INVESTIGATION OF ANTIMICROBIAL PROPERTIES OF *DIOSCOREA PENTAPHYLLA* FROM MID WESTERN GHATS, INDIA

Prakash.G* and **Hosetti. B. B.***
Toxicology Lab, Department of Applied Zoology, Kuvempu university, Shankaraghatta, India.

Abstract: Antibacterial and antifungal activity of crude extracts of medicinally important and traditionally used yam plant *Dioscorea pentaphylla* from mid Western Ghats was evaluated against 27 bacterial and 5 fungal clinical strains collected of the patients from infectious sources. The clinical strains belonging to their respective species showed concentration dependent susceptibility towards crude petroleum ether extract, chloroform extract and methanol extract at 100ìg/100ìL. All the extracts exhibited predominant antibacterial activity against *S. aureus* (ATCC-20852), *P. aeruginosa* (ATCC-29737) and *K. pneumoniae* (MTCC-618) respectively. and five clinically isolated pathogenic fungi, *T. rubrum, M. gypseum, T. tonsurans, M. audouini, and C. albicans* with antibacterial drug Ciprofloxacin and antifungal drug Fluconozole (50ìg/100ìL) as the standard drug. Out of three extracts, ethanol extracts possessed better minimum inhibition concentration against all the bacterial strains. All the three extracts showed significant result against all the five fungal pathogen strains. The results are promising and supported the traditional use of *D. Pentaphylla* for the treatment of bacterial and fungal infections.

Keywords : Antifungal; Antibacterial; Crude extracts Dioscorea pentaphylla; MIC and Traditional medicine.

INTRODUCTION

The practice of antimicrobial chemotherapy is one of constant challenges, particularly in view of the rapid evolutionary changes and wide variety of pathogens encountered. Many investigators evaluated the bioactivity of plant extracts and their constituents against the serious infectious organisms (Parekh and Sumitra, 2006; Kausik *et al.*, 2002).

Prevalence of antibiotic-resistant strains of bacteria due to the extensive use of antibiotics may render the current antimicrobial agents insufficient to control the bacterial diseases (Cown, 1999; Bax *et al.*, 2002). Numerous studies have been carried out in different parts of the globe to extract plant products for screening antimicrobial activity (Essawi and Srour, 2000; Rajanaik *et al*., 2005).

Dioscorea yam is a member of the Yam family. The yams are vining plants with 600 known species, 71 of which are native to North America (67 species in Mexico) (Hutchens Alma, 1991). In many species of yam, the rhizome (tuber) serves as both food and medicine. Many native Americans and south Asians used a syrup of the root to relieve labour pain and later physicians gave wild yam to patients with colic pain, morning sickness, asthma, hiccough, rheumatism and gastritis related to alcoholism (Foster and Duke, 2000). Modern herbalists value wild yam to treat intestinal colic, biliary colic, and flatulence as well as menstrual cramps and rheumatoid arthritis (Tierra, 1998; Grieve, 1971; Fleming *et al.*, 1998). Herbalists combine wild yam with black cohosh (and sometimes burdock root and motherwort) (Hutchens Alma, 1991) for rheumatic complaints. Chinese herbalists use wild yam as a tonic (Hutchens Alma, 1991).

In the present investigation, *Dioscorea pentaphylla* was selected, as one of the medicinally important plant, extensively consumed by local people as food. However, there is apparently no scientific reports on the antimicrobial properties of this plant. The lack of scientific knowledge has often exerted a major constraint on the use of traditional herbal remedies as an affordable alternative to orthodox medical treatment. Thus, the different solvent extracts of the tuber were screened for its activity against three bacterial pathogens and five fungal strains.

