Indian Journal of Plant Sciences ISSN: 2319–3824(Online)
An Open Access, Online International Journal Available at http://www.cibtech.org/jps.htm
2017 Vol.6 (2) April-June, pp.65-71/Nanna et al.
Research Article

ANTIFUNGAL ACTIVITY OF *TRICHOSANTHES CUCUMERINA* L AN ENDANGERED ETHNOMEDICINAL WILD HERB

***Rama Swamy Nanna, Shyamsundarachary Rudroju and Samatha Talari**
Department of Biotechnology, Plant Biotechnology Research Group, Kakatiya University, Warangal-506009 (TS), India
**Author for Correspondence*

ABSTRACT
The present work has been under taken to study the antifungal activity of stem, leaf, flower and seed extracts of *Trichosanthes cucumerina* L against disease causing fungi. Antifungal activity of different solvent (Water, Methanol, Chloroform, Petroleum ether, Acetone) extracts of stem, leaf, flower and seed of *T. cucumerina* has been studied to find out its activity against six important fungi *Penicillium, Fusarium chlmydosporum, Aspergillus flavus, Aspergillus terreus, Aspergillus fumigates* and *Curvularia lunata.* The antifungal activity of the stem, leaf, flower and seed extracts was done through well diffusion method and by measuring the inhibition zone around the disc. The results revealed that the seed extracts of *T. cucumerina* exhibited antifungal activity against all the microbes under study. The results provided evidence that the species *T. cucumerina* can be used as a potential source of antifungal agents.

Keywords: *Antifungal Activity, Trichosanthes Cucumerina, Different Extracts, Zone of Inhibition*

INTRODUCTION
It is well known that infectious diseases account for high proportion of health problems especially in the developing countries. The pathogenic microorganisms are always trying to develop resistance to various antibiotics. This resistance has increased due to indiscriminate use of commercial antibiotic drugs. Therefore, the chemotherapy of infectious diseases has proved to be a continuous struggle. Scientists are always in search of new antimicrobial substances from various sources to control the pathogenic microorganisms. Thus, it is of paramount importance for the scientists to develop new antimicrobial substances. The exploration of certain indigenous plants for their biological properties holds a great potential to solve the riddles caused by microorganisms.

Number of plant species have been tested for antimicrobial properties, the vast majority of have not been adequately evaluated (Balandrin *et al.*, 1985). Some of the plants showed antifungal activity: *Acacia nilotica, Withania somnifera* and *Sida cordifolia* (Mahesh and Satish, 2008). Infections induced by pathogenic fungi are increasingly recognized as an emerging threat to public health. Certain commensal fungi, such as *Candida* species, cause infections when their human hosts become immune compromised (Cannon *et al.*, 1995). Laurens *et al.*, (1985) worked on the antimicrobial activity of some Cucurbitaceae medicinal species of Dakar markets.

Several works have demonstrated in laboratory trials that different parts of plant, such as root, leaves, seeds and flowers possess inhibitory properties against bacteria, fungi and insects (Davicino *et al.*, 2007). In recent times, the rapid development of multi drug resistant bacterial and fungal strains of clinically important pathogens fetches the interest of scientists to develop newer broad spectrum antimicrobial agents.

The less availability and high cost of new generation antibiotics necessitates looking for the substances from alternative medicines with claimed antimicrobial activity. These developments and associated increase in microbial infections intensified the search for new, safe and more efficacious agents to combat serious microbial infections.

Based on review of literature, there appeared to be fewer studies performed on the antimicrobial activity of aqueous and organic extracts of *T. cucumerina*. The present investigation has been attempted to evaluate antifungal activity of different solvent extracts of various parts of *T. cucumerina* using some human and plant pathogenic and non-pathogenic fungi.

MATERIALS AND METHODS

Chemicals/Reagents

Chemicals and reagents used in this investigation were of analytical reagent grade and were purchased from Himedia (Mumbai, India), Sd-fine (Mumbai, India) and E-Merck (Mumbai, India).

Microorganisms Used: The following fungal species were used to assess the antifungal activity of extracts of *T. cucumerina.* These were obtained from Department of Microbiology, Kakatiya University, Warangal, Telangana State, India.

1.	*Penicillium spp.*	KUCC31
2.	*Fusarium chlamydosporum*	KUCC 27
3.	*Aspergillus flavus*	KUCC 24
4.	*A. terreus*	KUCC 38
5.	*A. fumigatus*	KUCC 25
6.	*Curvularia lunata*	KUCC 36

Media Used for the Assay: The following different specific growth media were used to culture the microorganisms.

