diseases in nearly all cultures (Bharti et al., 2018; Bharti et al., 2013). Recently there has been a shift in universal trend from synthetic to herbal medicine, which can be said *"back to nature"* (Bharti et al., 2016). Phytochemical screening of plants has revealed the presence of numerous chemicals including phenolic compounds, alkaloids, tannins, flavanoids, steroids, glycosides and saponins etc (Bharti et al., 2012-17; Salazar et al., 2008). The antimicrobial compounds of plants origin may inhibit bacterial growth by different mechanisms than those of synthetic antimicrobials and may have a significant clinical value in treatment of resistant microbial strains (Bharti et al., 2018; Houghton et al., 2007; Shankar et al., 1980). Therefore, the use of herbal products as antimicrobial agents may provide the better alternative of synthetic antibiotics (Berry et al., 2009; Falagas et al., 2009).

Based on the frequent usage in folk medicine and reported literature we selected *Asparagus resmosus (satavar; Family: Asparagaceae)* acclaimed for antibiotic and blood purifying properties (Ghosh and Saha, 2012). The *A. racemosus* plant (satavar, shatavari, shatamull, shatuli, kurilo or vrishya) is a species of asparagus commonly found in Himalayan range of India. The name "shatawari" means "curer of a hundred diseases" (shatum: "hundred"; vari: "curer"). Satawari is a woody climber growing to 1-2 m in height, with leaves like pine needles, small and uniform and the flowers white, in small spikes. It contains adventitious root system with tuberous roots. Stems are climbing, branched, up to 2 m; leaves are just modified stems, called cladodes. Flowers are white with a pink tinge and bell-shaped with 6 petals (Gomase et al, 2010). Within India, it is found growing wild in tropical and sub-tropical parts of India including the Andamans; and ascending in the Himalayas up to an altitude of 1500 m. The flowering occurs in October-November (Madhavan et al., 2010; Vichien, 2003). In Ayurvedic medicine, the root of Satavari is used in the form of juice, paste, decoction and powder to treat intrinsic haemorrhage, diarrhoea, piles, hoarseness of voice, cough, arthritis, poisoning, diseases of female genital tract, erysipelas, fever, as aphrodisiac and as rejuvinative. Thus, the present investigation has been carried out in order to study the possible antimicrobial effect of the two plants' extracts against isolated or procured microorganism's strains.

2. MATERIALS AND METHODS

2.1 Characterization and preparation of plants

The plant *Asparagus resmosus (satavar; Family: Asparagaceae)* was collected from different regions

in northern India in 2016 and identified according to the relevant monographs of Indian Pharmacopoeia (2012). The plant materials were botanically authenticated at botany department of Patna University; Patna. Voucher specimens of each of the plant's extract have been deposited in the department. The plant materials were washed under running tap water, blotted with filter paper, were dried in the shade at room temperature. It was then grounded in a mortar. Fifty gram of each of the freshly prepared plant material was extracted with 500 ml of two solvents; distilled water and ethanol by soaking for 48 h. The extracts were filtered using Whatman filter paper No. 1, and the filtrate was centrifuged at 12000 rpm for 10 min. The supernatant was again filtered using Whatman filter paper No. 1 under strict aseptic conditions. The collected filtrates were concentrated in vacuum at temperature below 40^0 C using a rotary evaporator (Buchi, Switzerland). The residue obtained was stored in freezer at -20^0 C until further test.

2.2 Phytochemical screening of plant extracts

Screening of chemical constituents was carried out qualitatively and quantitatively with ethanolic (EtOH) and aqueous (H_2O) extracts by using various chemical methods. For qualitative tests, different solvent extracts of two plants were analyzed for the presence of alkaloid, saponin, flavonoid, fixed oils and fats, tannins and phenolic compounds according to standard methods (Harborne, 1973). Flavonoids were determined by aluminium chloride colorimetric method (Chang et al., 2002). The absorbance of the reaction mixture was measured at 450 nm with a V-670 research grade UV-Vis spectrometer. Quercetin solutions at concentrations of 12.5–100 µg/ml in methanol were meant for calibration curve. The quantitative tests were performed for total phenol which was determined by Folin–Ciocalteu reagent and expressed as mg Gallic acid (GAL) equivalent/g dry weight. The standard curve was prepared using 0, 50, 100, 150, 200 and 250 mg/l solutions of Gallic acid in methanol: water mixture (50:50, v/v).

2.3 Selected micro-organisms:

In the present study, the bacteria selected are described in Table 1. Microbial pure cultures were obtained from MTCC (Microbial type culture collection), Chandigarh. The bacterial cultures were grown on nutrient agar medium (Hi Media, pH 7.4) at 37°C and potato dextrose agar medium (Hi Media, pH 5.6) at 27°C respectively. Both the cultures were maintained at 4°C.

Table 1. Selected Gram positive and Gram Negative microorganisms.

Types of microbes	Micro-organism strains	Causes
Gram positive	*Bacillus subtilis* (MTCC 6038)	Food poisoning
	Bacillus cereus (MTCC 1765)	Food poisoning, vomiting, Diarrhoea
Gram negative	*Escherichia coli* (MTCC 5946)	Bloody diarrhoea, kidney diseases
	Salmonella typhi (MTCC 8345)	Typhoid, enteric fever

2.4 Inoculum preparation:

A fresh microbial suspension was prepared by sub culturing the bacterial colonies in to the nutrient broth medium (Hi Media pH 7.4) and incubated at 37°C in order to maintain the uniform growth rate of each organism. The bacterial suspension of approximately 1×10^8 CFU/ml, which is equivalent to 0.5 Mc Farland turbidity standards to density (Perilla et al., 2003) was used throughout the experimentation.