2. MATERIAL AND METHODS

2.1. Plant material

Tubers of *Dioscorea pentaphylla* were collected from the Lakkavalli reserve forest in and around area of Bhadra wild life sanctuary of the Mid-western Ghats region of Karnataka, India and the species was identified by comparing with the authenticated specimen deposited at the Kuvempu University herbaria (Voucher specimen KUDB/ Ang/324). The leaves were washed in running tap water, shade dried, powdered

Author for Correspondence: Prakash.G, Toxicology Lab, Department of Applied Zoology, Kuvempu university, Shankaraghatta, India - 577451, Email: Prakash.geriyol@gmail.com.

mechanically and sieved (Sieve No. 10/44) and subjected to Soxhlet extraction using different solvents *viz.,* Petroleum ether, Chloroform and Ethanol. The extracts were concentrated under reduced pressure at 40 ± 5°C using a rotary flash evaporator (Buchi, Flawil, Switzerland).

2.2. Phytochemical analysis

Qualitative phyto-chemical analysis of *Dioscorea pentaphylla* tuber extracts was done as follows:

Tannins

20 mg extract was dissolved in 2 ml distilled water and filtered. 2 ml $FeCl_3$ was added to the filtrate, blue-black precipitate indicated the presence of tannins (Parekh *et al.,* 2006).

Alkaloids

20 mg extract was dissolved in 2 ml distilled water and filtered. To the filtrate, 2–4 drops of 1% HCl was added and steam was passed through it. To the 1 ml of this solution 6 drops of Wagner's reagent was added. Brownish-red precipitate indicated the presence of alkaloids (Parekh *et al.,* 2006).

Saponins

To 0.5 ml of the filtrate obtained in alkaloids test 5 ml distilled water was added. Frothing persistence indicated the presence of saponins (Parekh *et al.,* 2006).

Flavonoids

20 mg extract was dissolved in 10 ml ethanol and filtered. 0.5 ml conc. HCl and magnesium ribbon were added to 2 ml filtrate. Development of pink-tomato red color indicated the presence of flavonoids (Parekh *et al.,* 2006).

Terpenoids

Salkovski test was performed using a small amount of extract solution. To this solution 5 drops of conc. H_2SO_4 and 1 ml Chloroform were added. Change of yellow colour into red indicated the presence of terpenoids (Finar, 2003).

Phenols/polyphenols

A small amount of material was extracted in ethanol and evaporated to dryness. Residue was dissolved in distilled water and 0.5 ml Folin-ciocalteau reagent was added followed by 2 ml 20% Na_2CO_3 solution. Development of bluish colour indicated the presence of phenols (Sadasivam and Manickam, 1996).

2.3. Preparation of plant extracts for antimicrobial assay

100 ìg of all the crude extracts was dissolved in 100 ìl of 10% DMSO. The standard antibacterial drug Ciprofloxacin and antifungal drug Fluconozole were also tested at a concentration 50 ìg/100 ìl of each.

2.4. Evaluation of Minimal Inhibitory Concentrations (MIC).

The Minimal Inhibitory Concentrations (MIC) of the different solvent crude extracts was determined by micro dilution techniques in LB broth, according to Clinical and Laboratory Standards Institute (CLSI), USA guidelines. The bacterial inoculates were prepared in the same medium at a density adjusted to a 0.5 McFarland turbidity standard colony forming units and diluted 1:10 for the broth micro dilution procedure. The micro titre plates were incubated at 37°C and MIC was determined after 24 h of incubation. The highest activity of the isolated compounds compared to those of the crude extracts indicates that those compounds alone were solely responsible for antimicrobial activity.

2.5. Screening of Antimicrobial activity.

Twenty seven clinical strains of three of the most common bacterial pathogens, *S. aureus, P. aeruginosa and K. pneumoniae*, (*Staphylococcus aureus* – ATCC-29737; *Pseudomonas aeruginosa* - ATCC-20852 and *Klebsiella pneumoniae* - MTCC-618) strains of the corresponding bacteria and five clinically isolated pathogenic fungi, *T. rubrum, M. gypseum, T. tonsurans, M. audouini, and C. albicans* were used as test organisms. Different pathogens

Table 1: list of the clinical strains used for antimicrobial activity.

