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In vitro Antifungal activity of lemongrass (*Cymbopogon citratus*) leaf extracts

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Abstract
Colletotrichum musae is a plant pathogen primarary affecting the genus musa. which includes bananas and plantains. It is best known as to cause of anthracnose (the black and brown rots) indicating ripeness in banana and affecting the quality of fruits. *Aspergillus niger* contaminant of food and produces black mould on certain fruits and vegetables such as grapes apricots onions and peanuts. Lemongrass is an herb which belongs to gramineae family. Scientifically it is called as *Cymbopogon citratus*. The prefix lemon owes to its typical lemon like odour, due to presence of citral a cyclic monoterpene. Lemongrass has phytoconstituents such as tannins, flavanoids, alkaloids, and various essential oils in this herb. Secondary active metabolites of a number of components have also been implicated in the varied pharmalogical effects of this plant. Lemongrass possesses various antimicrobial properties. The extracts of lemon grass leaves (fresh and dried) with cold and hot water, solvents like ethanol and methanol were screened for its antifungal activity against *Aspergillus niger* (a soil habitat) and *colletotrichum musae* (a plant pathogen) by agar well method at three different concentration whereas methanol dried leaves extract shown a greater zone of inhibition (10.90 mm) followed by methanol fresh leaves extract (10.20 mm) in *Aspergillus niger* whereas least zone of inhibition was observed in ethanol fresh leaves extract (5.20mm) in *colletotrichum musae*.

Keywords: aspergillus Niger, colletotrichum musae, antifungal activity

Introduction
The world's demand for banana increases annually (Food and Agriculture Organization of the United Nation, 2006) [8]. The control of damaging fungal disease in banana fruit, especially anthracnose, relies mainly on the use of synthetic fungicides. The frequent use of these fungicides may ultimately result in fungicide resistant pathogens. In addition, there is also a potential problem of fungicide contamination of both fruits and the environment. Thus, there is an obvious and increasing need for alternative control strategies. So we want to manage the diseases in ecofriendly manner. It can be concluded from the results of this work that of lemongrass essential oil treatments with refrigeration at 5^0C may serve as alternative to conventional chemical preservatives in the preservation of yoghurt by hurdle technology (Shaaban *et al.*2010) [19]. The cinnamon oil concentration of 0.4% proved the best among all other treatments and inhibited the conidial germination up to (83.2%) followed by 0.3, 0.2 and 0.1% (80.1, 72.3 & 69.6%), respectively.(Maqbool *et al.*,2010). According to Sangeetha *et al.*, (2010), *O. sanctum, C. citratus* and *C. martinii* were fungistatic at 0.04% (v⁄v) and fungicidal at 0.06–0.08% (v⁄v) against both *L. theobromae* and *C. musae* pathogens. Basil oil (0.12-0.16% v/v) in a liquid bioassay indicated fungistatic and fungicidal efficacy on *C. gloeosporioides, L. theobromae* (isolated from crown rot tissues) and P. caricae-papayae isolated from 'Red Lady' and 'Rathna' cultivars of papaya (Abeywikrama *et al.* 2012).
Silva *et al.,* (2008) [20] said that numerous essential oils have been tested for both *in vitro* and *in vivo* antifungal activity and some pose much potential as antifungal agents. By using disk diffusion assay, and they evaluated the antifungal activity of lemongrass oil and citral against yeasts of *Candida* spp (*Candida albicans, C. glabrata, C. krusei, C. parapsilosis* and *C. tropicalis*). They showed that the lemongrass oil and citral have a potent *in vitro* activity against *Candida* spp. Petrus *et al.* (2016) [13]. Stated the immersion of denture surfaces in lemongrass extract (LGE) was effective in reducing *C. albicans* biofilms without affecting mechanical and physical substratum properties. Furthermore, human cell outcomes showed that the concentration of the tested extract was effective and safe to use. In addition, these results demonstrated the potential to use lemongrass, as valid disinfectant either pre- or post-biofilm formation, as an alternative substance for controlling *C. albicans* biofilms development on denture surfaces. Lemongrass oil reduced spore germination and germ tube length in *C.coccodes, B. cinerea C.herbarum* and *R.stolonifer* (post harvest pathogens) with the effects dependent on oil concentration by Nikos and costas (2007) [12]. Somda *et al.*

(2007) [22] stated that essential oils from *Cymbopogon citratus* (Lemongrass) *Eucalyptus camaldulensis*, (Eucalyptus) and crude oil from *Azadirachta indica* (Neem) were tested *in vitro* for inhibitory activity against *Colletotrichum graminicola*, *Phoma sorghina* and *Fusarium moniliforme*. Plant extracts was also tested on naturally infected sorghum seeds for controlling the fungi above mentioned. Essential oil from *C. citratus* significantly inhibited the *in vitro* radial growth of *C. graminicola* (76.2% inhibition), compared to the fungicide Dithane M-45. *C. citratus* essential oil showed effectiveness in inhibiting the growth of fungi by disc diffusion and broth dilution bioassay. Minimum inhibitory and minimum fungicidal concentrations between 0.062 and 20 micro/ml were determined. The Clinical and Laboratory Standards Institute agar-based method was also applied *for A. niger* and *C. albicans*. Data show the strong antifungal properties of lemon grass oil (*C. citratus*) *in vitro* (Reyhan irikin and mihriban korukluogly, 2009) [17].

