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Phytochemical investigation and Antifungal activity of *Duranta erecta* L. and *Tragia involucrata* L. plant ethanol extracts against phytopathogenic fungus *Curvularia lunata* and *Alternaria alternata*

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Abstract

Introduction: More than 800 million people in developing countries do not have adequate food supplies and at least 10% of food is lost due to plant disease. Plant diseases are caused by pathogens such as fungi, bacteria, nematodes and viruses.

Material and Methods: The present investigation was aimed to study the antifungal activity and preliminary phytochemical analysis of *Duranta erecta* L. and *Tragia involucrata* L. The ethanol leaf extracts of *D. erecta* and *T. involucrate* were tested against two phytopathogenic fungi *Curvularia lunata* and *A. alternata*by poisoned food technique using eight different concentrations (0.1%, 0.2%, 0.4%, 0.6%, 0.8%, 1%, 2% and 3%).

Results: Assessment was carried out in terms of percentage of mycelial growth inhibition of the test fungus. The results of antifungal activity of ethanol extracts of *D. erecta* and *T. involucrata* against *C. lunata* and *A. alternata* were observed mycelia growth inhibition ranged from 27.77 to 100%. Phytochemical analysis of

ethanol extractsrevealed that thepresence of phytocompounds such as alkaloids, flavonoids, tannins, steroid, saponins and phenolic compounds which be responsible for the observed antifungal property.

Conclusion: The ability of the crude leaf ethanol extract of *D. erecta* to inhibit the growth of fungi is an indication of its broad spectrum antifungal property which may be employed in the management of fungal infection.

Key words: *Duranta erecta, Tragia involucrata,* Phytochemical analysis, Antifungal activity, Poisoned food technique.

Introduction

Plant diseases are a common occurrence, often having a significant economic impact on yield and quality. Thus managing diseases is an essential and crucial for production of most crops. Insect and pest control is one of the major problems faced by Indian agriculturists. According to a survey conducted by the International Crops Research Institute for the Semi-Arid Tropics (ICRISAT), 93% of Indian farmers use only chemical pesticides, fungicides to control the pests and diseases but the crops receive between 1-15 % sprayed pesticides. Despite the heavy use of pesticides farmers still lose 11-40% of their crop yield due to pest damage. [1]

Fungi are ubiquitous in the environment, and infection due to fungal pathogens has become more common. The genus *Curvularia* and *Alternaria* Nees is widely distributed in nature and its species are among the most common fungi on the phyllosphere [2]. It includes both plant-pathogenic and plant-saprophytic species that may damage crops in the field or cause post-harvest decay [3], causing considerable economic losses for farmers and food industries. In addition, the genus produces mycotoxins and phytotoxins, and studies in the last decade have emphasized its toxicogenic properties rather than simply those that cause spoilage. The toxins alternariol, alternariol methyl ether, altenuene, and tenuazonic acid are known as possible food contaminants with potential toxicological risk. [4]

Pathogenic fungi are the main infectious agents in plants, causing alterations during developmental stages including post-harvest. In fruit and vegetables, there is a wide variety of fungal genera causing quality problems related to aspect, nutritional value, organoleptic characteristics, and limited self-life. [5]In addition, in some cases fungi are indirectly responsible for allergic or toxic disorders among consumers because of the production of mycotoxins or allergens. Generally, plant pathogenic fungi are controlled by synthetic fungicides. There is an increased demand for production and regulations on the use of synthetic pesticides and the emergence of pathogens resistant to the products employed. Thus, there is a need to search for alternative approaches to control plant diseases in an ecofriendly method. It is revealed that plant extracts of many higher plants have exhibited antibacterial, antifungal and insecticidal properties under laboratory trails.[6,7,8] Plant metabolites and plant based pesticides appear to be one of the better alternatives as they are known to have minimal environmental impact in contrast to the synthetic pesticides. [9]

