Journal of Medicinal Plants Research Vol. 5(21), pp. 5247-5253, 9 October, 2011
Available online at http://www.academicjournals.org/JMPR
ISSN 1996-0875 ©2011 Academic Journals

Full Length Research Paper

Antimicrobial potential of the selected plant species against some infectious microbes used

Rabia Naz, Asghari Bano*, Humaira Yasmin, Samiullah and Uzma Farooq

Plant Physiology Laboratory, Department of Plant Sciences, Quaid-i-Azam University Islamabad, Pakistan.

Accepted 15 June, 2011

During the present investigation, *in vitro* studies were carried out to evaluate the antibacterial and antifungal activity of two selected plant species viz, *Calotropis procera* and *Citrullus colocynthis*. Ethanolic, methanolic and aqueous extracts of leaf were examined for their antibacterial, antifungal activities. Methanolic leaf extracts were found to be more active against gram positive bacteria (*Bacillus subtilis*: ATCC 6059 and *Staphylococcus aureus*: ATCC 6538) as well as gram negative bacteria (*Pseudomonas aeruginosa*: ATCC 7221 and *Klebsiella pneumoniae*) than water and ethanol extracts of leaves. The leaf extracts from *C. colocynthis* showed greater inhibitory activity against gram positive and gram negative bacteria as compared to that of *C. procera* leaf extract. Antifungal activity of the two plant species was performed with both methanolic and aqueous extract against *Aspergillus fumigatus*. The results revealed that methanolic as well as aqueous extract of *C. procera* were found to be most effective in inhibiting the growth of selected fungal strain. It is inferred from the present investigation that the ability of extracts of *C. colocynthis and C. procera* to inhibit the growth of bacteria and fungi is an indication of their broad spectrum antimicrobial potential which may be implicated in the management of microbial infections.

Key words: Crude extract, aqueous extract, minimum inhibition concentration.

INTRODUCTION

The traditional folk medicinal system uses the plant products for the treatment of various infectious diseases. In recent times, plants are being extensively explored for harboring medicinal properties. Studies by various researchers have proved that plants are one of the major sources for drug discovery and development (Rates, 2001), Gordon and David (2005). Plants are reported to have antimicrobial, anticancer, anti inflammatory, antidiabetic, hemolytic, antioxidant, larvicidal properties etc. A proper health care system can be established with supplying low cost medicine to population by using various medicinal plants. Recent discovery shows that these plants have fewer side effects than the Allopathic medicine. The herbal medicine is gaining popularity all over the world (Yesmin et al., 2008). Traditional medicine using plant extracts continues to provide health coverage for over 80% of the world's population, especially in developing world (WHO, 2002). *Calotropis procera* Linn. A wild growing plant of family Asclepiadaecae is known to posses multifarious medicinal properties. Different parts of the plant have been used in Indian traditional system of medicine for the treatment of leprosy, ulcers, tumors, piles and diseases of spleen, liver and abdomen (Kirtikar and Basu, 1935). Chemical investigations on *C. procera* L. resulted in the isolation of octacosanoic acid, semiarenone and trematol, a triterpenoid alcohol and Column chromatography of the n-hexane extract of *C. procera* followed by recrystallization afforded white needle shaped crystals, which was chemically b-sitosterol on the basis of its 1HNMR and IR spectral data.

The root of the *C. procera* Linn is used as a carminative in the treatment of dyspepsia (Kumar and Arya, 2006). Further, the root bark and leaves of *C. procera* are used by various tribes of central India as a curative agent for jaundice (Samvatsar and Diwanji, 2000). *Citrullus colocynthis* belongs to the family Cucurbitaceae. Members of this family are generally dioecious herbs

*Corresponding author. E-mail: banoasghari@gmail.com. Tel: + 92-05190643096.

