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Antimicrobial activity of Lemon Grass (*Cymbopogan citratus*) extract against microbes of environment and food origin

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Abstract

In this investigation Lemon grass (*Cymbopogan citratus*) used for determination of antibacterial activity against food and soil bacteria, and for determination of antifungal activity against fungal pathogens (*A. niger*, *A. flavus*, *Candida sps.*,and *Trycophyton sps.*). The antimicrobial activity of the Lemon Grass in different solvents (n-Hexane, Chloroform, Acetone) was examined. In antibacterial activity of the Lemon Grass extract showed maximum zone of Inhibition was observed in Chloroform extract (3.1cm) and minimum zone of inhibition was observed in Acetone extract (1.2cm). The MIC of the Lemon extracts showed that MIC ranged between 3.1 mg/mL. The ethanolic extract of *E. coli* appeared to be the most effective with the lowest inhibitory concentration being 2.75 mg/mL against *Bacillus sps*. N-Hexane extracts of *Pseudomonas sps*. was the second most effective plant extracts with MIC of 2.55 mg/mL. In the Antifungal assay, *Candida sps*. was showed maximum antifungal activity (3cm) and *Trycophyton sps* was showed minimum antifungal activity (0.9 cm).

Introduction

The name Cymbopogon is derived from the Greek words “kymbe” (boat) and “pogon” (beard), referring to the flower spike arrangement. The biologically active constituent of lemon grass is citral constituting more than 75% (w/w) of its essential oil (Huynh *et al.*, 2008). Lemongrass essential oil is applied for its medicinal value to cure acne, oily skin, scabies, flatulence,

headaches, blood circulation problems (Pearson 2010) and excessive perspiration due to its antimicrobial and antibacterial activities (Lawless 1995).

It is used widely as an essential ingredient in Asian cuisines due to the sharp lemon flavour. *C. citratus*, which belongs to the family of Gramineae, is commonly used in folk medicine for treatment of nervous and gastrointestinal disturbances (Bassole *et al.*, 2011**).** It is also used as antispasmodic, analgesic, anti-inflammatory, anti-pyretic, diuretic and sedative (Bassole *et al.*, 2011; Francisco *et al.*, 2011).

Several studies have shown that the lemon grass has antibacterial and antifungal properties. It can be used in cleaning wounds and in food poisoning, staphylococcal infections, and other common infections of the colon, stomach, and urinary tract (Bhoj *et al.*, 2011). *Cymbopogon citratus* (DC) Stapf (Poaceae) (lemon grass) is an aromatic perennial tall grass with rhizomes and densely tufted fibrous root. The plant is a native herb from India and is cultivated in other tropical and subtropical countries (Dama *et al.*, 2011; Gore *et al.*, 2010; Huynh *et al.*, 2008).

Essential oils are organic substances produced by plants which can be obtained from roots, stem, bark, leaves, flowers, fruits and seeds (Pearson 2010). Lemongrass is a rich source of citral, which is used in perfumery, pharmaceutical industries, and bioactive compounds (flavonoids and vitamin C) as shown in figure. 1.

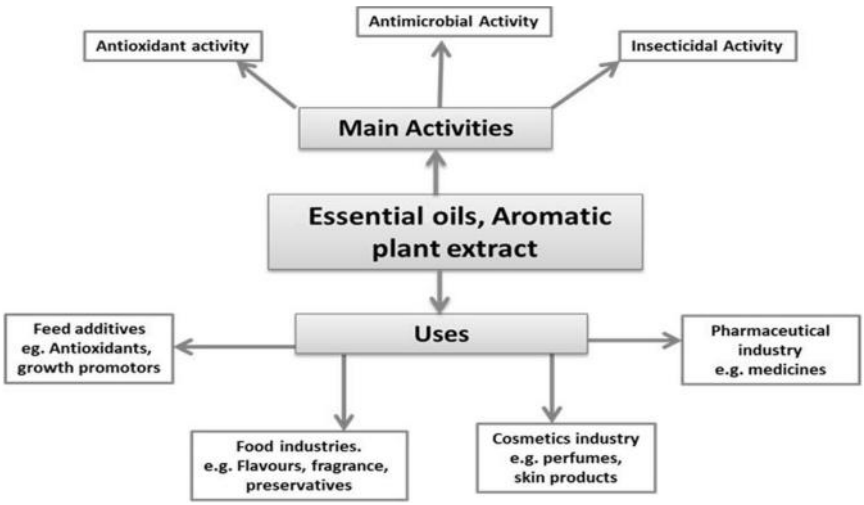


Figure 1: Different activities and uses of essential oils

These natural antioxidants occur in all higher plants, and in all parts of plants. Typical compounds that exhibit antioxidant activity includes vitamins, carotenoid and phenolic

compounds. Plants also possess enzymatic systems that protect them against H_2O_2 and other harmful reactive oxygen species; these include superoxide dismutase (SOD) and catalase. Human body is subject to numerous biologic stresses. This may be due to the environmental or pathological. The mode of stress which has been a speculative subject of interest in the recent times is "oxidative stress phenomenon" this is believed in part to be responsible for the inflammatory conditions (Sodipo *et al.,* 1991).

