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Antibacterial and Phyto-chemical Analysis of Some Medicinal Plants and their Efficacy on Multidrug Resistant Bacteria

Sanjay Kumar[1], Atul Kumar Singh[2], Santosh Kumar Verma[3], Richa Misra[4] and Chandrabhan Seniya[4]*

[1]Department of Botany, Nagaland University, Headquarter Lumami, Nagaland - 798 601, India.
[2]Centre for Research in Nanotechnology and Science,
Indian Institute of Technology, Bombay - 400 076, India.
[3]Department of Life Sciences, ITM University, Gwalior, India.
[4]Department of Biotechnology, Madhav Institute of Technology & Science Gwalior - 474 005, India.

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Plants are the most important resource of herbal medicines and the occurrence of secondary metabolites with many therapeutic activities A major portion of the general inhabitants in developing countries still make use of long-established folk medicine against ailments since Vedic time. The herbal drugs popularity increased and used widespread. The research is still lagging behind to get the efficacy of plant derived medicines on microorganisms. The eight different medicinal plants (*Nyctanthes arbortristis, Vitex negundo, Phyllanthus amarus, Adhatoda vasica, Hemidesmus indicus, Asparagus racemosus, Terminalia arjuna and Terminalia chebula*) were exploited in support of phytochemical test and to revise their antibacterial effectiveness on *E. coli* Dk1 and *Staphyllococus aureus* MRS901.The screening for antibacterial activity of plant part extract was done by agar well diffusion method. The inhibitory effect was analyzed by calculating Zone of Inhibition (ZOI) and minimum inhibitory concentration (MIC) values. The phytochemical screening of bioactive compounds for presence or absence was done. The result showed the maximum ZOI by *Terminalia arjuna* and *Terminalia chebula* (ranges 9-13mm) in both aqueous and methanolic extract than by other plant extracts. The phytochemical screening of bioactive compounds showed the presence of alkaloids, tannins, flavonoids, saponins, terpenoids, glycosides, cardiac glycosides and steroids in aqueous and methanolic extract both. It is suggested that test plants have some antibacterial activity and could be applied on other bacterial strains other than *E. coli* and *S. aureus*.

Key words: Medicinal Plants, *Escherichia coli*, *Staphylococcus aureus,* Phytochemical, Plant Extract.

Historically, plants have provided a source of inspiration for novel drug compounds, as plant derived medicines have made large contributions to human health and well being. The concept of healing the human beings illness with plants appeared and developed between 2500 and 500 BC in India. It was mainly focused on man and his illness and turned into the 'Ayurvedic concept' or 'Science of Life'. The practice of Ayurvedic therapeutics concept consisted of 8 sections divided into 180 chapters and listed 314 plants[1].

The Indian subcontinent is a huge reservoir of therapeutic plants that are utilized in time-honored health cure[2]. There were approximately 20,000 curative vegetation had been documented in India[3] and the traditional communities are using only 7000-7500 plants for curing different diseases[4,5]. The most important constituent of the sum population in developing nations silently exercising established folk medicines against different diseases which found in different plant resources[6].

* To whom all correspondence should be addressed.
Tel.: +91-751-2409320 (O); Fax: +91-751-2664684;
E-mail: chandrabhanseniya@gmail.com

The positive health means metabolically well balanced human beings. The disease evolves from the body due to external factors. Plant derived medicines have been the first line of defense in maintaining health and combating diseases[7,8]. The majorities of medicines were prepared from the plant and animal products, minerals and metals. These local medicinal plant preparations should be scientifically evaluated and disseminated properly to the indigenous population so that could be given better access to efficacious drug treatment and improved health status[9].

Now a days, multiple drug resistance (MDR) has developed among microbes due to the indiscriminate use of commercial antimicrobial drugs commonly used in the treatment of infectious disease [10]. In addition to this, antibiotics are sometimes associated with adverse effects on the host, including hypersensitivity, immune suppression and allergic reactions[11]. It was suggested about the disturbing frequency of antibiotic confrontation in therapeutically significant bacteria[12]. Also, There were quite a lot of information on the antimicrobial action of different herbal extort in diverse area of the globe[13].

An increase in MDR microbes has triggered immense interest in search of new drug or preparations from the natural sources. Antimicrobial activity of medicinal plants is widely spread and a large number of its secondary metabolites showed antimicrobial activity. There is a great structural diversity exist among antimicrobial phytocompounds[14]. Major groups of phytocompounds include essential oils and isolated compounds such as alkaloids, flavonoids, lactones, diterpenes, napthoquinones[15]. The considerable amounts of work have been published on antimicrobial activity of medicinal plants from different parts of the world[2,16-17].

Hasegawa *et al.*,[18] demonstrated that various genoside and aglycones isolated from Panx ginseng adversely affected tetracycline resistance *Staphylococcus aureus* and was reversed by non-toxic concentrations of these compounds. A compound (z-4,5,7,9 trithiadeo 1, 6 diane 9-oxide) was isolated from oil macerated garlic extract and showed broad antimicrobial activity against bacteria and yeast[19]. Ahmad and Beg[20] demonstrated the broad spectrum antimicrobial activity of various plants (*Lawsonia inermis, Eucalyptus sp., Holarrhena antidysenterica, Hemidesmus indicus, Casuarina equisetifolia, Terminalia bellerica, Terminalia chebula, Embelica officinalis, Camellia sinensis, Syzygium aromaticum, Plumbago zeylanica* and *Punica granatum*) against MDR human pathogens. The antimicrobial activity of *Allium sativum* was reported on *Staphylococcus aureus*, *Salmonella typhimurium* and *Yersinia enterocolitica*[21]. The extracts (Petrol, Acetone, Chloroform, and Methanol) of leaves and stems of *Hypericum mysorence* and *Hypericum patulum* showed broad spectrum activity against some tested microbes[22]. *Plumbago zeylanica* (ethyl acetate whole extract) showed the bactericidal activity against *Helicobacter pylori*[23]. The roselle calyx and protocatechuic acid inhibited the growth of methicillin resistant *S. aureus, Klebsiella pneumonia, Pseudomonas aeruginosa and Acinetobactor brumannii*.[24] The studies were directed to see the activity against a variety of test microbes including both pathogenic and non-pathogenic strains. The active compounds for antibacterial activity are yet to be determined in most of the cases.

The situations illustrated above forced scientists to search for new antimicrobial substances. It is a requirement to build up a substitute antimicrobial drugs for the healing of contagious infections from medicinal flora or a regular demand for innovative and valuable remedial resources[25,26].

At present, eight different medically important shrubbery were hold for phytochemical test and examined their antibacterial effectiveness (*In vitro*) on MDR strains of microbes i.e. *E. coli* Dk1 and *S. aureus* 901.

MATERIALS AND METHODS

Collection of medicinal plants

The plant foliage, roots, barks and fruits were collected from Sanjeevani Ayurveda City Centre, Gwalior (Table 1). The Plants were additionally identified for physical distinctiveness of flower, leaf, root and fruit morphology in Department of Botany, Jiwaji University, Gwalior (India).

