

# Studies on Antibacterial Activity and Phytochemical Analysis of *Datura metel* L against Bacterial Pathogens Associated with HIV

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## Abstract

*Datura metel* Linn is an Indian medicinal plant widely used in Phytomedicine to cure diseases such as asthma, cough, convulsions and other various human ailments. Antimicrobial activity of ethyl acetate and methanol extracts of plant was investigated by agar disc and well-diffusion method against HIV associated opportunistic infections causing bacterial pathogens. The plant extracts showed better inhibitory activity against the tested organisms. Phytochemical screening of the plant revealed the presence of Saponins, Tannins, Phenolic compounds, Alkaloids, Carbohydrates, Anthraquinones, Protein and aminoacids, fixed oil and fats, Glycosides. This study creates social awareness among the HIV patients who were infected by various opportunistic bacterial pathogens.

**Keywords:** *Datura metel*; HIV associated opportunistic infections; Antibacterial activity; Social awareness

## Introduction

Medicinal plants used as sources for extracts or pure products for therapeutic use represent a rapidly expanding area of health science (Chopra *et al.*, 1956). Higher plants, as sources of medicinal compounds have continued to play a dominant role in the maintenance of human health since ancient times. It is reported that over 50% off all modern clinical drugs are of natural product origin and natural products play an important role in drug development programs in the Pharmaceutical industry.

The AIDS epidemic is one of the most destructive health crises of modern times, ravaging families and communities throughout the world. People living with AIDS are prone to developing other illnesses and infections because of their suppressed immune systems. Many of these illnesses are very serious, and they need to be treated. Some can be prevented. People with HIV can get many infections called opportunistic infections or OIs (Onorato *et al.*, 1999). Many bacterial pathogens, including *Mycobacterium*, *Staphylococcus*, *Streptococcus*, *Shigella*, *Camphylobacter*, *Listeria* and *Leginoella*, *Haemophilus*, *Pseudomonas*, *Rhodococcus* and *Salmonella* are most common in persons infected with HIV.

## Plant Description

*Datura metel* Linn (Thorn-apple, Devil trimpet solanaceae) particularly the leaves and seeds are used as anesthetic, anodyne, anti-asthmatic, anti-pasmodic, anti-tussive, Bronchodilator, and Hallucinogenic (Awadh *et al.*, 2004). The plant finds application in the treatment of diarrhea and skin diseases. It is used in the treatment of catarrh, epilepsy, insanity, hysteria, rheumatic pains, hemorrhoids, painful menstruation,

skin-ulcers and wounds. It is also used in the treatment of burns. It is used to calm cough and to treat laryngitis and Treacheries (Dabur *et al.*, 2004).

## Taxonomic Hierarchy of the Plant (*Datura metel*)

Domain	Eukaryota
Kingdom	Plantae
Subkingdom	Viridiaeplantae
Phylum	Tracheophyta
Subphylum	Euphylllophytina
Class	Magnoliopsida
Subclass	Lamidae
Order	Solanales
Family	Solanaceae
Subfamily	Solanoideae
Tribe	Solaneae
Genus	<i>Datura</i>
Species	<i>metel</i>

It is a native of tropical Asia, now pantropic in distribution. This is a coarse, erect, branched, smooth or slightly hairy herb or short-lived shrub 0.5–2 meters in height. The leaves are ovate to oblong-ovate, 9–18 centimeters long, with inequilateral base, pointed tip, and irregularly and shallowly lobed margins. The flowers are very large, white or nearly purple, axillaries, and solitary. Seeds along with other substances are used as a remedy for the symptoms of madness base don homeopathic principle, and detection of seeds is said to be useful in eye diseases (Jain, 2001).

Leaves of *D. metel* contain a total amount of 0.16% of alkaloid; and the seeds contain 0.50% scopolamine, hyoscyamine, and atropine. The seeds contain hyoscyamine 0.041%; a little atropine (0.05% as a chloride); scopolamine, 0.216%; fatty oil 11%; palmitic acid 6.18%; oleic acid, 60.80%; d-linolic, 23.55%; B-linolic acid 2.92; capronic acid, 1%; and phytosterin, 1% (Afsharypuor *et al.*, 1995). *Datura alba* contains considerable quantity the alkaloids, hyoscyamine, hyoscyamine, and atropine. The air-dried leaves from Philippine specimens of the plant contains 0.21% of total alkaloids, in the seeds 0.465%, and in the wood and roots (ground up together) 0.17% (Chatterji and Lahiri, 1949).