Abbreviations: DMSO, Dimethyl sulfoxide; **MIC,** minimum inhibitory concentration.

which may be prostate or climbing by means of tenderils. Fruit is fleshy and many fruits are used as vegetable or as edible fruits. *C. colocynthis* is a small scarbid perennial creeping herb with prostate or climbing stem, bearing smooth spherical fruits which are mottled green when young and some what yellow when ripe (Shah and Qadry, 1985). Colocynth was well known to Greeks and Romans. The drug was equally known to the Arabian Physicians and was produced in Cyprus and Spain during the ages. The main chemical constituents of *C. colocynthis* reported in the literature are docosan-1-ol acetate, 13-dimethyl-pentadec-13-en-1-al, 11-14-dimethyl hexadecane, 14-ol 2-one,10-14-dimethyl hexadecane, 14-ol-2-one,linoleic acid, oleic acid, carbohydrate, amino acid, organic acid, lipid, sterols and phenols (Ayub and Yankov 1981; Basalah et al., 1985; Habs et al., 1984 and Navot and Zamir, 1986). The present work was designated to investigate further antibacterial and antifungal activities of methanolic, ethanolic and aqueous leaf extracts of *C. procera* and *C. colocynthis*.

MATERIALS AND METHODS

Plant materials

Fresh leaves of *C. procera and C. colocynthis* were collected from arid region of Punjab, Pakistan during 2009. The plants were identified by the National Herbarium, Department of Plant Sciences, Quaid-i-Azam University Islamabad.

Processing of the plant

Plant leaves were washed properly with distilled water. The leaves were shade dried at room temperature. The dried leaves of *C. procera and C. colocynthis* were uniformly grinded using mechanical grinder and stored in air tight containers for further use. About 250 g of pulverized plant materials were extracted in ethanol and methanol separately. Another 250 g of plant material was extracted in water for 4 days with occasional shaking (Harbone, 1998). Ethanol and methanol used were of analytical grade. The separated extracts were then filtered through Whatman's No 1 filter paper and the ethanol and methanol filterate were separately reduced to dryness using rotary evaporator. The viscous mass was then dried in air at room temperature. Dried extract was collected in an air tight container and stored at 4°C. The extract thus obtained was used for antimicrobial activities.

Test microorganism

The test microorganisms used in this study (bacteria: *S. aureus*: ATCC 6538*, P. aeruginosa*: ATCC 7221*, K. pneumonia, B. subtilis*: ATCC 6059; fungi: *A. fumigatus*). The bacterial isolates were first subcultured in a nutrient broth (SIGMA) and incubated at 37°C for 18 h while the fungal isolates were subcultured on a Sabouraud dextrose agar (SDA) (MERCK) for 72 h at 25°C.

Positive and negative control

Penicillin (1 mg/ml) was used as positive control for the test microorganisms. Sterilized distilled water and Dimethyl sulfoxide (DMSO) were used as negative control.

Assay for antibacterial activity

Antibacterial activity of the methanolic, ethanolic and aqueous extract of selected plant extracts was determined in accordance with the agar-well diffusion method described by Irobi et al. (1994). The bacterial isolates were first cultured in a nutrient broth for 18 h prior to use and standardized to 0.5 McFarland standards (10^6 $cfuml^{-1}$). The 0.5 McFarland standards were prepared by adding 0.5 ml 0.048 M Barium chlorides to 99.5 ml 0.36 N sulphuric acid. Barium sulphate turbidity standard (4 to 6 ml) and was taken in screw capped test tube. Nutrient agar medium was prepared by adding nutrient agar (MERCK) 2.3 g in 100 ml of distilled water; pH was adjusted at 7.0 and was autoclaved. It was allowed to cool up to 45°C. Petri plates were prepared by pouring 75 ml of seeded nutrient agar and allowed to solidify. Wells were bored into the agar using a sterile 6 mm diameter cork borer. Approximately 100 µl of the crude extract at 12 $mgml^{-1}$ were added into the wells, allowed to stand at room temperature for about 2 h and incubated at 37°C. Controls were set in parallel in which case the respective solvents were used to fill the well. The plates were observed for zones of inhibition after 24 h. The effects were compared with those of penicillin at a concentration of 1 mg/ml.