The natural flavonoids are also attracting more and more attention not only due to their antioxidant properties, but also as anti-carcinogenic and anti-inflammatory agents because of their lipid anti-peroxidation effects (Martin *et al.,* 2002). Lemongrass biomass is steam distilled for the extraction of essential oil, a natural product with wide application in the food and pharmaceutical industries, perfumery and cosmetics, and eco-friendly pesticides (Ganjewala & Luthra 2010; Weiss 1997). Lemongrass oil has a pleasant and refreshing aroma and antifungal and antibacterial properties (Anaruma *et al.,* 2010; Guynot *et al.,* 2003; Kumar *et al.,* 2009; Pandey *et al.,* 2003)**.**

Materials and Methods

Isolation and Characterization of Fungal and Bacterial Isolates

The fungal isolates used in this study were obtained from Sabouraud‟s dextrose agar to evaluate their cellulolytic and hemicellulolytic activities and nutrient agar media used for isolation of Bacterial sps. The morphological and biochemical characteristics of the isolates were examined according to the Bergey's manual of determinative Bacteriology (Kumar *et al.,* 2015).

Preparation of Lemongrass Extracts

Selected *Cymbopogan citratus* was dried in shade and then powdered with the help of grinding machine. 2 grams of shade dried powder was filled in the collection tube and extracted with n- Hexane, Acetone, Acetone successively upto 48 hrs and will be used for antibacterial assays.

Antibacterial Activity Assay

Agar well diffusion method used to evaluate the antimicrobial activity of plants extracts (Owen *et al.,* 2004; Magaldi *et al.,* 2004) similarly to the procedure used in disk-diffusion method, the

agar plate surface is inoculated by spreading a volume of the microbial inoculums over the entire agar surface. Then, a hole with a diameter of 6 to 8 mm is punched aseptically with a sterile cork borer or a tip, and a volume (20-100 mL) of the extract solution at desired concentration is introduced into the well. Then, agar plates are incubated under suitable conditions depending upon the test microorganism. The antimicrobial agent diffuses in the agar medium and inhibits the growth of the microbial strain Tested

Minimum Inhibitory Concentration (MIC) Test

Minimum inhibitory concentration was determined according to the method described earlier (Kalita *et al.,* 2016; Sharma *et al.,* 2011) by adding various concentrations of essential oil (6.25–100 µg) in Sabouraud dextrose broth medium and Nutrient Broth. Further, 100 µl of inoculum was added to each tube and incubated the tubes at 28°C for 7 days. The MIC was regarded as the lowest concentration of the oil that did not permit any visible growth after 7 days of incubation.

Antifungal activity Assay

Antifungal activity of solvents extract of all the selected lemongrass extracts was determined by agar well diffusion method on CDA medium. Solvent extracts of Lemon Grass was screened for antifungal properties against *Aspergillus niger, Aspergillus flavus, Candida sps*. and *Trycophyton sps.* The 24-h-old cultures of separate strains grown in nutrient broth were spotted on the fungal test cultures prepared on the CDA medium (Kumar *et al.,* 2015). The plates were incubated at room temperature for 7 days and fungal growth inhibition was measured (Kumar *et al.,* 2015; Pearson 2010).