The present study was undertaken to make a survey of prevalent opportunistic bacterial infections in the HIV populations, to find out the antimicrobial susceptibility of *Datura metel* L against the pathogens and to create awareness among HIV patients for taking control and preventive measures against the opportunistic bacterial infections.

## Materials and Methods

### Plant collection

Fresh leaves, stems and root materials of *Datura metel* L were collected from the Herbal Garden of PRIST University, Thanjavur, Tamil Nadu, India. The plant materials were cleaned and shade dried and powdered. Fresh plant material were washed with tap water, air dried, homogenized to a fine powder and stored in air-tight bottles.

### Plant Extraction

Three-hundred milligrams of the extract was weighed and 5 ml of acetone was added to dissolve the extract and 0.2 ml of Tween 80 was used as an emulsifier. It was made up to 300 ml by adding distilled water in a standard flask. This is considered as a stock solution. From stock solution different ppm solutions such as 1000, 500, 250, 125 and 62.5 were prepared. The crude plant extracts were obtained by using Soxhlet apparatus. Two types of solvents has been used namely ethylacetate and methanol. For the collection of crude extract classical method was performed by using rotatory shaker.

### Bacterial Strains

Bacterial strains used in this study were isolated from clinical cases of suspected symptomatic HIV positive patients such as *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, *Escherichia coli*, *Staphylococcus aureus* and *Salmonella typhi*. All the strains were confirmed by cultural and biochemical characteristics and maintained in slants for further use; 3 types of concentration were used namely 10mg/10ml, 10mg/1ml and 50mg/1ml of plant extract was dissolved in dimethylsulfoxide. In 10mg/10ml has three types of concentration such as 10–100mg (10 mg (10 µl), 50 mg (50 µl), 100 mg (100 µl)). In 10mg/1ml has three types of concentration such as (100–1000mg), 250 mg (25 µl), 500 mg (50 µl), 1000 mg (100 µl). In 50mg/1ml has three types of concentration such as (1000–2000 mg), 1250 mg (25 µl), 1500 mg (30 µl), 2000 mg (40 µl) were used.

### Antibacterial Activity

The antibacterial assay of ethyl acetate and methanolic extracts was performed by two methods namely the agar well diffusion method (Perez *et al.*, 1990) and agar disc diffusion method (Parekh and Chanda, 2007).

### Phytochemical Screening

Qualitative chemical tests were carried out on the ethylacetate and methanolic extract of the powdered specimens using standard

procedures to identify the phytoconstituents as described by Trease and Evans (1989) and Sofowora (1993).

### Thin-layer Chromatography (TLC)

TLC is used to separate the compound present in the crude plant extract. The separation of the compound depends on the usage of the solvent. Here solvents used were 5% and 10% methanol in chloroform; 1mg/ml concentration of the crude stem extract was spotted on the TLC plates and dried. It was then run with both ultraviolet and iodine chamber. The Relative Factor (RF) value was calculated by using the following formula.

RF = Distance traveled by solute/Distance traveled by solvent. The compounds from the spots were scrapped and used for further screening.

### Bioautography

Bioautography is a rapid aid in the bioassay guided isolation and fractionation of antibacterial compounds and fractions. The activity of plant extract against bacteria is determined on chromatograms in accordance with the bioautography procedure of Begue and Kline (1972).

### Estimation of Radical Scavenging Activity using DPPH assay

The Radical Scavenging Assay activity of different extract was determined using DPPH assay (Bauer *et al.*, 1966).

The ability of scavenge DPPH radical was calculated by the following equation:

$$\% \text{ of DPPH radical scavenging activity} = \frac{\text{Abs control} - \text{Abs sample}}{\text{Abs control}} \times 100$$

Abs control = Absorbance of DPPH radical and ethanol

Abs sample = Absorbance of DPPH radical and stem methanol extract of *Datura metel* L.

## Results

Isolated bacteria with CD4 counts are represented in Table 1. Five major bacterial pathogens viz., *Pseudomonas aeruginosa* showed highest incidence followed by *Klebsiella pneumoniae*, *Escherichia coli*, *Staphylococcus aureus* and *Salmonella typhi* known to be found among the HIV patients (Figure 1). Morphology, staining and biochemical characteristics of the isolated bacterial pathogens are shown in Table 2.

### Antimicrobial Activity of *Datura metel*

Tables 3 and 4 represent the results for methanolic and ethylacetate root-extract of *Datura metel* respectively. Tables 5 and 6 represent the results for methanolic and ethylacetate stem-extract of *Datura metel* respectively. Tables 7 and 8 represent the results for methanolic and ethylacetate leaf-extract of *Datura metel* respectively. Figure 2 represents the antibacterial activity of *Datura metel* L against bacterial pathogens.