Determination of relative percentage inhibition

The relative percentage inhibition of the test extract with respect to positive control was calculated by using the following formula (Kumar et al., 2010; Ajay et al., 2003):

$$\text{Relative percentage inhibition of the test extract} = \frac{100 \times (X-Y)}{(Z-Y)}$$

Where, X: total area of inhibition of the test extract, Y: total area of inhibition of the solvent, Z: total area of inhibition of the standard drug. The total area of the inhibition was calculated by using area = πr^2; where, r = radius of zone of inhibition.

Determination of minimum inhibitory concentration (MIC)

The determination of MIC of the methanolic, ethanolic and aqueous extracts was carried out by using the method of Akinpelu and Kolawole (2004). Two-fold dilutions of the plants crude extract was prepared and 2 ml aliquots of different concentrations were added to 18 ml of pre-sterilized molten nutrient agar for bacteria at 40°C to have the final concentration of 12 mg/ml. The medium was then poured into sterile Petri dishes and allowed to settle. The surface of the medium was allowed to solidify under laminar flow prior to streaking with 18 h old bacterial cultures. The plates were incubated at 37°C for 24 h, after which they were examined for the presence or absence of growth. Antimicrobial activity of the leaf extract was evaluated by measuring the zone of inhibition. Experiment was carried out in triplicates for each test organism. The MIC was taken as the lowest concentration that prevented the growth of the test microorganism.

Assay for antifungal activity

The agar tube dilution method was used for the determination of antifungal activity of extract (Washington and Sutter, 1980). The samples were prepared by dissolving 12 mg extract in 1 mL of DMSO. Culture media was prepared by dissolving 6.5 gm of

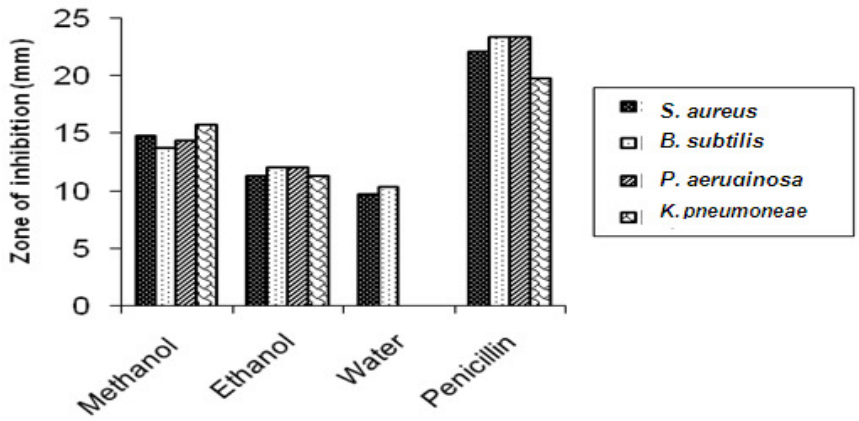

Figure 1. Antibacterial activities profile of methanol, ethanol and water extracts from Leaf tissue of *C. procera*.