Plant Parts decoction and Extract Preparation

500g leaves/bark/root/fruits of plant were

collected and washed with tap water, dried in oven (80°C) and crushed to powder form with the help of electric grinder. The powder was wrapped in aluminum foil and stored for further use.

The powdered form of plant parts was suspended in 45 ml of water and methanol (H_2O+CH_3OH) solution to prepare a 10% (5g powder) and 15% (7.5g powder) extort in a 100 ml flask. The flask container was wrapped through the aluminium foil and kept back on rotating shaker (500 rpm) for 48 h. The extract suspensions were funneled twice with cheese cloth (4 -fold) and then with Whattman's paper. The filtrates were saved in falcon tube to make it thick in the form of paste on incubator at 35°C. The thick and dried paste was further liquefied in distilled water for the concentration of 10mg/ml and 15mg/ml for 10% and 15 % extract.

Bacterial culture preparation

Multi Drug Resistant (MDR) Strains of *S. aureus* MRS901 and *E. coli* Dk1 were collected from DRDE (Defence Research & Development Establishment), Gwalior. The strains were revived in Muller Hinton media (Beef infusion solids- 4.0 gm, Starch- 1.5 gm, Casein hydrolysate- 17.5 gm, Agar- 15.0 gm and Distilled water- 1 L, pH 7.4) by streaking and incubated at 37°C for 24 h. The bacterial colony was picked from streaked plate and frequently sub cultured or maintained in Nutrient Broth (Peptone- 10.0 gm, Beef extract- 10.0 gm, Sodium chloride- 5.0 gm and Distilled water- 1 L, pH 7.2). The broth nutrient media along with the bacterial strains were kept in incubator for 24h at 37°C for their complete growth.

For Antimicrobial as-say, microbial cultures were freshly grown at 37°C and appropriately diluted 5 times in normal saline to obtain the cell suspension of 10^5 CFU/ml.

Determination of Zone of inhibition (ZOI) by Agar well diffusion method

The six sterile petriplates were prepared with M H Agar medium for bacterial culture. The two different concentrations (10% and 15%) of water and methanol extracted bacterial solutions were added for *S. aureus* and *E. coli* and incubated for bacterial growth. After bacterial growth in bacterial culture plates, the round wells were made in the bacterial culture M H agar plate by the aid of a microtip (6 mm diameter) and 50 microlitre plant extract s (10% and 15%) was poured in the wells through micropipette and inoculated for 24h at 37°C.

[*ZOI represented in tables as = Total inhibition zone (in mm) – 6mm diameter of well*]

Determination of Minimum Inhibitory Concentration

Broth dilution susceptibility testing method was used to determine the minimal concentration of antimicrobials to inhibit or kill the test strains. The Minimum Inhibitory Concentration (MIC) was determined by measuring about 5ml of nutrient broth into empty sterile tubes. One milliliter of the different concentrations of extract was then added and the test organism. This was then incubated for 24 hours at 37°C. The tubes were then observed for visible growth with the help of a spectrophotometer. The tube with the least concentration of the extract that showed no growth (-ve) was determined as the "MIC". MIC values were taken as the lowest dilution/ concentration of plant extracts, which inhibit growth in the test tube observed by lack of turbidity after incubation and confirmed by spectrophotometrically.

Preliminary Phytochemical Analysis of tested medicinal plants

Some important qualitative experiments were executed for the occurrence or lack of phytochemicals in leaves, barks, roots, and fruits extract (Trease and Evans, 1983 & 1989) such as alkaloids, tannins, hydrolysable tannins, flavonoids, saponins, terpenoides, glycosides, cardiac glycosides and steroids[27].

Test for Alkaloids (Mayer's test)

When picric acid solution was added to the plant parts extract (1:1v/v) and development of orange coloration showed the presence of alkaloids.

Test for Tannins ($FeCl_3$ Test)

3g of the powdered sample was boiled in 50ml distilled water for 3minutes on a hot plate. The mixture was filtered and a portion of the filtrate diluted with sterile distilled water in a ratio of 1:4 and 3 drop of 10% ferric chloride solution added. A blue or green colour indicates the presence of tannins.

Test for hydrolysable tannins

he mixture of 10% ammonia and plants extract in equal volume showed the emulsion on shaking indicates the presence of hydrolysable tannins.

Test for Glycosides

Add dilute H_2SO_4 (25ml)to plant extract (5ml) and heat for 15 minutes, cool the mixture and add 10% NaOH to neutralise the mixture. Add fehling solution A and B (5ml) and appearance of brick red precipitate suggested the presence of glycosides.

Test for Terpenoids (Salkowski test)

5ml of each plant part extract was mixed in 2 ml of chloroform, and concentrated H_2SO_4 (3 ml) was carefully added to form a layer. Formation of reddish brown coloration at the interface shows the positive results for presence of terpenoids.

Test for Cardiac glycosides (Keller-Killani test)

5ml of each plant part extracts was treated with 2 ml of glacial acetic acid containing one drop of ferric chloride solution. A brown ring of the interface indicates a deoxysugar characteristic of cardenolides. A violet ring may appear below the brown ring, while in the acetic acid layer, a greenish ring may form just gradually throughout thin layer which shows the presence of Cardiac glycosides.

Test for Saponins

Froth test for saponins was used. 1g of the sample was weighed into a conical flask in which 10ml of sterile distilled water was added and boiled for 5 min. The mixture was filtered and 2.5ml of the filtrate was added to 10ml of sterile distilled water in a test tube. The test tube was stopped and shaken vigorously for about 30 second. It was then allowed to stand for half an hour. Honeycomb froth indicated the presence of saponins.

Test for Flavonoids

5 ml of dilute ammonia solution were added to a portion of the aqueous filtrate of each plant part extract followed by addition of concentrated H_2SO_4. Formation of yellow color observed in each extract indicated the presence of flavonoids.

Test for Steroids

Two ml of acetic anhydride was added to 0.5 ml methanolic extract of each sample (plant parts) with 2 ml H_2SO_4. The color changed from violet to blue or green in some samples indicating the presence of steroids.

RESULTS AND DISCUSSION

The 10% aqueous extract of *A. racemosus, H. indicus, T. arjuna* and *T. chebula* contains the antibacterial bioactive compounds which inhibits the growth of *E. coli* Dk1, while the growth of *S. aureus* was inhibited by *H. indicus, T. arjuna and T. chebula*. On the other hand, 15% aqueous extract of *A. racemosus, T. arjuna* and *T. chebula* has bioactive compounds to inhibit the growth of both *E. coli* Dk1 and *S. aureus* MRS901 (Table 2-3).

Similarly, 10% methanolic extract of *A. racemosus, T. arjuna* and *T. chebula* contains the antibacterial bioactive compound which inhibit the growth of *E. coli* Dk1, while the growth of *S. aureus* was checked by almost all the plants extract except *V. negundo*. On the other hand, 15% methanolic extract of *A. racemosus, T. arjuna* and *T. chebula* has bioactive compounds to inhibit the growth of *E. coli Dk1* but *S. aureus* MRS901 was inhibited by almost all the plant extracts (Table 4-5).