### Phytochemical Screening of *Datura metel*

Table 9 shows the phytochemical screening results for *Datura metel*. The highest antimicrobial activity shows stem methanol extract of the plant was subjected to phytochemical analysis. The plant showed positive

results for Saponins, Tannins, Alkaloids, Flavanoids and carbohydrates. Negative results for Ferric chloride test of phenolic compounds, anthraquinones, Gelatin and lead acetate tests for Phenolic compounds, millions and biuret tests for protein and aminoacids and Glycosides.

### TLC

RF value of the highest antimicrobial activity shows root methanol extract of *Datura metel* L was calculated by the TLC method as 0.83 (Fig. 3).

### Discussion

AIDS is a life threatening disease caused by a virus HIV. Antibacterial activity of the plant materials showed different inhibition spectrum

Bacteria	Number of bacteria isolated	CD <sub>4</sub> Count/ $\mu$ l
<i>Pseudomonas aeruginosa</i>	13	300380
<i>Klebsiella pneumoniae</i>	11	350470
<i>Escherichia coli</i>	9	480530
<i>Staphylococcus aureus</i>	2	500-670
<i>Salmonella typhi</i>	1	300-380
Total	36	

Table 1. Isolated bacteria from HIV positive patients

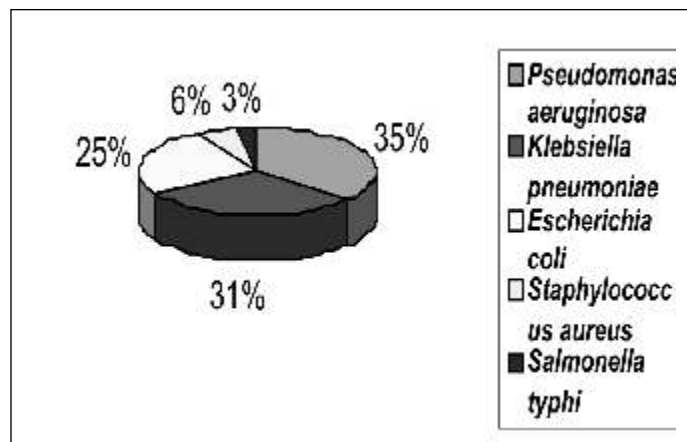


Figure 1. Isolated bacteria from HIV positive patients

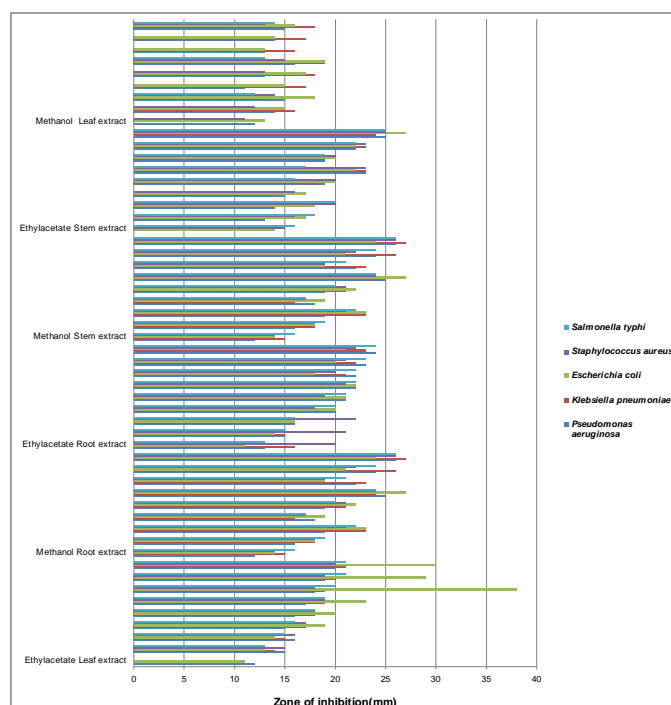


Figure 2. Antibacterial activity of *Datura metel* L against bacterial pathogens

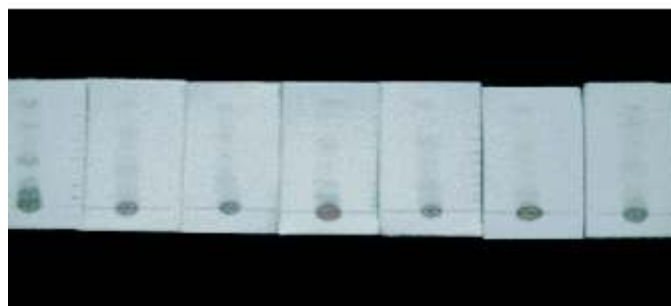


Figure 3. Thin-layer Chromatography for root methanol extract of *Datura metel* L