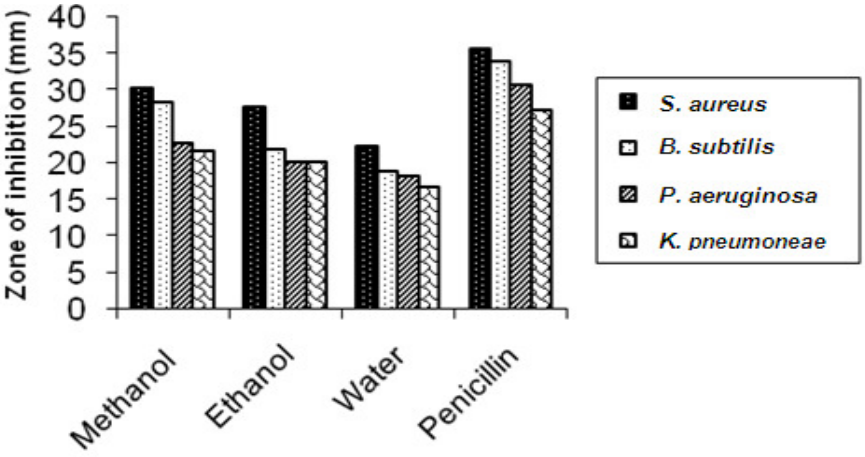

Figure 2. Antibacterial activities profile of methanol, ethanol and water extracts from leaf tissue of *C. colocynthis*.

Sabouraud dextrose agar per 100 ml distilled water (pH 5.6). The 10 mL of Sabouraud dextrose agar (MERCK) was dispensed in screw capped tubes or cotton plugged test tubes and was autoclaved at 121°C for 21 min. Tubes were allowed to cool at 50°C and Sabouraud dextrose agar was loaded with 67 µl of extract pipetted from the stock solution. The tubes containing the media were then allowed to solidify in slanting position at room temperature. Three slants of the extract sample were prepared for fungus species. The tubes containing solidified media and plant extract were inoculated with 4 mm diameter piece of inoculum, taken from seven days old culture of fungus. One test tube of each extract was prepared, used as positive control. Slants without extract were used as negative control. The test tubes were incubated at 28°C for 7d. Cultures were examined twice weekly during the incubation. Reading was taken by measuring the linear length (mm) of fungus in slant and growth inhibition was calculated with reference to negative control. Percentage inhibition of fungal growth for each concentration of compound was determined as;

$$\text{Percentage inhibition of fungal growth} = \frac{100 - \text{Linear growth in test (mm)} \times 100}{\text{Linear growth in control (mm)}}$$

Statistical analysis

The values of antimicrobial activities of the methanolic, ethanolic and aqueous leaves extract of *C. procera* and *C. colocynthis* were expressed as mean ± standard deviation (n= 3) for each sample.

RESULTS

Antibacterial activity

The usage of these plants for medicinal purpose was reported by several researchers. The methanolic, ethanolic and aqueous extract of *C. procera* and *C. colocynthis* leaves exhibited the antibacterial activity against four isolates of bacteria (Figures 1 and 2) and the results were expressed as mean ± standard deviation (n=3). The antibacterial activities of the methanolic, ethanolic and aqueous extracts compared favourably with that of standard antibiotic (Penicillin).