2.5 Bioassay of crude plant extracts:

In the present study, the antimicrobial activity of various plant parts *i.e.* leaf, stem, fruit, inflorescence and whole plant extracts in different solvents (ethanol and distilled water) were screened by agar well diffusion method (Perez et al., 1990). The prepared agar plates were marked with organism and extract name. Fresh bacterial culture inoculum of 100 µl having 1×10^8 cfu/ml cell density was spread on agar plates with sterile glass spreader. A well of 8 mm diameter punched off at previously marked Petri plates into nutrient agar medium with sterile cork borer and then filled with l00µl of each plant extract. Plates were placed in a refrigerator for 30 minutes for pre-diffusion of plant extracts and then incubated at 37 °C for bacteria until the appearance of inhibition zone. After incubation, plates were examined and zone of inhibition (excluding well diameter) was measured as a property of antimicrobial activity.

2.6 Assay of antibacterial activity

The antimicrobial activities of plant extracts were determined by disc diffusion test (Perez et al., 1990) and micro-dilution assay (Okeke et al., 2001). The two separated fractions of plant extracts *i.e.* ethanol and water fractions were pooled together and concentrated with the solvent recovering assembly. Concentrated fractions were dried and checked for presence of antimicrobial activity by modified agar well diffusion method. Bacterial strains were

cultured overnight in Nutrient agar (HiMedia, Mumbai) at 37±2°C. Overnight grown culture of microorganisms was used for inoculums preparation. A loopful of isolated colony was inoculated in 4ml of Peptone water (HiMedia, Mumbai) at 37°C for 2 h. The turbidity of resulting suspension was compared to 0.5 McFarland turbidity standards. The level of turbidity was equivalent to approximately 3.0×10^5 cfu/ml. The Mueller Hinton Agar media (HiMedia, Mumbai) was prepared and poured into Petri dishes. Once the media solidifies it was then inoculated with micro-organism suspended in peptone water. The media was then punched with 6 mm diameter hole and filled with extract and control (positive and negative). Vancomycin (20µg/ml) was taken as positive control for bacterial strains and 100% DMSO was used as negative control. The experiment was performed at two different concentrations (8 and 10 mg). The bioassay was carried out in triplicates to eliminate any error. The Petri dishes were incubated for 24 h at 37±2°C for bacteria. The antimicrobial activity was calculated by measuring the diameter of zone of inhibition in millimetres around the well.

2.7 Determination of minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC)

In the present study, minimum inhibitory concentration (MIC) was evaluated by serial broth dilution method (Subramanian et al., 2002) for the plant extracts showing more than 7mm 30ml of inhibition. Density of bacterial suspension was maintained uniformly throughout the experiment at 1×108 CFU/ml by comparing with 0.5 Mc-Farland turbidity standards. About 40µl of plant extract from stock solution (l00mg/ml) was taken into the first dilution tube and added 960µl of nutrient broth and mixed well. About 500µl of solution from first dilution tube was taken and added 500µl of nutrient broth into second tube, this step was repeated 5 times and from last tube 500µl solution was discarded. Final volume was made up to 1ml by adding 500µl of test organism in each tube. The MIC was tested in the concentration range between 8mg/ml to 0.250 mg/ml. Tubes were incubated at 37°C for 24 hours in an incubator. About l00µl (0.1%) 2,3,5- triphenyl tetrazolium chloride solution as a growth indicator was incorporated in each tube to find out the bacterial inhibition and tubes were further incubated for 30 minutes at 37°C. Bacterial growth was visualized when colourless 2, 3, 5-triphenyl tetrazolium chloride was converted into red color formation in the presence of live bacteria. MIC assay was repeated thrice by using DMSO and nutrient broth as controls.

To determine the minimum bacterial concentration (MBC), l00µl of broth was collected from those tubes tested for determination of MIC which did not show any growth and spread on sterile nutrient agar plate for any bacterial growth. Plates were incubated at 37°C for 24 hours. After incubation the concentration at which no visible bacterial growth was observed considered as the minimum bactericidal concentration (Doughari, 2008).

2.8 Statistical analysis

Data were expressed as the mean ± S.E.M. For statistical analysis of the data, group means were compared by one-way ANOVA with *Post Hoc* analysis. The Tukey–Karmer *Post Hoc* test was applied to identify significance among groups. Graphs are plotted using MATLAB version 7.8.0 R2009a, Natick, Massachusetts: The Math works Inc. 2009. The *p*-value 0.001 was considered to be statistically significant.

RESULTS

3.1 Phytochemical screening of plant extracts

Phytochemical screening of the selected plant extract showed the presence of different percentage of active phytochemicals such as alkaloid, flavonoid, phenolic, saponin, tannin etc. (Table 2). The phenolic content of ethanolic extract was found to be 9.4 mg GAL/g while 6.8 mg GAL/g per gram dry weight basis for aqueous extract (Table 3). The flavonoid content of ethanolic extract in terms of quercetin equivalent was found to be 0. 38 mg/g while for aqueous extract it was found to be 0.46 mg/g (Table 3). The difference in total phenolic and flavonoid content of EtOH, and aqueous extracts between two plants was found statistically significant ($p < 0.01$). The IC_{50} value of *A. racemosus* in ethanol was found to be 0.2016 µg/ml while that in water was found to be 0.2248 µg/ml (Table 6 and Figure 2).

Table 2. Qualitative determinations of active ingredients in alcoholic and water extracts of *Asparagus racemosus* plants

Plant constituents	Extract of *Asparagus racemosus*	
	EtOH	Aq
Alkaloids	+	+
Saponins	+	+
Flavonoids	+	+
Fixed oils and fats	+	+
Tannins and phenolic compounds	+	+

Table 3. Total phenolic content (expressed as mg Gallic acid (GAL) equivalent/g dry weight) and flavonoid content (expressed as mg Quercetin solution equivalent/g dry weight) of *Asparagus racemosus* plant extracts.