Clinical strains	Clinical condition	Source
P. aeruginosa		
Ps-1	Bronchitis	Wounds
Ps-2	Otitis media	Pus
Ps-3	Burns	Sputum
Ps-4 and Ps-5	Upper UTI	Stool
Ps-6	Food poisoning	Hospital effluent
Ps-7	Cross infections in UTI	Hospital effluent
Ps-8	Septicemia	Old wounds
Ps-9	Unknown	Ear swab
K. pneumoniae		
Kp-1	Pneumonia	Mucus
Kp-2	Gram negative	Follicullitis stipules
Kp-3	Burns	Pus
Kp-4	UTI	Urine
Kp-5	Septicemia	Sputum
Kp-6	Cross infections in UTI	Urine
Kp-7	Abscess in immunodeficiency	Wounds
Kp-8	Upper UTI	Urine
Kp-9	Unknown	Hospital effluent
S. aureus		
Sa-1	Abscess in immunodeficiency	Wounds
Sa-2	Burns	Pus
Sa-3	Septicemia	Old wounds
Sa-4	Food poisoning	Stool
Sa-5	Burns	Pus
Sa-6 and Sa-7	Unknown	Hospital effluent
Sa-8	Abscess in immunodeficiency	Sputum
Sa-9	Otitis media	Ear swab
Fungal strains		
T. rubrum	Cutaneous mycoses	Skin
T. tonsurans	Scaring of the scalp	Scalp ringworm
M. gypseum	Ringworm	Infections Skin
M. audouini	Cutaneous mycoses	Skin and hairs
C. albicans	Opportunistic mycoses candidosis	Lungs

Ps = clinical strains of pseudomonas aeruginosa, Kp = clinical strains of Klebsiella pneumoniae, Sa = clinical strains of Staphylococcus aureus.

and their serotype were isolated from infected patients in the district health centre of Annamali nagar, and were identified in the Department of Zoology, Annamali University, India in support with National Chemical Laboratory, Pune, India. The profile of bacterial species and their strains of different clinical origin are shown in Table 1**.** All the bacterial pathogens were maintained at -30°C in Brain Heart Infusion (BHI) containing 17% (v/v) glycerol. Before testing, the suspensions were transferred to LB broth and incubated overnight at 37°C. Inocula were prepared by adjusting the turbidity of the medium to match the 0.5 McFarland standards. Dilutions of this suspension in 0.1% peptone (w/v) solution in sterile water inoculated on LB agar, to check the viability of the preparations. In case of fungal stocks cultures were stored on Brain Heart Infusion (BHI, Merck) culture media (pH 6.5).

2.6. Antimicrobial assay

The agar radial well diffusion method (Mukherjee *et al.,* 1995) was used for the assessment of antimicrobial activity of the extracts of *D. pentaphylla*. Nutrient agar medium (tryptone 10 g/l, yeast extract 5 g/l, sodium chloride 10 g/l, agar-agar 15 g/l, pH 7.2) was poured into sterilized petri dishes (90 mm diameter). LB broth containing 100ìl of 24 h incubated cultures of the respective clinical isolates and the ATCC and MTCC strains were spread separately on the agar medium. Wells were created using a sterilized cork borer under aseptic conditions.

In order to identify antifungal activity of total extracts and fractions against fungal pathogens the agar diffusion assay was performed in BHI culture media (pH 6.5). Fungal spores were obtained by centrifugation at 1500 × g/4°C for 15 min and diluted in phosphate buffer saline (PBS), pH 7.2. Spore count was performed using haemocytometer. After loading 10ìl of the cell suspension in PBS and number of spores/ml was calculated, the final concentration of each strain was identified to be 106 spores/ml. Cultures were incubated for 72 h at 24°C. 100ìl of fungal inocula was spread on the BHI agar plates and wells were made using cork borer and 50ìl of test compounds were loaded to each wells. The plates were refrigerated for 2 h in order to stop fungal growth and facilitate diffusion of the substances. The reference antibacterial agent Ciprofloxacin and antifungal agent Fluconozole were loaded in the corresponding wells in the bacterial and fungal culture plates. Bacterial culture plates were then incubated at 37°C for 24 h, and fungal culture plates were incubated at 24°C for 48 h. At the end of the incubation period, inhibition zones were observed mesured.