Lemon grass (*Cymbopogon Flexuosus*) and *(Cymbopogon Citraus)* is a native aromatic tall sedge/grass (Rangari vinod, 2009) [14]. Family (Poaceae / Gramineae) with diverse medicinal value and grown in many parts of tropical and subtropical south east Asia and Africa. It was grown in India a century back and now commercially cultivated in different parts of India. The oil has been found to posses bactericidal, anti-bacterial and anti-fungal properties, which is comparable to Penicillin in its effectiveness (Lutterodt *et al.*, 1999) [14]. Ewanishi *et al*., (2012) stated that the cold maceration and agar diffusion technique were employed to assess phytochemical properties and the antimicrobial potency of *Cymbopogon citratus* (lemongrass) against selected microbial pathogens using hexane, chloroform and methanol as extracting solvents. Antimicrobial property of lemongrass (*Cymbopogon citratus*) oil against pathogenic bacteria isolated from pet turtles (De Silva *et al*., 2017) [6] lemongrass oil is used to control the turtle borne pathogens. The lemongrass oil and citral may change the activities of drug metabolizing enzymes and reduce oxidative stress in the liver (Chinen-chun *et al*., 2018) [5]. The antimicrobial activity of many plants have been reported by many researchers (Reddy *et al*., 2001; Atleb and Erdourul, 2003) [3]. However the studies of antimicrobial activities of lemongrass are very limited towards the pathogenic bacteria and fungi.

Materials and Methods

Location

The in *vitro* experiments were conducted out in the microbiology laboratory Department of microbiology Faculty of Agriculture, Annamalai University. Stalks and leaves are used the essential oil is extracted from fresh plant material by means of steam distillation.

Fresh leaves extract

Weigh 25 g of lemongrass and cut it into small pieces. The pieces were taken in 500 ml of round bottomed flask. Add 300 ml of distilled water to the flask containing the grass and set the apparatus for distillation. Boil the mixture vigorously and collect the distillate until no more oily drops can be seen passing over. More water should be added if necessary to avoid charring of flasks contents. Extract the distillate with hexane, dry them over sodium sulfate and remove the solvent on rotary evaporator with external heating at 45°C. Finally 2 ml of yellow to ochre colored oily liquid with fresh lemon like tone with a hint of rose was obtained. (Arputha bibiana *et al*., 2012) [2].

Dried plant extract

The dried samples were grinded and sieved. The powdery sample were then partitioned in two parts

a. Ethanol Extract

(12 & 16 gm) powdered samples were extracted with 100ml of ethanol. The powdered sample was soaked in the solvent for 24 hours and plant extract was prepared using reflux and steam distillation method. In this method, plant material is immersed in a solvent in the round- bottomed flask, which is connected to a condenser. The solvent is heated up to its boiling point. As the vapors are condensed, the solvent is recycled into the flask.

b. Methanol Extract

(12 & 16 gm) powdered sample were extracted with Methanol extract. The powdered sample was soaked in 100 ml water for 24 hours and plant extract was prepared using the same above mentioned method.

c. Cold Water Extract

(12 & 16 gm) powdered sample were extracted with cold water. The powdered sample was soaked in 100 ml water for 24 hours and plant extract was prepared using the same above mentioned method.

d. Hot Water Extract

(12 & 16 gm) powdered sample were extracted with hot water. The powdered sample was soaked in 100 ml water for 24 hours and plant extract was prepared using the same above mentioned method.

Antifungal activity of lemongrass

Agar well diffusion method was followed to determine the antimicrobial activity of lemon grass of fresh leaves and dry leaves extract.

Antifungal activity was studied by agar well diffusion method. The Potato Dextrose Agar (PDA) medium poured into sterile Petri plate and allowed to solidify. The fungal inoculum seeded on PDA medium. Then wells (5mm in diameter) were made in the medium using sterile cork borer. 200µl each of the different extracts of different concentrations 250ppm, 500ppm and 1000ppm were transferred into separate well indicating antifungal activity. For each treatment three replicates were maintained in millimeters (mm).