Plant	Extracts	Total phenol	Total flavonoid
Tinospora cardifolia	Ethanol	9.4	0.38
	Water	6.8	0.46

3.2 Antimicrobial activity of plant extracts

The plant extracts showed different degrees of antimicrobial activity depending on the concentration of extracts, type of solvent used for extraction and the bacterial strains tested for susceptibility assay (Table 4 and Figure 1). The collective analysis of antimicrobial activity of extract indicated that among the two medicinal plants used in the study the ethanolic extracts exhibited better antibacterial activities than aqueous extracts. The ethanolic extract of *A. racemosus* showed maximum antibacterial activity with maximum diameter of zone of inhibition against the four strains (Table 4 and Figure 1). At 500 mg/ml concentration, the ethanolic extract of *A. racemosus* on *Bacillus subtilis*, *Bacillus cereus, Escherichia coli* and *Salmonella typhi* showed maximum zone of inhibition of 6.0 mm, 7.0 mm, 7.5 mm and 7.0 mm respectively (Table 4 and Figure 1).

Again the aqueous extracts of the plant showed different degrees of antimicrobial activity depending on the concentration of extracts and the bacterial strains tested for susceptibility assay (Table 4 and Figure 1). The aqueous extract of *A. racemosus* showed maximum antibacterial activity with maximum diameter of zone of inhibition against the four strains (Table 4 and Figure 1). At 500 mg/ml concentration, the ethanolic extract of *A. racemosus* on *Bacillus subtilis*, *Bacillus cereus, Escherichia coli* and *Salmonella typhi* showed maximum zone of inhibition (Table 4 and Figure 1). The result also showed that both the ethanolic extract and aqueous extracts of *A. racemosus* in agar diffusion test at the concentrations of 250 mg/ml also showed an effectual zone of inhibition of all the tested micro-organisms.

Table 4. Antimicrobial activities of the investigated plant *A. racemosus* in agar diffusion test.

Plant	Extracts	Concentration in mg/ml	Zone of inhibition (mm)			
			B. s.[a]	B. c.[b]	E. c.[c]	S. t.[d]
Asparagus racemosus	Ethanol	500	6.0	7.0	7.5	7.0
		250	–	4.0	4.0	3.5
		125	–	–	–	–
		60	–	–	–	–
		30	–	–	–	–
		15	–	–	–	–
	Water	500	5.5	4.0	7.5	7.0
		250	–	3.5	4.0	3.5
		125	–	–	–	–
		60	–	–	–	–
		30	–	–	–	–
		15	–	–	–	–

Note: [a] **B. s. *Bacillus subtilis;*** [b] **B. c., *Bacillus cereus*;** [c] **E. c., *Escherichia coli;*** [d] **S. t., *Salmonella typhi*.**

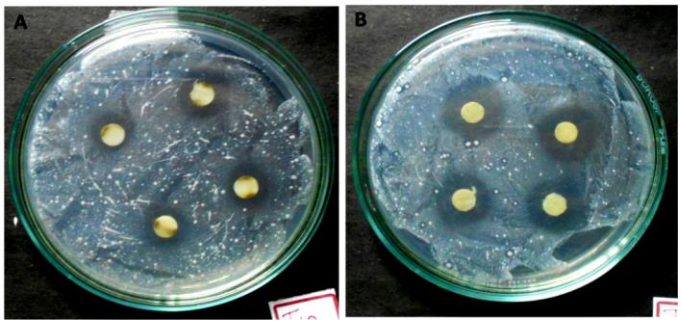

Figure 1: Antimicrobial activities of the investigated plant *Asparagus racemosus* in agar diffusion test at 500 mg/ml concentration of ethanol (A) and water (B).

3.3 Minimum inhibitory concentration (MIC)/ Minimum bactericidal concentration (MBC)

The minimum inhibitory concentration (MIC) in µg/ml minimum bactericidal concentration (MBC) in µg/ml of the plant extract on four bacterial strains was carried out by Broth dilution method (Table 5). The statistically significant results were obtained with *p* value of 0.001 ($p<0.05$). Data obtained from the MIC indicated that plant extracts in ethanolic medium had the same effects as of aqueous extract on the solid medium (disc diffusion agar) and were observed to be concentration dependent (Table 5). The minimum inhibitory concentration (MIC) as well as minimum bactericidal concentration (MBC) in case of the investigated plant extract of *Asparagus racemosus* against bacterial strains of *E. coli* had been found to be 250 µg/ml.

DISCUSSIONS

Plants produce a diverse range of bioactive molecules, making them rich source of different types of medicines. Most of the drugs today are obtained from natural sources or semi synthetic derivatives of natural products and used in the traditional systems of medicine. It has been reported that between the years 1983 and 2016 (Cragg et al., 2017), the systematic screening of antibacterial plant extracts represents a continuous effort to find new compounds with the potential to act against multi-resistant bacteria. Plants have been reported to contain large varieties of chemical substances that possess important preventative and curative therapies. Despite the presence of various approaches to drug discovery, plants still remain the main reservoir of natural medicines (Bharti et al., 2018).

Phytochemical constituents are responsible for medicinal and antimicrobial activity of plant species (Parveen and Sharma, 2014). Secondary metabolites found in plants are the main reason for their antimicrobial potential. Several workers throughout the world have carried out antimicrobial studies on some medicinal plants including *Asparagus racemosus* (Madhavan et al., 2010). Other names of the plant include Satavare (Hindi), Buttermilk root, Climbing asparagus and Wild asparagus (English) etc. In Nepal it is called kurilo. The name "shatawari" means "curer of a hundred diseases" (shatum: "hundred"; vari: "curer") (Sharma et al., 2009; Hayes et al., 2008; Saxena and Chourasia 2001).