Duranta erecta Linn. (Syn. *Durantaplumieri* Jacq., *D. repens* Linn. and Eng: Golden dewdrop) is commonly known as pigeon berry and locally called 'Kata mehedi' belongs to the family Verbenaceae. The plant is not browsed by cattle and is believed to be poisonous. [10] Ethyl acetate and aqueous extracts of leaves showed significant antimalarial activity when administered to mice. The fruits are used in the treatment of malaria and intestinal worms. [11] The leaves are used in the treatment of abscess. [12] From the genus *Duranta* several iridoid glycosides as durantosides I, II, III, IV, and lamiide were isolated. [13] Flavonoids and C-alkylated flavonoids Salama et al.[14]and some alkaloids were isolated. *Tragia involucrata* is one of the major constituents of all the antidiabetic formulations available in the market. [15] The plant is being used predominantly to treat asthma[16], diarrhoeaexcessive urination, vomiting, dermatosis, and itching of the skin, migraine [17] and piles [18]. It is also used in treating baldness, leprosy, tumor and elephantiasis. Decoction of leaves is taken with the leaves of *Cipadessa baccifera* and *Aristolochia talaga* to cure scorpion, insect and snake bites. [19]

Hence, present investigation aims to find out the antifungal activity of the ethanol crude extracts of two medicinal plants against the tested phytopathogenic fungus.

MATERIALS AND METHODS

Plant materials

The leaves of *Durantaerecta* (Verbenaceae) and *Tragiainvolucrata* (Euphorbiaceae) were collected from the botanical garden, puducherryand Abisekapakkam field respectively during the month of August 2014.

The collected plants were immediately brought to the laboratory using separate polythene bags, washed with tap water, then surface sterilized with10 per cent sodium hypochlorite solution and rinsed with sterile distilled water and shade dried. After shade drying, the leaves were packed in brown cover and kept in an oven at 60°C for an hour to make grinding easy. After an hour, the leaves and seeds were ground using electric blender. The powdered plant materials were then packed in air lock zip pouch.

Preparation of solvent extracts

Five hundred grams of powder of leaves *Duranta erecta* and *Tragiainvolucrata* was loaded in separate Soxhlet apparatus and extracted with ethanol. The solvent was evaporated using rotary evaporator (Heidolph, Germany) under reduced pressure at 40°C and the crude extracts were kept at 4°C for antifungal screening.

Phytochemical screening

The ethanol extracts of *D. erecta*and *T. involucrata*were used for qualitative phytochemical studies. Phytochemicals like Terpenoids, Tannin, Cardic glycosides, Steroids, Alkaloids, Phenolic compound and Coumarins were carried out according to the standard methods.[20, 21]

In vitro Studies

Microorganisms used

The plant pathogen *Curvularialunata* and *Alternaria alternate* were obtained from the Department of Botany, Annamalai University, Annamalainagar. The pure fungal cultures were maintained on Potato Dextrose Agar medium at 28 ± 2 º C. *In viro* antifungal activity was determined by using Potato dextrose Agar.

Preparation of inoculum

Fungal isolates were maintained on Potato dextrose Agar, stored at room temperature and sub cultured periodically. Twenty ml of medium was dispensed in sterile petri dishes and allowed to cool. The isolates were grown for seven to ten days before use.

Antifungal assay

Antifungal activity of plants was determined by food-poisoned technique[22].Different concentration of the plant extract *viz.,* 0.2%,0.4%,0.6%,0.8%,1.0%,2.0% and 3.0% was prepared by mixing the dried evaporated solvent extract in 100 ml of sterilized PDA medium and transferred equally into five Petri plates. The media was allowed to solidify. The seven day old fungal culture disk of 6 mm diameter was taken and inoculated upside downat the center of Petri plates containing plant extracts in aseptic condition. All plates were incubated at 28 ± 2 ^{0}C and radial growth of colony was measured after seven day of incubation. Each test was performed in triplicate.

The average diameters of the fungal colonies were measured on the 7th day of incubation and percentage of mycelial growth inhibition was calculated. [23]

$$Mycelial\ growth\ inhibition\ (\%) = \frac{g_c - g^+}{g_c}\ x\ 100$$

Where,

gc = growth of mycelial colony in control set after incubation period subtracting the diameter of inoculum disc.

gt = growth of mycelial colony in treatment set after incubation period subtracting the diameter of inoculum disc.

Statistical analysis

The results are expressed as the mean ± SD. All statistical analyses were performed using SPSS version 16.0 statistical software (SPSS Inc., Chicago, IL, USA). Student's t-test was performed to determine any significant ethanol solvents extracts for *in vitro* antifungal assays.

Results and Discussion

The bio pesticides derived from the plants are expected to play an increasingly significant role in crop protection strategies. Exploitation of naturally available chemicals from plants, which retards the reproduction of undesirable microorganisms, would be a more realistic and ecologically sound method for plant protection and will have a prominent role in the development of future commercial pesticides for crop protection strategies, with special reference to the management of plant diseases[9, 24]. The present study phytochemical analysis of *D. erecta*and *T. involucrata* revealed that the plant possessed phytoconstituents as alkaloids, flavonoids, tannins, steroids, saponins and phenolic compounds and the tables are presented in Table 1.

