SCIENCE PARK
ISSN: 2321-8045
IMPACT FACTOR : 3.4218(UIF)
VOLUME - 5 | ISSUE - 17 | 16 NOVEMBER – 2017

STUDIES ON ANTIBACTERIAL ACTIVITIES OF ESSENTIAL OIL FROM LEMON GRASS (*CYMBOPOGON FLEXUOSUS* L AND *CYMBOPOGON CITRATUS*) (DC.) STAPF.

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ABSTRACT

Lemongrass oil shows antibacterial activities the experiments conducted in the present study shows the effect of lemongrass oil on test organisms. The result obtained from Agar diffusion assay and broth dilution method support the general indication that gram positive organism are more sensitive to the oil then gram negative bacterial. All the test organisms showed difference in their sensitivity against different antibiotics gram positive organisms S. *aureus*, *B. cereus* and *B. subtilis* were formed more susceptible then Gram negative organisms *(E. Coli, K. Pneumonia, P. aeurginosa)*. The antibacterial activity was formed progressively increasing with increasing oil concentration.

KEYWORDS: Lemon grass, antibacterial activity, essential oil, gram negative organisms, gram positive organisms.

INTRODUCTION

Excess and imbalance use of industrial waste water, cultivation of heavy feeder crop like sugarcane have resulted in to increase in soil pH and salt content of the soil low productivity. Lemon grass essential oil yielding crop is good option for farmers.

Lemon grass (*Cymbopogon flexuosus*) is a native aromatic tall sedge (family: Poaceae) which grows in many parts of tropical and sub-tropical South East Asia and Africa in India, it is cultivated along Western Ghats (Maharashtra), Karnataka and Tamil Nadu states besides foot-hills of Arunachal Pradesh and Sikkim. It was introduced in India about a century back and is now commercially cultivated in these States.

The genus *Cymbopogon* includes about 140 species of aromatic grasses in India. Lemongrass makes wild growth in many tropical and semi tropical parts of Asia, Africa and in parts of Central America and South America. For the extraction of oil, wild growth grass is now rarely used, as cultivation of the grass has become a common practice. The major constituent of lemongrass oil is citral.

It varies from 60 to 92 per cent depending on the variety of the grass, type of soil, application of manure and fertilizer and climatic conditions. The value of lemongrass oil is determined by the percentage of citral in the oil. The common method for the estimation of citral content in lemongrass oil is the sodium bisulphite addiction method. By adopting column chromatographic method citral

having 99 percent purity can be separated.

The use of whole herbs and extractives has remained the main approach of folk medicine practitioners in the treatment of ailments and debilitating diseases. They usually claimed that such whole herbs and extractives are efficacious against several ailments and diseases without recourse to scientific proofs. Increased cases of opportunistic diseases emanating from side effects associated with synthetic drugs continue to necessitate incremental efforts in searching for effective biological substitutes with little or no side effects. Therefore, efforts are being directed towards elucidating potential sources such as ethno-medicinal plants (Patil, 2010). New, robust and less cumbersome extraction techniques assisted by recent developments in biotechnology have enhanced investigation of natural compounds faster with more precision than before leading to isolation of bioactive compounds with intense health benefits (Wang and Weller, 2006). According to folk medicine, several plants possess ethno medicinal benefits and *Cymbopogon citratus* Stapf, also known as lemongrass remained one medicinal benefits.

REVIEW OF LITERATURE

Because a host of medicinal properties have been ascribed on *Cymbopogon* species have been used as a blood purifier, in rheumatism and cholera, the essential oils of some of their species have been reported to be used as carminatives, stimulatives, antiseptics, suedorofics and also in rhenumatism and neuralgia.

