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RESEARCH ARTICLE

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Antifungal Activities of Essential Oils from Four Commonly Used Ethno-Medicinal Plants.

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Abstract

Essential oils from plants may provide potential alternatives to the chemical control agents currently used because the compositions of essential oils are rich of bioactive chemicals. The antifungal activities of *Ageratum conyzoides, Lantana camara, Litsea cubeba* and *Piper mullesua* were tested against five phyto-pathogenic fungi viz, *Alterneria alternate, Mucor haemilis, Helminthosporium solani, Humicola grisea* and *Botrytis cinerea*. Among the selected entho-medicinal plants tested, *Litsea cubeba* was found to be most effective in controlling the growth of phyto-pathogenic fungi under controlled environment. Culture media containing *Litsea cubeba* oil extract showed no visible growth of *Alterneria alternate* while *Mucor haemilis, Helminthosporium solani, Humicola grisea* and *Botrytis cinerea* showed visible growth after 92 hrs of incubation. *Ageratum* conyzoides oil was found effective against *Alterneria alternate* and *Helminthosporium solani*. Similarly, *Lantana camara* was effective against *Alterneria alternate*, *Mucor haemilis* and *Humicola grisea*. *Piper mullesua* was also found to restrict the growth of sample *Alterneria alternate*, *Helminthosporium solani* and *Mucor haemilis*. Essential oils from *Ageratum, Lantana, Litsea* and *Piper* tested above can form an integral part of integrated pest management in for controlling phytopathogenic fungus.

Keywords: Antifungal properties, essential oils, growth inhibition, fungal biomass, MIC.

Introduction:

In the past few years, scientific investigation on plants as a source of new bio-molecules for human disease management has increasing rapidly (Grierson and Afolayan 1999). Although, traditionally plants have been well exploited by man for the treatment of human diseases (as in Ayurveda), but not much information is available on the exploitation of plant wealth for the management of plant diseases, especially against phytopathogenic fungi. Several fungi, which cause severe damage to stored food commodities, were generally managed by synthetic chemicals, which were considered both efficient and effective (Miller 1995; Janardhana et al. 1999; Galvano et al. 2001). The continuous use of these synthetic fungicides started unraveling non biodegradability and known to have residual toxicity to cause pollution (Pimentel and Levitan 1986). Pesticide pollution of soil and water bodies is well documented (Nostro et al. 2000). Hence in recent time application of plant metabolites for plant disease management has become important viable component of Integrated Pest Management, as plant metabolites are eco-friendly. In recent years, there has been a gradual revival of interest in the use of medicinal and aromatic plants in developed as well as in developing countries, because plant-derived drugs have been reported to be safe and without side-effects.

Essential oils play an important role in the protection of the plants as antibacterials, antivirals, anti-fungals, and insecticides and also against herbivores by reducing their appetite for such plants. Essential oil extracted from various aromatic plants represents an important part of the traditional pharmacopoeia mostly in tropical and temperate countries. They are liquid, volatile, limpid and rarely colored, lipid soluble and soluble in organic solvents with a generally lower density than that of water. They can be synthesized by all organs of aromatic plants i.e. buds, flowers, leaves, stems, twigs, seeds, fruits, roots, wood or bark, as secondary metabolites and are stored in secretory cells, cavities, canals, epidermis cells or glandular trichomes (Bakkali et al. 2008). Plant having high content of essential oils may provide potential alternatives to the control agents currently used because the compositions of essential oils are rich of bioactive chemicals (Isman 2000). These oils are also used in embalmment, preservation of foods and as antimicrobial, analgesic, sedative, anti-inflammatory, spasmolytic and locally anesthesic remedies. Up to the present day, these characteristics have not changed much except that more is now known about some of their mechanisms of action, particularly at the antimicrobial level.

A survey of literature reveals that there are many essential oils which possess antifungal activity (Soliman and Badeaa 2002; Thoppil et al. 2003; Govinden-Soulange et al. 2004; Romagnoli et al. 2005; Pinto et al. 2006; Tabanca et al. 2007; Tullio et al.

2007; Dutta et al. 2007). They were reported to have antimicrobial activities such as antifungal (Soliman and Badeaa 2002), antibacterial (Dorman et al. 2000), insecticidal (Isman 2000) and nematicidal effects (Pandey et al. 2000). Much work has been done on ethno medicinal plants in India (Maheshwari et al. 1986; Negi et al. 1993). Interest in a large number of traditional natural products has increased (Taylor et al. 1996).

India is largely agriculture dependent and several phyto-pathogenic fungi are principally responsible for crop loss, low production, on farm deteriorating and post harvest loss of crop/agricultural products. Present work in therefore attempted to test the sensitivity of few important and common phyto-pathogenic fungal species against the oil extracts of four commonly used ethnomedicinal plants (i.e, *Ageratum conyzoides, Lantana camara, Litsea cubeba* and *Piper mullesua*).

Ageratum conyzoides, Lantana camara, Litsea cubeba are terrestrial weeds found abundant in degraded and secondary forest of tropical and subtropical region of India. *Piper mullesua* is wild creeper found to be cultivated by several tribal people for commercial purposes. All these selected plants are having medicinal importance and are used by the different tribes of northeastern part of India (Table 1).