It could be suggested that *T. arjuna* and *T. chebula* has the maximum effect on *E. coli* Dk1 and *S. aureus* MRS901 with a range of 8-11 and 7-13 ZOI at 10 and 15% of aqueous and methanolic extract respectively (Table 2-5). It might be suggested that *T. arjuna* and *T. chebula* has almost

Table 1. List of medicinal plants and their parts used in the present study

S. No.	Plants Name	Family	Plant Parts used
1	*Nyctanthes arbortristis*	Oleaceae	Leaves
2	*Vitex negundo*	Lamiaceae	Leaves
3	*Phyllanthus amarus*	Euphorbiaceae	Leaves
4	*Adhatoda vasica*	Acanthaceae	Leaves
5	*Hemidesmus indicus*	Asclepiadaceae	Roots
6	*Asparagus racemosus*	Asparagaceae	Roots
7	*Terminalia arjuna*	Combretaceae	Barks
8	*Terminalia chebula*	Combretaceae	Fruits

all phytochemical compounds with ZOI activity than *H. indicus* and *A. racemosus* (Table 7-9).

Minimum inhibitory concentrations (MICs) are defined as the lowest concentration of an antimicrobial that will inhibit the visible growth of a microorganism after overnight incubation. MICs are used by diagnostic laboratories mainly to confirm resistance, but most often as a research tool to determine the *in vitro* activity of new antimicrobials, and data from such studies have been used to determine MIC breakpoints. The MICs for 10 and 15% extracts both in aqueous and methanolic extracts of all selected plants have been determined by standard method and as per our previous study[92]. Table 6 represents expected MIC values for all selected plants against *E. coli* Dk1 and *S. aureus* MRS901.

Phytochemical analysis of leaves, bark, root and fruit extract revealed the presence or absence of phyto-chemicals viz., alkaloids, tannins, hydrolysable tannins, flavonoids, saponins, terpenoids, glycosides, cardiac glycosides and steroids (Table 7-9). Alkaloids are a group of naturally occurring chemical compounds which mostly contain basic nitrogen atoms. Alkaloids, the largest groups of chemical produced by plant, showed antibacterial activities. Tannin is an astringent, bitter plant polyphenolic compound that binds to and precipitates proteins along with various other organic compounds including amino acids and alkaloids[28]. Tannins have been found to form irreversible complexes with proline rich proteins resulting in the inhibition of the cell protein synthesis, play an important role of potent antioxidant[29]. A hydrolysable tannin or pyrogallol type tannin is a type of tannin that on heating with HCl and H_2SO_4 yields gallic or ellagic acid. Nonaka *et al.*,[30] suggested that ellagic acid inhibits the HIV replication in lymphocyte cells. Flavonoids or bioflavonoids are also known as Vitamin P or Citrin. These are plant secondary metabolites or yellow pigments having a structure similar to that of flavones. Flavonoids are phenolic structures containing one carbonyl group complexes with

Table 2. Antibacterial activity of 10% aqueous extracts on *E. coli* Dk1 and *S. aereus* MRS901

S. No	Name of Medicinal Plants	*E. coli* Dk1		*S. aureus* MRS 901	
		Activity	ZOI (mm)	Activity	ZOI(mm)
1.	*Adhatoda vasica*	R	-	R	-
2.	*Asparagus racemosus*	S	6	R	-
3.	*Hemidesmus indicus*	R	6	S	7
4.	*Nyctanthes arbortristis*	R	-	R	-
5.	*Phyllanthus amarus*	R	-	R	-
6.	*Terminalia arjuna*	S	8	S	8
7.	*Terminalia chebula*	S	10	S	9
8.	*Vitex Negundo*	R	-	R	-

Table 3. Antibacterial activity of 15% aqueous extracts on *E. coli* Dk1 and *S. aereus* MRS901

S. No	Name of Medicinal Plants	*E. coli* Dk1		*S. aureus* MRS 901	
		Activity	ZOI (mm)	Activity	ZOI(mm)
1.	*Adhatoda vasica*	R	-	R	-
2.	*Asparagus racemosus*	S	8	S	7
3.	*Hemidesmus indicus*	R	-	R	-
4.	*Nyctanthes arbortristis*	R	-	R	-
5.	*Phyllanthus amarus*	R	-	R	-
6.	*Terminalia arjuna*	S	9	S	8
7.	*Terminalia chebula*	S	11	S	10
8.	*Vitex Negundo*	R	-	R	-

extra-cellular and soluble protein which exhibits antibacterial activity[14], anti-allergic, anti-inflammatory, anti-microbial[31], anti-cancer[32] and anti-oxidant[33]. Saponins are amphipathic glycosides formed soap-like foaming, when shaken in aqueous solutions. Structurally, saponins composed of hydrophilic glycoside moieties which combined with lipophilic triterpene derivatives[34]. The properties may be helpful in anti-bacterial activity. Terpenoids are modified terpenes where methyl group have been moved or removed and oxygen atom added. A glycoside is a molecule in which a sugar is bound to a non-carbohydrate moiety. Most of the plants store this chemical in the form of inactive glycosides and many such plant glycosides are used as medications. Cardiac glycosides are formed as secondary metabolites in several plants and mainly used in treatment of congestive heart failure and cardiac arrhythmia. A steroid is a type of organic compound that contains a specific arrangement of four cycloalkane rings that are joined to each other.

Each medicinal plant tested for their efficacy against MDR *E. coli* Dk1 and *S. aureus* MRS901 was illustrated briefly.

Phyllanthus amarus

P. amarus primarily contains lignans, geranin, flavonoids[35], hydrolyseable tannins[36], amariin[37] and amarulone[38]. The phytochemical screening of *P. amarus* revealed the presence of alkaloids, tannins, hydrolysable tannins, flavonoids, saponins, terpenoids, glycosides, cardiac glycosides and steroids in methanol leaf extract but glycosides and steroids are absent in aqueous leaf extract (Table 7-9). It supports the findings of others. According to Okwu and Josiah[39], flavonoids are antioxidants and might be contributed to antioxidant activity of the plant. It also showed the similar effect with Vitamin E which is also a powerful antioxidant[40]. The pharmacological applications of *P. amarus* includes antibacterial,[41] antioxidant[42] and antiviral[43] activity.