Table 1. Phytochemical analysis of ethanol extracts of *Duranta erecta* and *Tragia involucrata*

S.no	Secondary metabolites	*Duranta erecta*	*Tragia involucrata*
1	Alkaloids	++	+
2	Flavonoids	+	+
3	Tannins	+	+
4	Cardic glycosides	_	-
5	Steroids	++	++
6	Terpenoids	-	_
7	Phenolic compounds	++	+
8	Saponins	++	+
9	Coumarins	_	-

- = Absence, + = weak, ++ = medium, +++ = strong

In the present investigation different concentrations of ethanol extracts of *D. erecta* and *T. involucrata* were tested against two plant pathogens *viz.*,*C. lunata* and *A. alternata.* The results of our study on the inhibition of *C. lunata* mycelial growth revealed that the highest percentage of inhibition was observed at 1-3% concentration of ethanolic extract of *D. erecta* and 2-3% concentration of ethanolic extract of *T. involucrata.* For *A. alternata* 100% inhibition of the mycelial growth was observed only at 3% concentration of the *D. erecta* extract. The ethanolic extract of *T. involucrata* found to inhibit 75% of mycelial growth at 3% concentration. Among the two pathogens tested for its sensitiveness to ethanolic extract of*D. erecta* and*T. involucrata, C. lunata* was much more sensitive to both the plant extracts than that of *A. alternata.* Out of the two plants tested the *D. erecta* ethanol extract showed stronger antifungal activity against *C. lunata* and *A. alternata* than that of *Tragia involucrata* and are presented in table 2. Various studies have shown a similar strategy of using crude plant extract against plant pathogens. [25,26]. The crude extracts of *Allium sativum, Capsicum annuum, Artimesia vulgaris, Eupatorium adenophorum, Gaultheria fragrantissima* and *Phyllanthus emblica* were assessed *in vitro* for activity against *Fusarium solani*. The extract of *A. sativum* completely inhibited the mycelial growth of the test fungus at the concentration of 40%. [27]

Sikarwar *et al.*[28] investigated the antifungal activity of *D. repens* aqueous and methanol leaf extracts of *D. repens* were tested against three fungi *viz., Aspergillus niger, Candida albicans* and *Microsporum gypseum.* The result showed the promising antifungal activity against the tested fungi. Methanol extract was found to possess a more potent inhibitory effect when compared to the aqueous one. *In vitro* antifungal activity of *Coixlacrymajobi* showed that the aqueous extract inhibits higher percentage of *Rhizoctonia solani* mycelia growth (70.53%) than that of control.In field trials aqueous extract of *Lantana camera* showed the highest decrease (61.07%) in the root rot disease incidence. [26] Gitika *et al.*, 2019 [29] reported that the curcumin (200 mg/disc) has retained the highest antifungal activity on *Aspergillus flavus* and *Aspergillus fumigatus* in comparison to synthetic derivatives used as antifungal agents. The ethyl acetate extract of *Piper nigrum* showed excellent antimicrobial activity against dandruff causing fungi.

The present investigation antifungal activity of *D. erecta* and *T. involucrata* against *C. lunata* and *A. alternata* are presented in the table 2. The average mycelia growth inhibition ranges from 27.77 to 100%. Among the different concentrations of the *T. involucrata* ethanol extracted tested 2% and 3% extract completely inhibits the growth of the pathogen. The other lower concentrations moderately inhibited the growth of *C. lunata* when compared to control. The methanol extracts of five plants were tested for their antifungal activity against 10 phytopathogenic fungi and *C. albicans* B017. Among all extracts, *Lawsonia inermis* showed greatest percent inhibition of mycelial growth of target fungi (76.47 - 87.77 %) followed by *Withania somnifera* (54.44 - 78.88 %). [30] The effect of essential oil extracted by hydrodistillation from two species of Eucalyptus on the mycelia growth of *Phaeoramularia angolensis* and found that the essential oils were fungicidal. [31] The ethanolic leaf extracts of medicinal plants, *Aristolochia indica, Coleus strobilifer, Lepidagathis cristata, Rhinacanthus nasutus, Spatholobus parviflorus* and *Tarenna asiatica* against the dermatophytic fungal pathogens, *Trichophyton mentegrophytes* and *Candida albicans*. [32]. *Amaranthus spinosus* extracts are reported as a good antifungal source against *Candida albicans, Saccharomyces cerevisia, Aspergillus niger, Fusarium oxysporium* and *Aspergillus flavus*. [33]