Plant species	Tribe	Uses	References
Ageratum conyzoides Linn.	Lepcha Tribe of Sikkim	Part use: Leaf Disease: Cut, wounds, diarrhoea, dysentery, intestinal colic with flatulence	Pradhan and Badola (2008), Kala (2005)
	Apatani Tribe	Part use: Leaf Disease: Cuts, wounds	Sajem and Gosai (2006)
	Jaintia Tribe	Part use: Leaf Disease: Cuts, wounds	
Lantana camara Linn.	Lepcha Tribe of Sikkim	The juice of crushed leaves is applied to the fresh cut and wounds to heal. Crushed leaves are tied over the sprain to relieve pain.	Pradhan and Badola (2008)
Litsea cubeba (Lour) Pers	Adi tribe	Fresh ripe and unripe fruits are taken as a remedy for cold and cough and also for good sleep. Seeds are chewed in case of thread worm infection.	Srivastava and Adi (2009), Bhuyan (2007)
Piper mullesua Buch.-Ham.	Adi, Nyshi, Galo and Apatani tribes of Arunachal Pradesh	Leaves and inflorescence are used for cold and cough, stomach upset, case of thread worm infection.	Hussain and Hore (2008)

Table 1- Ethno-medicinal uses of four medicinal plants by different tribes of North East India.

A scientific and systematic investigation on antifungal properties the oil extract of the above selected plant species are lacking. Present study is an attempt to investigate the antimicrobial properties of essential oils of above ethno-medicinal plants on five phytopathogenic fungal species.

Material and Methods

Collection of Plant Materials

Fresh leaves of *Ageratum conyzoides, Litsea cubeba, Lantana camara, Piper mullesua* free from diseases were collected from Arunachal Pradesh (India), washed thoroughly (2-3 times) with running tap water and finally with sterile distilled water. A voucher specimen of the plant is deposited in the herbarium of Department of Botany, Rajiv Gandhi University, Arunachal Pradesh, India for future reference.

Collection of Essential Oil

The leaves of above plants were cut into pieces and dried at room temperature before extraction. Above dried leaves were grounded to semi-powdered state using mixer grinder (USHA). The air-dried aerial parts (100g) were hydro distilled in a Clevenger apparatus (Borosil) with 250ml distilled water for 5 hrs in accordance with the British pharmacopoeia (1997). The plant material was placed in the round-bottom flask of the Clavengers apparatus. Water was heated to produce steam that carried the most volatile fractions of the aromatic material with it. The steam was then chilled (in a condenser) using running water and the resulting distillate was collected. The essential oil was found to float on the top of the hydrosol (the distilled water component) and was separated off. The yield of oil varies from 0.5ml to 2ml per 100g dried plant depending on the species selected. Extraction was done several times to collect the required quantity of essential oil. Oil was separated from water using disposable syringe (1ml). The oils were stored in a sealed glass vial in a refrigerator at 4ºC until required. Essential oil was not dried and was used directly after required dilution.

Collection of Phyto-pathogenic fungi

For the above study, five Phyto-pathogenic fungi viz, *Alterneria alternate, Mucor haemilis, Helminthosporium solani, Humicola grisea* and *Botrytis cinerea* were selected. The above selected Phyto-pathogenic fungi were purchased from IMTECH, Chandigarh, India. Accession numbers of the above samples are given in table 2.

Samples	Accession No.	Sample no.
Alternaria alternata	MTCC 149	S149
Mucor hiemalis	MTCC 157	S157
Helminthosporium solani	MTCC 1899	S1899
Humicola grisea	MTCC 352	S352
Botrytis cinerea	MTCC 359	S359

Table 2- Phyto-pathogenic fungal species purchased from IMTECH, Chandigarh

Literature review showed that all these fungal species are plant pathogen and cause diseases like Leaf spots, Rot diseases, Blights, Silver Scurf, Wilt disease and Gray mold disease (Table 3).

These phyto-pathogens are maintained as fresh culture in potato dextrose agar medium (200 g, scrubbed and diced potato in 1000 ml distilled water, 15 g agar, 20 g dextrose, pH ± 5.6) and as dry preservation at -20°C

with 20% glycerol water. A seven-day-old culture of each fungal species was used during investigation.

Samples	Diseases	Target species
Alternaria alternata	Leaf spots, rots and blights on many plant parts	Kiwi, Papaya, Citrus, Pomegranate, Prunus
Mucor hiemalis	Rot disease	Guava
Helminthosporium solani	Silver Scurf	Potato
Humicola grisea	Wilt disease	Artemisia
Botrytis cinerea	Gray mold disease/ (Leaf spot, Damping-off disease, Flower blight, Botrytis blight)	Kiwi, Grapes, Citrus, Strawberries

Table 3- Plant diseases caused by tested phyto-pathogenic fungi

Anti Fungal Activity Assessment

The antifungal activities of *Ageratum conyzoides, Lantana camara, Litsea cubeba* and *Piper mullesua* were analyzed using three different techniques for proper interpretation.

