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Research Article

IN VITRO ANTIFUNGAL ACTIVITY OF LEAF AND ROOT EXTRACTS OF THE MEDICINAL PLANT, *HYPOCHAERIS RADICATA* L.

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ABSTRACT

Objective: To assess the antifungal activity of the successive extracts (petroleum ether, chloroform, ethyl acetate, methanol and water) of leaf and root parts of *Hypochaeris radicata* and to determine the minimum inhibitory (MIC) concentration against nine human pathogenic fungi.

Methods: The antifungal activities of the leaf and root extracts against nine pathogenic fungi were tested by using disc diffusion method. Tetracycline was used as positive control. MIC for the methanolic leaf and root extracts of this species was assessed for antifungal susceptibility using broth micro dilution method.

Results: From the evaluation it was found that ethyl acetate extracts inhibited the fungal growth effectively than the other solvent extracts. The fungal species, *Aspergillus niger* and *Mucor* sp. were very sensitive to the ethyl acetate extract of both leaf (24.83 ± 0.28) and root (20.96 ± 0.25). Among the two parts studied, the root part showed higher antifungal activity than the leaf part. MIC for the methanolic leaf and root extracts of this species was ranging between 200 and 500µg/mL and 200 and 600µg/mL respectively.

Conclusion: The obtained results provide a support for the use of this plant in traditional medicine and it is a potential antiseptic source for the prevention and treatment of fungal infections.

Keywords: *Hypochaeris radicata,* Antifungal activity, Disc diffusion, MIC.

INTRODUCTION

Generally, the fungal infections are the most common cause of many skin diseases in developing countries [1]. Opportunistic fungal infections, mainly resulting from the species of *Candida*, *Cryptococcus* and *Aspergillus* are life-threatening in immunocompromised patients (with AIDS, cancer, or organ transplant) [2]. Due to the increasing number of individuals of this category, fungal infections have increased in the last two decades, affecting millions of people worldwide [3]. Using of synthetic chemicals for controlling these skin diseases is not ecofriendly and they are not providing environmental security. Therefore, it is necessary to search for more effective and less toxic novel antifungal agents that would overcome these disadvantages. Interestingly, plants are widely employed in folk medicine, mainly in communities with inadequate conditions of public health and sanitation. The antimicrobial compounds produced by plants are active against plant and human pathogenic microorganisms [4]. Several medicinal plants have been extensively studied in order to find more effective and less toxic compounds [5].

The species, *Hypochaeris radicata* (Asteraceae) commonly known as cat's-ear, is native to Europe also distributed in high hills of Nilgiris, the Western Ghats, India (above 2200m msl). It has been extensively used in traditional medicine for its anticancer, anti-inflammatory, anti-diuretic and hepatoprotective activities and to treat kidney problems. The leaves and roots of this species also possess antioxidant property [6] and antibacterial activity [7] also. The milky sap is bitter and the plant is suspected by some of being unwholesome as fodder [8]. It is high in protein and low in fibre. The calcium content is exceptionally high and it is rich in copper, sulphur and chloride. The seed is an important constituent in the diet of many farmland birds including linnets (*Carduelis cannabina*) [9]. The objective of the present study was to evaluate the antifungal activity and minimum inhibitory concentration of leaf and root extracts of *H. radicata* against certain pathogenic fungal species.

MATERIALS AND METHODS

Plant collection and identification

The fresh plant material was collected from Kattabettu, Nilgiris, the Western Ghats, India (above 2200m above msl). The authenticity of the plant was confirmed in Botanical Survey of India Southern Circle, Coimbatore by referring the deposited specimen. The voucher number of the specimen is BSI/SRC/5/23/2010-11/Tech.153.

Preparation of plant extracts

Fresh plant material of leaf and root parts were washed under running tap water, shade dried and then homogenized to fine powder and stored in airtight bottles. About 50g of coarsely powdered leaves and roots (50g/250mL) were extracted separately in a soxhlet extractor for 8 to 10 hours (50-85°C) sequentially with petroleum ether, chloroform, ethyl acetate, methanol and water separately in order to extract non-polar and polar compounds [10].