2.7. Statistical analysis

The results of these experiments are expressed as mean ± SE of three replicates in each test. The data were evaluated by one-way Analysis of Variance (ANOVA) and mean separations were carried out using Duncan's Multiple Range Test (DMRT, Gomez and Gomez 1984). Followed by Tukey's multiple comparison tests to assess the statistical significance. P=0.05 or less was considered as statistically significant leval.

3. RESULTS

Results of phytochemical analysis of *Dioscorea pentaphylla* tuber extracts are printed in Table 2. All the three extracts were tested for the presence of phenols/polyphenols, flavonoids, terpenoids, tannins, alkaloids and saponins. Phenols and Saponins were invariably present in all the solvent extracts. The analysis of different tuber extracts (*viz.*, petroleum ether, chloroform and ethanol) also showed presence of combinations of other phytochemical constituents as well.

Table 2: Phytochemial analysis *Dioscorea pentaphylla* tuber extracts

Phytochemicals	Pet. ether	Chloroform	Ethanol
Phenol/polyphenols	+	+	+
Terpenoids/ Steroids	-	+	+
Flavonoids	+	-	+
Saponins	+	+	+
Alkaloids	+	-	-
Tannins	-	+	+

The results of antimicrobial investigation revealed that the Minimal Inhibitory Concentrations (MIC) of the petroleum ether, Chloroform and ethanol extracts was 100ìl/100ìl for each. The results of antibacterial activity of all crude extracts a synchronizing effect on clinical strains of pathogenic

Table 2. Antibacterial activity of the crude extract and their pure compounds of *Dioscorea pentaphylla* against clinical strains of *Pseudomonas aeruginosa*.

Clinical Strains	Diameter of zone of inhibition (mm)			
	Pet. Ether extract	Chloroform Extract	Ethanol Extract	Ciproflaxin
***Ps*-1**	07.40 ± 0.10	12.83 ± 0.20	17.23 ± 0.14	23.30 ± 0.15
Ps-2	09.00 ± 0.12	10.16 ± 0.16	16.33 ± 0.24	20.50 ± 0.28
Ps-3	11.23 ± 0.15	11.73 ± 0.33	19.23 ± 0.14	22.23 ± 0.14
Ps-4	10.23 ± 0.15	13.50 ± 0.17	18.43 ± 0.23	20.20 ± 0.26
Ps-5	12.30 ± 0.10	13.23 ± 0.14	18.06 ± 0.18	23.30 ± 0.15
Ps-6	7.40 ± 0.10	17.33 ± 0.20	20.50 ± 0.28	24.33 ± 0.20
Ps-7	10.60 ± 0.12	16.00 ± 0.28	17.00 ± 0.28	20.00 ± 0.28
Ps-8	08.53 ± 0.29	12.13 ± 0.13	19.23 ± 0.17	23.16 ± 0.16
Ps-9	11.37 ± 0.19	13.33 ± 0.16	19.12 ± 0.00	24.50 ± 0.28

Clinical strains of *Pseudomonas aeruginosa* from different clinical sources.
The values are the mean of three experiments ± S.E.
Means followed by the same letter was not significantly different by Tukey's test at the 0.05% probability level.

Table 3. Antibacterial activity of the crude extract and their pure compounds of *Dioscorea pentaphylla* against clinical strains of *Klebsiella pneumoniae*.