Asparagus racemosus is an important plant in traditional medicine in tropical and subtropical India. Its medicinal use has been reported in the Indian and British Pharmacopoeias and in traditional systems of medicine such as Ayurveda, Unani and Siddha (Gomase et al., 2010). The root of the plant is used to promote milk secretion and as demulcent, diuretic, aphrodisiac, antiseptic antiparasitic, antitumor and antidiarrhea. It is also used to treat debility, (especially in women), infertility, and impotence, menopause problems, for stomach ulcers, hyperacidity, dehydration, cough and chronic fevers. The appropriate dose of *Asparagus racemosus* depends on several factors such as the user's age, health, and several other conditions (Sharma et al., 2009; Hayes et al., 2008; Saxena and Chourasia 2001). The plant is also used in dyspepsia, constipation, stomach spasms and stomach ulcers. It is also used for fluid retention, pain, anxiety, cancer, bronchitis, tuberculosis, dementia and diabetes. The antidiabetic properties have been shown due to its stimulation in insulin secretion. Some people use it to ease alcohol withdrawal. Women use various parts of plants for premenstrual syndrome and uterine bleeding; and to start breast milk production. *Asparagus racemosus* is also used to increase sexual desire (as an aphrodisiac). The mechanism of action of *Asparagus racemosus* for above properties is not well defined. However some scientific research documents suggest that *Asparagus racemosus* has antioxidant and antibacterial effects, and might improve the immune system. *Asparagus racemosus*

might have an effect like a water pill or diuretic (Sharma et al., 2009; Hayes et al., 2008; Saxena and Chourasia 2001).

Kumar et al., (2013) suggested that the presence of alkaloids and phenols in leaves, corn and roots of *Ensete superbum* may be tested for antimicrobial activities. With this knowledge in background the present study was designed with the aim to evaluate and compare the effects of ethanolic (EtOH) and aqueous (H_2O) extracts of *Asparagus racemosus* according to phytochemical aspects for antibacterial efficacies. Phytochemical screening of the selected plant extracts showed the presence of different percentage of active phytochemicals such as alkaloid, flavonoid, phenolic, saponin, tannin etc. (Table 4.1 and Table 4.2). The chemical constituents of *Asparagus racemosus* include a polycyclic alkaloid Asparagamine A, which has been isolated from the dried roots. In addition, Steroidal saponins, Shatavaroside A, Shatavaroside B, Filiasparoside C, Immunoside, Shatavarins, and Schidigerasaponin D5 (or asparanin A) have also been isolated from the roots of *Asparagus racemosus*. An isoflavone 8-methoxy-5,6,4'-trihydroxyisoflavone 7-*O*-β-D-glucopyranoside has also been isolated from the plant (Goyal et al., 2003; The Ley Group, 2012; Sekine, 2010; Sharma et al., 2009; Hayes et al., 2008; Saxena and Chourasia 2001).

Table 5. Minimum inhibitory concentration (MIC) (µg/ml) and minimum bactericidal concentration (MBC) (µg/ml) of the investigated plant extract of *Asparagus racemosus* against bacterial strains.

Plant	Extract	B. s.[a]		B. c.[b]		E. c.[c]		S. t.[d]	
		MIC[i]	MBC[j]	MIC[i]	MBC[j]	MIC[i]	MBC[j]	MIC[i]	MBC[j]
Asparagus racemosus	E	1000	500	500	250	250	250	500	250
	W	1000	500	500	500	250	250	500	500

Note: [a] **B. s. *Bacillus subtilis;*** [b] **B. c., *Bacillus cereus*;** [c] **E. c., *Escherichia coli;*** [d] **S. t., *Salmonella typhi*;** [i] **MIC, minimum inhibitory concentration;** [j] **MBC, minimum bactericidal concentration.**

Table 6. IC_{50} values (µg/ml) for DPPH radical scavenging activity of the selected plant extract of *Asparagus racemosus* and phytochemical screening.

Plant	Extracts	10 µg/ml	50 µg/ml	100 µg/ml	500 µg/ml	1000 µg/ml	IC_{50}
Asparagus racemosus	Ethanol	40.82	49.90	56.20	57.00	60.35	0.2498
	Water	35.79	39.65	39.85	41.80	46.67	0.2890

Note: **IC, inhibitory concentration**

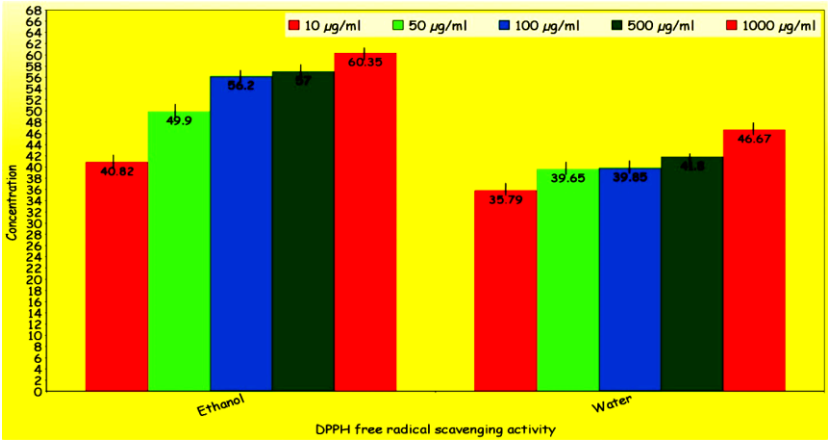

Figure 2: DPPH radical scavenging activity of the selected plant extract of *Asparagus racemosus*.Ishaku et al., (2013) investigated the antibacterial activity of *G. arborea* fruit methanol and hexane extracts against several isolates from diagnostic labs and found significant activities in tested organisms. El-Mahmood et al., (2010) screened leaf, stem bark extracts for presence of phytochemicals alkaloids, saponins, tannins and anthraquinones which showed antimicrobial activity against a number of pathogenic microorganisms. The prepared leaf extracts in this study also showed similar results. These results are almost comparable with the reference antibiotics activity.