Results

Table 1: Inhibition effect of cold and hot water extract of lemongrass against *Aspergillus Niger*

S. No	Lemongrass	Zone of inhibition (mm) Concentration in PPM		
		250	500	1000
1	Cold water fresh leaves extract	7.50	8.10	9.00
2	Cold water dried leaves extract	8.40	9.30	10.00
3	Hot water fresh leaves extract	5.70	6.40	7.30
4	Hot water dried leaves extract	6.90	7.60	8.50

Table 2: Inhibition effect of cold and hot water extract of lemongrass against *Colletotrichum musae*

S. No	Lemongrass	Zone of inhibition (mm) Concentration in PPM		
		250	500	1000
1	Cold water fresh leaves extract	6.50	7.40	9.00
2	Cold water dried leaves extract	7.00	7.80	9.80
3	Hot water fresh leaves extract	5.40	6.10	7.00
4	Hot water dried leaves extract	6.00	7.10	8.70

Table 3: Inhibition effect of ethanol and methanol extract of lemongrass against *aspergillus Niger*

S. No	Lemongrass	Zone of inhibition (mm)		
		Concentration in PPM		
		250	500	1000
1	Ethanol fresh leaves extract	5.50	6.20	7.70
2	Ethanol dried leaves extract	7.70	8.60	9.60
3	Methanol fresh leaves extract	7.80	8.50	10.20
4	Methanol dried leaves extract	9.00	9.20	10.90

Table 4: Inhibition effect of ethanol and methanol extract of lemongrass against *Colletotrichum musae*

S. No	Lemongrass	Zone of inhibition (mm)		
		Concentration in PPM		
		250	500	1000
1	Ethanol fresh leaves extract	5.20	6.30	7.10
2	Ethanol dried leaves extract	6.30	7.40	9.00
3	Methanol fresh leaves extract	7.20	8.30	9.20
4	Methanol dried leaves extract	8.00	8.50	9.50

The maximum area of inhibition zone were observed 10.90 mm in the dried leaves of methanol extract in *Aspergillus niger* and followed by fresh leaves methanol extract 10.20 mm at 1000ppm. The least inhibition zone were observed in ethanol extract of fresh leaves in *Colletotrichum musae* of *Cymbopogon citratus* 5.20 mm at 250 ppm. (Table-3) and (Table-4).

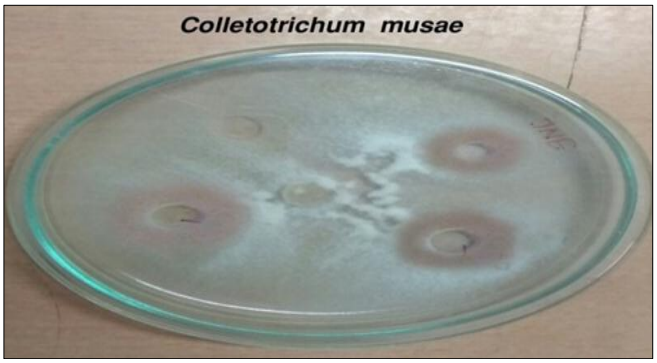

Fig 1: *Colletotrichum musae* showing different zones of inhibition

Discussion

Among the two fungal cultures were tested with hot and cold water extracts of lemongrass *Aspergillus Niger* showed the maximum zone of inhibition when compared to the *colletotrichum musae* in hot water dried leaves extract followed by fresh leaves extract. Among the two fungal cultures tested with ethanol and methanol extracts of lemongrass *Aspergillus Niger* showed the maximum zone of inhibition when compared to the *colletotrichum musae* in ethanol dried leaves extract followed by fresh leaves extract. Similar results reported by *C.citratus* can be used as an antifungal agent in syrups and tonic. Plant extract have a role as preservatives. This study indicated that plant extract and essential oils may possess antifungal activity and can be exploited as an ideal treatment for future herbal drug contaminant for eliminating fungal spread. Recently, there has been a considerable interest in extracts and essential oils from aromatic plants with antimicrobial activities for controlling pathogens and/or toxin producing microorganisms in foods (Reddy *et al*., 1998 [16]; Soliman & Badeaa, 2002 [21]; Valero & Salmeron, 2003) [23]. In addition, the antimicrobial activity of ethanol dried leaves extract might be due to the presence of steroids (Nagamani *et al.,* 2012) [11]. Among the two fungal cultures tested with methonal extracts of lemongrass *Aspergillus Niger* showed the maximum zone of inhibition when compared to the *colletotrichum musae* in methanol dried leaves extract followed by fresh leaves extract.antifungal activity of leaves extracts of *Cymbopogon citratus* were also noted by Bhavya trivedi and Padma singh (2014).

Conclusion

Lemongrass is a medicinal herb it contains several antifungal properties due to its high citral content, flavanoids and tannins may be responsible for this antimicrobial activity.

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