Clinical Strains	Diameter of zone of inhibition (mm)			
	Pet. Ether extract	Chloroform Extract	Ethanol Extract	Ciproflaxin
Kp-1	09.23 ± 0.14	13.23 ± 0.15	16.23 ± 0.14	25.00 ± 0.12
Kp--2	08.56 ± 0.12	14.33 ± 0.17	15.40 ± 0.10	20.23 ± 0.15
Kp-3	09.23 ± 0.14	12.30 ± 0.15	14.23 ± 0.14	21.37 ± 0.09
Kp-4	10.23 ± 0.14	12.77 ± 0.09	14.50 ± 0.28	20.20 ± 0.26
Kp-5	09.40 ± 0.10	14.27 ± 0.18	17.73 ± 0.12	23.37 ± 0.09
Kp-6	12.23 ± 0.14	10.43 ± 0.23	18.30 ± 0.15	22.53 ± 0.18
Kp-7	12.30 ± 0.10	12.33 ± 0.17	19.26 ± 0.14	24.37 ± 0.19
Kp-8	11.16 ± 0.16	14.30 ±0.17	16.17 ±0.17	23.43 ± 0.12
Kp-9	14.06 ± 0.06	13.30 ± 0.15	18.43 ± 0.03	24.43 ± 0.12

Clinical strains of Klebsiella pneumoniae from different clinical sources.
The values are the mean of three experiments ± S.E.
Means followed by the same letter was not significantly different by the DMRT test at 0.05 % probability level.

Table 4: Antibacterial activity of the crude extracts and their pure compounds of *Dioscorea pentaphylla* against clinical strains of *Staphylococcus aureus*.

Clinical Strains	Diameter of zone of inhibition (mm)			
	Pet. Ether extract	Chloroform Extract	Ethanol Extract	Ciproflaxin
Sa-1	19.40 ± 0.23	13.33 ± 0.17	18.37 ± 0.19	28.33 ± 0.17
Sa-2	18.43 ± 0.23	14.23 ± 0.15	20.60 ± 0.10	26.90 ± 0.21
Sa-3	14.27 ± 0.18	14.87 ± 0.37	16.70 ± 0.10	21.50 ± 0.29
Sa-4	12.40 ± 0.21	12.17 ± 0.17	20.63 ± 0.09	24.50 ± 0.29
Sa-5	13.47 ± 0.24	14.33 ± 0.17	17.27 ± 0.12	20.43 ± 0.23
Sa-6	16.30 ± 0.15	12.17 ± 0.17	19.33 ± 0.22	27.10 ± 0.21
Sa-7	17.60 ± 0.15	13.17 ± 0.17	20.30 ± 0.25	25.50 ± 0.29
Sa-8	12.57 ± 0.07	16.13 ± 0.12	19.57 ± 0.12	23.50 ± 0.29
Sa-9	11.43 ± 0.23	13.33 ± 0.17	18.60 ± 0.10	23.83 ± 0.44

Clinical strains of Staphylococcus aureus from different clinical sources.
The values are the mean of three experiments ± S.E.
Means followed by the same letter was not significantly different by DMRT at the 0.05% probability level.

Table 5. Antifungal activity of the crude extract and their pure compounds of *Dioscorea pentaphylla* against clinically isolated fungal pathogens.

Clinical Strains	Diameter of zone of inhibition (mm)			
	Pet. Ether extract	Chloroform Extract	Ethanol Extract	Fluconozole
Trichophyton rubrum	10.63 ± 0.09	13.30 ± 0.15	14.37 ± 0.19	15.43 ± 0.23
Microsporum gypseum	12.37 ± 0.09	15.37 ± 0.09	20.37 ± 0.09	21.37 ± 0.19
Tricophyton tonsurans	11.60 ± 0.10	16.23 ± 0.15	16.15 ± 0.09	16.57 ± 0.12
Microsporum audouini	14.42 ± 0.09	12.77 ± 0.15	10.37 ± 0.19	16.40 ± 0.10
Candida albicans	10.06 ± 0.13	14.37 ± 0.19	18.13 ± 0.09	18.23 ± 0.15