Antimycotic Assay by Disc Diffusion Technique: Oils were screened for their antifungal activity against *Alterneria alternate*, *Mucor haemilis, Helminthosporium solani, Humicola grisea* and *Botrytis cinerea*, by disc different method (Boyer 1976). The mycelial mat of 7-day old culture sample were washed, suspended in normal saline solution and then filtered through glass wool aseptically. The colony forming units (CFU/ml) of suspension of the test fungus was determined and test inoculum was adjusted 0.5 OD at 660nm using sterilized distilled water. These conidia were used for antifungal assay tests. Inocula (100µl) were applied on the surface of the PDA plate and spread by using sterile glass spreader. The sterile filter discs (12mm diameter, Whatman filter paper No. 42) were soaked in added concentrations of essential oils. Different size of paper disc was used by several workers To load higher concentration of essential oil per paper disc, the disc were dried after soaking and the process was repeated for 3-4 times. 100µl (1:200x diluted) standardized essential oil was soaked/disc. These dishes were incubated at 27ºC and Zone of inhibition (mm) were recorded from 6 days upto 32 days. The test was performed in triplicate. Standard antibiotics Fluconazole (Himedia) was used as positive control. Distilled water is used as negative control. The growth inhibition zone was measured using HiAntibiotic zone scale-C (HiMedia).

Antimycotic Assay by Biomass estimation:

Anti-fungal activity was also analyzed by measuring dry weight biomass from regular growth intervals (i.e, 48hrs to 288 hrs). Peptone broth media (Peptone 0.5%, dextrose 1%, Yeast extract 0.1%, $MgSO_4$ 0.05%, $CuSO_4$ 0.03%, Tween-80 0.5%) was used as growth media. 10ml of broth media was added in separate test tubes and sterilized at 15lb for 30 minutes. After autoclave, test tubes were cooled and 100µl of essential oil was added to each tube (1:200x diluted) and mixed well. After proper mixing, 100µl of culture suspension (OD 0.5) was added and mixed well. Test tubes were incubated in incubator shaker (Scigenics) at 27°C with continuous shaking at 150rpm. After requisite incubation, cultures were taken out and filtered using Wattman Paper No.1 and the suspension was dried at 50°C for 3-4 hrs. The difference of initial dry weight and final dry weight of filter paper gives the value of fungal dry weight. Dry weight was measured from 48 hrs incubation upto 288 hrs of incubation. The incubation period for different testes was standardized on the basis of growth pattern of fungi in selected growth media.

Antimycotic Assay by Spectrophotomeric method:

Although spectrophotometric method is not commonly used for fungal growth analysis however, in the present study anti fungal activity was also evaluated by measuring fungal growth after different incubation period starting from 72 hrs to 360 hrs using spectrophotometer (Scigenics). Peptone broth media (Peptone 0.5%, dextrose 1%, Yeast extract 0.1%, MgSO4 0.05%, CuSO4 0.03%, Tween-80 0.5%) was used as growth media added with 0.1% (v/v). 10ml of broth media was added in separate test tubes and sterilized at 15lb for 30 minutes. After autoclave, test tubes were cooled and 0.1% (v/v) of essential oil extract was added to each tube (1:200x diluted) and mixed well. After proper mixing, 100µl of culture suspension (OD 0.5) was added and mixed well. Incubation was done in incubator shaker at 27°C with continuous shaking at 150rpm to avoid clumping of fungal mycelia. Growth was estimated by measuring optical density at 660nm. Absorbance was calculated after 10 seconds of pouring the broth culture in cuvette to minimize estimation error.

Determinations of Minimum Inhibitory Concentration (MIC)

A micro-dilution method was used to determine MIC (NCCLS 1999). All tests were performed in PDA Media supplemented with Tween-80 at final concentration of 0.5% (v/v). 20 ml of media was poured per plate. After drying, 100µl of fungal broth culture was added to it and spread evenly using sterile glass spreader. The final concentration of each fungal strain was adjusted to 0.5 OD before use. Various quantity of essential oil (1:200x diluted) ranging from 15µl to 300µl of was added per plate and mixed well. Plates were incubated at 27°C for 7 days. The MIC is defined as the minimum concentration at which no visible growth of fungal mycelium was recorded.

Results

Among the selected ethno-medicinal plants tested for antifungal activities, *Litsea cubeba* showed best result. Fungal sample S149 showed no growth in culture media containing 1:200 times diluted oil extract of *Litsea cubeba*. Sample number 157, 1899 and 352 showed no visible growth upto 92 hrs of incubation and after 92 hrs slow growths was recorded upto 288

hrs. Sample 359 also showed growth after 216 hrs of incubation. *Ageratum* oil showed good result against sample 149 and 1899. *Lantana camara* effective against 149, 157 and 352. Piper was found to restrict the growth of sample 149, 1899 and 157.

Zone of inhibition on agar plate was found maximum with *Litsea cubeba* (upto 40mm) (Photo 1). Best result was found against the fungal sample 149, 1899, 352 and 359 respectively. *Lantana camara* was found effective against the fungal samples 149, 157, 352 and 359 respectively. Similarly, *Piper mullesua* extract was found effective against the fungal samples 149, 352 and 359 respectively (Table 4).

Growth analysis of phytophogenic fungal species by measuring dry weight biomass at different growth intervals is shown in Fig. 1.