In addition, Rahman et al., (2009) proposed that the aqueous and ethanolic extracts of *Moringa oleifera* have antimicrobial activity against *Microsporum canis, Aspergillus fumigatus*, *Candida albicans*, *Escherichia coli* and *Staphylococcus aureus* by disc diffusion method. The data pertaining to the antibacterial and antifungal potential of the plant extracts and the inhibition zone formed by extracts and minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) values against bacteria are presented in Table 4.5. The results obtained reveal that the medicinal plant shows different degree of inhibition against the selected micro-organisms in different solvent *viz.,* ethanol and aqueous. The diameter of zone of inhibition produced depends on several factors broadly classified as extrinsic and intrinsic parameters. However, intrinsic factors such as nature of medicinal and aromatic plants including its components, solubility and diffusing property are predetermined. Due to variable infusibility, the antibacterial activity with very high potency may not be demonstrated a zone of inhibition commensurate to its efficacy (Prasai et al., 2004).

In classifying the antimicrobial activity of gram-positive and gram-negative micro-organisms, it would generally be expected that a much greater number of antimicrobial agents would be active against Gram-positive than Gram-negative bacteria. In case of bacteria the basis for their differences in susceptibility might be due to the differences in the cell wall composition of gram-positive and gram-negative bacteria (Saranraj et al., 2011). The Gram negative bacteria having an outer phospholipid membrane carrying the structural lipopolysaccharide components, makes the cell wall impermeable to lipophilic solutes, while protein constitutes a selective barrier to the hydrophilic solutes (Nikaido and Vaara, 1985). Gram-positive bacteria should be more susceptible having only an outer permeability barrier. In this study, the extract showed some degree of activity against one or more of the bacterial and fungal strains extracts. The antimicrobial activities of plant extracts against different selected organisms are broadly classified in to five categories as: a) better activity with more than 20 mm of zone of inhibition, b) good activity with 15 mm to 20 mm of zone of inhibition, c) moderate activity with 6 mm to 15 mm of zone of inhibition, d) least activity with 1 mm to 5 mm of zone of inhibition, e) no activity with zero zone of inhibition. The zone of inhibition (mm) of various plant extracts against the tested organisms was shown in Table 4 as well as in Figure 1.

On the same track, Yagoub (2008) screened petroleum ether, ethanol and aqueous extracts against clinical isolates of *E. coli* from urine samples and found ethanol extract was more effective than other extracts. The antimicrobial activity of crude ethanolic and aqueous stem and root bark extracts of *Adansonia digitata* and stem bark extract is found to be more active against *E. coli* and *Salmonella* sp. in comparison with root bark extract. The antibacterial activity of ethanol and chloroform extracts against a number of pathogenic micro-organisms was found to be more active than chloroform extract. In our study ethanolic extracts have shown better activity against selected gram positive bacteria than gram negative bacteria. However, aqueous extract has shown moderate activity against pathogenic micro-organisms.

Bioactive compounds are normally accumulated as secondary metabolites in all plant cells but their concentration varies according to the plant parts, season, climate and particular growth phase. Fruit is one of the highest accumulator plant parts of such compounds and people are generally preferred it for therapeutic purposes. Some of the active compounds inhibit the growth of disease causing microbes either singly or in combination (Cowan, 1999; Murari et al., 2016). They can inhibit the growth of microbes by binding their surface proteins, breaking the peptide bonds, acting as chelating agents, altering their biochemical systematic or by preventing utilization of available nutrients to the microorganisms. Some compounds also cause lyses of microbial cells (Cowan, 1999). In the present study different solvent extract (ethanolic and aqueous) of *Asparagus resmosus* was evaluated according to phytochemical aspects showed the presence of different percentage of active phytochemicals such as alkaloid, favonoid, phenolic, saponin, tannin etc. The correlation between antibacterial and antioxidant activity and chemical composition: (phenols, quinones, flavones, tannins, terpenoids, and alkaloids) has been well documented (Akgul and Gulshen, 2005, Doughari and Manzara, 2008).

Plant's phenolics and flavonoids have also been documented as free radical scavengers and antioxidants (Amal et al., 2009; Pourmorad et al., 2006). The alcoholic extracts of the plant showed an effectual free radical scavenging in the DPPH assay in comparison with aqueous extracts (Table 6). The antioxidant activity of extract strongly depends on the extraction solvent. The ethanol and water are the main solvents for extraction of poly phenolic compound compared to other solvents such as acetone, ethyl-acetate that are the causes of the

efficiency reduction of the plant extraction methods (Akgul and Gulshen 2005). The alcoholic extracts of the plant showed an effectual free radical scavenging in the DPPH assay in comparison with aqueous extracts.

Datta et al., (2011) found that ethanol extract showed strong free radical scavenging activity and significant antimicrobial activity against clinical isolates of *Pseudomonas aeruginosa, Klebsiella pneumonia, Proteus vulgaris* and *Staphylococcus aureus.* The minimum inhibitory concentration of the leaf extract was observed in the range of 25 µg to 100 µg for the bacterial strains. In this study ethanolic extracts and aqueous extracts of the plant extracts have shown considerable high antibacterial activity against most of the test organisms. Based on the results the extracts which showed better to good activity contain antibacterial principles which can be evaluated further.

The plant extracts showed different degrees of antimicrobial activity depending on the concentration of extracts, type of solvent used for extraction and the bacterial strains tested for susceptibility assay (Table 4 and Figure 1). The collective analysis of antimicrobial activity of extract indicated that the ethanolic extracts exhibited better antibacterial activities than aqueous extracts. The ethanolic extract of *Asparagus resmosus* showed maximum antibacterial activity with maximum diameter of zone of inhibition against the four strains (Table 4 and Figure 1).

Accordingly, the result also showed that both the ethanolic extract and aqueous extracts of *Asparagus resmosus* in agar diffusion test at the concentrations of 250 mg/ml also showed an effectual zone of inhibition of all the tested micro-organisms. The ethanol extracts exhibited better antibacterial activities than aqueous extracts. The differences in bacterial susceptibility to the extracts is perhaps due to the differences in cell wall and/or genetic composition (Karaman et al., 2003) or due to the differences in the composition, concentrations and the mechanism of action of the bioactive compounds. In addition, the possibilities of interaction with other constituents are also likely (Karaman et al., 2003).