Clinically isolates fungal pathogens from different clinical sources.
The values are the mean of three experiments ± S.E.
Means followed by the same letter was not significantly different by DMRT at the 0.05% probability level.

bacteria and dermatitis fungi was detected. The zones of inhibition of the microbial colony are depicted in Tables 3 to 5. The pet ether extract demonstrated antibacterial activity against all the clinical strains of bacteria. It showed maximum activity against *S. aureus* (16.13 mm) followed by *P. aeruginosa* (12.30 mm), *K. pneumoniae* (12.23 mm) and among fungal strains *Microsporum audouini* (14.42 mm) when compared to standard. Chloroform extract showed least inhibition activity against all the strains of bacteria and fungi. It was 19.40 mm against *S. aureus*, *P aeruginosa* (16.00 mm), *K pneumoniae* (14.33 mm) and in fungal strain *Tricophyton tonsurans* (16.23 mm) respectively. Whereas, ethanol extract showed significant inhibition zone as similar to standard. Ethanol extract illustrated inhibition zone against S. aureus (20.63 mm), *P aeruginosa* (20.50 mm), *K pneumoniae* (19.26 mm) and in fungal strain *Microsporum gypseum* (20.37 mm) and *Candida albicans* (18.13 mm) respectively.

Among all the tested extracts ethanol proved to be most potent bactericidal agent against all the strains as compared to other extracts, but it is not up to the standard drug

Ciprofloxacin. Among the five dermatitis fungi cultured for antifungal assay, all the crude extracts showed the zone of inhibition of the colony was found to be very good. The ethanol extracts showed significant inhibition against in *C. albicans, T. rubrum, M. gypseum* and *T. tonsurans* at par with standard drug Fluconozole.

4. DISCUSSION

The results obtained in the hitherto study showed wide spectrum of antimicrobial properties for the petroleum ether, chloroform and ethanolic extracts of *D. pentaphylla*. The organic solvent extracts of *D. Pentaphylla* tubers studied in the current work showed remarkable antibacterial activities against *Pseudomonas aeruginosa , Klebsiella pneumoniae* and *Staphylococcus aureus* responsible for causing diseases in animals and humans. These microorganisms pose an important public health and economic concerns for human society. However, the solvent extracts proved to be significant in their activity against the above bacterial strains.

There are reports showing that alkaloids and flavonoids are the responsible for the antifungal activities in higher plants (Cordell *et al.,* 2001). Moreover, secondary metabolites such as tannins and other compounds of phenolic nature are also classified as active antimicrobial compounds. Phenols, the aromatic compounds with hydroxyl groups are widespread in plant kingdom. They occur in all parts of plants. Phenols are said to offer resistance to diseases and pests in plants. Grains containing high amount of polyphenols are resistant to bird attack (Sadasivam and Manickam, 2006). Interestingly, phytochemical screening of the current investigation revealed that extracts from both the plant parts and the tuber possess at least three to four of the following classes of secondary metabolites: phenols, flavonoids, terpenoids, tannins, alkaloids and saponins. Therefore, the presence of these phytochemicals, could justify the observed antifungal activities in the current study. These results are in agreement with earlier studies realized on other plant species belonging to the euphorbiaceae (Mahomoodally *et al.,* 2005) and asteraceae (Boussaada *et al.,* 2008). Dioscorea (Sautor *et al.,* 2003) attributing antimicrobial activities to the presence of secondary metabolites. Diosgenyl saponins are one of the most abundant steroid saponins, with diosgenin as the steroidal sapogenin, are reported to exert a large variety of biological functions, such as anti-fungal, anti-bacterial, and anticancer (Li *et al.,* 2001).