Phyto- pathogens	Incubation period	Zone of inhibition (mm)				
		Ageratum conyzoides	*Lantana camara*	*Litsea cubeba*	*Piper mullesua*	*Positive Control*
Alterneria alternata	6 days	10±1.00	16±0.60	40±0.60	20±2.00	14±1.53
	12 days	10±0.00	16±2.00	40±0.00	14±1.00	12±1.53
	18 days	8±1.00	14±3.5	40±0.00	12±1.51	12±2.52
	24 days	0±0.00	14±1.00	40±2.30	12±0.60	10±1.00
	32 days	0±0.00	10±2.00	40±5.20	18±2.52	10±0.60
Mucor haemilis	6 days	18±2.00	16±0.58	10±1.00	12±2.00	12±3.00
	12 days	18±0.58	14±0.58	16±0.60	12±1.53	12±1.00
	18 days	18±0.60	12±0.58	24±3.51	12±0.58	12±2.00
	24 days	16±1.53	12±1.53	40±0.60	12±2.00	12±3.00
	32 days	16±1.00	16±0.00	40±1.15	10±0.60	12±2.52
Helminthosporium solani	6 days	12±0.58	10±2.00	40±0.00	12±0.60	14±3.00
	12 days	12±2.00	10±1.00	36±3.50	12±2.52	14±0.58
	18 days	12±0.58	10±1.53	40±1.15	10±2.00	14±1.53
	24 days	12±1.53	10±0.60	40±0.00	10±0.58	14±0.58
	32 days	12±0.00	10±1.36	40±.60	12±1.00	12±2.52
Humicola grisea	6 days	16±0.57	16±0.62	34±0.58	18±1.50	16±2.31
	12 days	16±2.00	16±.058	36±1.50	18±3.00	14±2.08
	18 days	16±1.00	16±0.58	38±2.00	18±0.60	14±0.58
	24 days	14±2.58	16±2.00	40±2.25	18±2.00	14±2.00
	32 days	12±0.60	12±3.12	40±0.58	12±0.00	13±3.51
Botrytis cinerea	6 days	16±0.58	18±0.58	30±2.00	14±2.00	16±5.51
	12 days	16±2.51	14±1.53	40±0.00	14±1.00	16±3.31
	18 days	16±1.50	14±0.60	40±0.60	14±0.60	16±1.53
	24 days	16±3.00	12±2.50	40±1.16	12±0.00	14±2.52
	32 days	16±1.00	14±2.50	40±0.61	0±0.00	14±4.00

Table 4: Zone of inhibition (mm) of fungal growth by essential oil of four plant species (1:200x oil extract). Fluconazole was used as +ve control.

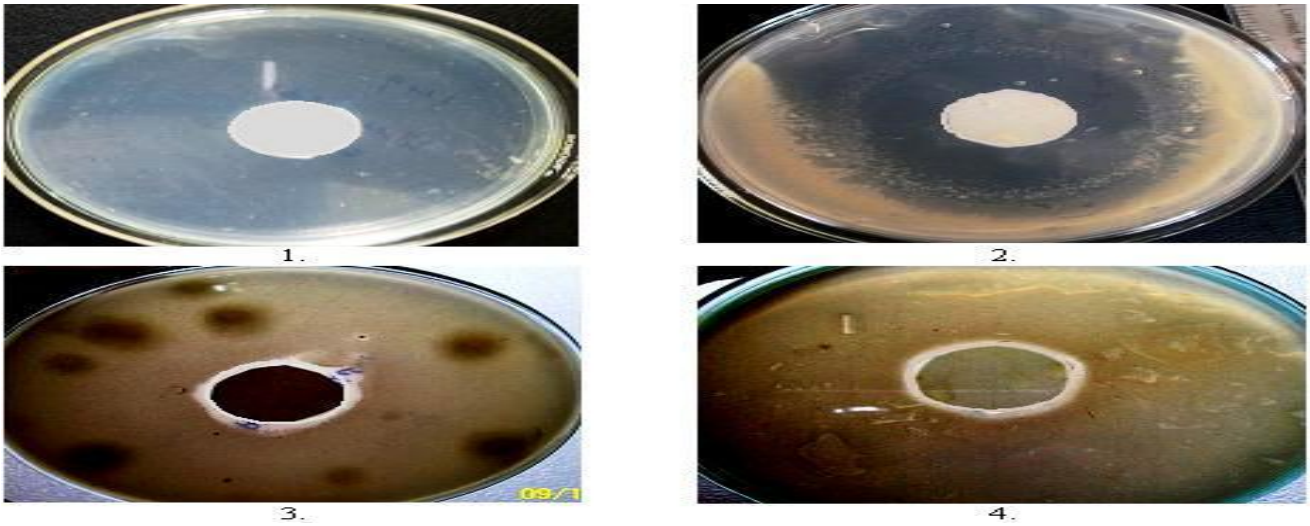

Photo 1: Zone of inhibition of different oil extracts on the selected phyto-pathogenic fungal species.