Table 2. Mycelial growth inhibition in (Percentage) ethanol extract of *Duranta erecta* and *Tragia involucarata* against *Curvularia lunata* and *Alternaria alternata*

S.No	Concentration (%) Mycelial growth inhibition [a]								
	Plants	**0.2%**	**0.4%**	**0.6%**	**0.8%**	**1%**	**2%**	**3%**	***P* values**
Curvularialunata		Control- Nil							
1	***D. erecta***	73.3±0.03	74.4±0.21	75.0±0.02	90.5±0.25	100.0±0.15**	100.0±0.32**	100.0±0.53**	0.002
2	***T.involucarata***	27.7±0.05	41.1±0.02	54.4±0.21	64.3±0.48	74.32±0.50	100.0±0.41**	100.0±0.62**	0.045
Alternaria alternata									
1	***D. erecta***	56.2±0.04	62.5±0.12	65.0±0.12	67.5±0.42	68.75±0.28	91.25±0.53	100.0±0.43**	0.003
2	***T.involucarata***	43.7±0.76	50.0±0.01	53.5±0.04	56.2±0.13	59.15±0.62	62.30±0.72	75.7±0.28	0.048

[a]-mean of three assays; ± - standard deviation ** significant at $p < 0.05$

D. erecta- Duranta erecta; T.involucarata- Tragia involucarata

The present study phytochemical analysis of *D. erecta*and *T. involucrata* revealed that the plant possessed phytoconstituents as alkaloids, flavonoids, tannins, steroids, saponins and phenolic compounds. The *Zanonia indica* different extracts showed the presence of phytochemical constitution such as phenols, alkaloids, terpenoids, flavonoids, steroids, carbohydrates, tannins, saponins, glycosides quinones, resins and proteins [34] Phytochemical constituents such as alkaloids, flavonoids, tannins, phenols, saponins and several other aromatic compounds are secondary metabolites of plants that serve a defense mechanism against prediction by many microorganisms, insects and other herbivores. [35, 36]The phytochemical analysis of *D. erecta*and *T. involucrata* revealed that the plant possessed phytoconstituents as alkaloids, flavonoids, tannins, phenols, steroids, triterpenoids and saponins. [37]Alkaloids, which are one of the largest groups of phytochemicals in plants, have amazing effects on humans and this has led to the development of powerful painkiller medication. [38]The antimicrobial compounds may be found as alkaloids, flavonoids, phenolic compounds, saponins, steroids, tannins and triterpenoids, whose presence may be attributed to the medicinal properties of plants. [39]The phytoconstituents have been found to inhibit bacteria, fungi, viruses and pests.[40]The presence of such phytoconstituents in the leaf extract might be responsible for its activity.The present study showed that various active constituents in the ethanol extracts of *D. erecta* and *T. involucrata* were responsible for antifungal activity. And the findings of the study also correlated with the previous studies which state that the active compounds found in the higher plants may play an important role in controlling the plant disease causing organisms. [41, 42]

Conclusion

To conclude the two plants tested for antifungal activity against *C. lunata* and *A. alternata* ethanol extract of *D. erecta* showed antifungal activity stronger than that of *T. involucratata.* Out of the two plant pathogen tested for their sensitiveness *C. lunata* was more sensitive to both the plant extracts than that of *A. alternata.* Finally it can be concluded that the ethanol extract of *D. erecta* can be used as a source of antifungal agent.