Preparation of inoculums

The nine fungal cultures obtained from TNAU, Coimbatore such as *Paecilomyces lilacinus, Mucor* sp., *Trichoderma viride, Verticillium lecanii, Candida albicans, Fusarium* sp., *Penicillium* sp., *Aspergillus fumigatus* and *A. niger* were was grown at 27°C on potato dextrose agar (PDA) medium. Spores of the each fungus species was collected from cultures on agar plates after 7 days [11]. PDA broth prepared by transferring a loop full of cells from the stock cultures was diluted with fresh potato dextrose broth. The sporangial suspension concentration was adjusted to $2x10^5$ (CFU/mL) spores [12].

Antifungal activity

Antifungal activity was investigated by the disc diffusion method [13]. Fungal suspension ($2x10^5$) was streaked on the potato dextrose agar (PDA) medium containing Petri plates. Then, sterile discs (made from Whatman filter paper) each about 5mm diameter impregnated with the leaf and root extracts separately were placed on the inoculated plates. Similarly, each plate was placed with a sterile disc, tetracycline as positive control. All the plates were incubated at 28°C for 24-48 hours. The zones of growth inhibition around the disc were measured after 48 hours. The sensitivity of the fungal species to the plant extracts was determined by measuring the sizes of inhibition zones (diameter of the zone) on the agar surface around the disc.

Minimum Inhibitory Concentration (MIC)

The minimum inhibitory concentration (MIC) was determined through the broth dilution method [14]. The fungal inoculum (10^{-5} dilution) was taken in test tubes with (1800µl) nutrient broth

supplemented with eight different concentrations of both leaf and root extracts 100-800µl/mL separately. The results of the extracts were compared with a standard, positive control (tetracycline 100µg/mL) and negative control (methanol 100µg/mL). All the test tubes were incubated at 35°C for 24-48 hours. The tubes were examined for visual turbidity. The MIC values were taken as the lowest concentration that inhibited the visual growth of the tested organisms [15, 16].

Statistical analysis

The antibacterial activity of *H. radicata* leaf and root extracts was indicated by clear zones of growth inhibition. All experiments were performed in triplicates and the results are presented as mean ± SD (Standard Deviation) according to New Duncan's Multiple Range Test [17].

RESULTS

Exploitation of the evaluation of antifungal activity of the present study revealed that the *H. radicata* possess potential antifungal activity against nine pathogenic fungal species. From the evaluation it is found that ethyl acetate extract inhibited the growth of the colonies of large number of fungal species than the other solvent extracts studied.

Table 1: Antifungal activity of various alcoholic and aqueous leaf extracts of *Hypochaeris radicata.*

Control/ Extracts	Zone of inhibition (mm)								
	PL	**Ms**	**TV**	**VL**	**CA**	**Fs**	**Ps**	**AF**	**AN**
PC	-	20.56 ± 0.51^{a}	24.66 ± 0.35^{a}	25.86 ± 0.15^{a}	16.73 ± 0.25^{b}	9.40 ± 0.36^{c}	37.23 ± 0.25^{d}	33.50 ± 0.50^{d}	31.36 ± 0.56^{d}
PE	-	8.76 ± 0.25^{a}	-	-	-	-	-	8.83 ± 0.15^{a}	10.23 ± 0.40^{b}
CF	-	12.96 ± 0.57^{a}	12.83 ± 0.15^{a}	7.86 ± 0.15^{b}	8.56 ± 0.60^{b}	-	7.83 ± 0.15^{b}	13.50 ± 0.50^{a}	12.73 ± 0.25^{a}
EA	9.93 ± 0.11^{a}	14.66 ± 0.61^{b}	14.76 ± 0.25^{b}	9.93 ± 0.11^{a}	9.83 ± 0.15^{a}	10.16 ± 0.15^{ab}	8.83 ± 0.28^{a}	14.66 ± 0.30^{b}	24.83 ± 0.28^{c}
ME	-	14.60 ± 0.40^{a}	7.73 ± 0.25^{b}	7.60 ± 0.52^{b}	15.53 ± 0.47^{a}	7.73 ± 0.25^{b}	-	12.96 ± 0.57^{a}	19.96 ± 0.57^{c}
WA	-	-	-	-	11.26 ± 0.25^{a}	-	-	-	15.56 ± 0.51^{b}

'-' indicates no activity.

PC = Positive control (Ampicillin), PE = Petroleum ether, CF = Chloroform, EA = Ethyl acetate, ME = Methanol, WA = Water.