Result and Discussion

Isolation of Bacterial and Fungal sps

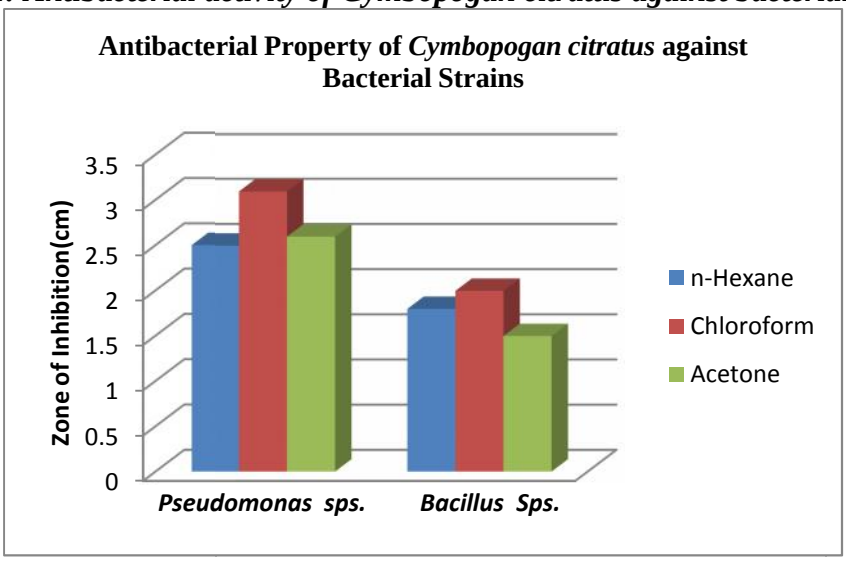
The fungal isolates used in this study were obtained on Sabouraud‟s dextrose agar (SDA) are *Aspergillus niger*, *Aspergillus flavus*, *Candida sps.* and *Trycophyton sps*.. The bacterial isolates examined in this study, which were obtained on Nutrient Agar Media (NAM) are *Bacillus subtilis, Pseudomonas aueroginosa*.

Antibacterial test

In the present study, the antimicrobial activity of the *Cymbopogan citratus* in different solvents (n-Hexane, Chloroform, Acetone) was examined (as shown in figure 2). There was significant variation ($p<0.05$) observed between n-Hexane, Chloroform, Acetone solvents for the antimicrobial activities of each of the tested plant extracts and microorganisms.

Figure 2: Antibacterial activity of Cymbopogan citratus Extract

Graph 1: Antibacterial activity of Cymbopogan citratus against bacterial strains



In Antibacterial Activity of the *Cymbopogan citratus* extract showed maximum zone of Inhibition was observed in Chloroform extract (3.1cm) and minimum zone of inhibition was observed in Acetone extract (1.2cm) as shown in Graph 1

Minimum Inhibitory Concentration (MIC) Test

The minimum inhibitory concentration was determined for *Cymbopogan citratus* extracts which show antimicrobial activity against microbes associated. The MIC of the Lemon extracts showed that MIC ranged between 3.1 mg/mL and 50 mg/mL for the organic extracts and between 25 and 50 mg/mL for the extracts. When comparing the MIC values of the tested plants, the ethanolic extract of *E. coli* appeared to be the most effective with the lowest inhibitory concentration being 2.75 mg/mL against *Bacillus sps*. N-Hexane extracts of *Pseudomonas sps.* were the second most effective plant extracts with MIC of 2.55 mg/mL. Plant extracts show MIC in the range of 25-50 mg/mL and were thus shown to be less effective against microbes. It has been established that MIC results do not always correlate well with the DIZ values but, in present investigation, the observation of MIC related with the DIZ value might be due to the adoption of disc diffusion assay for both antimicrobial activity and MIC determination of plant extracts as shown in Table 1.

Table 1: MIC of plant extracts in different solvents

Plant	**Solvent**	***Pseudomonas sps.***	***Bacillus sps.***	***A.niger***	***A. flavus***	***Candida sps***	***Trcophyton sps.***
Cymbopogon Citrates	N-Hexane	2.1	1.7	2.1	3	1.7	1.2
	Acetone	2.5	1.8	1.2	1.8	2.6	1.4
	Chloroform	2.9	1.3	1.9	2.0	3.2	2.3