against the isolated opportunistic bacterial pathogens (Hammer *et al.*, 1997). Administered orally, the antibacterial compounds may be able to control wide range of microorganisms but there is also the possibility that they may cause an imbalance in the gut microflora allowing opportunistic pathogenic coliforms to become established in the gastrointestinal tract with result deleterious effects. Among them leaf methanol extract showed good antibacterial activity (Dorman, 1999). Phytochemical screening of leaf methanol extract of *Datura metel*

Name of the Organism	Gram staining	Acidfast staining	Morphology	Motility	Biochemical tests							
					Indole	MR	VP	Citrate	Urease	Nitrate reduction test	H <sub>2</sub> S Production Test	Carbohydrate fermentation test
<i>Pseudomonas aeruginosa</i>	-	-	Bacilli	+	-	-	-	+	-	+	+	-
<i>Klebsiella pneumoniae</i>	-	-	Bacilli	+	-	-	+	+	+	+	-	+(A,G)
<i>Escherichia Coli</i>	-	-	Bacilli	+	+	+	-	-	-	+	-	+(A,G)
<i>Staphylococcus aureus</i>	+	-	Cocci	-	-	+	-	-	+	+	-	+(A)
<i>Salmonella typhi</i>	-	-	Bacilli	+	-	+	-	+	-	+	-	+(A,G)

Table 2. Morphology, staining and biochemical characteristics of isolated bacteria

Organisms	Concentration(mg/ml)						
	10mg/10ml(10-100mg)			10mg/10ml(100-1000mg)			10mg/10ml(1000-2000mg)
	10mg 10µl	50mg 50 µl	100mg 100 µl	250mg 25 µl	500mg 50 µl	1000mg 100 µl	1250mg 25 µl
<i>Pseudomonas aeruginosa</i>	12	16	19	18	19	25	22
<i>Klebsiella pneumoniae</i>	15	18	23	16	21	24	23
<i>Escherichia coli</i>	14	18	23	19	22	27	19
<i>Staphylococcus aureus</i>	14	18	21	17	21	24	19
<i>Salmonella typhi</i>	16	19	22	17	20	24	21

Table 3. Antibacterial activity of *Datura metel* L methanolic root-extracts Zone of inhibition (mm); R: Resistant

Organisms	Concentration(mg/ml)						
	10mg/10ml(10-100mg)			10mg/10ml(100-1000mg)			10mg/10ml(1000-2000mg)
	10mg 10µl	50mg 50 µl	100mg 100 µl	250mg 25 µl	500mg 50 µl	1000mg 100 µl	1250mg 25 µl
<i>Pseudomonas aeruginosa</i>	13	15	16	20	21	22	22
<i>Klebsiella pneumoniae</i>	16	15	16	16	17	19	21
<i>Escherichia coli</i>	11	14	16	20	21	22	18
<i>Staphylococcus aureus</i>	20	21	22	18	19	21	20
<i>Salmonella typhi</i>	13	15	16	20	21	22	22

Table 4. Antimicrobial activity of *Datura metel* L ethyl acetate root-extract Zone of inhibition (mm); R: Resistant

Organisms	Concentration(mg/ml)						
	10mg/10ml(10-100mg)			10mg/10ml(100-1000mg)			10mg/10ml(1000-2000mg)
	10mg 10µl	50mg 50 µl	100mg 100 µl	250mg 25 µl	500mg 50 µl	1000mg 100 µl	1250mg 25 µl
<i>Pseudomonas aeruginosa</i>	12	16	19	18	19	25	22
<i>Klebsiella pneumoniae</i>	15	18	23	16	21	24	23
<i>Escherichia coli</i>	14	18	23	19	22	27	19
<i>Staphylococcus aureus</i>	14	18	21	17	21	24	19
<i>Salmonella typhi</i>	16	19	22	17	20	24	21

Table 5. Antimicrobial activity of *Datura metel* L methanolic stem-extracts Zone of inhibition (mm); R: Resistant