Determination of minimum inhibitory concentration (MIC) involves exposing the test organism to serially diluted extract and determining the minimum concentration that inhibits growth (Bharti et al., 2018). The four micro-organisms were tested for their ability to produce visible growth in presence of serially diluted antimicrobial agents. The samples were removed, serially diluted and the numbers of surviving bacteria were determined by plating on agar media. The results are almost comparable with the reference antibiotics tested in this study. Data obtained from the MIC indicated that plant extracts in liquid medium had the same effects on the solid medium (disc diffusion agar) and were observed to be concentration dependent (Table 5). Comparison of minimum inhibitory concentration (MIC) in µg/ml minimum bactericidal concentration (MBC) in µg/ml of the plant extracts on four bacterial strains was carried out by Broth dilution method (Table 5). The statistically significant results were obtained with *p* value of 0.001 ($p<0.05$). Data obtained from the MIC indicated that plant extracts in ethanolic medium had the same effects as of aqueous extract on the solid medium (disc diffusion agar) and were observed to be concentration dependent (Table 5). The minimum inhibitory concentration (MIC) as well as minimum bactericidal concentration (MBC) in case of the investigated plant extract of *Asparagus resmosus* against bacterial strains of *E. coli* had been found to be 250 µg/ml. The inhibitory activity of *Asparagus resmosus* extract compared to other extracts against the pathogenic bacteria confirms the anti-infective potential of this plant.

Masola et al., (2009) obtained MIC values *A. digitata* stem bark extracts have shown varied sensitivity (1.5 mg/ml to 12 mg/ml) against various pathogenic microorganisms. In this study the ethanol and aqueous extracts have shown sensitivity between 2-8 mg/ml which appeared to be significant. No antimicrobial activity was seen in lower concentration of aqueous extracts against tested bacterial strains. Thankamani et al., (2011) found no antimicrobial activity in hexane flower extracts of *Alstonia schlorais* against *Salmonella typhi* and *Lactobacillus* sp. as well as in water extract against *Pseudomonas aerouginosa* and *S. typhi.* In this study also no activity was noticed against the tested organisms for all the extracts. Kumar et al., (2013) evaluated antimicrobial activity against twenty pathogenic microorganisms and tested leaf extracts prepared in various solvents i.e. benzene, chloroform, acetone, methanol and distilled water and found solvent extracts were more effective at variable inhibition zone against tested organisms than distilled water extracts. The MIC values of ethanolic extract revealed significant activity and lowest MIC values were obtained against the tested microorganisms. In this study, both gram positive and gram negative bacteria appeared to be more susceptible to ethanolic extracts of plants in comparison with other organisms.

CONCLUSION

Thus, the present investigation report systematically and validate that the investigated plant extracts put forth the antibacterial effect. Such a method may provide a treatment that is simple, relatively inexpensive and could be incorporated into the normal diet of the patient, which is highly favorable. Clinical data validate can be used as a kind of validation of the ethno pharmacological and the clinical phytotherapeutic use while more advanced computational techniques can explicate the antimicrobial property of their phytochemicals. Further studies on pharmacokinetics and clinical efficacy have to be performed to prove the antimicrobial effects under *in vitro* and *in vivo* conditions.