Media for Fungal Cultures

Sabourad's Dextrose Agar Medium (SDA)

1.	Peptone	10.00g
2.	Dextrose	40.00g
3.	Agar	20.00g
4.	Distilled water	1000ml
5.	pH	6.8

Asthana and Hawker's Medium (AH)

1.	Glucose	5.00 g
2.	KN03	3.50g
3.	KH2P04	1.75g
4.	MgS04	0.75g
5.	Agar	20.0g
6.	Distilled water	1000ml

A medium without agar was employed as broth medium.

Plant Material: The plant material (stem, leaf, flower and seeds) of *T. cucumerina* was collected from the Research field, Department of Biotechnology, Kakatiya University, Warangal, Telangana State, India. This collected plant material was washed thoroughly with distilled water to remove the surface contaminants and shade dried at room temperature for 30-45 days. Later finely powdered using an electric blender and stored in air tight containers for future use.

Preparation of the Extracts

The powder (25gm) was used for the extraction with 150 ml 80% methanol for 24 hours by Soxhlet equipment and filtered through 0.45 µm membrane filter. This filtrate was evaporated under reduced pressure and dried in a rotator evaporator at 55°C.

Dried extracts were stored in screw cap bottles at -20°C and used as stock. Further, the same was diluted by using distilled water to arrive at different concentrations (1:1, 1:2 and 1:3). Fresh extracts were prepared for every third day.

Preparation of Sample/Test Solution for Antifungal Activity

A concentration of 200mg/ml of each solvent extract of different plant parts was prepared in DMSO (which did not influence the microbial growth).

Preparation of Spore Suspension

From the fresh cultures, spores (fungi) were collected and transferred in a test tube containing sterilized distilled water and Sabourad's dextrose broth. The spore suspension thus, obtained was used for testing antifungal activity.

Cultivation Techniques

a) *Slant Preparation:* Agar slants were prepared by dispensing 10 ml of aliquots of molten medium into 30 ml test tubes and sterilized. The test tubes were then laid on a 30 angle and allowed to set.

b) *Plate Preparation:* Using sterile technique, 20ml aliquots of sterile molten medium were transferred to sterilized petridishes. After solidifying, the plates were used for the assay.

c) *Sub-Culturing:* Subcultures were prepared by transferring loopful of inoculums from culture slants to freshly prepared agar slants. These were incubated in the desired conditions (Sabourad's Dextrose Agar medium, 22^{o}C for 5-7 days in dark).

Antifungal Assay: The antifungal activity of petroleum ether, chloroform, ethyl acetate, acetone methanol and aqueous extracts was determined by agar well diffusion method.

The culture plates inoculated with test organisms were allowed to solidify and punched with sterile cork borer (7.0 mm diameter) to make open wells.

The open well was filled with 0.05ml or 50µl of the extract. For normal fungi, the test was carried out on Sabourad's Dextrose Agar medium. Plates were incubated at 30^{o}C and 22^{o}C respectively for 72 hrs. The zones of inhibition were measured and recorded.