1. *Litsea cubeba* oil extracts showing almost zero growth of *Alterneria alternata* (after 12 days of incubation).
2. *Litsea cubeba* oil extracts showing growth restriction of *Mucor haemilis* (after 12 days of incubation).
3. *Piper mullesua* oil extracts showing growth restriction of *Alterneria alternata* (after 12 days of incubation).
4. *Ageratum conyzoides* oil extracts showing growth restriction of *Humicola grisea* (after 12 days of incubation)

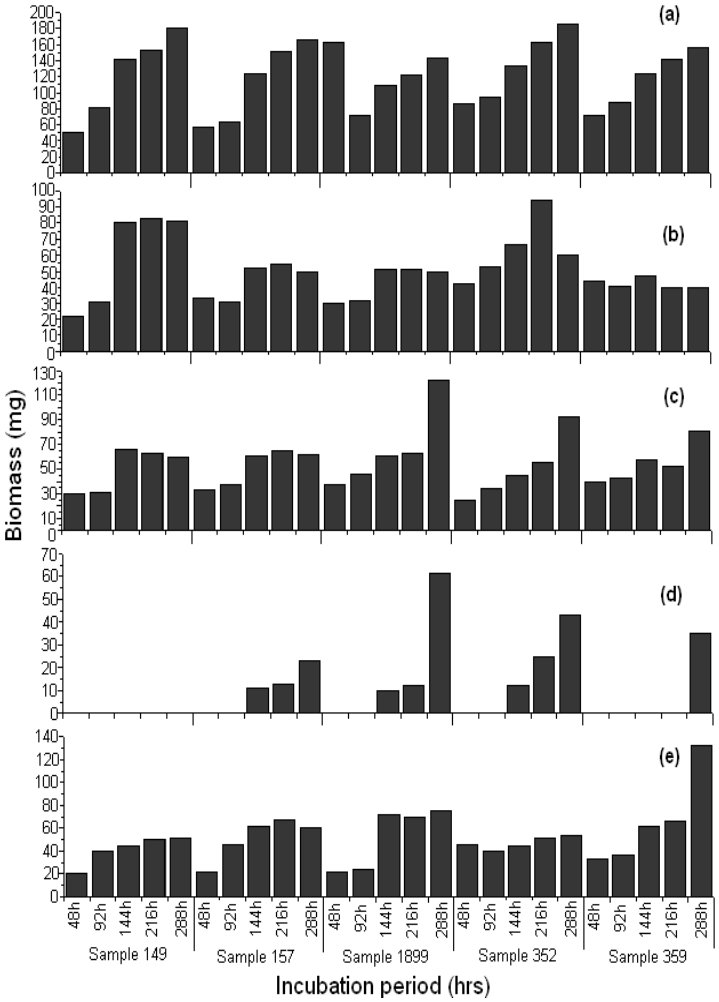

Figure 1-Graphical representation of growth activity in phytopathogenic fungi against four essential oils of ethnomedicinal plants estimated by dry weight biomass method.
Abbreviations. S149 = *Alternaria alternata*, S157 = *Mucor hiemalis*, S1899 = *Helminthosporium solani*, S352 = *Humicola grisea*, S359 = *Botrytis cinerea* (a)Control, (b) *Ageratum conyzoides* (c) *Lantana Camara* (d) *Litsea cubeba* (e) *Piper mullesua*

Litsea cubeba was most effective in suppressing the growth of all the selected fungi upto 288hrs of incubation as estimated by dry weight biomass. However, it was most effective against *Alterneria alternate* and *Botrytis cinerea*. *Ageratum* was effective against *Helminthosporium solani* and *Mucor haemilis* while *Lantana camara* was effective against *Alterneria alternate* and *Mucor haemilis*. Similarly, *Piper mullesua* was effective against *Alterneria alternate, Mucor haemilis* and *Helminthosporium solani*.

Antifungal activities were also analyzed spectrophotometrically by measuring growth of fungi at 660nm after regular periods of incubation. Phyto-pathogenic fungal species tested showed minimum growth in the broth culture containing *Litsea cubeba* oil extract followed by the oil extracts of *Piper mullesua* and *Ageratum conyzoides* respectively. Fungal sample 149 was most to *Litsea* and *Ageratum*. Similarly, fungal samples 352 and 359 were *Piper mullesua* while fungal species 1899 was most *Ageratum conyzoides* and 155 against *Lantana camara* as compared to other fungal species tested (Fig. 2).