Roots and stems of these species are used in the antidotal treatment of snake bite and scorpion sting [Heiba, H.I. and Rizk, A.M. (1986)]. It is also reported that *Cymbopogon flexuosus* is believed by the Kukuto people in the eastern highlands of Papua New Guinea to prevent sickness. Rauber, *et. al (*2005*)* reports *Cymbopogon* is an important medicinal plant finding use in native medicine. The oils and infusions of many of its species are used in treating a variety of human diseases which include interalia, gout, rheumatism, sprains, cough, cold, fevers, various stomach troubles including cholera and leprosy. Citral an important acyclic monoterpene present int he essential oils of many *Cymbopogon* species is a starting material for synthetic violet and non-toxic adhesives besides Vitamin A. An infusion of fresh leaves is administered as a diaphoretic and stimulant in catarrh and febrile conditions. The oil is used as a carminative find application in chronic rheumatism and other painful conditions [Krishnan Nambiar, V.P., Sasidharan N, Renuka C and Balagopalan N (1985)].

In addition to the above uses there are other uses also for the oil and the grass. Spent lemongrass is use for manuring in combination with organic manures and also for making pulp for paper industry. The Nizam Paper Mill at Puthukottai in Tamilnadu is reported to have used lemongrass collected from forest as raw material. Also it has to be noted that spent grass, after distillation of oil, can be converted into silage by addition of gram flour. It contains about 7-8 per cent crude proteins with calcium and phosphorus. It can also be used as fuel for distillation process or could be made use as farm yard manure. It is observed that if eggs are placed on dry lemongrass for hatching, it can protect chicken from the attack of insects. A spray of the infusion of lemongrass or a light spray of lemongrass oil can also serve the above purpose. In Maharashtra, spent grass is popularly used as mulching material for ginger and there is a popular belief that lemongrass mulch reduces the incidents of rot and other fungal disease. Tamil Nadu Agricultural University, Coimbatore showed that spent grass in combination with cow dung could generate reasonable amount of methane under anaerobic decomposition results confirm that heifers can achieve weight gain with the use of plant residues from lemongrass (West Indian) following their distillation.

Experiments have also proved that lemongrass oil is a good mosquito repellent and so it is a constituent in some mosquito repellent creams.Thus the grass and the oil have diversified uses.

Lemon grass (*Cymbopogon flexuousus*) is one of the most important essential oil yielding genera of the Poaceae. The genus comprises ~140 species that are widely distributed in semi-temperate to tropical regions of Asia, Africa and America. Approximately 45 species have been reported to occur in India. Lemongrass is an aromatic plant belonging to the Grami-neae family. The *Cymbopogon* species that produce volatile oils are called aromatic grasses (Rao, 1997). Different types of essential oils, such as palmarosa oil,

lemongrass oil, citronella oil and gigner grass or rusa oil, are very popular in perfumery (Rao, 1997, Singh & Sangwan *et al*. 2001 a). *Cymbopogon* species display wide variation in morphological attributes and essential oil composition at inter- and intra-specific levels. (Rao, 1997). Knowledge of germplasm diversity is important for plant conservation and improvement, therefore there is interest in determining the genetic diversity in *Cymbopogon* germplasm.

Lemon grass (*Cymbopogon flexuosus*) in India is cultivated along with western ghats (Maharashtra) Karnataka and Tamil Nadu states besides foot-hills of Arunachal Pradesh and Sikkim. It was introduced in India about a century back and is now commercially cultivated in these states. But recently it's cultivation is gaining importance in Uttar Pradesh, Bihar, M.P. and Chhatish Garh. It is widely grown in many parts of tropical and sub-tropical and sub-tropical South East Asia and Africa. Lemon grass is an important aromatic grass, but the area under cultivation is very less. For the Successful cultivation of aromatic crops, there are several cultural practices like use of organic and inorganic sources.

Cymbopogon flexuosus L. (Lemongrass) is an economically important plant that has been used for centuries, as a medicine because of its wide-ranging therapeutic properties included relief of rheumatic and other pain and healing effect on ulcers (Fenwick *et al.*, 1990). Flavonoids extracted from other pain and healing effect on ulcers (Francisco *et al.*, 2011). Flavonoids extracted from Lemongrass are of considerable interest as natural plant components with antioxidant and antifungal activity of the flavonoids present in Lemongrass, licochacone A and lcochacone B which have equal antioxidant activity of vitamin E, and glabrene which is 3 times as active when compared with vitamin E.