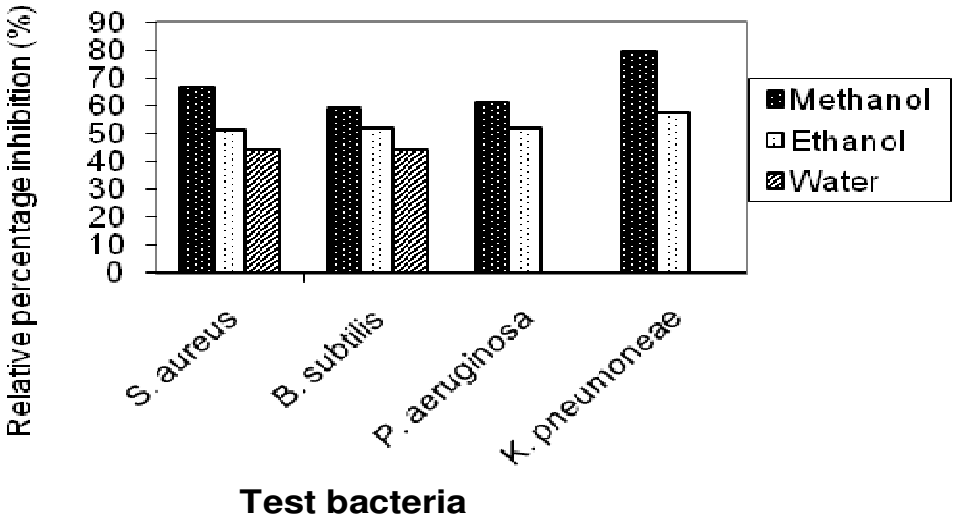

Figure 3. Determination of relative % age inhibition of methanol, ethanol and water extracts from leaf tissue of *C. procera* compared to standard antibiotic.

The *C. procera* methanolic leaf extract showed maximum (15.7 ± 0.33) antibacterial activity as compared to ethanolic and aqueous extracts against *K. pneumonia* and lowest activity against *B. subtilis* (13.7 ± 0.88). The water extract showed lowest antibacterial activity against *S. aureus* and B. *subtilis* and showed no activity against *P. aeruginosa* and *K. pneumoneae.* The methanolic leaf extract of *C. colocynthis* showed maximum (30.3 ± 0.33) inhibition against *S. aureus* and minimum inhibition against *K. pneumoneae. Ethanolic* and water extracts also showed maximum inhibition against *S.aureus* and minimum inhibition against *K. pneumoneae* (Figure 2).

Relative percentage inhibition

The results of antimicrobial activity of *C. procera* and *C. colocynthis* methanol, ethanol and water extracts were compared with the positive control (Standard drugs) for evaluating their relative percentage inhibition (Figures 3 and 4), the *C. procera* methanol extract exhibits maximum (79.7%) relative percentage inhibition against *K. pneumoneae* followed by *S. aureus* (66.8%), *P. aeruginosa* (61.4%), *B. subtilis* (58.8%). While the *C. colocynthis* methanol extract showed maximum relative percentage inhibition as compared to ethanol and water extracts (Figure 4). Methanol leaf extracts exhibit maximum (84.9%) percentage inhibition against *S. aureus* followed by *B. subtilis* (83.2%), *K. pneumonia*(79.5%) and *P. aeruginosa* (73.9%).

Minimum inhibition concentration (MIC)

Results of MIC are reported in Tables 1 and 2. The *C. procera* methanol extract showed 5 mg/ml, MIC values for *S. aureus, B. subtilis, P. aeruginosa* and *K. pneumoneae*. Ethanol leaf extract MIC values for different bacteria tested ranged between 6.5 to 8 mg/ml, while that of water extract ranged between 10 to 12 mg/ml. The *C. colocynthis* methanol extract showed 2.5 to 5 mg/ml MIC values for *S. aureus, B. subtilis, P. aeruginosa* and *K. pneumoneae*. Ethanol leaf extract MIC values for different bacteria tested ranged between 2.5 to 8 mg/ml, while that of water extract ranged between 8 to 10 mg/ml.

Antifungal activity

The methanol and water extracts of *C. procera* and *C. colocynthis* showed broad antimycotic activity against the tested fungal isolate at a final concentration of 12 $mgml^{-1}$ (Tables 3 and 4) and the performance of the extracts were similar to the antibacterial activity. The methanolic extract of *C. procera* and *C. colocynthis* inhibited the mycelia growth of *A. fumigatus* by 94 and 66%, respectively. While the water extract of both the plants exhibited inhibition by 84 and 58%, respectively.