Phyllanthus amarus are highly valued for the treatment of array of human diseases including

Table 4. Antibacterial activity of 10% aqueous extracts on *E. coli* Dk1 and *S. aereus* MRS901

S. No	Name of Medicinal Plants	*E. coli* Dk1		*S. aureus* MRS 901	
		Activity	ZOI (mm)	Activity	ZOI(mm)
1.	*Adhatoda vasica*	R	-	S	7
2.	*Asparagus racemosus*	S	6.5	S	6
3.	*Hemidesmus indicus*	S	6	S	8
4.	*Nyctanthes arbortristis*	R	-	S	6
5.	*Phyllanthus amarus*	R	-	S	6
6.	*Terminalia arjuna*	S	7	S	10
7.	*Terminalia chebula*	S	10	S	12
8.	*Vitex Negundo*	R	-	R	-

Table 5. Antibacterial activity of 15% aqueous extracts on *E. coli* Dk1 and *S. aereus* MRS901

S. No	Name of Medicinal Plants	*E. coli* Dk1		*S. aureus* MRS 901	
		Activity	ZOI (mm)	Activity	ZOI(mm)
1.	*Adhatoda vasica*	R	-	S	5
2.	*Asparagus racemosus*	S	8	S	7
3.	*Hemidesmus indicus*	S	6	S	8
4.	*Nyctanthes arbortristis*	S	6	S	6
5.	*Phyllanthus amarus*	R	-	S	5
6.	*Terminalia arjuna*	S	9	S	11
7.	*Terminalia chebula*	S	12	S	13
8.	*Vitex Negundo*	S	6	S	7

hepatic and urolithic or other renal diseases. In an experiment, a single oral 100-400mg/kg/day of the leaf and seed aqueous extract of *P. amarus* showed their protective effects in acetaminophen and gentamicin induced nephrotoxic Wistar rats for 14 days. In the acetaminophen nephrotoxic rats, 100–400 mg/kg/day significantly ($p < 0.05$, $p < 0.01$, $p < 0.001$) attenuated elevations in the serum creatinine and blood urea nitrogen levels in dose related fashion, as well as, attenuation of acetaminophen-induced tubulonephrosis. Similar effects were also recorded in the gentamicin model of acute renal injury[44]. Results suggest that the nephroprotective effect of *P. amarus* could be due to the inherent antioxidant and free-radical-scavanging principle(s) contained in the extract. In the near future, *P. amarus* could constitute a lead to discovery of a novel drug for the treatment of drug-induced nephrotoxicity.

Adhatoda vasica

A. vasica mainly consists of pyrroquinazoline alkaloids[45,46]. The phytochemical screening of *A. vasica* revealed the presence of alkaloids, tannins, hydrolysable tannins, flavonoids, saponins, terpenoids, glycosides, cardiac glycosides and steroids in methanol leaf extract but flavonoids and cardiac glycosides are absent in aqueous leaf extract (Table 7-9). All the parts of plant have been used for the treatment of various ailments of respiratory tract in both children and adult[47]. The plant is used as an ingredient of numerous popular formulations (Bisolvon, Kada, Fermiforte, Spirote) including cough syrup used in combination with ginger and tulsi [48,49]. It exerts its action as an expectorant and antispasmodic. The pharmacological application of *A. vasica* includes antibacterial[50], anti-inflammatory[51] and antitussive[52] activity.

Vinothapoosan and Sundar[53] reported that the methanolic, chloroform and diethyl ether extract ointment (10% w/w) of *A. vesica* has significant wound healing activity.

Hemidesmus indicus

The roots of *H. indicus* contain steroids, terpenoids, flavonoids and saponins but alkaloids are absent[54,55]. The phytochemical screening of *H. indicus* revealed the presence of alkaloids, tannins, hydrolysable tannins, flavonoids, saponins, terpenoids, glycosides, cardiac glycosides and steroids in both methanol and aqueous root extract (Table 7-9). The phytochemical screening for compounds supports the others findings. The roots are useful in biliousness, blood diseases, dysentery, diarrhea, respiratory disorders, skin diseases, syphilis, fever, asthma, eye disease, kidney and urinary disorders[56]. The pharmacological application of *H. indicus* includes antibacterial[57,58], anti-inflammatory[59], anti-oxidant, free radical scavenger[60] and anti venome activity.

The methanolic extract of *H. indicus* roots showed antihepatotoxicity activity when administered into the paracetamol and CCl_4 induced hepatotoxicity in Wistar rats at the rate of 100-500mg/kg body weight[61].

Asparagus racemosus

The major active constituents of *A. racemosus* are steroidal saponins in roots, quercitin, rutin, hyperoside in flower and fruits and

Table 6. Minimum Inhibitory Concentration of selected plants against *E. coli* Dk1 and *S. aureus* MRS901

S. No.	Plants Name	MIC (Methanolic extracts)		MIC (Aqueous extracts)	
		E. coli Dk1	*S. aureus* MRS901	*E. coli* Dk1	*S. aureus* MRS901
1	*Nyctanthes arbortristis*	30.00 mg/ml	50.00 mg/ml	50.00 mg/ml	60.00 mg/ml
2	*Vitex negundo*	35.00 mg/ml	40.00 mg/ml	40.00 mg/ml	40.00 mg/ml
3	*Phyllanthus amarus*	10.00 mg/ml	30.00 mg/ml	20.00 mg/ml	40.00 mg/ml
4	*Adhatoda vasica*	35.00 mg/ml	40.00 mg/ml	50.00 mg/ml	60.00 mg/ml
5	*Hemidesmus indicus*	25.00 mg/ml	35.00 mg/ml	30.00 mg/ml	40.00 mg/ml
6	*Asparagus racemosus*	20.00 mg/ml	25.00 mg/ml	25.00 mg/ml	35.00 mg/ml
7	*Terminalia arjuna*	8.00 mg/ml	10.00 mg/ml	15.00 mg/ml	20.00 mg/ml
8	*Terminalia chebula*	10.00 mg/ml	15.00 mg/ml	15.00 mg/ml	20.00 mg/ml

diosgenin, quercitin-3-glucuronide in leaves. The phytochemical screening of *A. racemosus* revealed the presence of alkaloids, hydrolysable tannins, saponins, terpenoids, glycosides, cardiac glycosides and steroids in methanol root extract but tannins and flavonoids are absent. Similarly, alkaloids, hydrolysable tannins, saponins, terpenoids and cardiac glycosides are present in aqueous root extract but tannins, flavonoids, glycosides and steroids are absent (Table 7-9). The