REFRERNCES

1. Akgul C, Gulshen S. Antibacterial activity of crude methanolic extract and its fractions of aerial parts of *Anthemis tinctoria*. Ind J Biotech Biophys 2005; 42: 395–397.
2. Amal A, Ashraf A, Hossam ES. Chemical compositions and antioxidant/ antimicrobial activities of kaffmaryam (*Anastatica hierochuntica*) and doum palm (hyphaenethebaica) cultivated in Egypt. J Bio Bilimleri Arastirma Dergisi 2009; 2(2): 71–79.
3. Berry RE, Klumpp DJ, Schaeffer AJ. Urothelial cultures support intracellular-bacterial community formation by uropathogenic *Escherichia coli*. Infect Immun 2009; 77(7):2762-72.
4. Bharti SK, Krishnan S, Kumar A, Kumar A (2018). Antidiabetic phytoconstituents and their mode of action on metabolic pathways. Therapeutic Advances in Endocrinology and Metabolism. 9(3) 81–100.
5. Murari K, Sharma NK, Bharti SK (2016). The coming age of future of medicine: Next Frontier. Biochemistry & Analytical Biochemistry, 5(297): Doi:10.4172/2161-1009.1000297.
6. Bharti SK, Krishnan S, Kumar A, (2016). Phytotherapy for diabetes mellitus: back to nature. Minerva Endocrinologica, 41(1):143-6.
7. Bharti A, Krishnan S, Bharti SK (2015). Personalized Diabetes Clinics Revisited. Biochemistry & Analytical Biochemistry, 4(4): 1000e159.
8. Bharti SK, Krishnan S, Kumar A, Gupta AK, Kumar A (2015). Mechanism-based antidiabetic activity of Fructo- and isomalto-oligosaccharides: Validation by *in vivo, in silico* and *in vitro* interaction potential. Process Biochemistry, 50(2):317–327.
9. Bharti SK, Krishnan S, Sharma NK, Kumar A, Prakash O, Gupta AK, Kumar A (2015). *In vivo* and *in silico* investigation of antidiabetic activity of fruit of *Withania coagulans* Dunal. Current Hypertension Reviews, 2015;11(2):143-58.
10. Bharti SK, Krishnan S, Kumar A, Razak KK, Murari K, Bharti BK, Gupta AK (2013). Antidiabetic activity and molecular docking of Fructooligosaccharides produced by *Auridobasidium pullulans* in poloxamer-407 induced T2DM rats. Food Chemistry, 136(1):813-821.
11. Bharti SK, Krishnan S, Kumar A, Razak KK, Murari K, Bharti BK, Gupta AK (2012). Antihyperglycemic Activity with DPP-IV Inhibition of Alkaloids from seed extract of *Castanospermum australe*: Investigation by experimental validation and molecular docking. Phytomedicine, 20(1):24–31.
12. Bharti SK, Kumar A, Sharma NK, Prakash O, Krishnan S, Gupta AK, Kumar A (2013). Tocopherol from seeds of *Cucurbita pepo* against diabetes: Validation by *in vivo* experiments supported by computational docking. Journal of the Formosan Medical Association, 112(11):676–690.
13. Bharti SK, Kumar A, Prakash O, Krishnan S, Gupta AK (2013). Essential oil of *Cymbopogon citratus* against diabetes: Validation by *in vivo* experiments and computational studies. Journal of Bioanalysis and Biomedicine, 5(5):194-203.
14. Bharti SK, Sharma NK, Gupta AK, Padamdeo SR (2012). Antidiabetic effect of aqueous extract of *Withania coagulans* flower in Poloxamer-407 induced type 2 diabetic rats. Journal of Medicinal Plants Research, 6(45):5706-5713 (Academic Journals publishing).
15. Chang C, Yang M, Wen H, Chern J (2002) Estimation of total flavonoid content in propolis by two complementary colorimetric methods. J. Food Drug Analaysis, 10: 178-182.
16. Cowan, M.M., (1999). Plant Products as Antimicrobial Agents. *Clinical Microbiology Reviews,* 12:564-582.
17. Cragg GM, Boyd MR, Khanna R, Kneller R, Mays TD, Mazan KD, Newman DJ, Sausville EA (2017). International collaboration in drug discovery and development: the NCl experience. Pure Appl. Chem. 71: 1619-1633.
18. Datta, A., Ghosh, S. and Singh, M. (2011). Antimicrobial property of *Piper betel* leaf against clinical isolates of bacteria. *International Journal of Pharma Sciences and Research,* 2(3): 104-109.
19. Doughari J, Manzara, S. (2008). *In vitro* antibacterial activity of crude leaf extracts of *Mangifera indica* Linn. Afr J Microbiol Res; 2: 67–72.
20. Einstein, B.I., Mandell, G.L., Bennett, J.E. and Dolin, E. (2000). (Eds.) Douglas and Bennett's Principles and Practice of Infectious Diseases. Churchill Livingstone, New York, USA.
21. El-Mahmood, A.M., Doughari, J. H. and Kiman, H.S. (2010). *In vitro* antimicrobial activity of crude leaf and stem bark extracts of *Gmelina arborea* (Roxb) against some pathogenic species of *Enterobacteriaceae. African Journal of Pharmacy and Pharmacology,* 4(6): 355-361.
22. Falagas ME, Kotsantis IK, Vouloumanou EK, Rafailidis PI. (2009). Antibiotics versus placebo in the treatment of women with uncomplicated cystitis: a meta-analysis of randomized controlled trials. J Infect; 58:91-102.
23. Ghosh, S. & Saha, S. Tinospora cordifolia: One plant, many roles. Anc. Sci. Life 2012; 31(4): 151-9.