DISCUSSION

Methanol, ethanol and water extracts were used in this study to evaluate the antifungal, antibacterial activities. Generally the methanol extract was more active than other extracts against the selected bacterial isolates and fungus, because most of the antimicrobial agents in plants are soluble in methanol (Chandrasekaran and Venkatesalu, 2004). This may be attributed to the presence of soluble phenolic and polyphenolic compounds (Kowalski and Kedzia, 2007). The inhibitory

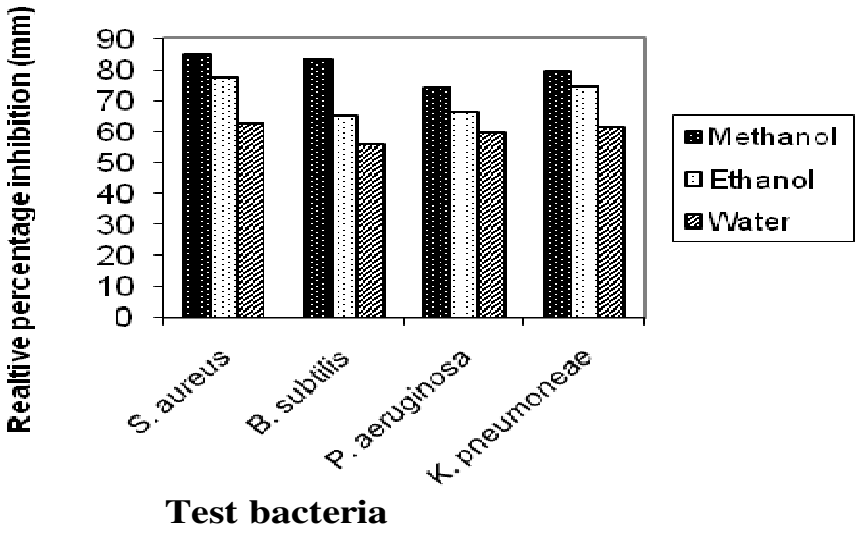

Figure 4. Determination of relative percentage age inhibition of methanol, ethanol and water extracts from leaf tissue of *C. colocynthis* compared to standard antibiotic.

Table 1. MIC values of methanol, ethanol and water extracts from Leaf tissue of *C. procera.*

Test bacteria	MIC in mg/ml			
	Methanol mgml^{-1}	Ethanol mgml^{-1}	Water mgml^{-1}	PC mgml^{-1}
Staphylococcus aureus	5.0	8.0	12.0	0.05
Bacillus subtilis	5.0	6.5	10.0	0.05
Pseudomonas aeruginosa	5.0	8.0	Nd	0.05
Klebsiella pneumoneae	5.0	8.0	Nd	0.05

PC- positive control (penicillin), Nd- not determines.

Table 2. MIC values of methanol, ethanol and water extracts from Leaf tissue of *C. colocynthis.*

Test bacteria	MIC in mg/ml			
	Methanol mgml^{-1}	Ethanol mgml^{-1}	Water mgml^{-1}	PC mgml^{-1}
Staphylococcus aureus	2.5	2.5	8.0	0.05
Bacillus subtilis	2.5	5.0	10	0.05
Pseudomonas aeruginosa	5.0	8.0	10	0.05
Klebsiella pneumoneae	5.0	8.0	10	0.25

PC- positive control (penicillin).

Table 3. Antifungal activity of methanol and water extracts from Leaf tissue of *C. procera.*

Test fungi	Linear growth (cm)		Percentage inhibition (%)	
	Methanol 12 mgml^{-1}	Water 12 mgml^{-1}	Methanol 12 mgml^{-1}	Water 12 mgml^{-1}
Aspergillus fumigatus	0.59±0.01	1.57±0.003	94.1	84.6
Positive control	0.17±0.003	0.19±0.003	98	98

effect of the extract of *C. procera* and *C. colocynthis* against pathogenic bacterial strains can introduce the plants as a potential candidate for drug development for the treatment of ailments caused by these pathogens. The non activity or low activity of the water extract against most bacterial strains investigated in this study is

Table 4. Antifungal activity of methanol and water extracts from Leaf tissue of *C. colocynthis.*