Earlier chemical investigation of yam tubers afforded two norclerodane diterpenoids (Murray *et al.,* 1984). Clerodane class of diterpenes is a group of compounds that has attracted considerable interest because of problems associated with their stereochemistry and because of the diverse biological activities shown by some members (Roengsunran *et al.,* 2002). They are known to possess anti-tumor, anti-bacterial, anti-feedant, and anti-fungal activities (Biswanath *et al.,* 2005; Harding *et al.,* 2006). The studies of Quan *et al* (2006) reported efficient antibacterial activity against *Bacillus subtilis* and *Staphylococcus aureus* of diosgenin deratives like 2,6-iodopseudogiosgenin and 2,6- iodopsuedogiosgenone. Sautour *et al* (2004) showed Steroidal Saponin from *Dioscorea cayenensis* showed a positive results aganist *Candida albicans* (IP 1180-79), *C. glabrata* and *C. tropicalis* (clinical isolates). The CH_2Cl_2-soluble portion of the crude extract and the two clerodanes were showed significant activities against *P. aeruginosa, S. typhi, S. paratyphi A and S. paratyphi B,* was reported by Teponno *et al* (2006).

In the heitherto the traditional use of tubers of *Dioscorea pentaphylla* for the treatment of bacterial and fungal infections is realised. For follow-up research, it is needed to determine the active components in each extract and confirm their mechanismof action.

REFERENCES

1. Bax, R., Mullan, N. and Verhoef, F. 2002. The millennium bugs- the need for and development of new antibacterials. *International Journal of Antimicrobial Agents,* **16:** 51-59.
2. Biswanath, D., Reddy, M.R., Ramu, R., Ravindranath, N., Harish, H., Ramakrishna, K.V.S., Rao, Y.K., Harakishore, K., Murthy, U.S.N., 2005. Clerodane diterpenoids from Pulicaria wightiana. *Phytochemistry,* **66:** 633–638.
3. Boussaada, O., Chriaa, J., Nabli, R., Ammar, S., Saidana, D., Mahjoub, M.A., Chraeif, I., Helal, A.N. and Mighri, Z. 2008. Antimicrobial and antioxidant activities of methanol extracts of *Evax pygmaea* (Asteraceae) growing wild in Tunisia. *World J Microbiol Biotechnol,* **24:**1289-1296.
4. Cordell, G. A., Quinn-Beattie, M. L. and Farnsworth, N. R. 2001. The potential of alkaloids in drug discovery. *Phytother Res,* **15:**183-205.
5. Cown, M. M. 1999. Plant products as antimicrobial agents. *Clinical Microbiology Reviews,* **12:** 51-59.
6. Essawi, T. and Srour, M. 2000. Screening of some Palestinian medicinal plants for antibacterial activity. *Journal of Ethnopharmacology,* **70:** 343-349.
7. Finar, I. L. 2003. Organic chemistry-vol 2, Stereochemistry and the chemistry of natural products. Vth edition. Delhi: Pearson Education (Singapore) India branch; 769-71.
8. Fleming Thomas., Guenwald Joerg., Brendler Thomas., Jaenicke Christof and Mehtoa Mukesh (eds.) .1998. PDR for Herbal Medicines. Montvale, NJ, Medical Economics Company, Inc.
9. Foster, S. and Duke, J. 2000. A Field guide to medicinal plants and herbs of eastern and central North America. New York, Houghton Mifflin.
10. Grieve, M. 1971. A Modern Herbal. New York, Dover Publications, Inc.
11. Harding, W.W., Schimidt, M., Tigewell, K., Kannan, P., Holden, K.G., Gilmour, B., Mavarro, H., Rothman, R.B., and Prisinzano, T.F. 2006. Synthetic studies of neoclerodane diterpenes from Salvia divinorum: semisynthesis of salvinicins A and B and other chemical transformations of salvinorin A. *Journal of Natural Products* ,**69:** 107–112.
12. Hutchens Alma, R. 1991. Indian Herbology of North America. Boston, Shambhala Publications, Inc.