24. Gomase, V.S and Sherkhane, A.S. (2010). Isolation, structure elucidation and biotransformation studies on secondary metabolites from Asparagus racemosus. Inter. J. Microbio., 2:7-9.
25. Goyal, R. K.; Singh, J; Lal, H (2003). *"Asparagus racemosus*- an update". *Indian journal of medical sciences. **57** (9): 408–14.*
26. Harborne JB (1991). Phytochemical methods**.** Chapman and Hall, London.
27. Houghton, P.J., Howes, M.J., Lee, C.C. and Steventon, G. (2007). Uses and abuses of *in vitro* tests in ethnopharmacology: visualizing an elephant. *Journal of Ethnopharmacology,* 110:91-400.
28. Hayes, Patricia Y.; Jahidin, Aisyah H.; Lehmann, Reg; Penman, Kerry; Kitching, William; De Voss, James J. (2008). "Steroidal saponins from the roots of *Asparagus racemosus". Phytochemistry. **69** (3): 796.*
29. Hugo, W.B. (1992). Biology of microorganisms. IN Hugo, W.B. and Russell, A.D. (Eds.) *Pharmaceutical Microbiology*. Blackwell Scientific Publications, Oxford, UK.
30. Ishaku. A., Ishakeku, D. and Agwale, S. (2013). Phytochemical screening and antibacterial activity of *Gmelina arborea* fruit extracts. *International Journal of Microbiology and Immunology Research,* 1 (2): 026-031.
31. Kalayou, S., Haileselassie, M., Gebre-egziabher, G., Tiku, T., Sahle, S., Taddele, H. and Ghezu, M. (2012). In-vitro antimicrobial activity screening of some ethnoveterinary medicinal plants traditionally used against mastitis, wound and gastrointestinal tract complication in Tigray Region, Ethiopia. *Asian Pacific Journal of Tropical Biomedicine,* 2(7): 512-522.
32. Karaman I, Shahin F, Gulluce M, Ogutsu H, Sngul M, Adiguzel A. Antimicrobial activity of aqueous and methanol extracts of Juniperus oxycedrus L. J Ethnopharmacol 2003; 85: 231-235.
33. Kumar, A., Jha, K.K., Kumar, D., Agrawal, A. and Gupta, A. (2012). Preliminary phytochemical analysis of leaf and bark (mixture) extract of *Ficus Infectoria* plant. *The Pharma Innovation,* 1(5): 71-76.
34. Kumar, M., Nehra, K. and Duhan, J.S. (2013). Phytochemical analysis and antimicrobial efficacy of leaf extracts of *Pithecellobium Dulce. Asian Journal of Pharmaceutical and Clinical Research,* 6(1): 70-76.
35. Madhavan, V., Tijare, R.D., Mythreyi, R., Gurudeva, M.R. and Yoganarasimhan, S.N. (2010). Pharmacognostical studies on the root tubars of *Asparagus gonoclados* Baker- Alternate source for the Ayurvedic drug satavari. Indian. J. nature. Resour., 1(1): 57-62.
36. Mariita, R.M., Ogol, C.K.P., Oguge, N.O., and Okemo, P.O. (2010). Antitubercular and Phytochemical Investigation of Methanol Extracts of Medicinal Plants used by the Samburu Community in Kenya. *Tropical Journal of Pharmaceutical Research,* 9(4): 379-385.
37. Masola, S.N., Mosha, R.D. Wambura, P. N. (2009). Assessment of antimicrobial activity of crude extracts of stem and root barks from *Adansonia digitata* (Bombacaceae) (African baobab). *African Journal of Biotechnology*. 8(19): 5076-5083.
38. Murray, P.R., Rosenthal, K.S., Kobayashi, G.S. and Pfaller, M.A. (1998). Medical Microbiology. Mosby Inc., Missouri, USA.
39. Najar MS, Saldanha CL, Banday KA. Approach to urinary tract infections. Indian J Nephrol 2009; 19:129-39
40. Nikaido, H., and Vaara, M. (1985). Molecular basis of bacterial outer membrane permeability. *Microbiology Reviews,* 49(1): 1-32.
41. Okeke MI, Iroegbu CU, Eze EN, Okoli AS, Esimone CO. Evaluation of extracts of the root of *Landolphia owerrience* for antibacterial activity. J Ethnopharmacol 2001; 78: 119-127.
42. Parveen, T. and Sharma, K. (2014). Phytochemical Profiling of Leaves and Stem Bark of *Terminalia arjuna* and *Tecomella undulate. International Journal of Pharmacy & Bioscience,* 1(1): 1-7.
43. Perez C, Pauli M, Bezevque P (1990). An antibiotic assay by agar well diffusion method. Act. Biol. Med. Exp., 15: 113-115.
44. Perilla MJ, Ajello MS, Bopp C, Elliott J, Facklam R, Popovic T, Wells J (2003) Manual for the Laboratory Identification and Antimicrobial Susceptibility Testing of Bacterial Pathogens of Public Health Importance in the Developing World. Centre for Disease Control and Prevention: National Centre for Infectious Diseases, Atlanta, Georgia, USA.
45. Pourmorad F, Hosseinimehr SJ, Shahabimajd N. Antioxidant activity, phenol and flavonoid contents of some selected Iranian medicinal plants. Afr J Biotechnol 2006; 5(11): 1142–1145.
46. Prasai, T., Lekhak, B. and Baral, M.P. (2004). Antibacterial property of medicinal plants against gram negative bacteria isolated from water. *Proceedings of IV National Conference on Science and Technology,* 2: 410-415.
47. Rahman, M.M., Mominul, M., Sheikh, L, Sharmin, S.A., Islam, S.M., Rahman, A.M., Rahman, M.M. and Alam, M.F. (2009). Antibacterial Activity of Leaf Juice and Extracts of *Moringa oleifera* Lam. against some human pathogenic bacteria. *Journal of Natural Sciences,* 8(2): 219-227.
48. Rathee, D., Rathee, D., Rathee, P. and Rathee, S. (2012). Phytochemical screening and antimicrobial activity of Picrorrhiza kurroa, an Indian traditional plant used to treat chronic diarrhea. *Arabian Journal of Chemistry,* in press.
49. Salazar, R., Pozos, M.E., Cordero, P., Perez, J., Salinas, M.C. and Waksman, N. (2008). Determination of the antioxidant activity of plants from Northeast Mexico. *Pharmaceutical Biology,* 465:166-170.
50. Saranraj P, Siva Sakthi S and Geetha M, (2011). Pharmacological screening of Datura metel and Acalypha indica for its Antifungal activity against fungal pathogens, International Journal of Pharmaceutical Science and Health Care, 1 (2), 15 - 30.
51. Saxena, V. K.; Chourasia, S (2001). A new is flavone from the roots of *Asparagus racemosus. Fitoterapia.*

***72** (3): 307–9.*
52. Sekine, T. (2010). "ChemInform Abstract: Structure of Asparagamine A (I), a Novel Polycyclic Alkaloid from *Asparagus racemosus". ChemInform.* ***26** (5).*
53. Sharma, U; Saini, R; Kumar, N; Singh, B (2009). "Steroidal saponins from *Asparagus racemosus".* *Chemical & pharmaceutical bulletin.* **57** (8): 890–3.
54. Sleigh, D.J. and Timburg, M.C. (1998). Notes on Medical Bacteriology. Churchill Livingstone, Edinburgh, UK.
55. Subramanian M, Chintalwar GJ, Chattopadhyay S. Antioxidant properties of a *Tinospora cordifolia* polysaccharide against iron-mediated lipid damage and gamma-ray induced protein damage. Redox Rep. 2002; 7:137–43.
56. Thankamani, V., James, J., Veettil, A.K.T. and Sagadevan, L.D.M. (2011b). Phytochemical screening and anti-microbial activity of *Alstonia scholaris* flowers (L) R.BR. Fam: Apocynaceae. *International Journal of Pharmaceutical Research and Development,* 3(3): 172-178.
57. The Indian Pharmacopoeia, 2012. http://ipc.nic.in
58. The Ley Group: Combinatorial Chemistry and total synthesis of natural products Archived May 25, 2012, at the Wayback Machine.
59. Vichien
2003. http://wwwpharmchulaacth/vichien/crude-45/cardiac/asparghtm.
60. Yagoub, S.O. (2008) Anti-microbial activity *of Tamarindus indica* and *Adansonia digitata* extracts against *E. coli* isolated from urine and water specimens. *Research Journal of Microbiology,* 3 (3): 193.