Test fungi	Linear growth (cm)		Percentage inhibition (%)	
	ethanol 12 mgml^{-1}	Water 12 mgml^{-1}	Methanol 12 mgml^{-1}	Water 12 mgml^{-1}
Aspergillus fumigatus	3.43±0.006	4.33±0.007	66	58
Positive control	0.17±0.003	0.19±0.003	98	98

in agreement with previous works which show that aqueous extracts of plant generally showed little or no antibacterial activities (Koduru et al., 2006; Aliero et al., 2006; Ashafa et al., 2008; Aiyegoro et al., 2008). The reputation of *C. procera* as a remedy for different microbial diseases traditionally including diarrhoea and dysentery was supported by the antibacterial screening tests (Yesmin et al., 2008). The methanolic extracts of Citrullus colocynthis exhibited an antibacterial activity against *P. aeruginosa* ATTC 27853 (Takhi et al., 2011).

The susceptibility of selected fungus *A. fumigatus* to *C. procera* and *C. colocynthis* extracts is significant, as *A. fumigatus* spores caused Aspergillosis due to its inhalation. *A. fumigatus* is an opportunistic pathogen which usually affects cavities that have formed in the lungs from preexisting lung diseases. The fungus mass gradually enlarges, destroying lung tissue in the process, but usually does not spread to other areas (Bansod and Rai, 2008). Generally the methanol extract had the highest activity against both bacterial and fungal isolates. This was followed by the ethanol extract and the least was observed in the water extract. The ability of the extracts to inhibit the growth of several bacterial and fungal species is an indication of the broad spectrum anti-microbial potential of *C. procera* and *C. colocynthis*, which makes the plant a candidate for bioprospecting for antibiotic and antifungal drugs. Fungal infection seems especially controllable due to good hygienic condition, but development of fungal resistance to antifungal drugs is almost an inevitable consequence of their application (Ekhaise and Okoruwa, 2001). The result in this study revealed that the crude, methanol, ethanol extracts of the plant have antifungal activity against the test organisms. Similarly, the methanol crude extract of *C. procera* have antifungal activity against *Microsporum canis* and *Trichophyton rubrum* at 5.0 mg/ml concentration (Kuta, 2006).

Conclusion

From the present investigation it has been revealed that *C. procera* and *C. colocynthis* have the ability to provide protection against *B. subtilus*, *P. aeruginosa*, *S. aureus*, *K. pneumoneae* and *A fumigates.* The leaves extract of 12 mg/ml showed maximum inhibition against all the bacterial and fungal strains similar to the antibiotic Penicillin. *The C. procera* and *C. colocynthis* represents a rich source of valuable medicinal compounds and leaves of *C. procera* and *C. colocynthis* contain high antibacterial and antifungal property and can be further explored for the isolation of its bioactive compound.

REFRENCES

Aiyegoro OA, Akinpelu DA, Afolayan AJ, Okoh AI (2008). Antibacterial activities of crude stem bark extracts of Distemonanthus benthamianus Baill; J. Biol. Sci., 8(2): 356-436.

Ajay KK, Lokanatha RMK, Umesha KB (2003). Evaluation of antibacterial activity of 3,5-dicyano-4,6-diaryl-4-ethoxycarbonyl-piperid-2-ones, J. of Pharm. Biomed. Anal., 27: 837-840.

Akinpelu DA, Kolawole DO (2004). Phytochemistry and antimicrobial activity of leaf extract of *Piliostigma thonningii* (*Schum*). Sci. Focus, 7: 64-70.

Aliero AA, Grierson DS, Afolayan AJ (2006). Antifungal activity of *Solanum pseudocapisum* Res. J. Bot., 1: 129-133.

Ashafa AOT, Grierson DS, Afolayan AJ (2008). Antimicrobial activity of extract from Felicia muricata Thunb J. Biol. Sci., 8(6): 1062-1066.

Ayoub SMH, Yankov LK (1981). On the constituents of the peel of *Citrullus colcynthis*. Part-2. *Fitoterpia,* 52(1): 17-18.