Lemon grass may not be preservative in nature; it has preservative properties, which may useful in built-in safety systems in food. In addition, Lemongrass herb is cheap, safe, and had medical functions. Citral, the main constituent of lemongrass essential oil, is used in perfumery, confectionery and infusions, and used as raw material in the synthesis of ionone, aromatic substances, and vitamin A. In Europe, leaves are used in teas and infusions. In Mexico, lemongrass tea is used traditionally as a sleep aid, tranquilizer, digestive, anti-influenza and antispasmodic (Rauber *et al*.2005).

Cheel et al. (2005) lemongrass (*Cymbopogon citratusl.*), a tall perennial grass comprising of about 55 species, is native to warm region and grows in almost all tropical and subtropical countries. The biologically active constituent of lemon grass is citral constituting more than 75% (w/w) of its essential oil. Lemongrass is widely used as an essential ingredient in Asian countries because of its sharp lemon flavour. Herbal tea of lemongrass is used as seadives, febrifuge and immune stimulant in India.

The lemongrass essential oil is applied for its medicinal value to cure acne, oily skin, scabies, flatulence, headaches, blood circulation problem. Ganjewala *et al* (2012) have reported that it has also been used as carminative, stimulant, emmenagogue, diuretic and antiseptic. Lemon grass is used for stomach problem and it is also used in combination with few other plants for effective treatment of malaria.

Lemongrass (*Cympopogon citratus* L.) is a plant in the grass family that contains 1 to 2% essential oil on a dry basis with widely variation of the chemical composition as a function of genetic diversity, habitat and agronomic treatment of the culture. Lemon grass essential oil is characterized by a high content of citral (composed of neral and geranial isomers (c. 69%)), which is used as a raw material for the production of ionone, vitamin A and betacarotene. Souza *et al*. (1986) observed It is a tall, clumped perennial grass growing to a height of 1 m. The leaf-blade is liner, tapered at both ends and can grow to a length of 50 cm and width of 1.5 cm.

Procurement of lemongrass oil

Materials and Methods:

The essential oil of lemongrass *Cymbopogon citratus* and *C. flexuosus* and some other species obtain after the steam distillation of the lemongrass plant material cultivated for the project work.

Hydrodistrillation unit of the project work which was purchased from Swaraj Herbal Pvt. Ltd. was established in village Margasni in Tahsil Velha District Pune which is 50km away from Pune. And the

Lemongrass is cultivated in 50 acres of land. After the plantation in one year 4-5 harvest were taken. 1st harvest was possible after 120 days from the day of plantation.

Test organisms/ Bacterial organisms

The test organisms used in this study was obtained from the Culture Collections of the National Chemical Laboratory Pune. The Organism Used in this study were: *S. aureeus, B. cereus, B. subtilis, E.coli, K.Pneumoniae, and P. aeruginosa*.

Propagation and maintenance of text organisms

Test organism were streaked on the Nutrient Agar slants and were incubated overnight at (37±1)°C. The cultures were kept under refrigerated conditions and were sub cultured after every fifteen (15) days. Preparation of concentrations of lemongrass oil for experimental purpose the different concentrations (v/v) of lemongrass oil viz 5%, 10%, 15%, 20%, 25%, 30% were prepared aseptically in sterile tween-80.

Methods to Observe Antibacterial activity of Lemon grass Oil

The testing of the bacterial cultures for the inhibitory effect of essential oil of lemon grass for different concentration (5%, 10%, 15%, 20%, 25%, 30%) were performer by using agar well diffusion method as described by Southwell *et al.* 1993. The Nutrient agar media containing 0.5% tween-80 was melted and 20 mL of media was added to individual sterilized petriplates separately on a level plate form and allowed to solidify. 1 mL of active cell suspension of organisms was spread with the help of sterilized swabs on the agar surface uniformaly. Three wells of 5 mm diameter each were made in agar partiplates of the solidified agar medium using sterilized hollow stainless steel gel cutter.