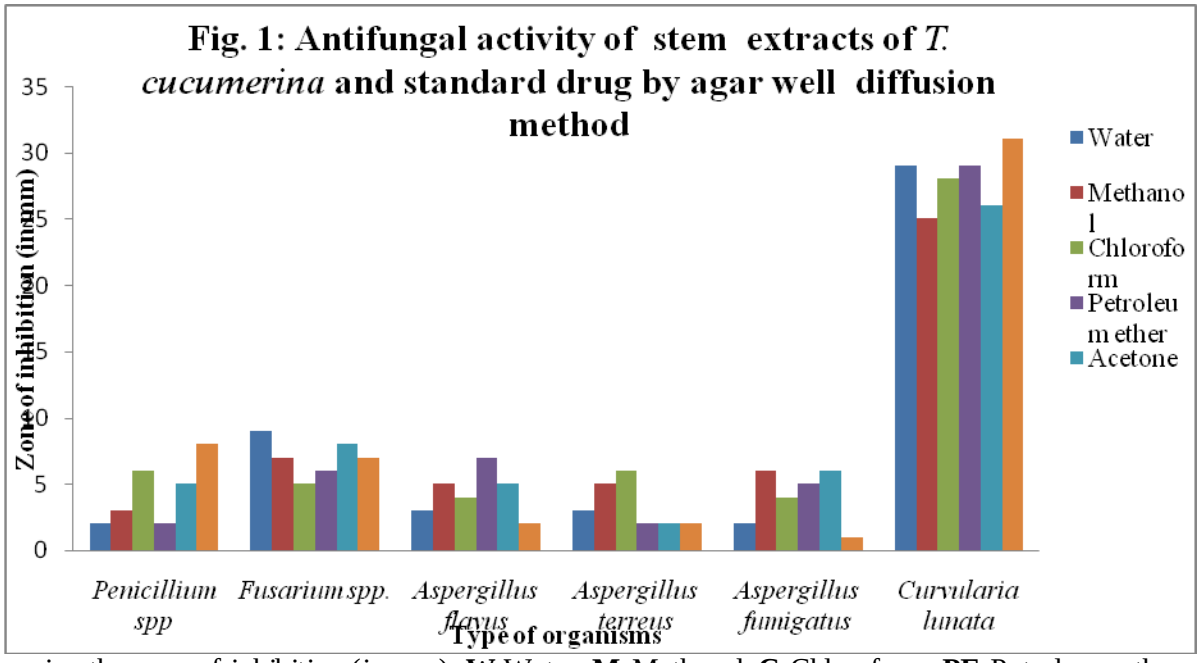
RESULTS AND DISCUSSION

The present investigation evaluated the antifungal activity of crude extracts of stem, leaf, flower and seed prepared by using different solvents in a soxhlet apparatus. The organisms used in the study were *Penicillium, Fusarium, Aspergillus flavus, Aspergillus terreus, Aspergillus fumigatus* and *Curvularia lunata.* The antifungal activity of stem, leaf, flower and seed was analyzed using agar well diffusion method and the results are presented in Table 1, Figure 1. The results obtained from the investigations showed that almost all the extracts exhibited considerable antifungal activity against all microorganisms tested.

Table 1: Antifungal Activity of Different Extracts of *T. Cucumerina* and Standard Drug by Agar Well Diffusion Method

Plant Extracts																								
	Stem						Leaf						Flower						Seed					
Test Organism	**W**	**M**	**C**	**PE**	**A**	**S**	**W**	**M**	**C**	**PE**	**A**	**S**	**W**	**M**	**C**	**PE**	**A**	**S**	**W**	**M**	**C**	**PE**	**A**	**S**
Penicillium spp.	02	09	03	03	02	29	05	55	04	02	06	35	02	04	02	04	05	35	07	09	02	02	05	32
Fusarium chlmydosporum	03	07	05	05	06	25	06	05	05	06	03	36	06	05	06	03	04	31	06	07	06	06	05	35
Aspergillus flavus	06	05	04	06	04	28	02	06	06	04	04	39	04	06	04	05	03	32	08	05	04	04	06	32
A. Terreus	02	06	07	02	05	29	02	04	05	05	02	38	05	05	05	02	02	35	04	06	05	05	04	32
A. fumigatus	05	08	05	02	06	26	05	06	05	06	05	34	06	05	06	02	03	32	05	08	06	06	06	31
Curvularia lunata	08	07	02	02	01	31	06	05	02	01	01	35	01	02	01	04	06	29	04	07	01	01	05	31

W- Water; M- Methanol; C-Chloroform; PE- Petroleum ether; A-Acetone; S-standard



Showing the zone of inhibition (in mm), **W**-Water, **M**-Methanol, **C**-Chloroform, **PE**-Petroleum ether, **A**-Acetone, **S**-Standard (Nystatin)

The wild variety of *T. cucumerina* stem extracts were found significantly by inhibiting almost all the organisms as evidenced by zone of inhibition ranging from 01-09mm (Table 1). The order of extracts showing antifungal activity was recorded as: methanol>chloroform>Petroleum ether>acetone>Aqueous extracts. Methanolic extracts showed maximum zone of inhibition against *Penicillium spp, A. fumigatus* (8-9mm) (Figure 1).

Chloroform extracts showed moderate activity against *Aspergillus terreus* (07mm). Acetone extracts showed least activity (01mm) against the fungal strain *Curvularialunata*. The extracts from leaves of *T. cucumerina* showed ranging of 1-6mm zone of inhibition indicating that the organisms were moderately sensitive to the different solvent extracts (Table 1).