Table 7: Studies on Intravenously Safety of Plant Extract

S. No.	Plants Name	Intravenously Use
1	*Nyctanthes arbortristis*	1. M Kannan and A.J.A. Ranjit Singh, An Immuno-Pharmacological Investigation of Indian Medicinal Plant *Nyctanthes arbortristis* Linn; *World Applied Sciences Journal* 2010; **11**(5):495-503. 2. Harleen Kaur Sandhar, Mohanjit kaur, Bimlesh Kumar and Sunil Prasher. An update on *Nyctanthes arbortristis* Linn, *Internationale Pharmaceutica Sciencia,* 2011; **1**(1): 77-86.
2	*Vitex negundo*	R. K. Gupta and V. R. Tandon. Antinociceptive Activity of *Vitex negundo* Linn Leaf Extract, *Indian J Physiol Pharmacol*, 2005; **49**(2): 163-170.
3	*Phyllanthus amarus*	K. N. S. Sirajudeen, Siti A. Sulaiman, M. Madhavan, Z. Ismail, M. Swamy, Md. Lukmi Ismail and Musa Yaacob. Safety Evaluation of Aqueous Extract of Leaves of a Plant *Phyllanthus Amarus* in Rat Liver, 2006; **3**(4): 78-93.
4	*Adhatoda vasica*	G. Vinothapooshan and K. Sundar. Wound Healing Effect of Various Extracts of *Adhatoda vasica*, *International Journal of Pharma and Bio Sciences*, 2010; **1**(4): 530-536.
5	*Hemidesmus indicus*	1. J R Baheti, R K Goyal and G B Shah. Hepatoprotective activity of *Hemidesmus indicus* R. Br. In rats, *Indian Journal of Experimental Biology*, 2006; **44**: 399-402. 2. Nadana Saravanan and Namasivayam Nalini. Inhibitory effect of *Hemidesmus indicus* and its active principle 2-hydroxy 4-methoxy benzoic acid on ethanol-induced liver injury, *Fundamental & Clinical Pharmacology*, 2007; **21**(5): 507–514.
6	*Asparagus racemosus*	**1.** N.P. Visavadiya and R.L. Narasimhacharya. Hypolipidemic and antioxidant activities in *Asparagus racemosus* in hypercholesteremic rats, *Indian Journal of Pharmacology,* 2005; **37**: 376–380. 2. Manish Gautam, Santanu Saha, Sarang Bani, A. Kaul, Sanjay Mishra, Dada Patil et al. Immunomodulatory activity of Asparagus racemosus on systemic Th1/Th2 immunity: Implications for immunoadjuvant potential, *Journal of Ethnopharmacology*, 2009; **121**(2): 241-247.
7	*Terminalia arjuna*	1. A. Bharani, A. Ganguly and K.D. Bhargava. Salutary effect of *Terminalia arjuna* in patients with severe refractory heart failure, *International Journal of Cardiology,* 1995; **49**(3): 191-199. 2. Jaspal Singh Sandhu, Biren Shah, Shweta Shenoy, Suresh Chauhan, G. S. Lavekar and M. M. Padhi. Effects of *Withania somnifera* (Ashwagandha) and *Terminalia arjuna* (Arjuna) on physical performance and cardiorespiratory endurance in healthy young adults, *Int J Ayurveda Res.*, 2010; **1**(3): 144–149.
8	*Terminalia chebula*	1. H.N. Shivaprasad, M.D. Kharya, A.C. Rana and S. Mohan. Preliminary Immunomodulatory Activities of the Aqueous Extract of *Terminalia chebula, Pharmaceutical Biology*, 2006; **44**(1): 32-34. 2. Praveen Sharma, T. Prakash, D. Kotresha, Md Asif Ansari, Uday Raj Sahrm, Bimlesh Kumar, Jeevan Debnath and Divakar Goli. Antiulcerogenic activity of *Terminalia chebula* fruit in experimentally induced ulcer in rats, *Pharmaceutical Biology*, 2011; **49**(3): 262-268.

phytochemical screening supports the presence of compound steroidal saponins in methanol root extract. The new compound like racemofuran (a,a-diphenyl-b-picrylhydrazyl), isoflavone (8-methoxy-5,6,4-trihydroxy-isoflavone), asparagamine (polycyclic compound), racemosol (9,1-dihydrophenenthrene), sarsapogenin and kaempferol were isolated from various parts of the *A. racemosus*[62-64]. The pharmacological application of *A. racemosus* includes antibacterial[65], anti-diarrhoeal[66] and anti-dyspepsia[67] activity.

Visavadia and Narasimhacharya[68] reported that addition of *A. racemosus* root powder at 5g% and 10g% level as feed supplement reduces the plasma and hepatic lipid (cholestrol) levels and also decreases lipid peroxidation in hypercholesteremic rats.

Terminalia arjuna

T. arjuna bark contains a very high level of flavonoids (arjunolone, flavones, bicalein, quercitin, kaempferol, pelorgonidin) and tannins[69]. The phytochemical screening of *T. arjuna* revealed the presence of alkaloids, tannins, hydrolysable tannins, flavonoids, saponins, terpenoids, glycosides, cardiac glycosides and steroids in both methanol and aqueous bark extract (Table 7-9). The screening supports the presence of compounds of flavonoids and tannins with others findings. The pharmacological application of *T. arjuna* includes antimicrobial[70, 71], antioxidant[72] and anti-inflammatory[73].

The efficacy of *T. arjuna* as an anti-ischemic agent and as a potent antioxidant preventing LDL cholesterol oxidation and reperfusion ischemic injury to heart and its potential to reduce atherogenic lipid levels have been amply demonstrated in various experimental and clinical

Table 8. Phytochemical screening of aqueous extracts of eight medicinal plants

Species → ↓Tests	*Phyllanthus amarus*	*Adhatoda vasica*	*Hemidesmus indicus*	*Asparagus racemosus*	*Terminalia arjuna*	*Terminalia chebula*	*Nyctanthes arbortristis*	*Vitex Negundo*
Alkaloids	+	+	+	+	+	+	+	+
Tannins	+	+	+	-	+	+	+	+
Hydrolysable Tannins	+	+	+	+	+	+	-	+
Flavonoids	+	-	+	-	+	+	+	+
Saponins	+	+	+	+	+	+	-	-
Terpenoids	+	+	+	+	+	+	-	-
Glycosides	-	+	+	-	+	+	+	-
Cardiac glycosides	+	-	+	+	+	+	-	+
Steroids	-	+	+	-	+	+	+	-

where, + Presence of bioactive compounds; - Absence of bioactive compounds

Table 9. Phytochemical screening of methanolic extracts of eight medicinal plants

Species → ↓Tests	*Phyllanthus amarus*	*Adhatoda vasica*	*Hemidesmus indicus*	*Asparagus racemosus*	*Terminalia arjuna*	*Terminalia chebula*	*Nyctanthes arbortristis*	*Vitex Negundo*
Alkaloids	+	+	+	+	+	+	+	+
Tannins	+	+	+	-	+	+	+	+
Hydrolysable Tannins	+	+	+	+	+	+	+	+
Flavonoids	+	+	+	-	+	+	+	+
Saponins	+	+	+	+	+	+	+	+
Terpenoids	+	+	+	+	+	+	+	+
Glycosides	+	+	+	+	+	+	+	+
Cardiac glycosides	+	+	+	+	+	+	+	+
Steroids	+	+	+	+	+	+	+	-

Where, + Presence of bioactive compounds; - Absence of bioactive compounds

studies at different concentrations and formulations[74].

Terminalia chebula

T. chebula fruit contains high phenolic content, especially hydrolysable tannins[75]. The phytochemical screening of *T. chebula* revealed the presence of alkaloids, tannins, hydrolysable tannins, flavonoids, saponins, terpenoids, glycosides, cardiac glycosides and steroids in both methanol and aqueous fruit extract (Table 7-9). The screening supports the presence of compounds of hydrolysable tannins with others findings. The pharmacological application of *T. chebula* includes antibacterial[76], antiviral, adaptogenic[77] and antioxidants[78].

The efficacy of *T. chebula* has been amply demonstrated in various experimental and clinical studies at different concentrations and formulations[79].

Nyctanthes arbortristis

The main constituents of the leaf extract includeβ-sitosterol, flavonol glycosides and iridoid glycosides[80]. The phytochemical screening of *N. arbortristis* revealed the presence of alkaloids, tannins, hydrolysable tannins, flavonoids, saponins, terpenoids, glycosides, cardiac glycosides and steroids in methanol and alkaloids, tannins, flavonoids, glycosides and steroids in aqueous leaf extract (Table 7-9). The hydrolysable tannins, saponins, terpenoids and cardiac glycosides are absent in aqueous leaf extract[81]. It is suggested that leaf juice with honey useful for dry cough, skin diseases, dandruff and chronic fever.