Organisms	Concentration(mg/ml)						
	10mg/10ml(10-100mg)			10mg/10ml(100-1000mg)			10mg/10ml(1000-2000mg)
	10mg 10µl	50mg 50 µl	100mg 100 µl	250mg 25 µl	500mg 50 µl	1000mg 100 µl	1250mg 25 µl
<i>Pseudomonas aeruginosa</i>	R	13	14	15	19	23	19
<i>Klebsiella pneumoniae</i>	R	13	14	14	18	23	19
<i>Escherichia coli</i>	14	17	18	17	20	22	20
<i>Staphylococcus aureus</i>	15	16	20	16	20	23	20
<i>Salmonella typhi</i>	16	18	20	R	16	17	19

Table 6. Antimicrobial activity of *Datura metel* L ethylacetate stem-extracts Zone of inhibition (mm); R: Resistant

showed positive results for tannins, saponins, flavonoids, alkaloids, oils and soaps etc., Thin layer chromatography was then applied to get the RF value for the plant extract produced better antibacterial activity. Hence, these studies definitely create some sort of social awareness among the HIV patients. The growth of many fungi, yeasts, bacteria, and viruses were inhibited by the phytochemical compounds found in *Datura* (Youdim et al., 1999). Phytochemical investigation of the 50% ethanol eluate fraction of macroporous resin for the flower of *Datura metel* L. led to the isolation of a new compound named yangjinhualine A(1) and five known megastigmane sesquiterpenes through repeated silica gel and ODS column chromatography and semipreparative HPLC (Sharma et al., 1970). Five known megastigmane sesquiterpenes were also isolated from *D. metel* L. for the first time. Hyoscyne amounts to over 90% of the total alkaloids present. In the air-dried leaves from Philippine specimens of the plant he found 0.21% of total alkaloids, in the seeds 0.465%, and in the wood and roots (ground up together) 0.17% (Hugo and Russell, 1984). Phytotherapeutically, phytochemicals containing plants are used to treat nonspecific diarrhoea, inflammations of mouth, throat and slightly injured skins (Sharma et al., 1970).

**Conclusion**

Antibacterial activity of the plant-extracts show different inhibition spectrum against the isolated opportunistic bacterial pathogens. Among them leaf methanol extract shows good antibacterial activity. Phytochemical screening of leaf methanol extract of *Datura metel* L showed positive results for all phytochemical constituents namely tannins, saponins, flavonoids, carbohydrates, alkaloids, anthraquinones, protein and aminoacids, mixed oils and fats. Hence, this study provided a good medicinal plant based treatment strategy and will create

Organisms	Concentration(mg/ml)								
	10mg/10ml(10-100mg)			10mg/10ml(100-1000mg)			10mg/10ml(1000-2000mg)		
	10mg 10µl	50mg 50 µl	100mg 100 µl	250mg 25 µl	500mg 50 µl	1000mg 100 µl	1250mg 25 µl	1500mg 30 µl	2000mg 40 µl
<i>Pseudomonas aeruginosa</i>	12	14	15	11	13	16	13	14	15
<i>Klebsiella pneumoniae</i>	R	16	13	17	18	19	16	17	18
<i>Escherichia coli</i>	13	15	18	15	17	19	13	14	16
<i>Staphylococcus aureus</i>	11	12	14	R	13	15	R	R	13
<i>Salmonella typhi</i>	R	R	12	R	R	13	R	R	14

Table 7. Antimicrobial activity of *Datura metel* L methanolic leaf-extracts Zone of inhibition (mm); R: Resistant.

Organisms	Concentration(mg/ml)								
	10mg/10ml(10-100mg)			10mg/10ml(100-1000mg)			10mg/10ml(1000-2000mg)		
	10mg 10µl	50mg 50 µl	100mg 100 µl	250mg 25 µl	500mg 50 µl	1000mg 100 µl	1250mg 25 µl	1500mg 30 µl	2000mg 40 µl
<i>Pseudomonas aeruginosa</i>	12	15	16	15	16	17	18	19	20
<i>Klebsiella pneumoniae</i>	R	14	15	17	18	19	19	20	21
<i>Escherichia coli</i>	11	13	14	19	20	23	38	29	30
<i>Staphylococcus aureus</i>	R	15	16	17	18	19	18	19	20
<i>Salmonella typhi</i>	R	13	15	16	18	19	20	21	21

Table 8. Antimicrobial activity of *Datura metel* L ethylacetate leaf-extracts Zone of inhibition (mm); R: Resistant