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Reference

1. Roa GVR, Rao VR. Status of IPM in Indian Agriculture: A Need for Better Adoption. *Ind J Plant Prot*. 2010; 38: 115-121.
2. Lopes MC, Martins VC. Fungal plant pathogens in Portugal: *Alternariadauci*. *RevIbero americana de Micol*. 2008; 25:254-256.
3. Campo S, Gillbert KB, Carrirgfon JC. Small RNA –Based Antiviral Defense in the Phytopathogenic fungus *Colletotrichum higginsianum*. *PLos pathogens* 2016; 2: 1-36.
4. Pose G, Ludemann V, Segura J, Fernández Pinto V. Mycotoxin production by *Alternaria* strains isolated from tomatoes affected by Blackmoldin Argentina. *Mycotoxin Res.* 2004; 20:80-86.
5. Agrios GN. Losses caused by plant diseases. 2004; p. 29-45. Plant Pathology. Elsevier, Oxford, UK.
6. Adaikala Raj G, Chandrasekaran M, Krishnamoorthy S, Venkatesalu V. Screening of phytochemical and antibacterial potential of different organic solvent extracts of *Stoechospermum marginatum* (Ag) Kutz. From Manappad coast, Tuticorin District, South India. *J Med Herbs Ethnomed*. 2015a; 1(1): 50-58
7. Shariff N, Sudarshana MS, Umesha S, Hariprasad P. Antimicrobial activity of *Rauvolfia tetraphylla* and *Physalis minima* leaf and callus extracts. *Afri J Biotechnol*. 2006; 5: 946-950.
8. Bouamama H, Noel T, Villard J, Benharref A, Jana M. Antimicrobial activities of the leaf extracts of two Moroccan *Cistus* L. species. *J. Ethnopharmacol*. 2006; 104: 104-107.
9. Verma J, Dubey NK. Prospecdtives of botanical and microbial products as pesticides of tomorrow. *Current Sci*. 1999; 76: 172-179.
10. Nelson G: The shrubs and woody vines of Florida. Pineapple Press, Inc. Sarasota 1996.
11. Castro O, Barrios M, Chinchilla M and Guerrero O. Chemical and biological evaluation of the effect of plant extracts against *Plasmodium berghei*. *Rev De Biol Trop*. 1996; 44: 361-367.
12. Xiao P: A pictorial Encyclopaedia of Chinese Medical Herbs, Chuokoron-Sha, Inc.1992.
13. Takeda Y, Morimoto Y, Matsumoto T, Ogini C, Hirata E, Takushi A, Otsuka H. Iridiod glycosides from the leaves and stem of *Duranta erecta*. *Phytochem.* 1995; 39(4): 829-833.
14. Salama OM, Amer MM, Lahloub MF, Spengel S.Repennoside. A new iridiodglucoside from *Duranta repens* Fruits. Mans. J Pharm Sci 1992; 8: 212-221.
15. Kar A, Choudhary BK, Bandyopadhyay NG. Comparative evaluation of hypoglycaemic activity of some Indian medicinal plants in alloxan diabetic rats. *J Ethnopharmacol*. 2003; 84: 105-108.

16. Savithramma N, Sulochana K, Rao N. Ethnobotanical survey of plants used to treat asthma in Andhra Pradesh, India. *J Ethnopharmacol.* 2007;113: 54-61.
17. Udayan PS, Harinarayanan MK, Tushar KV, Balachandran I. Some common used by *Kurichiar*tribes of Tirnelli forest, Wayanad district, Kerala in medicine and other traditional uses. *Ind J Trad Knowl.* 2008; 7: 250-255.
18. Silja VP, Varma KS, Mohanan KV. Ethanomedical plant knwoledge of the Mullukuruma tribe of Wayanad district, Kerala. *Ind J Trad Knowl.* 2008; 7: 604-612.
19. Ayyanar M, Ignacimuthu S. Traditional knowledge of Kanitribals in Kouthalai of Tirunelveli hills, Tamil Nadu, India. *J Ethnopharmacol*. 2005; 102: 246-255.
20. Harborne IB. Phytochemical methods: A guide to modern techniques of plant analysis. 2nd edn, Chapman and Hall, New York. **1973;** 88–185.
21. Trease GE, Evans WC. Textbook of Pharmacognosy. 12th edn. Balliese Tindall and Company Publisher, London. **1983;** 343–383.
22. Rao GP, Srivastava AK. Toxicity of essential oils of higher plants against fungal pathogens of sugarcane. *Current Trend Sugar Pathol*. (eds.) Rao, G.P., A.G. Gillasple, P.P. Upadhaya, A. Bergamin, V.P. Agnihotri and C.T. Chen. International Books and Perodicals Supply Service, Pitampura, Delhi, 1994; pp. 347-365.
23. Schmitz H. Food poisoned technique. Industrial Engineering Chemical Analyst Education 1930; p.361-363.
24. Gottlieb OR, Borin MR, Brito NR. Integration of ethnobotany and phytochemistry: dream or reality. *Phytochem.* 2002; 60: 145-152.
25. Ambikapathy V, Gomathi S, Panneerselvam A. Effect of antifungal ctivity of some medicinal plants against *Pythium debaryanum* (Hesse) *Asian J Plant Sci Res.* 2011; 1 (3): 131-134.
26. Mangang HC, chhetry GKN. Antifungal Properties of certain Plant Extracts against *Rhizoctonia solani* causing root rot of French bean in organic soil of Manipur. *Int J Sci Res Pub*. 2012; 2: 5.
27. Shrestha AK, Tiwaris RD. Antifungal activity of crude extracts of some medicinal plants against *Fusariumsolani* (Mart.) Sacc. Ecoprint. *An Int J Ecol.* 2009; 16: 75-78.
28. Sikarwar MS, Hui BJ, Subramanium K, Valeisamy, Yean LK, Balaji K. A Review on *Artocarpus altilis* (Parkinson) (breadfruit). *J Appl Pharma Sci.* 2014; 4 (8): 91-98.
29. Gitika, A., Mishra, R., Kumar Panda, S., Mishra, C., Sahoo, P. R., Evaluation of antifungal activity of curcumin against *Aspergillus flavus*. *Int. J. Curr. Microbiol. App. Sci.* 2019; 8, 2323–2329.
30. Dongmo PMJ. Antifungal Potential of Eucalyptus saligna and Eucalyptus camaldulensis Essential Oils from Cameroon against *Phaeoramularia angolensis*. *European J Sci Res.* 2008; 24 (3): 348-357.
31. Khan ZS, Nasreen S. Phytochemical analysis, antifungal activity and mode of action of methanol extracts from plants against pathogens. *J Agricul. Technol.* 2010; 6(4): 793-805.