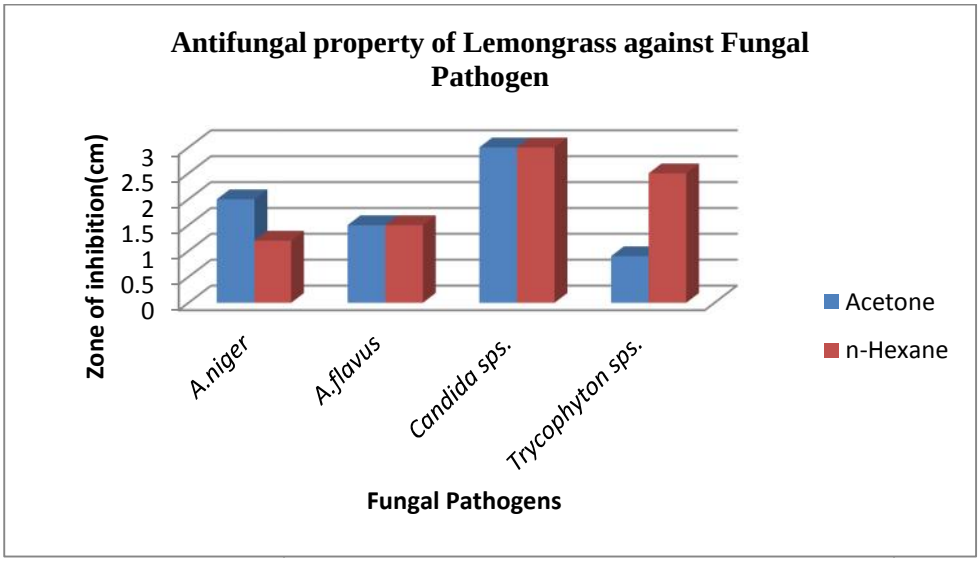
Antifungal activity test

Antifungal activities of lemongrass species were observed against fungal isolates *Aspergillus niger, Aspergillus flavus, Candida sps, Trycophyton sps* as shown in Figure 3. The *Candida sps.* was showed maximum Antifungal Activity (3cm) and *Trycophyton sps* was showed minimum Antifungal activity (0.9 cm).

Figure 3: Antifungal Activity Test

The extracts of Lemon Grass showed antifungal activity against fungal sps. with inhibition zone diameter range of 0.9-3 cm/15µL **(**Graph 2**)**. The acetone and n-Hexane extracts of Lemon grass showed higher antifungal activity against *Candida albicans* with inhibition zone diameters of 3 cm/15µL. The acetone extracts of Lemon Grass showed the Minimum antifungal activity against Trcophyton sps. with inhibition zone diameters of 0.9 mm/15µL.

Graph 2: Antifungal property of Lemongrass against fungal Pathogens



Conclusion

Plant extracts have been used for many thousands of years, in food preservation, pharmaceuticals, alternative medicine and natural therapies. It is necessary to investigate those plants scientifically which have been used in traditional medicine to improve the quality of

healthcare. Essential oils are potential sources of novel antimicrobial compounds especially against bacterial pathogens. In vitro studies in this work showed that the essential oils inhibited bacterial growth but their effectiveness varied. The antimicrobial activity of many essential oils has been previously reviewed and classified as strong, medium or weak. World wide investigations carried out on *Cymbopogan citratus* have motivated researchers to focus their interest toward the study of botanical antimicrobials. It is apparent that the use of essential oils and their derivatives has been widely described and essential oils have been used against a wide range of bacteria and fungus. Accordingly, this review provides a brief overview of essential oils, their active constituents and their potential as sources of antibacterials and antifungals activity. The relevant literature summary shows that essential oils exhibit a diverse range of antimicrobial properties, and indicates their natural sustainability when used as potential biocontrol agents against fungal and bacterial pathogens. Extract was made using solvents of acetone, n-hexane and chloroform. N-hexane extracts of lemongrass shows MIC of 10^{-3} for *A.niger*, 10^{-2} for *A. flavus* and 10^{-3} for *Candida sps.*

References

Anaruma, ND, Schmidt, FL, Duarte, MCT, Figueira, GM, Delarmelina, C, Benato, EA and Sartoratto A Control of *Colletotrichumgloeosporioides* (Penz.) Sacc. in yellow passion fruit using *Cymbopogoncitratus* essential oil. *Braz. J. Microbiol*. 41 (2010): 66-73.

Bassole, IHN, Lamien-Meda, A, Bayala, B, Obame, LC, Ilboudo, AJ, Franz, C and Dicko, MH Chemical composition and antimicrobial activity of Cymbopogon citratus and Cymbopogongiganteus essential oils alone and in combination. *Phytomedicine*. (2011).

Bhoj RS, Singh, RK, Singh, V. and Ebibeni, N Antimicrobial activity of lemon grass (*Cymbopogon citratus*) oil against microbes of environmental, clinical and food origin, 1.9 (2011): 228-236.

Dama, GY, Tare, HL, Gore, MS, Deore, SR, Bidkar, JS Comparative hemintholytic potential of extracts obtained from *Cymbopogon citratus* and Wrightiatinctoria leaves. *Int. J. Pharm. Bio. Sci*. 2.1 (2011): 321-327.

De, F, Melo, S, Soares, SF, da Costa, R.F, da Silva, CR., de Oliveira, MBN, Bezerra, RJAC and Bernardo Filho, M Effect of the *Cymbopogoncitratus*, *Maytenusilicifolia* and *Baccharisgenistelloides* extracts against the stannous chloride oxidative damage in *Escherichia coli*. *Mutation Research/Genetic Toxicology and Environmental Mutagenesis*. (2001).