PL = *Paecilomyces lilacinus,* Ms = *Mucor* sp., TV = *Tricoderma viride,* VL = *Verticillium lecanii,* CA = *Candida albicans,*

Fs = *Fusarium* sp., Ps = *Penicillium* sp., AF = *Aspergillus fumigatus,* AN = *Aspergillus niger.*

Values were performed in triplicates and represented as mean ± SD.

Mean values followed by different superscript in a column are significantly different ($p<0.05$).

Table 2: Antifungal activity of various alcoholic and aqueous root extracts of *Hypochaeris radicata.*

Control/ Extracts	Zone of inhibition (mm)								
	PL	**Ms**	**TV**	**VL**	**CA**	**Fs**	**Ps**	**AF**	**AN**
PC	-	20.56 ± 0.51^{a}	32.16 ± 0.76^{b}	24.40 ± 0.52^{a}	19.66 ± 0.25^{a}	10.23 ± 0.25^{c}	34.63 ± 0.40^{b}	34.46 ± 0.47^{b}	34.66 ± 0.51^{b}
PE	-	7.73 ± 0.25^{a}	9.66 ± 0.30^{b}	8.56 ± 0.51^{ab}	-	-	7.86 ± 0.75^{a}	10.76 ± 0.20^{c}	10.66 ± 0.50^{c}
CF	6.66 ± 0.57^{a}	19.50 ± 0.50^{b}	20.90 ± 0.36^{b}	12.76 ± 0.25^{ab}	13.73 ± 0.25^{ab}	9.30 ± 0.60^{a}	11.66 ± 0.30^{ab}	13.66 ± 0.54^{ab}	17.66 ± 0.23^{b}
EA	7.50 ± 0.50^{a}	20.96 ± 0.25^{b}	12.80 ± 0.26^{ab}	8.23 ± 0.68^{a}	11.73 ± 0.25^{ab}	14.83 ± 0.15^{ab}	15.46 ± 0.56^{ab}	15.30 ± 0.60^{ab}	15.66 ± 0.15^{ab}
ME	-	9.73 ± 0.25^{a}	14.83 ± 0.15^{b}	9.73 ± 0.25^{b}	9.73 ± 0.25^{a}	6.66 ± 0.57^{c}	7.16 ± 0.15^{ca}	11.66 ± 0.57^{ab}	14.65 ± 0.12^{b}
WA	-	-	10.73 ± 0.64^{a}	-	8.93 ± 0.83^{b}	-	-	12.56 ± 0.51^{c}	10.50 ± 0.50^{a}

'-' indicates no activity.

PC = Positive control (Ampicillin), PE = Petroleum ether, CF = Chloroform, EA = Ethyl acetate, ME = Methanol, WA = Water.

PL = *Paecilomyces lilacinus,* Ms = *Mucor* sp., TV = *Tricoderma viride,* VL = *Verticillium lecanii,* CA = *Candida albicans,*

Fs = *Fusarium* sp., Ps = *Penicillium* sp., AF = *Aspergillus fumigatus,* AN = *Aspergillus niger.*

Values were performed in triplicates and represented as mean ± SD.

Mean values followed by different superscript in a column are significantly different ($p<0.05$).

Effect of leaf extract

Among the five solvents attempted, the ethyl acetate extract showed higher inhibitory activity (24mm) followed by methanol extract (19mm) and chloroform extract (12mm) against the fungus, *Aspergillus niger.* The chloroform and methanol extracts showed significant activity against all the tested fungal species which was ranging between 7mm and 13mm, and 6mm and 19mm respectively. However, the petroleum ether and water extracts showed moderate activity against the fungal species *viz., Mucor* sp.,

A. fumigatus, A. niger and *Candida albicans,* and *A. niger* respectively (Table 1).

Effect of root extract

The greater zone of inhibition was produced by ethyl acetate and chloroform extracts of root of *H. radicata* against the fungi, *Mucor* sp. and *Tricoderma viride* (20mm) followed by methanol, water and petroleum ether extracts (Table 2). Methanol extract showed highest activity against *T. viride* and *Aspergillus niger* (14mm) and minimum activity against the fungus, *Fusarium* sp. The activity of petroleum ether and water extracts the fungal growth was not noteworthy.

Minimum inhibitory concentration

Table 3 presents the data on minimum inhibitory concentration (MIC) of methanolic leaf and root extracts of *H. radicata.* The leaf and root extracts exhibited remarkable antifungal activity which was ranging between 200 and 500µg/mL and 200 and 600µg/mL respectively.