The fungus *Curvularia lunata* was the exception as it showed only 1mm zone of inhibition in petroleum ether and acetone extracts. Aqueous, ethanolic, chloroform, petroleum ether, acetone extracts have shown the maximum zone of inhibition (6mm) (Table 1; Figure 1), which can be used as antifungal agents. The flower extracts of *T. cucumerina* were found significantly by inhibiting the almost all the organisms tested as evidenced by the zones of inhibition ranging from 1-6mm. Maximum zone of inhibition (6mm) was observed with aqueous and petroleum ether extracts against *Fusarium chlamydosporum*, acetone extracts against *Penicillium* spp., methanol and chloroform extracts against *A. flavus* and methanol and petroleum ether extracts against *A. fumigates*.

The seed extracts showed the inhibition zone ranging from 1-9mm (Table 1). Methanolic extract showed maximum zone of inhibition against *Penicillium spp* (09mm). Aqueous extracts showed moderate activity against *A. flavus*. Petroleum ether and chloroform extracts showed least zone of inhibition (1mm) against *Curvularia lunata*.

Based on the results, it was found that the seed extracts were most significant with larger zones of inhibition and inhibiting a broad spectrum of fungi followed by remaining extracts. The results obtained from this work showed that the plant extracts of medicinally important *T. cucumerina* exhibited antifungal activity against *Penicillum spp, Fusarium chlmydosporum A. flavus, A. terreus, A. fumigatus* and *Curvularia lunata*.

In particular, the plant extracts of *T. cucumerina* offer effective bioactive compounds for growth inhibition of the fungi.

Discussion

Antimicrobial properties of certain Indian medicinal plants were reported based on folklore information (Srinivasan *et al*., 2001; Parekh and Chanda, 2008; Patel and Coogan, 2008; Gupta and Banerjee, 2008; Duraipandiyan *et al.,* 2010). The screening of plant extracts and plant products for antimicrobial activity has shown that higher plants represent a potential source of new anti-infective agents. Infectious diseases account for high proportion of health problems in the developing countries (Sashikumar *et al.,* 2003). Resistance to antibiotics emerges in bacteria due to genetic mutations and consecutive selection of resistant mutants through selective pressure of antibiotics present in large amount in soil, plants, animals and humans (Andrasevic, 2004). Problems with drug resistant micro organisms and side effects of modern drugs and emerging diseases where no medicines are available have stimulated renewed interest in plants as significant source of new medicines (Patwardhan *et al.,* 2004). Traditionally, used medicinal plants produce a variety of compounds of known therapeutic properties. These substances that can inhibit pathogens and have little toxicity to host cells are considered candidates for developing new antimicrobial drugs (Aquil and Ahmad, 2003). The Cucurbitaceous plants especially seeds show the antimicrobial properties (Obi *et al*., 2009).

So far, there are no reports on antifungal activity of *T.cucumerina* by using different solvents as we have experimented in the present study, but similar work was done by other researchers on *T. anguina*. The crude methanol extracts of *T. anguina* displayed lower activities. The ethyl acetate extracts of seed of *T. anguina* showed maximum antifungal activity against *Aspergillus flavus* as found in the present investigation (Ali-Shtayeh and Abu Ghdeib, 1999). Cucurmoschin (8kDa), a novel anti-fungal peptide abundant in arginine, glutamate and glycine residues was purified from black pumpkin seeds. It is reported to inhibit 6 mycelia growth of fungi *Botrytis cinerea*, *Fusarium oxysporum and Mycosphaerella oxysporum* and the treatment of dermatophytic infections. Hugo *et al.,* (2005) investigated the methanol and ethyl acetate extracts of mixture of stem and leaves of *Coccinia indica*, against *Aspergillus fumigatus*, *C. albicans*, *Erwinia amylovora* and *Fusarium culmorum* and found that they were more effective.

Methanol extract of leaves was investigated by Deewanjee *et al.,* (2007) for antifungal activity against *C. albicans*, *Aspergillus niger, Penicillium notatum Penicillium funiculosum*. Antifungal activity of hexane, dichloromethane, ethyl acetate and ethanol extracts of *Luffa operculata* against. *C. albicans* was investigated by Jagessar *et al.,* (2007). Hussain and Nand Deeni (1991) screened the antimicrobial activity of Cucurbitaceous plants and their alkaloids. Investigations were carried out on total extracts of the dried powder combination of fruits of *Momordica charantia, Emblica officinalis* and *Coccinia indica* by Sankanarayanan and Jolly (1993). Similar studies were carried out by Kumar *et al.,* (1997) on the seeds of Cucurbitaceous plants. Antimicrobial activities of *Momordica charantia, Mentha piperata* and *Pisum sativum* were reported by Subhan and Tariq (2005). Ethanol extracts from leaves of *Cayaponia podantha*. (Cucurbitaceae) and four other Brazilian plants were screened for antimicrobial activity by Maria *et al*., (2006). Alessandra *et al.,* (2008) studied the chemical composition and antimicrobial activity of *Momordica charantia* seed essential oil.