Basalah MO, Ali Whaibi MH, Sher M (1985). Comparative study of some metabolities of *Citrullus colocynthis* Schrad and *Cucumis prophetarum* L. J. Biol. Sci. Res., 16(1): 105-123.

Chandrasekaran , Venkatesalu V (2004). Antibacterial and antifungal activity of *Syzygium jambolanum* seeds, J. Ethnopharmacol., 91: 105-108.

Ekhaise FO, Okoruwa P (2001). Antibacterial Activity of Vera extract on *Staphylococcus aureus*. Trop. J. Environ. Sci. Health, 4: 28-31.

Kumar Gaurav, Karthik L, Bhaskara Rao KV (2010). *In vitro* anti-*Candida* activity of *Calotropis gigantea* against clinical isolates of *Candida*, J. Pharm. Res., 3: 539-542.

Gordon MC, David JN (2005). Biodiversity: A continuing source of novel drug leads, Pure Appl. Chem., 77: 7-24.

Habs M, Jahn SAA, Schmaehl D (1984). Carcinogenic activity of condensate from colquint seeds *(Citrullus colcynthis)* after chronic eipcutaneous administration to mice. J. Cancer Res. Clin. Oncol., 108(1): 154-156.

Harborne JB (1998). Phytochemical Methods - A Guide to Modern Techniques of Plant Analysis. Chapman and Hall, London, pp. 182-190.

Irobi ON, Moo-Young M, Anderson WA, Daramola, SO (1994). Antimicrobial activity of the bark of *Bridelia ferruginea* (Euphorbiaceae). Intern. J. Pharmacog., 34: 87-90.

Kirtikar KR, Basu BD (1935). Indian Medicinal Plants, Lolit Mohan Basu, Allahabad, India, p. 1606.

Koduru S, Grierson DS, Afolayan AJ (2006). Antimicrobial activity of Solanum aculeastrum (Solanaceae). Pharamacol. Biol., 44: 284-286.

Kowalski R, Kedzia B (2007). Antibacterial activity of *Silphium perfoliatum* extracts Pharm. Biol., 45: 495-500.

Kumar VL, Arya S (2006). Medicinal uses and pharmacological properties of *Calotropis procera*. In: J.N. Govil, Editor, Recent Progress in Medicinal Plants 11, Studium Press, Houston, Texas, USA, pp. 373-388.

Kuta FA (2006). *In Vitro* investigation on the Effect of *Calotropis procera* leaves extract on *Microsporum canis* and *Trichophyton rubum.* Sch. Sci. Sci. Edu. Conf. Proceed., pp. 27-29.

Navot N, Zamir D (1986). Linkage relationships of 19 protein-coding genes in watermelons. Theor. Appl. Genet., 72(2): 274-278.

Rates SMK (2001). Plants as source of drugs, Toxicon, 39: 603-613.
Samvatsar S, Diwanji VB (2000). Plant sources for the treatment of jaundice in the tribals of Western Madhya Pradesh of India. J. Ethnopharmacol., 73: 313-316.
Shah CS and Qadry JS (1985). A Text book of Pharmacognosy (5thEdition). B.S. Shah, Prakashan, Pankore Naka, Ahmedabad, India, p. 284.
Takhi D, Ouinten M, Yousfi M (2011). Study of Antimicrobial Activity of Secondary Metabolites Extracted Fromspontaneous Plants from the Area of Laghouat, Algeria. Adv. Environ. Biol., 5(2): 469-476.
Washington JA and Sutter VL (1980). Dilution Susceptibility Test, Agar and Microbroth Dilution Procedure, Manual of Clinical Microbiology, 3rd ed. Am. Soc. Microbiol., Washington 56, 279⁀285. D. C., p. 453.
WHO (2002). Traditional Medicine: Growing Needs and Potential. WHO Policy Perspectives on Medicines. World Health Organization, Geneva, pp. 1-6.