Table 3: Minimum inhibitory concentration (MIC) of methanolic leaf and root extracts of *Hypochaeris radicata.*

Plant parts	Minimum inhibitory concentration (µg/ml)							
	PL	TV	VL	CA	Fs	Ps	AF	AN
Leaf	300	400	300	400	200	300	200	300
Root	300	400	300	400	200	600	300	200

PL = *Paecilomyces lilacinus,* Ms = *Mucor* sp., TV = *Tricoderma viride,* VL = *Verticillium lecanii,* CA = *Candida albicans,* Fs = *Fusarium* sp., Ps = *Penicillium* sp., AF = *Aspergillus fumigatus,* AN = *Aspergillus niger.*

DISCUSSION

The results obtained from the present investigation revealed that the highest antifungal activity was exhibited by the ethyl acetate extract and the least by the petroleum ether and water extracts. The basis of varying degree of sensitivity of test organisms of fungi may be due to the intrinsic tolerance of microorganisms and the nature and combinations of phytocompounds presents in the crude extracts.

The antifungal activity of ethyl acetate extract of *H. radicata* leaf showed highest inhibitory activity against the fungus, *Aspergillus niger.* It is a filamentous ascomycete fungus that is ubiquitous in the environment and has been implicated in opportunistic infections of humans [18]. It causes various diseases in plants and animals. In plants it causes black mould and rot diseases and in human beings it causes aspergillosis by which pulmonary allergy, bronchopulmonary aspergillosis and pulmonary aspergilloma are made [19, 20]. Raji and Raveendran [21] reported that water extracts of Asteraceae members showed strongest effect on reduction in growth of *A. niger* than the species of certain other families. They explained that certain specific compounds of unknown functional group may present in Asteraceae member which may played role in the inhibition of fungal colonies. This fact perhaps be a reason for the fungal inhibitory property of the study species of Asteraceae family, *H. radicata.* As in the present, Duraipandiyan and Ignacimuthu [22] reported that in majority of the species, out of 45 plants studied, ethyl acetate extract exhibited more pronounced antifungal activity than the other solvents. Similarly, Saheb *et al.*[23] assayed various extracts like aqueous, alcoholic and ethyl acetate extracts of leaves of five *Terminalia* species against five plant pathogenic fungi like *A. flavus, A. niger, Alternaria brassicicola, A. alternate* and *Helminthosporium tetramera* and found that the ethyl acetate extract showed better inhibitory effect against all the fungi tested. In the present study, chloroform and methanol extracts of leaf showed significant antifungal activity against seven fungi *viz., Mucor* sp., *Tricoderma viride, Verticillium lecanii, Candida albicans, Penicillium* sp., *A. fumigates* and *A. niger* and instead of *Penicillium* sp. the *Fusarium* sp. was inhibited by methanol extract. Water and petroleum ether extracts of leaf inhibited less number of two to three fungi only (*C. albicans* and *A. niger,* and *Mucor* sp., *A. fumigates* and *A. niger*). This could be due to the lack of specific active compounds in the extracts. However, all the extracts of *H. radicata* leaf inhibited the growth of *A. niger.*

The ethyl acetate and chloroform extracts of root of *H. radicata* exhibited greater zone of inhibition against the fungi *Mucor* sp. and *Tricoderma viride* which are causing many infectious diseases in human beings. On the other hand, the methanol extract generally inhibited the growth of all fungal species except *Paecilomyces lilacinus*. Petroleum ether and water extracts inhibited only few fungal species (*Mucor* sp., *T. viride, Verticillium lecanii Penicillium* sp., *Aspergillus fumigates* and *A. niger* and *T. viride, Candida albicans, A. fumigates* and *A. niger*). The most susceptible organisms to root extracts of the study species were determined to be *A. fumigatus, A. niger* and *T. viride,* and the most resistant organism was *P. lilacinus.*

The present results showed that the ethyl acetate extracts of leaf and root were more effective than the other extracts tested. In another Asteraceae member, *Stevia rebundiana* also the ethyl acetate extract has reported to have higher antifungal activity than that of the other alcoholic solvent extracts [24]. Among the two parts studied, the root part showed higher antifungal activity than the leaf part. In earlier report also it has been known that the root part of *Hypochaeris radicata* displayed prodigious antibacterial activity [7]. This may be attributed to the presence of variety of flavonoids and phenolic compounds in the roots [25] which may have the capacity to rupture the cytoplasmic membrane of the fungal cells and damage the intracellular compounds [26] or they may interact with lipid bilayers or inhibit the protein and nucleic acid synthesis of the fungal cell [27]. Various publications have documented the effective antifungal activity of Asteraceae members [28, 29, 30, 31]. It was further observed that the inhibitory activities of ethyl acetate extract of leaf against *Fusarium* sp. and ethyl acetate extract of root against *Mucor* sp. and *Fusarium* sp. were significantly greater than that of the standard drug, tetracycline, which indicates the effectiveness and specific inhibitory function of ethyl acetate solvent by deriving specific compounds against these fungi.