The immuno-pharmacological and haemagglutination properties of ethanolic extract of *N. arbortristis* were observed after injecting peritonially dose of 0.25g and 0.5g/kg body weight in to the rats[82]. The ethanol extracts of leaves, flowers, seeds and barks (600mg/kg) showed significant and dose-dependant CNS depressant activity in mice (prolongation of sleeping time induced by pentobarbital sodium) may be due to decrease in dopamine and increase in serotonin level[83].

Vitex negundo

The principal constituents of leaf extract are casticin, isoorientin, chrysophenol D, luteolin, p-hydroxy benzoic acid and D-fructose. The phytochemical screening of *V. negundo* revealed the presence of alkaloids, tannins, hydrolysable tannins, flavonoids, saponins, terpenoids, glycosides, and cardiac glycosides in methanol and alkaloids, tannins, hydrolysable tannins, flavonoids, and cardiac glycosides in aqueous leaf extract (Table 7-9). The saponins, glycosides and steroids are absent in aqueous leaf extract. The plant has anti-inflammatory[74], antibacterial[4], antifungal[85] and analgesic[86] activity.

The *Vitex negundo* possesses both central and peripheral analgesic activity at the rate of 100, 250 and 500 mg/kg employed in mice[86].

Today natural products derived from the plants are being tested for presence of new drugs with new modes of pharmacological action. A special feature of higher plants is their capacity to produce a large number of secondary metabolites[87]. Plants have been major source of medicine and the presence of plant secondary metabolites has been implicated for most plants therapeutic activities[88]. Phytomedicines derived from plants have shown great promise in the treatment of intractable infectious diseases[89]. Also, it has been suggested that aqueous and methanolic extracts from plants used in allopathic medicine are prospective resources of antiviral, antimicrobial representatives[90]. The higher plants as a supply of curative amalgam had sustained to cooperate a leading role in the safeguarding of human fitness since olden times[91].

CONCLUSION

The phytochemical analysis suggested the bioactive complex consistent for the in vitro antibacterial action of *T. arjuna* and *T. chebula* over MDR bacteria (i.e. *E. coli* Dk1 and *S. aureus* MRS901) in aqueous and methanolic extracts. The Zone of Inhibition (ZOI) was recorded and found that *T. arjuna* and *T. chebula* inhibited the growth of both strains *E. coli* Dk1 and *S. aereus* MRS901 with highest ZOI range 9-13mm. It may be accomplished that the bark and fruit extracts of *T. arjuna* and *T. chebula* might be used for the cure of illness caused by *S. aureus*.

REFERENCES

1. Narayana A, Subhose V. Standardization of Ayurvedic formulations: a scientific review. *Bull*

Indian Inst Hist Med Hyderabad 2005; 35: 21.
2. Aqil F, Ahmad I. Broad-spectrum antibacterial and antifungal properties of certain traditionally used Indian medicinal plants. *World J. Microbiol. Biotechnol.* 2003;**19**: 653-57.
3. Chopra RN, Nayar SL, Chopra IC. Glossary of Indian Medicinal Plants. CSIR, New Delhi, India 1956.
4. Perumal SR, Ignacimuthu S. Screening of 34 Indian medicinal plants for antibacterial properties. *J. Ethnopharmacol*. 1998; **62**: 173.
5. Kamboj VP. Herbal medicine – Some comments. *Current Science* 2000;**78**: 35.
6. Srivastava J, Lambert J, Vietmeyer N. Medicinal Plants: An Expanding Role in Development. The World Bank, Washigton, D.C. 1996;8.
7. John D. One hundred useful raw drugs of the Kani tribes of Trivandrum Forest division, Kerala, India. *Int. J. Crude Drug Res*. 1984; **22**: 17.
8. Veale DJH. South African traditional herbal medicines used during pregnancy and childbirth. *J. Ethnopharmacol*. 1992; **36**: 185.
9. Manandhar NP. Traditional medicinal plants used by tribals of Lamjung district, Nepal. *Int. J. Crude Drug Res*. 1987; **25**: 236.
10. Service RF. Antibiotics that resist resistance. *Science.* 1995; **270**: 724-27.
11. Ahmad I, Mehmood Z, Mohammad F. Screening of some Indian medicinal plants for their antimicrobial properties. *J Ethnopharmacol.* 1998; **62**: 183-93.
12. Monroe S, Polk R. Antimicrobial use and bacterial resistance. *Curr Opin Microbiol*. 2000; **3**: 496-501.
13. De Boer HJ, Kool A, Broberg A. Antifungal and antibacterial activity of some herbal remedies from Tanzania. *J Ethnopharmacol*. 2005; **96**: 461-69.
14. Cowan MM. Plant products as antimicrobial agents. *Clin Microbiol Rev*. 1999; **12**: 564-82.
15. Rios JL, Recio MC. Medicinal plants and antimicrobial activity. *J Ethnopharmacol.* 2005; **100**: 80-4.
16. Dorman HJD, Deans SG. Antimicrobial agents from plants: antibacterial activity of plant volatile oils. *J Appl Microbial*. 2000; **88**: 308-16.
17. Bonjar S. Evaluation of Antibacterial Properties of Some Medicinal Plants Used in Iran. *J Ethnopharmacol*. 2004; **94**: 301-5.
18. Hasegawa H, Matsumya S, Yamasak K. Reversal of Efflux-mediated Tetracycline Resistance in *Staphylococcus aureus* Clinical Isolates by Ginseng Prosapogenins. *Phytotherapy Research*. 1995; **9**: 260-3.
19. Yoshida H, Iwata N. Antimicrobial activity of a compound isolated from an oil-macerated garlic extract, *Biotech and Biochem.* 1998; **62**: 1014-17.
20. Ahmad I, Beg AZ. Antimicrobial and phytochemical studies on 45 Indian medicinal plants against multi-drug resistant human pathogens. *J Ethnopharmacol.* 2001;**74**:113-23.
21. Elizabeth KM. Antimicrobial activity of *Allium sativum* on some pathogenic bacteria. *Ind J Microbiol.* 2001;**41**:321-3.
22. Mukherjee PK, Saritha GS, Suresh B. Antimicrobial potential of two different *Hypericum* species available in India. *Phytother Res*. 2002; **16**: 692-5.
23. Wang YC, Huang TL. Anti- *Helicobacter pylori* activity of *Plumbago zeylanica* L. FEMS *Immunol Med Microbiol.* 2005; **43**: 407-12.
24. Liu IX, Durham DG, Richards RME. Bacterial synergy with β-lactam resistant strains of *S. aureus*. *J Pharmaceutical Pharmacolol.* 2000; **52**: 361-6.
25. Bhavnani SM, Ballow CH. New agents for Gram-positive bacteria. *Curr Opin Microbil.* 2000; **3**: 528-34.
26. Cordell GA. Biodiversity and drug discovery a symbiotic relationship. *Phytochemistry* 2000; **55**: 463-80.
27. Trease GE, Evans WC. Text book of Pharmocognosy.13th (eds). Alden Press; Oxford 1989;pp.512-3.
28. McGee H. On food and cooking: the science and lore of the kitchen. New York, Scribner 2004;714.
29. Trease GE, Evans WC. Textbook of Pharmacognosy. 12th Edn. Balliere, Tindall, London 1983; pp:55-9.
30. Nonaka G, Nishioka I, Nishizawa M. Anti-AIDS agents, 2: Inhibitory effect of tannins on HIV reverse transcriptase and HIV replication in H9 lymphocyte cells. *J Nat. Prod*. 1990; **53**(3): 587-95.
31. Cushnie TPT, Lamb AJ. Antimicrobial activity of flavonoids. *International J. Antimicrobial Agents* 2005; **26**(5): 343-56.
32. De Sousa RR, Queiroz KC, Souza AC, Gurgueira SA, Augusto AC, Miranda MA, Peppelenbosch MP, Ferreira CV, Aoyama H. Phosphoprotein levels, MAPK activities and NFkappa ^{2} expression are affected by fisetin. J Enzyme inhib. *Med. Chem.* 2007; **22**(4): 439-44.
33. Bagchi M, Mark M, Casey W, Jaya B, Xumei Y, Sidney S, Debasis B. Acute and chronic stress-induced oxidative gastrointestinal injury in rats and the protective ability of a novel grape seed proanthocyanidin extract. *Nutrition Research*