In vitro antimicrobial activity of fruit and leaf extracts of *Momordica charantia* was investigated by Mwambete (2009). Similar work was done by Belsem *et al.,* (2009) on Tunisian, *Citrulllus colocynthis*. Antimicrobial activity of ethanol extract of leaf, stem, fruit and seed of *Brynopsis laciniosa* was investigated by Bonyadi *et al.,* (2009). Alessandra *et al.,* (2008) have reported that the antifungal activity of *Momordica charantia* seed essential oil. Hadizadeh *et al.,* (2009) have worked on ethanol extracts of *Colocynthis* against *Alternaria alternate, F. oxysporum, F. solani, Rhizoctonia solani*. Belsem *et al*., (2009) have investigated the anticandidal activity of *Tunisian, Citrullus colocynthis*. Petroleum ether and methanol extracts of leaf and fruit of *M. charantia* were investigated for antifungal activity against *C. albicans* and it was found that methanolic fruit extracts showed higher activity than leaf extracts (Mwambete, 2009).

Our results reported the significant antifungal activity of seed extracts when compared to leaf, stem and flower extracts which correlate with previous findings (Mwambete, 2009).

Conclusion

Natural plant-derived fungicides may be a source of new alternative active compounds, in particular with antifungal activity. From the present studies, it can be concluded that antifungal activity of *T. cucumerina* plant extracts against microorganisms indicate that their medicinal values and supports the claim of the traditional healers that it has been used to relieve some type of fungal diseases. Susceptibility of various microbes to the organic extracts of this plant suggests an immense scope for developing antifungal and therapeutic agents to cure many diseases of natural herbal agents. Further studies are needed to determine the chemical identity of the bioactive compounds responsible for the observed antifungal activity in *T. cucumerina*.

REFERENCES

Alessandra B, Siciliano T, Arrigo MD and Germano MP (2008). Chemical composition and antimicrobial activity of *Momordica charantia* seed essential oil. *Fitoterapia* **79** 123.

Ali-Shtayeh MS and Abu Ghdeib SI (1999). Antimycotic activity of twenty-two plants used in folkloric medicine in the Palestinian area for the treatment of skin diseases suggestive of dermatophyte infection. *Mycoses* **42** 665-672.

Andrasevic AT (2004). Antibiotic resistance bacteria fight back. *Acta Medica Creation* **58** 245-250.

Aquil F and Ahmad I (2003). Broad spectrum antibacterial and antifungal activity of certain traditionally used Indian medicinal plants. *World Journal of Microbiolgy* **19** 653-657.

Balandrin MF, Klocke JA, Wutule ES and Bollinger WH (1985). Natural plant chemical sources of industrial and medicinal materials. *Science* **228** 1154-1160.

Belsem M, Marzouk Z, Décor R, Edzir H, Haloui E, Fenina N and Aouni M (2009). Antibacterial and anticandidal screening of Tunisian from *Citrullus colocynthis* Schrad. *Journal of Ethnopharmacol*ogy **125** 344-349.

Bonyadi RE, Vita A and Bipinraj NK (2009). Antimicrobial activity of the ethanolicextract of *Bryonopsis laciniosa* leaf, stem, fruit and seed. *African Journal of Biotechnology* **8** 3565-3567.

Cannon RD, Holmes AR, Mason AB and Monk BC (1995). Oral *Candida*: clearance, colonization, or *candidiasis*. *Journal of Dental Research* **74**(5) 1152-1161.

Davicino R, Mattar MA, Casali YA, Graciela S, Margarita E and Micalizzi B (2007). Antifungal activity of plant extract used in folk medicine in Argentina. *Revista Peruana de Biologia* **14** 247-251.

Deewanjee S, Kundu M, Maiti A, Majumdar R, Majumdar A and Mandal SC (2007). *In vitro* evaluation of antimicrobial activity of crude extract from plants *Diospyros peregrine*, *Coccinia grandis* and *Swietenia macrophylla. Tropical Journal of. Pharmaceutical Research* **6** 773-778.