Minimum inhibitory concentration (MIC) of methanolic leaf extract was ranging between 200 and 400µg/mL. The most susceptible species for this extracts were *Aspergillus niger* and *Fusarium* sp. (200µg/mL) and most resistant species were *Trichoderma viride* and *Candida albicans* (400µg/mL). MIC of methanolic root extract of the study species was ranging between 200 (against *Fusarium* sp. and *A. niger*) and 600µg/mL (against *Paecilomyces lilacinus*). From the above results it is known that *Fusarium* sp. and *Aspergillus* sp. were susceptible to both the leaf and root extracts.

CONCLUSION

The present study revealed that the leaf and root extracts *Hypochaeris radicata* of possess significant antifungal activity and it leads to discover novel antifungal drugs. It is interesting to note that the ethyl acetate extract of this species, could be used mainly against the two fungi, *Aspergillus niger and Mucor* sp. to control the infectious diseases aspergillosis and zygomycosis respectively in an effective manner.

REFERENCES

1. Ping-Hsein, Chi-Weilee, Jia-Ying Chou, Murugan M, Bor-Jinn Shieh, Hueih-Mir Chen. Antifungal activity of crude extracts and essential oil of *Moringa oleifera* L. Bioresource Technology 2007; 98: 232-236.

2. Liu WD, Lian CH. The early stage diagnosis of deep-seated fungal infections. Chinese Journal of Laboratory Medicine 2003; 26: 583-584.
3. Wong B, Klei B, Kozel T. Immunologic approaches and metabolite detection. The second NIAID Workshop in Medical Mycology, University of Arizona, Northern Arizona University, Flagstaff, AZ, June 8-11, 1994.
4. Ramezani H, Singh HP, Batish DR Kohli, Dargan JS. Fungicidal effect of volatile oils from *Eucalyptus citriodora* and its major constituent citronetial. New Zealand plant protection 2002; 55: 327-330.
5. Nakamura CV, Ishida K, Faccin LC, Filho BPD, Cortez DAG, Rozental S, de Souza W, Ueda-Nakamura T. *In vitro* activity of essential oil from *Ocimum gratissimum* L. against four Candida species. Research in Microbiology 2004; 155: 579-586.
6. Jamuna S, Paulsamy S, Karthika K. Screening of *in vitro* antioxidant activity of methanolic leaf and root extracts of *Hypochaeris radicata* L. (Asteraceae). Journal of Applied Pharmaceutical Science 2012; 2(7): 149-154.
7. Jamuna S, Paulsamy S, Karthika K. *In-vitro* antibacterial activity of leaf and root extracts of *Hypochaeris radicata* L. (Asteraceae) – a medicinal plant species inhabiting the high hills of Nilgiris, the Western Ghats. International Journal of Pharmacy and Pharmaceutical Sciences 2013; 5 (1): 175-178.
8. Aarssen LW. The biology of canadian weeds. *Hypochaeris radicata* L. Canadian Journal of Plant Science 1981; 61: 365-381.
9. Moorcraft D, Bradburry RB, Wilson JD. The diet of nestling linnets *carduelis cannabina* before and after agricultural intensification. Proceedings of the Btighton crop protection conference-weeds, 1997. p. 923-928.
10. Elgorashi EE, Van Staden J. Pharmacological screening of six Amaryllidaceae species. Journal of Ethnopharmacology. 2004; 90: 27-32.
11. Broekaert WF, Terras FRG, Cammue BPA, Vanderleyden J. An automated quantative assay for fungal growth inhibition. FEMS Microbiology Letters 1990; 69: 55-60.
12. Abril M, Curry KJ, Smith BJ, Wedge DE. Improved microassays used to test natural product-based and conventional fungicides on plant pathogenic fungi. Plant Diseases 2008; 92: 106-112.
13. Alzoreky NS, Nakahara K. Antibacterial activity of extracts from some edible plants commonly consumed in Asia. International Journal of Food Microbiology 2003; 80: 223-230.