13. Kausik, B., Ishitha, C., Banerji R.K. and Uday. B. 2002. Biological activities and medicinal properties of neem (*Azadirachta indica*). *Cur. Sci.* **82:** 11.

14. Li, B., Yu, B., Hui, Y., Li, M., Han, X., and Fung, K. P. 2001. Salt-assisted acid hydrolysis of starch to D- glucose under microwave irradiation. *Carbohydr. Res.* 331, 1.

15. Mahomoodally, M. F., Gurib-Fakim, A., and Subratty, A. H. 2005. Antimicrobial activities and phytochemical profiles of endemic medicinal plants of Mauritius. *Pharmaceutical Biol*, **43:**237-242.

16. Mukherjee, P.K., Balasubramanian. R., Saha, K., Saha, B.P., and Pal, M. 1995. Antibacterial efficiency of *Nelumbo nucifera* (Nymphaeaceae) rhizomes extract. *Indian Drugs* **32:** 274-276.

17. Murray, R.D.H., Jorge, Z., Khan, N.H., Shahjahan, M., and Quaisuddin, M., (1984). Diosbulbin D and 8-epidiosbulbin E acetate, norclerodane diterpenoids from *Dioscorea bulbifera* tubers. *Phytochemistry* **23:** 623– 625.

18. Parekh, J., Karathia, N.anf Chanda, S. 2006 Antibacterial activity of Bauhinia variegata. *J Biomed Res*, **9:**53-56.

19. Parekh, J. and Sumitra, C. 2006. In vitro antimicrobial activities of extracts of *Launaea procumbens Roxb*. (Labiateae), Vitis vinifera L. (Vitaceae) and *Cyperus rotundus L.* (Cyperaceae). *Afr. J. of Biomed Res*, **9:** 89-93.

20. Quan. H.J., Koyanagi.J., Hagiwara. K., Cui. X R., Isshiki. Y., Kondo. S., Komada. F., and Saito. S., (2006) Reactions of 26-iodopseudodiosgenin and 26-iodopseudodiosgenone with various nucleophiles and pharmacological activities of the products. *Chem. Pharm. Bull*. **54(1):** 72-79

21. Raja Naika, H., Krishna, V., Harish, B. G., Khadeer Ahamed, B. M., and Mahadevan, K. M. 2007. Antimicrobial Activity of Bioactive Constituents Isolated from the Leaves of *Naravelia zeylanica* (L.) DC. *International Journal of Biomedical and Pharmaceutical Sciences.*

22. Roengsunran, S., Musikul, K., Petson, A., Vilaivan, T., Sangvanich, P., Pornpakakul, S., Puthong, S., Chaichantipyuth, C., Jaiboon, N. and Chaichit, N. 2002. Croblongifolin, a new anticancer clerodane from *Croton oblongifolius*. *Planta Medica* **68:** 274–277.

23. Sadasivam, S and Manickam, A. 1996. Biochemical Methods, 2nd edition. New Delhi: New Age International (P) Ltd: 192-93.

24. Sautour, M., Mitaine-A. C. O., Miyamoto. T., Dongmo.A., and Lacaille-Dubois. M. A. 2004. A new steroidal saponin from *Dioscorea cayenensis. Chem. Pharm. Bull.* **52(11):** 1353—1355

25. Teponno, R. B., Tapondjou, A. L., Gatsing, D., Djoukeng, J. D., Mansour, E., Tabacchi, R., Tane P., Stoekli-Evans H., and Lontsi D. 2006. Bafoudiosbulbins A, and B, two anti-salmonellal clerodane diterpenoids from *Dioscorea bulbifera L.* var sativa, *Phytochemistry* **67:**1957–1963

26. Tierra Michael (1998): The Way of Herbs. New York, Pocket Books.

***