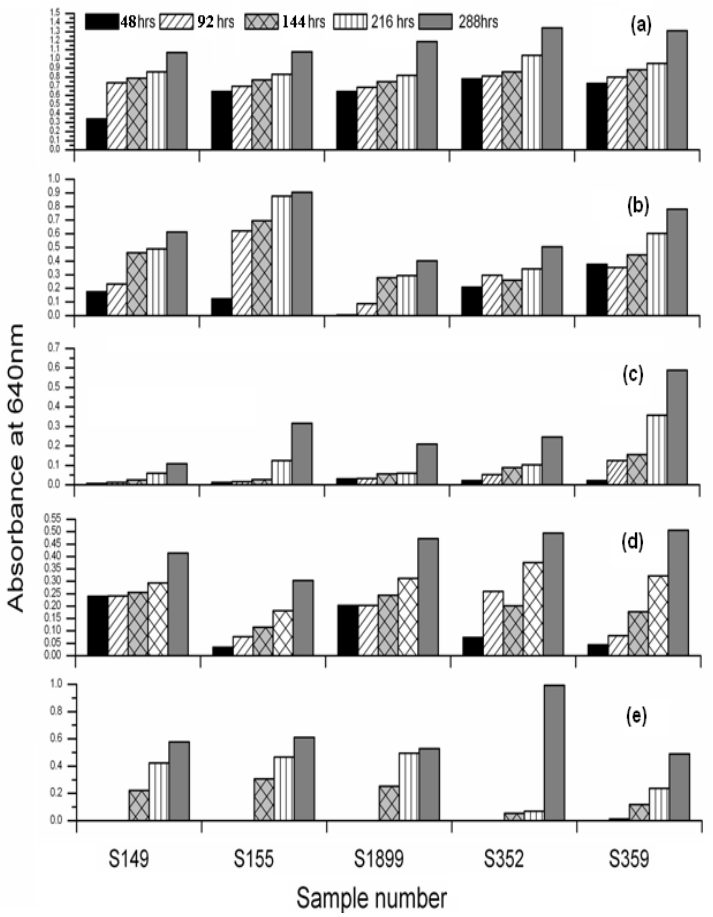

Figure 2-Growth estimation of phytopathogenic fungi against essential oils of four ethno-medicinal plants by spectrophotometric method.
Note: S149 = *Alternaria alternata*, S157 = *Mucor hiemalis*, S1899 = *Helminthosporium solani*, S352 = *Humicola grisea*, S359 = *Botrytis cinerea* (a)Control , (b) *Ageratum conyzoides,* (c) *Litsea cubeba* (d) *Lantana Camara* (e) *Piper mullesua*

Different range of MIC was recorded for different phyto-pathogenic fungi (Table 5).

Sample	*Ageratum conyzoides*	*Lantana camara*	*Litsea cubeba*	*Piper mullesua*
S149 (MTCC 149)*	159.7±3.5µl	120.3±11.50µl	14.7±2.52µl	135±0.6µl
S157 (. MTCC 157)	168.7±9.07µl	155±5.00µl	35±4.00µl	110.1±0.9µl
S1899 (MTCC 1899)	195±8.00µl	229.7±8.5µl	70±1.00µl	140±4.00µl
S352 (MTCC 352)	155±4.51µl	125±1.50µl	50.1±2.15µl	269.6±2.9µl
S359 (MTCC 359)	260±3.00µl	280±7.50µl	22.1±1.1µl	190.3±1.52µl

Table 5- MIC (µl/ml) to effect visible growth in phyto-pathogenic fungi by the essential oil of four ethno-medicinal plants at 1:200x dilutions.

Litsea cubeba was found to show inhibitory effects at the concentration however *Lantana camara* and *Ageratum conyzoides* require higher concentration for inhibitory effects on selected fungal samples. 1:200 times diluted *Litsea cubaba* oil showed visible inhibitory effects on selected phytopathogenic fungi at the quantity ranging between 15µl to 70µl. Similarly, *Lantana camara* oil was at 120-280µl in various fungal pathogens. *Piper mullesua* showed MIC between 110-270µl and *Ageratum conyzoides* at 155-260µl depending upon fungal species tested.

Alterneria alternate is most sensitive to *Litsea cubeba* oil followed by *Lantana camara* oil, *Piper mullesua* oil and least sensitive to *Ageratum conyzoides* oil. Similarly, *Mucor haemilis* is most sensitive to *Litsea cubeba* oil followed by *Piper mullesua* and *Lantana camara* and least sensitive to *Ageratum conyzoides* oil respectively. Nevertheless, *Helminthosporium solani* is highly sensitive to *Litsea cubeba* oil followed by *Piper mullesua, Ageratum conyzoides* and least sensitive to *Lantana camara*. *Humicola grisea* is also highly sensitive to *Litsea cubeba* oil followed by *Lantana camara*, *Ageratum conyzoides* and least sensitive to *Piper mullesua*. Lastly, *Botrytis cinerea* is most sensitive to *Litsea cubeba* followed by *Piper mullesua* and least sensitive to *Ageratum conyzoides* and *Lantana camara* oil respectively.

Discussion

Litsea cubeba oil extract was most effective in controlling the growth of the above selected phytopathogenic fungal species followed by *Piper mullesua, Ageratum conyzoides* and *Lantana camara* respectively. Importance of *Litsea cubeba* as antifungal has already been reported on few common human disease causing fungal species like *Aspergillus fumigatus, Trichophyton mentagrophytes* var. interdigitale and *Candida albicans*. The citral compound produce by *Litsea* is known to be having antifungal and antitumoral properties and help to prevent experimental atherosclerosis (Chen et al. 1994; Wang et al. 1999). However its significance as anti phyto-pathogenic agent has not been reported before. *Litsea cubeba* fruit, twig and leaves oils showed inhibitory properties against the growth of *Aspergillus niger, Penicillium citrinum* and *Trichoderma viride* at the concentrations of 200-300µg/ml (Chiang et al. 2007). In the above study, *Litsea cubeba* showed significant growth inhibitory effects of tested fungal species at the concentration of 20-70µl/ml at 1:200x dilutions. Among the compounds isolated and reported earlier from *Litsea cubeba*, geraniol is found to perform the best antifungal activity with minimum inhibitory concentration (MIC) values of 88, 256 and 275 µg/ml against *P. citrinum, T. viride and A. niger,* respectively (Chiang et al. 2007). It is also reported to be having insecticidal properties (Ko et al. 2009).