14. Koneman EW, Allen SD, Janda WN, Schreckenber PC, Winn WC. Jr. Color Atlas and Textbook of Diagnostic Microbiology, 5th Ed. Lippincott. Raven Publishers, Philadelphia.1997.
15. Bishu J, Sunil LL, Anuja S. Antibacterial property of different medicinal plants. *Occimum sanctum, Cinnamomum zeylanicum, Xanthoxylum armatum and Origanum majorana.* Kathmandu University J. Science, Engineering and Technol 2009; 5: 143-150.
16. Wayne PA. National committee for clinical laboratory standards. Performance standards for antimicrobial disk susceptibility tests; approved standard, 7th ed. NCCLS document M2-A7, 2000.
17. Gomez KA, Gomez KA. Statistical procedure for agricultural research with emphasis of Rice (Los Bans, Philippines International Rice Research Institute), 1976.
18. Perfect JR, Cox GM, Lee XY, Kauffman CA, De Repentigny L, Chapman SW, Morrison VA, Pappas P, Hiemenz JW, Stevens DA. Mycoses study group. Clinical Infectious Diseases 2001. 33(11): 1824-1833.
19. Young RC, Bennett JE, Vogel CL, Carbone PP, Vita VT. Aspergillosis, the spectrum of diseases in 98 patients. Medicine 1970. 49: 147-173.
20. Londero RT, Guadalupe JM. Aspergilloses Pulmonares. Journal Brasileiro de Pneumologia 1990. 16: 78-90.
21. Raji R, Raveendran K. Antifungal activity of selected plant extracts against phytopathogenic fungi *Aspergillus niger*. Asian Journal of Plant Science and Research 2013. 3(1): 13-15.
22. Duraipandiyan V, Ignacimuthu S. Antifungal activity of traditional medicinal plants from Tamil Nadu, India. Asian Pacific Journal of Tropical Biomedicine 2011. S204-S215.
23. Saheb SL, More Sm, Junne SB, Wadje SS. The antifungal activity of five *Terminalia species* checked by paper disc method. International Journal of Pharma Research and Development 2011. 3(2): 36-40.
24. Sathishkumar Jayaraman, Muthu Saravanan Manoharan, Seethalakshmi Illanchezian. *In* vitro Antimicrobial and Antitumor activites of *Stevia rebaundiana* (Asteraceae) leaf extracts. Tropical Journal of Pharmaceutical Research 2008; 7(4): 1143-1149.
25. Zidon C, Schubert B, Stuppner H. Altitudinal differences in the contents of phenolics in flowering heads of three members of the tribe Lactuceae (Asteraceae) occurring as introduced species in New Zealand. Biochemical and Systematic Ecology 2005; 33: 855-872.
26. Chen HM, Chan SC, Lee JC, Chang CC, Murugan M, Jack RJ. Transmission electron microscopic observations of membrane effects of antibiotic cecropin B on *Escherichia coli*. Microscopy Research and Technique 2003; 62: 423-430.
27. Adetumbe A, Javor GT, Lau BH. *Allium Sativum* (garlic) inhibits lipid synthesis by *Candida albicans*. Antimicrobial Agents and Chemotheraphy. 1986; 30(3): 499-501.
28. Malarkodi E, Manoharan A. Antifungal activity of *Parthenium hysterophorus* L. Journal of Chemical and Pharmaceutical Research 2013; 5(1): 137-139.
29. Sarmad M, sahaya S, Servin W, chitra D. Antimicrobial activity of *in vitro* raised *Acmella calva* (dc.) R.k.jansen. International Journal of Pharmacy and Pharmaceutical Sciences 2012. 4(5): 124-127.
30. Prajakta PP, Babu VV, Ramesh TS, Ajit GD. Antimicrobial activity of essential oil of *Blumea eriantha* DC. Against skin pathogens. International Journal of Pharmacy and Pharmaceutical Sciences 2012. 4(4): 296-299.
31. Munmi B, Kakoty BB, Sikia LR. Proximate analysis and antimicrobial activity of *Eclipta alba* L. Hassk. – a traditionally used herb. International Journal of Pharmacy and Pharmaceutical Sciences 2013. 5(1): 149-154.