Francisco, V, Figueirinha, A, Neves, BM, GarciaRodriguez, C, Lopes, MC, Cruz, MT and Batista, MT Cymbopogoncitratus as a source of new and safe anti-inflammatory drugs: bio-guided assay using lipopolysaccharide-stimulated macrophages. *Journal of Ethnopharmacology*. (2011).

Ganjewala, D and Luthra, R Essential oil biosynthesis and regulation in the genus Cymbopogon. *Natural Product Commun*. 5 (2010): 163-172.

Gore, MS, Tare, HL, Deore, SR, Bidkar, JS, Dama, GY Hemintholytic potential of *Cymbopogon citratus* leaves extract and its formulation as an emulsion. *Int. J. Pharm. Sci. Res*. 1.10 (2010):174-177.

Guynot, ME, Ramos, AJ, Setó, L, Purroy, P, Sanchis, V, and Marín, S Antifungal activity of volatile compounds generated by essential oils against fungi commonly causing deterioration of bakery products. *J. Appl. Microbiol*. 94 (2003): 893-899.

Huynh, KP, Maridable, J, Gaspillo, P, Hasika, M, Malaluan, R, Kawasaki, J Essential oil from lemongrass extracted by supercritical carbon dioxide and steam distillation. (2008).

Kalita, S, Kandimalla, R, Sharma, KK, Kataki, AC, Deka, M, Kotoky, A Amoxicillin functionalized gold nanoparticles reverts MRSA resistance. *Mater. Sci. Eng. C* 61 (2016): 720-727.

Kumar, A, Shukla, R, Singh, P and Dubey, NK Biodeterioration of some herbal raw materials by storage fungi and aflatoxin and assessment of *Cymbopogon flexuosus* essential oil and its components as antifungal. *Int. Biodeterior. Biodegradation*. 63 (2009): 712-716.

Kumar, V, Kumar, A, Pandey, KD, Roy, BK Isolation and characterization of bacterial endophytes from the roots of Cassia tora L. *Ann Microbiol*. 65 (2015): 1391-1399.

Lawless, J Illustrated Encyclopedia of Essential Oil. The Complete Guide to use of Oil in Aromatheraphy and Herbalism. (1995).

Martin, FR, Frutos, MJ, Pérez- Alvarez, JA, Martinez-Sánchez, F, Tel Rio, JA Flavonoids as neutraceutical: structural related antioxidant properties and their role on ascorbic acid preservation. In: Atta- Ur- Rahman (Ed.) (2002): 324-389.

Magaldi, S, Mata-Essayag, S, Hartung de Capriles, C Well diffusion for antifungal susceptibility testing, *Int. J. Infect. Dis*. 8 (2004): 39-45.

Omotade, IO Chemical profile and antimicrobial activity of *Cymbopogon citratus* leaves. *J. Nat. Prod*. 2 (2009): 98-10.

Owen, NL, Hundley, N Endophytes the chemical synthesizers inside plants. *Sci Prog* 87 (2004): 79-99.

Pandey, AK, Rai MK and Acharya, D Chemical composition and antimycotic activity of the essential oils of corn mint (*Menthaarvensis*) and lemon grass (*Cymbopogon flexuosus*) against human pathogenic fungi. *Pharmaceut. Biol*. 41 (2003): 421-425.

Pearson, O The Antibacterial Properties of Essential Oils. Plant database. Cymbopogon citrates last accessed on 2003. (2010).

Sharma, KK, Saikia, R, Kotoky, J, Kalita, JC, Das, J Evaluation of antidermatophytic activity of piper betle, Allamanda cathertica and their combination: an in vitro and in vivo study. *Int. J. PharmTech. Res*. 3 (2011): 644-651.

Sodipo, O.A., M.A. Akanji, F.B. Kolawole and A.A. Odutuga. Saponin is Active antifungal principle in *Garcinia kola*, heckle seed. *Biosci. Res. Commun*. (1991): 171.

Tassou, CC, Nychas, E, Skandamis, PN Herbs and spices and antimicrobials, 2 (2004): 36-54.

Valgas, SM, De Souza, EFA Smânia, et al., Screening methods to determine antibacterial activity of natural products, *Braz. J. Microbiol*. 38 (2007): 369-380.

Weiss, EA Essential oil crops. Wallingford, UK: CAB International. (1997): 59-137.