The mesure quantity of 25uL of each concentration was pipette out with a sterilized pipett and filed in the wells aseptically. In the control plate only Tween-80 was added into the well. The oil was allowed to defuse in the well for a period of one hour and plates were incubated at at (37±1)°C for 24-48 hours. The zone of inhibition (mm) was measured with graduated scale after the period of incubation. The determination of MIC of the essential oil of lemongrass on the test bacterial strain was done using broth dilution method as explained by Hammer *et al*. 1999 with different concentrations of oil. The cultures of the test strains were prepared by, inoculating the test strain in sterilized test tube containing, 5 mL nutrient broth. The tubes were incubated overnight at (37±1)°C. The MIC (Minimum Inhibitory Concentration) was defined as the lowest concentration of the test compound to inhibit the growth of microorganisms and the Minimum Bactericidal Concentration (MBC) was defined as the lowest concentration of the test compound to kill the microorganisms. The test tubes containing 10 mL of sterilized tryptic soy broth (TSB) with 0.5% (v/v) tween-80 were inoculated with different concentration of lemon grass oil ranging rangin from 0.5@-0.015% (v/v). (TSB) with 0.5% tween 80 without oil was used as positive growth control.

Tryptic soy broth or Trypticase soy broth (frequently abbreviated as TSB) is used in microbiology laboratories as a culture broth to grow aerobic bacteria. It is a complex, general purpose medium that is routinely used to grow certain pathogenic bacteria, which tend to have high nutritional requirements. (i.e., they are fastidious.) Its agar counterpart is tryptic soy agar (TSA). One of the components of Tryptic soy broth is Phytone, which is an enzymatic digest of soybean meal.

An aliquot of bacterial suspension (25 uL) to each tube was adde uniformly. The tubes were incubated at at (37±1)°C for 24 hours than 48 hours. The tubes were observed for turbidity after the period of incubation. The lowest concentration at which no visible growth occurs in either culture tubes was taken as MIC. Then the tubes showing no increased in the turbidity at each time interval 24-48 hours were streaked on nutrient agar plates to check the bacterial growth. Each trial was repeated thrice.

The antibiotic susceptibility test was performed by using the Bauer and Kirby Disc Diffusion Method as per CLSI guidelines and the antibiotics (Hi Media) used in the present study were : azithromycin (15mcg), ceftriazone (10 mcg), chloramphenicol (30 mcg), cerbenecillin (100 mcg), gentamicin (10 mcg), kanamycin (30 mcg), tobramycin (10 mcg), nitrofuratioin (300 mcg), vancomycin (30 mcg), ciprofloxacin (30 mcg).

RESULTS AND DISCUSSION

The result of the present investigation on standardization of cultivation method of lemon grass and effect of different treatment and extraction essential oil form lemon grass were studied .

Lemongrass oil was found effective against all the test organisms except *P. aeruginosa*. Gram positive organisms (*S. aureus, B. cereus and B. subtilis*) were found more susceptible than gram negative organisms (*E. Coli, K. pneumonia, p. aeruginosa*). The antibacterial activity was found progressively increasing with the increase in concentration of oil. The maximum effect was found at 30% concentration and minimum effect was observed at 5% concentration of oil.