Leaf extract of *Lantana camara* contain alkaloid, phenolics, Terpenoids, Phutosterols, Tannins and Saponins due to which it is reported to have antifungal properties (Ganjewala et al. 2009). Phyto-pathogenic properties of *Lantana camara* has earlier been reported on fungal species like *Alterneria alternate*, *Curvularia lunata*, *Fusarium equiseti*, *Botryodiplodia theobromae, Candida albicans*, *Trichophyton mutagraphytes,* by filter paper disk diffusion method (Benson 1990; Begum et al. 2007, Sharma and Kumar 2009). However, phyto-pathogens tested in the present study are different from the above species except *Alterneria alternate*. *Lantana camara* was effective against the tested phytopathogenic fungi at the concentration of 120-270µl/ml at 1:200x dilutions. Various parts of this plant are reported to be used in the treatment of diseases like cuts, ulcers, swellings, bilious fever, catarrh, eczema, dysentery, chest complaints of children, fistula, pustules, tumours, tetanus, malaria, rheumatism, toothache, cold, headache, uterine haemorrhage, chicken pox, eye injuries, whooping cough, asthma, bronchitis and arterial hypertension (Ross 1999; Rastogi and Mehrotra1995; Deena and Thoppil 2000). Leaf juice of *Lantata camara* is also used as a medicine to cure skin diseases in ayurvedic and traditional medicine (Dabur et al. 2007 and Joy et al. 1998).

Supporting the above study, Tripathi et al. (2008) have also reported antifungal properties of *Ageratum conyzoides*. He has reported 60% growth inhibition of *Botrytis cinerea* by *Ageratum conyzoides* extract at 500ppm of concentration. In the present study, *Ageratum conyzoides* is more effective against *Helminthosporium solani* as compared to other fungal species. It has more than 50% growth restriction of *Helminthosporium solani* at 195µl/ml concentration. Biochemical analysis on antifungal properties of *Piper mullesua* has not been carried out and present study reveals the prospect of using *P.mullesua* as antimicrobial compound in agricultural industry, its insecticidal properties however has already been reported from India.

Essential oil from *Litsea cubeba* was found to be effective against all the tested fungal pathogens with significant level of action while the essential oil from *Ageratum, Lantana,* and *Piper* were effective against selected fungal pathogens. Essential oils from *Litsea cubeba*, *Ageratum conyzoides, Lantana camara,* and *Piper mullesua* could be used as bio-control agent on fungal pathogens and can form an integral part of integrated pest management in agriculture sector.

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References:

1. Sharma, B., & Kumar, P. (2009). Bioefficacy of *Lantana camara* L. against some human pathogens. Indian Journal of Pharmaceutical Science, 71, 589-93.
2. Drivastava, S., Gupta, M.M., Prajapati, V., Tripathi, A.K. & Kumar, S. (2001). Insecticidal activities of Myristicin from *Piper mullesua*. Pharmaceutical Biology, 39, 226-229.
3. Ko, K., Juntarajumnong, W. & Chandrapatya, A. (2009). Repellency, fumigant and Contact toxicity of *Litsea cubeba* (Lour.) Persoon against *Sitophilus Zeamais Motschulsky* and *Tribolium castaneum* (Herbst). Kesetsart Journal (Natural Science), 43, 56-63.
4. Joy, P.P., Thomas, J., Mathew, S. Skaria, B.P. (1998). Medicinal plants. In: Bose TK, Kabir J, Das P and Joy PP