1999; **19**: 1189-99.
34. Hostettman K, Marston A. Saponins. Cambridge, Cambridge University Press 1995; 3.
35. Sharma AK, Handa SS. Estimation of phyllanthin and hypophyllanthin by high performance liquid chromatography in *Phyllanthus amarus*. *Phytochem. Anal.* 1993; **4**: 226-9.
36. Houghton PJ, Woldemariam TZ, O'Shea S, Thyagarajan SP. Two securinega type alkaloids from *Phyllanthus amarus*. *Phytochem*. 1996; **43**: 715-7.
37. Foo LY. Amariin, a di-dehydro hexahydroxy diphenoyl hydrolysable tannin from *Phyllanthus amarus*. *Phytochem*. 1993; **33**:487-91.
38. Rao GS, Bramley R. Hypophyllanthin. *Tetrahedron Lett*. 1971; **34**: 3175-8.
39. Okwu DE, Josiah C. Evaluation of the chemical composition of two Nigerian Medicinal plants. *African Journal of Biotechnolgy* 2006; **5**(4): 357-61.
40. Adeneye AA, Benebo AS, Agbaje EO. Protective effect of the Aqueous Leaf and seed Extract of *Phyllanthus amarus* on Alcohol – induced hepatotoxity in rats. *West Afr. J. Pharmacol. Drug Res*. 2006; **22&23**: 42-50.
41. Akinjogunla OJ, Eghafona NO, Enabulele IO, Mboto CI, Ogbemudia FO. Antibacterial activity of ethanolic extracts of *Phyllanthus amarus* against extended spectrum beta-lactamase producing *Escherichia coli* isolated from stool samples of HIV sero-positive patients with or without diarrhoea. *Afr. J. Pharm. Pharmacol.* 2010; **4**: 402-7.
42. Lim YY, Murtijaya J. Antioxidant properties of *Phyllanthus amarus* extracts as affected by different drying methods. *LWT-Food Sci. Technol*. 2007; **40**: 1664-9.
43. Mehrotra R, Rawat S, Kulshreshtha DK, Goyal P, Patnaik GK, Dhawan BN. In vitro effect of *Phyllanthus amarus* on Hepatitis B virus. *Indian J. Med. Res*. 1991; **93**:71-3.
44. Adeneye A, Benebo AS. Protective effect of the aqueous leaf and seed extract of *Phyllanthus amarus* on gentamicin and acetaminophen-induced nephrotoxic rats. *J Ethnopharmacology.* 2008; **118**: 318-23.
45. Singh A. Theraputic monograph- *Adhatoda vasica*, Ind-Swift Ltd, Mohali, Chandigarh 1997; 25-45.
46 Francis M, Sane RT, Tipnis S. Simultaneous HPLC method for determination of vasicine and glycyrrhizin from herbal preparations, *Indian Drugs*. 2003; **40**(12): 712-5.
47 Atal CK. Chemistry and Pharmacology of *Vasicine* - A new oxytocic and abortifacient, *Indian Drugs*. 1980; **15**(2):15-18.
48 Shete AB, Femiforte, indigenous herbomineral formulation in the management of non- specific leucorrhoea, Doctor's News. 1993; **5**: 13-4.
49 Farnlof A. Naturlakemedel och Naturmedel, Halsokostra dets Forlag, *Stockholm.* 1998; **109**: 32.
50 Dey SK, Banerjee D, Chattapadhyay S, Karmakar KB. Antimicrobial Activities of Some Medicinal Plants of West Bengal. *Int J of Pharma and Bio Sciences*. 2010; **1**: 3.
51. Chakraborty A, Brantner AH, Study of alkaloids from *Adhatoda vasica* Nees on their antiinflammatory activity. *Phytother Res*. 2001; **15**(6): 532-4.
52. Dhuley JN. Antitussive effect of *Adhatoda vasica* extract on mechanical or chemical stimulation-induced coughing in animals. *J Ethnopharmacol.* 1999; **67**: 361-5.
53. G. Vinothapooshan and K. Sundar. Wound Healing Effect of Various Extracts of *Adhatoda vasica*, *International Journal of Pharma and Bio Sciences*, 2010; **1**(4): 530-536.
54. Mukherjee K, Ray LN. Screening of some Indian plant species. Q. *J Crude Drug Res*. 1980;**18**: 77- 82.
55. Nagarajan S, Rao LJ. Determination of 2-hydroxy-4-methoxybenzaldehyde in roots of *Decalepis hamiltonii* and *Hemidesmus indicus R. Br. J. AOAC Int*. 2003; **86**: 564-7.
56. Kirthikar KR, Basu BD. Indian medicinal plants. Vol 1-4. Bishan Singh Mahendra Pal Singh, Dehradun., India 1980.
57. Das S, Devaraj SN. Antienterobacterial activity of *Hemidesmus indicus* R. Br root extract. *Phytother Res.* 2006; **20**: 416-21.
58. Das S, Prakash R, Devaraj SN. Antidiarrhoeal effect of methanolic root extract of *Hemidesmus indicus* (Indian Sarasparilla) – an in vitro and in vivo study. *Indian J. Exp. Biol.* 2003; **41**: 363-6.
59. Dutt MK, Sen TK, Sikdar S. Some preliminary *Hemidesmus indicus* in rat. *Indain J. Pharmacol.* 1982; **14**:78.
60. Ravishankara MN, Shrivastava N, Padh H, Rajani M. Evaluation of antioxidant properties of root bark of *Hemidesmus indicus* (Anantmul). Phytomedicine 2002; **9**:153-60.
61. Baheti JR, Goyal RK, Shah GB. Hepatoprotective activity of *Hemidesmus indicus* R. Br. in rats. *Indian J. Exp. Biol.* 2006; **44**: 399-402.
62. Dinan L, Savchenko T, Whiting P. Phytoecdysteroids in the genus *Asparagus* (Asparagaceae). *Phytochemistry* 2001; **56**: 569-76.