S. No.	Phytochemical Analysed	Result	Observation
1.	Saponins	+	Appearance of foam
2.	Tannins	+	Appearance of blue black colour
3.	Test for Phenolic compounds		
	i) Ferric chloride test	+	Appearance of dark green colour.
	ii) Gelatin test	+	White Precipitate formation occur
	iii) Lead acetate test	+	Bulky white precipitate formed.
4.	Carbohydrate tests		Appearance of violet ring.
	Molish's test	+	
5.	Antraquinones	+	Pink color was observed
6.	Alkaloids	+	Appearance of white creamy precipitate
7.	Protein and aminoacids		
	(i) Millon's test	-	No precipitate formation
	(ii) Biuret test	+	Pink colour was observed
8.	Flavonoids		
	Alkaline reagent test	-	No color change observed
9.	Glycosides		
	Borntercigers test	+	No color change was observed
10.	Fixed oils and fats		
	Saponification	+	Minimal foam was observed

Table 9. Phytochemical screening of the methanol leaf-extract of *Datura metel* Linn; [(+): Positive; (-): Negative].

social awareness among the HIV patients.

## References

- Afsharypuor, S.A. Mostajeran and R. Mokhtary, 1995. Scopolamine and Atropine in different parts of *Datura metel* during development. *Planta Medica* **61**: 383-384.
- Awadh, A., N. Ali, K. Al-rahwi and U. Lindequist, 2004. Some medicinal plants used in Yemeni herbal medicine to treat Malaria. *Afr. J. Trad. Comp. Alt. Med.* **1**:72-76.
- Bauer, A.W., W.M.M. Kirby, J.C. Sherris and M. Turck, 1966. Antibiotic susceptibility testing by a standardized single disk method. *Am. J. Clin. Pathol.* **45**:493-496.
- Begue, W.J. and R.M. Kline, 1972. The use of tetrazolium salts in bioautographic procedures. *J. Chromatogr.* **64**:182-184.
- Chatterji, R. and J.K. Lahiri, 1949. Studies on Indian *Datura*. *J. Amer. Pharm. Sci. Assoc.* **38**: 388-92.
- Chopra, R.N., S.N. Nayar and I.C. Chopra, 1956. In Glossary of *Indian Medicinal Plants, CSIR*:New Delhi, Vol. 91.
- Dabur, R., M. Ali, H. Singh, J. Gupta and Sharma G., 2004. A novel antifungal pyrrole derivative from *Datura metel* leaves. *Pharmazie* **59**:568-570.
- Dorman H.J.D., 1999. Phytochemistry and bioactive properties of plant extracts antibacterial, antifungal and antioxidant activities. Ph.D Thesis.
- Hammer, S.M., K.E. Squires, M.D. Hughes, J.M. Grimes, L.M. Demeter, et al., 1997. AIDS Clinical Trials Group 320 Study Team. *N. Engl. J. Med.* **337**:725.
- Hugo, W.B., and A.D. Russel, 1984. *Pharmaceutical Microbiology*, 3rd edition. Blackwell Scientific Publications; Pp.179-200.
- Jain, S.K, 2001. Ethnobotany in Modern India. *Phytomorphology Golden Jubilee Issue: Trends Plant Sci.* **39**-54.
- Onorato, M, M.J. Borucki, G. Baillargeon, D.P. Paar, D.H. Freeman and C.P. Cole, 1999. Risk factors for colonization or infection due to methicillin-resistant *Staphylococcus aureus* in HIV positive patients: a retrospective case-control study. *Infection Control Hosp. Epidemiol.* **20**: 26-30.
- Parekh J.S. and Chanda, 2007. Antibacterial and phytochemical studies on twelve species of Indian medicinal plants. *Afr. J. Biol. Res.* **10**: 175-181.
- Perez, C., Paul M. and Bazerque P., 1990. An antibiotic assay by the agar well diffusion method. *Acta Biol. Med. Exp.* **15**:113-115.
- Sharma P.N, G. Srimannarayana and N.V.Subbarao 1970. *The Medicinal Plant Industry*, Vol. 205.
- Sofowora, A, 1993. Recent trends in research into African medicinal plants. *J. Ethnopharmacol.* **38**:209-214.
- Trease, G.S., and H.C. Evans, 1978. *Textbook of Pharmacognosy* 9 th edition. Bailliar Zindall and Co.: London.
- Youdim, K.A, H.J.D. Dorman and S.G Deans, 1999. The antioxidant effectiveness of thyme oil alpha tocopherol and ascorbyl palmitate on evening primrose oil oxidation. *J. Essential Oil Res.* **11**: 643-48.