(Eds), Tropical Horticulture vol.2 (pp. 449-632). Naya Prokash, Calcutta.
5. Dabur, R., Gupta, A., Mondal, J.K., Singh, D.D., Bajpai, V., Gurav, A.M. & Lvekar, G.S. (2007). Antimicrobial activity of some Indian Medicinal plants. African Journal of Traditional, Complementary and Alternative medicines, 4, 313-318.
6. Council of Europe. European Pharmacopoeia, 3rd edn. Strausbourg, 1997: 121–122.
7. Bobbarala, V., Katikala, P.K., Naidu, K.C. & Penumajji, S. (2009). Antifungal activities of selected plant extracts against pathogenic fungi *Aspergillus niger* F2723. Indian Journal of Science and Technology, 2, 87-90.
8. Begum, J., Yusuf, M., Chowdhury, J.U., Khen, S. & Anwar, M.N. (2007). Antifungal activities of fourty higher plants against phytopathogenic fungi. Bangladesh Journal of Microbiology, 24, 76-78.
9. Tripathi, P., Dubey, N.K. & Shukla, A.K. (2008). Use of some essential oils as post-hervest botanical fungicides in the management of grey mould of grapes caused by *Botrytis cinerea*. World Journal of Microbiology and Biotechnology, 24, 39-46.
10. Ganjewala, D., Sam, S. & Khan, K.H. (2009). Biochemical compositions and antibactericidal activities of *Lantana camara* plants with yellow, lavender and White flower. EurAsian Journal of Bioscience, 3, 69-77.
11. Bakkali, F., Averbeck, S., Averbeck, D. & Idaomar, M. (2008). Biological effects of essential oils – A review. Food and Chemical Toxicology, 46(2), 446-75.
12. Chopra, R.N., Nayar, S.L. & Chopra, I.C. (1956). Glossary of Indian medicinal plants. New Deli: CSIR, p. 149.
13. CSIR. Weath of India (1962). Council of Scientific and Industrial Directorate, New Delhi, vol. VI. p. 31.
14. Ross, I.A. (1999). Medicinal plants of the world. Humana Press, New Jersey: p.179.
15. Rastogi, R.P. & Mehrotra, B.N. (1995). Compendium of Indian medicinal plants, vol. 1. Central Drug Research Institute, New Delhi: Lucknow and Publication and Information Directorate, CSIR, p.238.
16. Benson, H.J. (1990). Microbiological applications, 5th ed. Wm. C. Brown Publ., USA: p.134
17. Deena, M.J. & Thoppil, J.E. (2000). Antimicrobial activity of the essential oil of *Lantana camara*. Fitoterapia, 71, 453-455.
18. Chiang, Yu-Chung , Hsieh, C., & Yen T. (2007). Antifungal properties of *Litsea cubeba* essential oils against molds. Botany and Plant Biology Joint Congress. Hilton Chicago, Chicago, Illinois.
19. Soliman, K.M. & Badeaa, R.I. (2002). Effect of oil extracted from some medicinal plants on different mycotoxigenic fungi. Food and Chemical Toxicology, 40(11), 1669-75.
20. Thoppil, J.E., Minija J., Tajo A. & Deena, M.J. (2003). Antimicrobial activities of *Eusteralis deccanensis* and *E. quadrifolia* essential oils. Journal of Environmental Biology, 24(2), 211-212.
21. Govinden-Soulange, J., Magan, N., Gurib-Fakim, A., Gauvin, A., Smadja, J. & Kodja, H. (2004). Chemical composition and in vitro antimicrobial activities of the essential oils from endemic *Psiadia* species growing in Mauritius. Biol Pharm. Bulletin, 27(11), 1814-8.
22. Romagnoli, C., Bruni R., Andreotti E., Rai M.K., Vicentini C.B. & Mares D. (2005). Chemical characterization and antifungal activity of essential oil of capitula from wild Indian *Tagetes patula* L. Protoplasma, 57-65.
23. Pinto, E., Pina-Vaz, C., Salgueiro, L., Goncalves, M.J., Costa-de-Oliveira, S., Cavaleiro, C., Palmeira, A., Rodrigues, A. & Martinez-de-Oliveira, J. (2006). Antifungal activity of the essential oil of *Thymus pulegioides* on Candida, *Aspergillus* and *Dermatophyte* species. Journal of Medical Microbiology, 55(10), 1367-73.
24. Tabanca, N., Demirci, B., Crockett, S.L., Baser, K.H. & Wedge, D.E. (2007). Chemical composition and antifungal activity of *Arnica longifolia*, *Aster hesperius* and *Chrysothamnus nauseosus* Essential Oils. Journal of Agriculture and Food Chemistry, 55(21), 8430-8435.
25. Tullio, V., Nostro, A., Mandras, N., Dugo, P., Banche, G., Cannatelli, M.A., Cuffini, A.M, Alonzo, V., & Carlone, N.A. (2007). Antifungal activity of essential oils against filamentous fungi determined by broth micro-dilution and vapour contact methods. Journal of Applied Microbiology, 102(6), 1544-50.
26. Dutta, B.K., Karmakar, S., Naglot, A., Aich, J.C., & Begam, M. (2007). Anticandidial activity of some essential oils of a mega biodiversity hotspot in India. Mycoses, 50(2), 121-4.
27. Conseil de l'Europe, Pharmacopee Europeenne 1, Maisonneuve S.A. Editions, Sainte Ruffine, 1996.
28. Boyer, J.M. (1976). Impregnated Disk Method for Antifungal Antibiotic Testing. Antimicrobial Agents Chemotherapy, 9(6), 1070-1071.
29. NCCLS. (1999). Performance Standards for Antimicrobial Disk and Dilution Susceptibility, Tests for Bacteria Isolated from Animals; Approved Standard. NCCLS Document M31-A (ISBN 1-56238-377-9).
30. Pradhan, B.K. & Badola, H.K. (2008). Ethnomedicinal plant use by Lepcha tribe of Dzongu valley, bordering Khangchendzonga Biosphere Reserve, in North Sikkim, Indian Journal of Ethnobiology and Ethnomedicine, 4, 1-22.
31. Kala, C.P. (2005). Ethnomedicinal botany of the Apatani in the Eastern Himalayan region of India. Journal of Ethnobiology and Ethnomedicine, 1, 1-11.
32. Sajem, A.L, & Gosai, K. (2006). Traditional use of medicinal plants by the Jaintia Tribes in North Cachar Hills district of Assam, northeast India. Journal of Ethnobiology and Ethnomedicine, 2, 20-33.
33. Srivastava, R.C. & Adi community. (2009). Traditional knowledge to Adi tribe of Arunachal Pradesh on plants. Indian Journal of Traditional Knowledge, 8(2), 146-153.
34. Bhuyan, L.R. (2007). Some ethnomedicinal plants of Arunachal Pradesh. Bulletin of Arunachal Forest Research, 23 (1&2), 45-50.
35. Hussain, S. & Hore, D.K. (2008). Collection and conservation of major medicinal plants of Arunachal Pradesh. Indian Forester, 25, 1163-1179.
36. Chen, W.Y., Ko, F.N., Wu, Y.C., Lu, S.T., & Teng, C.M. (1994). Vasorelaxing effect in rat thoracic aorta caused by laurotetanine isolated from *Litsea cubeba* Persoon. Journal of Pharmacy and Pharmacology, 46(5), 380-382.
37. Wang, F., Yang, D., Ren, S., Zhang, H., & Li, R. (1999). Chemical composition of essential oil from leaves of *Litsea cubeba* and its antifungal activities. Zhong Yao Cai, 22(8), 400-402.

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