63. Saxena VK, Chourasia S. A new isoflavone from the roots of *Asparagus racemosus*. *Fitoterapia* 2001; **72**: 307–9.
64. Wiboonpun N, Phuwapraisirisan P, Tip-pyang S. Identification of antioxidant compound from *Asparagus racemosus*. *Phytotherapy Research* 2004; **8**: 771–3.
65. Mandal SC, Nandy A, Pal M, Saha BP. Evaluation of antibacterial activity of *Asparagus racemosus* Willd. root. *Phytotherapy Research* 2000; **14:** 118–9.
66. World Health Organization, 2005. <http://www.who.int/watersanitationhealth/diseases/diarrhoea/en>.
67. Dalvi SS, Nadkarni PM., Gupta KC. Effect of *Asparagus racemosus* (Shatavari) on gastric emptying time in normal healthy volunteers. *J. Postgraduate Medicine* 1990; **36**: 91–4.
68. Visavadia N P and Narasimhacharya A V R L (2005). Hypolipidemic and antioxidant activities of *Asparagus racemosus* in hypercholesteremic rats. *Indian J Pharmacol* **37**(6): 376-380.
69. Lin TC, Chien SC, Chen HF, Hsu FL. Tannins and related compounds from Combretaceae plants. *Chinese Pharmaceutical Journal* 2001; **52**: 1-26. .
70. Aqil F, Kahn MS, Owais M, Ahmad I. Effect of certain bioactive plant extracts on clinical isolates of beta-lactamase producing methicillin resistant *Staphylococcus aureus*. *J Basic Microbiol*. 2005; **45**: 106-14.
71. Rani P, Khullar N. Antimicrobial evaluation of some medicinal plants for their anti-enteric potential against multi-drug resistant *Salmonella typhi* . *Phytother Res*. 2004; **18**:670-3.
72. Gauthaman K, Maulik M, Kumari R, Manchanda SC, Dinda AK, Maulik SK. Effect of chronic treatment with bark of *Terminalia arjuna*: a study on the isolated ischemic-reperfused rat heart. *J Ethnopharmacol.* 2001; **75**: 197-201.
73. Halder S, Bharal N, Mediratta PK, Kaur I, Sharma KK. Anti-inflammatory, immunomodulatory and antinociceptive activity of *Terminalia arjuna* Roxb bark powder in mice and rats. *Indian J Exp Biol*. 2009; **47**(7): 577-83.
74. Dwivedi S. Terminalia arjuna Wight & Arn. a useful drug for cardiovascular disorders. *J Ethnopharmacol*. 2007;**114**(2):114-29.
75. Juang LJ, Sheu SJ, Lin TC. Determination of hydrolyzable tannins in the fruit of *Terminalia chebula* Retz. by high-performance liquid chromatography and capillary electrophoresis. *J. Separation Science* 2004; **27**(9): 718-24.
76. Malekzadeh F, Ehsanifar H, Shahamat M, Levin M, Colwell RR. Antibacterial activity of black myrobalan (*Terminalia chebula* Retz) against *Helicobacter pylori*. *Int J Antimicrob Agents*. 2001; **18**(1): 85-8.
77. Shin TY, Jeong HJ, Kim DK, Kim SH, Lee JK, Kim DK, Chae BS, Kim JH, Kang HW, Lee CM, Lee KC, Park ST, Lee EJ, Lim JP, Kim HM, Lee YM. Inhibitory action of water soluble fraction of *Terminalia chebula* on systemic and local anaphylaxis. *J Ethnopharmacol.* 2001; **74**(2): 133-40.
78. Jagetia GC. The evaluation of the radio protective effect of Triphala in the mice exposed to ± radiation. *Phytomedicine* 2002; **9**: 99-108.
79. Chattopadhyay RR and Bhattacharya SK., *Terminalia chebula* : An Update. *Pharmocognosy Reviews* 2007; **1**(1): 151-156.
80. Saxena RS, Gupta B, Lata S. Tranquilizing, antihistaminic and purgative activity of *Nyctanthes arbortristis* leaf extract. *J Ethanopharmacol* 2002; **81**(3): 321-5.
81. Puri A, Saxena R, Saxena RP, Saxena KC, Srivastava V, Tandon JS. Immunostimulant activity of *Nyctanthes arbortristis* L. *J Ethanopharmacol* 1994; **42**(1): 31-7.
82. Kannan M and Singh AJAR., An immune-pharmocological investigation od Indian medicinal pant *Nyctanthes arbor-tristis Linn*. *World Applied Sciences J.* 2010; **11**(5): 495-503.
83. Das S, Sasmal D, Basu S P., Evaluation of CNS depressant activity of different plant parts of *Nyctanthes arbor-tristis* Linn. *Indian J Pharm. Sci*. 2008; **70**(6): 803-806.
84. Dharmasiri MG, Jayakody JR, Galhena G, Liyanage SS, Ratnasooriya WD. Anti-inflammatory and analgesic activities of mature fresh leaves of *Vitex negundo*. *J Ethanapharmacol* 2003; **87**(2-3): 199-206.
85. Sathiamoorthy B, Gupta P, Kumar M, Chaturvedi AK, Shukla PK, Maurya R. New antifungal flavonoid glycoside from *Vitex negundo*. *Bioorg. Med. Chem. Lett*. 2007; **17**(1): 239-42.
86. Gupta RK, Tandon VR. Antinociceptive activity of *Vitex negundo* Linn. Leaf extract. *Indian J Physiol Pharmacol* 2005; **49**(2):163-70.
87. Castello MC, Phatak A, Chandra N, Sharon M. Antimicrobial activity of crude extracts from plant parts and corresponding calli of *Bixa orellana* L. *Indian J. Exp. Biol.* 2002; **40**(12): 1378-81.
88. Aibinu I. Medicinal plants as Antimicrobials. In: Outlines and Pictures of Medicinal Plants

from Nigeria, Odugbemi, T. (Ed.). Univ. Of Lagos Press 2006; 53-64.

89. Idu M, Omonigho SE, Igeleke CL. Preliminary investigation on the Phytochemistry and antimicrobial activity of *Senna alata* L. flower. *Pak. J. Biol. Sci.* 2007; **10**(5): 806-9.

90. Vlietinck AJ, Van Hoof L, Totté J, Lasure A, Vanden Berghe D, Rwangabo PC, Mvukiyumwami J. Screening of hundred Rwandese medicinal plants for antimicrobial and antiviral properties. *J. Ethnopharmacol.* 1995; **46**: 31.

91. Farombi EO. African indigenous plants with chemotherapeutic potentials and biotechnological approach to the production of bioactive prophylactic agents. *African J Biotech.* 2003; **2**: 662-71.

92. Chandrabhan Seniya, Santosh Kumar Verma, Sumint Singh Trivedia, Richa Verma, H.S. Vijayarti, Shridutt Vyas. Metal Stress and Antibiotic Susceptibility Profile of Some Bacterial and Fungal Strains. *J Pure Appl Microbio,* 2012; **6**(4).