Duraipandiyan V, Ignacimuthu S and Gnanasekaran M (2010). Antifungal activity of triterpenoid isolated from *Azima tetracantha* leaves. *Folia Histochemica Cytolbiologica* **48** 311-313

Gupta SK and Banerjee AB (2008). Screening of selected West Bengal plants for antifungal activity. *Economic Botany* **26**(3) 255-259.

Hadizadeh I, Peivastegan B and Kolahi M (2009). Antifungal activity of Nettlle (*Urtica dioica L.*), Colocynth is (*Citrullus colocynthis L. Schrad*), Oleander (*Nereum oleander* L.) and Konar (*Ziziphus spina-christ*) extracts of plants pathogenic fungi. *Pakistan Journal of Biological Sciences* **12** 58-63.

Hugo DB, Kool JA, Broberg A, Mzira RW, Hedberg I and Levenfors JJ (2005). Antifungal and antibacterial activity of some herbal remedies from Tanzania. *Journal of Ethnopharmacol*ogy **96** 461-469.

Hussain HS and Nand Deeni YY (1991). Plants in Kano ethno medicine, screening for antimicrobial activity and alkaloids. *International Journal of Pharmacognsy* **29** 51-56.

Jagessar RC, Mohamed A and Gomes G (2007). Antibacterial and antifungal activity of leaf extracts of *Luffa operculata*, vs *Peltophorum pterocarpum*, against *Candida albicans, Staphylococcus aureus* and *Escherichia coli. Nature and Science* **5** 81-93.

Kumar S, Bagchi GD and Darokar MP (1997). Antibacterial activity observed in the seeds of some Coprophilous plants. *International Journal of Pharmacognosy* **35** 179-184.

Laurens A, Mboup S, Tignokpa M, Sylla O and Masquelier J (1985). Antimicrobial activity of some medicinal species of Dakar markets. *Pharmazie* **40** 482-485.
Mahesh B and Satish S (2008). Antimicrobial activity of some important medicinal plants against plant and human pathogens. *World Journal of Agricultural Sciences* **4**(5) 839-843.
Maria DC, Truiti T, Aparecida C, Filho BBPD, Sarragiotto MH and Souza MCD (2006). Screening of five Brazilian plants for anti-inflammatory and antimicrobial activities. *Pharmaceutical Biology* **44** 516-521.
Mwambete KD (2009). The *in vitro* antimicrobial activity of fruit and leaf crude extracts of *Momordica charantia*: A Tanzania medicinal plant. *African Health Sciences* **9** 34-39.
Obi RK, Nwanebu FC, Ndubuisi UU and Orji NM (2009). Antibacterial qualities and phytochemical screening of the oils of *Cucurbita pepo* and *Brassica nigra*. *Journal of Medicinal Plant Research* **3** 429-432.
Ogunlana EO and Ramstad E (1975). Investigations into the antibacterial activity of local plants. *Planta Medica* **27** 354-359.
Parekh J and Chanda S (2008). *In vitro* antifungal activity of methanol extracts of some Indian medicinal plants against pathogenic yeast and moulds. *African Journal of Biotechnology* **7**(23) 4349-4353.
Patel M and Coogan MM (2008). Antifungal activity of the plant *Dodonaca viscosa var. angustifolia* on Candida albicans from HIV-infected patients. *Journal of Ethnopharmacology* **118**(1) 173-176.
Patwardhan B, Vaidya ADB and Chorghade M (2004). Ayurveda and natural products drug discovery. *Current Science* **86** 789-799.
Sankaranarayanan J and Jolly CI (1993). Phytochemical, antibacterial and pharmacological investigations on *Momordica charantia* Linn., *Emblica officinalis* Gaertn. And *Curcuma longa* Linn. *Indian Journal of Pharmaceutical Sciences* **1** 6-13.
Sashi KJ, Ramya M and Janardhan K (2003). Antimicrobial activity of ethnomedicinal plants of Nilgiri Biosphere reserve and Western Ghats. *Asian Journal of Microbiology, Biotechnology and Environmental Sciences* **5** 183-185.
Srinivasan D, Nathan S, Suresh T and Lakshmana Perumalsamy P (2001). Antimicrobial activity of certain Indian medicinal plants used in folkloric medicine. *Journal of Ethnopharmacology* **74** 217-220.
Subhan S and Tariq P (2005). Antibacterial activities of *Mentha Piperita, pisum sativum and Momordica charantia*. *Pakistan Journal of Botany* **37** 997-1002.