In broth Dilution method the test organisms were found to be inhibited by lemongrass oil at very low concentration as compared to Agar Diffusion Method. Lemongrass oil was found to be effective against gram positive as compared to gram negative bacteria. *P. aeruginosa* was found to be highly resistant (even at neat). *S. aureus* and B. cereus was found to be more sensitive and got inhibited at 0.03% concentration (initial MIC) and at 0.06% concentration (Final MIC). The final MIC and MBC was found to be the same. *B. subtilis* and E. coli was found to be inhibited at a concentration of 0.06% (MIC) with an MBC of 0.12% concentration. Compared to other organisms k.pneumoniae showed a higher MIC (0.25%) and MBC (0.5%)
All the test organisms showed difference in their sensitivity against different antibiotics. Gram-positive organisms were found to be more susceptiable as compared to gram-negative organisms. Among grame-positive organisms s. aureus was found sensitive to all the antibiotics except nitrourantioin. Bcereus ws also found sensitive to almost all antibiotics tested except kanamycin and tobramycin. Bsubtilis was found to be more resistant to antibiotic as compared to *S. aureus* and *B. cereus*. Azithromycin, ceftriazone, chloramphenicol, carebenicillin were found ineffective against B. subtilis. Gram negative organisms like *K. peneumoniae* and *P. aeruginosa* showed maximum resistance to antibiotics. *E.coli* was found resistant to ceftriazone, chloramphenicol, carbenicillin and tobramycin.

From the present study it is clear that lemongrass oil possess a promising antibacterial activity against the test organisms. The results obtained from the Agar diffusion assay and broth dilution method support the general indication that gram positive organisms are more sensitive to the oil than gram negative bacteria. Similar observations were made by Onawunmi and Ongulana and *P. aeruginosa* were found resistant at all the concentraction of lemongrass oil including neat.

Similar result were reported by Pereira et al, Martra war et al., Torris et al, Alam et al, and Onawunmi *et al*., The test organisms were found inhibited by lemongrass oil at very low concentration in broth dilution method as compared to agar diffusion method, this is in accordance with the results of Tortorano *et al*. The results obtained by each of these methods differ due to many factors between assays. These include difference in microbial growth, exposure of microorganisms to the oil, the solubility of oil or oil components and the use and quality of an emulsifier etc.

The comparative effects of lemongrass oil and the standard antibiotic discs on the various test organisms are demonstrable indications of the oil as an antibacterial agent. Onawunmi 1984 and Ongulana 1986 had also reported the similar antibiotic susceptibility pattern and had suggested that the lest organisms particularly gram negative were found to be more susceptible to lemongrass than standard antibiotics (Onawunmi 1984, Ogunlana 1986). Thus, we conclude that in present era of emerging multidrug resistance among gram positive and gram negative organisms lemongrass oil will be helpful in treating such infections.

ACKNOWLEDGEMENT:-

The author is thankful to the chairman Ranadey Tech. Pvt. Ltd. Pune for providing the necessary assistance to complete the work. Author is also thankful to the Management and the Principal Poona College, Pune – 411001 and Head Department of Botany, S.P. Pune University and National Chemical Laboratory, Pune for providing the necessary facilities.