32. Arivalagan K, Prakash M. Antifungal Activity of Medicinal Plants Collected from Servarayan Hills, Eastern Ghats, against Dermatophytic Fungi. *Int.J.Curr.Microbiol.App.Sci.* 2021; 10(08): 305-311.
33. Jadhav V., Biradar S.D. Evaluation of antifungal activity of *Amaranthus spinosus* L. (Amaranthaceae). *Int. J. Curr. Microbiol. App. Sci.* 2016; 5, 38–4334.
34. Madhura SH, Shrishail C. Phytochemical evaluation and antioxidant activity of *Zanonia indica* fruit extracts. *Int. J Botany Stud*. 2021; 5, 67-74.
35. Adaikala Raj G, Chandrasekaran M, Krishnamoorthy S, Venkatesalu V. Antibacterial activity of different solvent extracts of *Caulerpa chemnitzia* (Esper) J.V. Lamououx, from Mandapam, Gulf of Mannar Southeast Coast, Tamil Nadu, India. *J Med Herbs Ethnomed.* 2015b; 1: 24-31.
36. Jayaraman M, Senthilkumar A, Adaikala Raj G, Venkatesalu V, Isolation of mosquito larvicidal molecule from the leaves of *Clausena anisata*. J Exp Sci2015; **6:** 12-16.
37. Basri DF, Shabry ASM, Meng CK. Leaves extracts from *Canarium odontophyllum* Miq (Dahai). Extracts Cytotoxic Activity against human colorectal Cancer cell line HCT 116. *Nat rod Chem Res*. 2015; 3 : 166. Doi: 10.4172/2329-6836. 1000166.
38. Kam PCA, Lie W, Traditional Chinese herbal medicine. *Anaesthesia*. 2002; 57(11): 1083-1089.
39. Santhi R, Alagesaboopathi C, Rajasekara Pandian M. Antibacterial activity of *Andrographis lineata* Nees and *Andrographis echioides* Nees of Shevaroy hills of Salem District, Tamil Nadu. *Ad Plant Sci*. 2006; 19(II): 371-375.
40. Marjorie MC. Plant products as antimicrobial agents. *Clin Microbiol Rev*.1999; 12: 564-582.
41. Kumar A, Shukla R, Singh P, Prasad CS, Dubey NK. Assessment of *Thymus vulgaris L.*essential oil as a safe botanical preservative against post-harvest fungal infestation of food commodities. *Food Sci Emerg*. 2008; 4: 575-580.
42. AL-Fatimi M, Wurster, M, Schroder, G and Lindequist, U. Antioxidant, antimicrobial and cytotoxic activities of selected medicinal plants from Yemen. *J Ethnopharmacol*. 2007; 111(3): 657-666.