REFERENCES :-

1) Alam K, Agua T, Maven H, Taie R, Rao KS, Burrows I, et al. Preliminary screening of seaweeds, sea grass and lemongrass oil from Papua New Guinea for antimicrobial and antifungal activity. Inter J Pharmacognosy 1994; 32 (4): 396-9.
2) Bauer AW, Kirby WMM, Shevis JC, Turck M. Antibiotic susceptibility testing by a standardized single disc method. Am J Clin Path 1966; 45:493-6.
3) Cheel, J., Theoduloz, C., Rodrguez, J. and Schmeda, -H. G. 2005. Free radical scavengers and antioxidants from lemongrass (*Cymbopogon citratus* (DC.) Stapf.). Journal of Agricultural and Food Chemistry 53:2511-2517.
4) Francisoc, V., Figueirinha, A., Neves, B.M., Garcai-Rodriguez, C., Lopes, M. C., Cruz, M. T. and Batista, M. T. 2011. Cymbopogon citratus as source of new and safe anti-inflamatory drugs: bio-guided assay using lipopolysaccharide-stimulated macrophages. Journal of Ethnopharmacology 133: 818-827.
5) Ganjewala D., Gupta A.K. and Muhury R., 2012. An update on bioactive potential of a monoterpene aldehyde citral. Journal of Biologically Active Products from Nature, 2:186-199.
6) Hammer KA, Carron CF, Relay TV. Antimicrobial activity of essential oils and other plant extracts J Appl Microbiol 1999; 86; 985- 90.
7) Heiba, H.I. and Rizk, A.M. (1986), Constituents of Cvmbopogon Species, Quatar University Science Bulletin, Vol 6, p.55.
8) Krishnan Nambiar, V.P., Sasidharan N, Renuka C and Balagopalan N (1985), Studies on the Medicinal Plants of Kerala Forest, Kerala Forest Research Institute, Peechi, p.147.
9) Marta War ON, Majra Rajra Rodriguez J, Gaston Garcia S, Celia Lierene R. Antimicrobial activity of the essential oil and cream of *Cymbopogon citratus* (DC.) stapf. Revcubana Plt Med 2004; 2: 44-7.
10) Onawunmi G.O., Ogunla na EO. A study of the antibacterial activity of the essential oil of lemongrass (*Cymbogogon citratus*). Inter J Crud Drug Res 1986; 24 (2): 64-8.
11) Onawunmi G.O., Yisak, W.A. and Ogunlana, E. O. 1984. Antibacterial constituents in the essential oil of *Cymbopugon citratus* (dc.) Stapf. Journal of Ethnopharmacology 12: 274-286.
12) Patil, A.S. 2010. Exploring Passiflora incarnata (L.): A medicinal plants secondary metabolites as antibacterial agent. Journal of Medicinal Plants Research 4: 1496-1501.
13) Pereira RS, Sumita TC, Furlan Mr, Jorge AOC, Ueno M. Antibacterial activity of essential oils on microorganisms isolated from urinary tract infections. Revista de Saude Pubica 2004; 38(2): 326-8.
14) Rao, B.R.R., K. Singh, P.N. Kaul, A.K. Bhattacharya and K. Singh. (1997), Response of Palmarosa (*Cymbopogon martinii* (Roxb). Wats. Var. Motia Burk.) to plant spacings and nitrogen fertilizer application. Intern. J. Trop. Agril. 8(3): 177-183.
15) Rauber, C.D.A.S., S.S. Guterres and E.E.S. Schapoval, 2005. LC determination of citral in *Cymbopogon citratus* volatile oil. Journal of Pharmaceutical and Biomedical Analysis, 37(3): 597-601.
16) Singh-Sangwan N, Farooqi AHA & Sangwan Rs. 2001. Regulation of essential oil production in plants. Plant Growth Regul. 34: 3-21.
17) SouthWell A, Hayes A, Markherm J, Leach D. The search for optimally bioactive Australian tea tree oil. Acta Horti 1993; 334:26-65.
18) Souza, F. M. L., Lodder, H. M., Gianotti, F., O., Ferreira, T. M. and Carlini, T. A. 1986. Pharmacology of Lemon grass (*Cymbopogon citratus* Stapf.). II. Effects of daily two month administration in male and female rats and in offspring exposed “in utero”. Journal of Ethnopharmacology 17:65-74.
19) Torres RC, Ontengco DC, Balgos NS, Villanuva MA, Lanto EA, Cruz MS, et al. (Antibacterial essential oils from some Philippine plants). Laguna: The Philippine society for Microbiol Inc; 2002, p.219-20.
20) Tortorano Am, Viviani MA, Barchiesi F, Scalise G. Comparison of three methods for testing Azole susceptibilities of Candida albicans strains isolated sequentially from oral cavities of AIDS patients. J clinc Microbiol 1998; 36(6): 1578-83.

21) Wang, L. and Weller, C.L. 2006. Recent advances in extraction of nutraceuticals from plants. Trends in Food Science and Technology 17:300-312.