ORIGINAL RESEARCH PAPER

Biotechnology

TLC Profiling of *Cymbopogon citratus* (Lemongrass) by using Soxhlet Apparatus

KEY WORDS: Lemongrass, TLC , Soxhlet apparatus, solvent extraction.

Rahul Anand Students, Aakriti Biotechnology, Ranchi

Pappu Kumar Students, Aakriti Biotechnology, Ranchi

Gaurav Kumar Students, Aakriti Biotechnology, Ranchi

Nilesh Kumar Students, Aakriti Biotechnology, Ranchi

Khushboo Kumari Students, Marwari College, Ranchi.

Neetu Prasad Students, Marwari College, Ranchi.

Ranjeeta Kumari Students, Marwari College, Ranchi.

Deepak Kumar Professor, Aakriti Biotechnology, Ranchi

ABSTRACT

Lemongrass is a popular herb known for its lemon-like aroma. Its herb parts also carry other essential oils such as *myrcene, citronellol, methyl heptanone, dipentene, geraniol, limonene, geranyl acetate, nerol,* etc. These compounds are known to have counter-irritant, rubefacient, insecticidal, antifungal and anti-septic properties. The study aims to obtain various different intra and extra cellular components of the herb by Thin Layer Chromatography.

Introduction

Cymbopogon citratus or Lemongrass is a well known multi-application herb. It is a tall perennial grass, native to temperate to tropical regions. It is widely distributed in the Indian Sub-continent, Africa, South America, Australia, Europe and North America. In India, they grow wild in all regions ranging from sea level to an altitude of 4,200 meters. Many of the species are native to India. It is cultivated well in Assam, Kerala, Maharashtra, and Uttar Pradesh. Lemongrass is cultivated on large scale at Chinnar wildlife sanctuary in the Western Ghats of India (Nair and Jayakumar, 1999). It is a very good source of vitamin A and C, folic acid, Magnesium, Zinc, Copper, Iron, Potassium, Calcium and Manganese. Traces of B vitamins have also been reported in lemongrass. Apart from its culinary applications, lemongrass is widely used as a medicinal herb to treat various minor health conditions like diarrhea, constipation, heat burn, bloating etc. Lemongrass tea is recommended for healthy digestive tract. It is used as a home remedy by many people for its properties like anti-inflammatory, antidepressant, anti-fungal, astringent, antibacterial, antipyretic, and insecticidal. It also has anti-cancer properties. Citral, a molecule found in lemongrass slows down the growth of breast cancer cells. There are several antioxidant properties of lemongrass that helps in lowering the risk of cancer by fighting free radicals. Every part of the plant is used in various herbal treatments. There are fifty five different species of lemongrass know to humans in the genus *Cymbopogon*. Among fifty five different species, *C. citratus* is a very common ingredient in Asian and Caribbean food. In Asia it can be found in Thai, Vietnamese and Indonesian cuisines which include tea, curry and flavoring agent. It has a typical lemon-like fragrance, hence the name Lemongrass. Since, the plant has multiple applications in various industries; lemongrass cultivation can prove to be a profitable venture for the farmers in India. The income can range from Rs 17,000 per ha in the first year to Rs. 70,000 in the subsequent years. (Sonu et al., 2014)

The average elemental composition of the herb is Nitrogen-0.74%, Phosphorous-0.07%, Potassium-2.12%, Calcium-0.36%, Magnesium-0.15%, Sulfur-0.19%, Iron-126.73 ppm, Manganese-155.82 ppm, Zinc-35.51 ppm and Copper-56.64 ppm (Joy, 2003). East Indian lemongrass oil contains 75-85% of aldehydes consisting largely of Citral. Citral (3,7-dimethyl-2,6-octadien-1-al) forms the major constituent of the lemongrass extract at 75-85% of the total biochemical composition. Other components of lemongrass oil include heptenone, dipentene etc. and other important essential oils. Due to its lovely fragrance, lemon grass is used as a flavouring ingredient in several products such as soaps, perfume, candle, mosquito and other insect repellents (Vanisha, 2012).

Lemongrass has been widely investigated to show various industrial and medicinal applicable properties like antibacterial (Behboud et al, 2012), anti-fungal (Paranagama et al, 2003), antidandruff (Chaisripipat et al., 2015), anti-oxidant activity(Jose et al, 2005) and anti-inlamatory activity (Olorunnisola et al, 2014); thus, it can prove to be an excellent source of drug for various diseases. It has been found that lemongrass has no toxic effects on humans(Jose et al, 1986). Low dose of lemongrass extract has been found to be lethal for *Salnonella typhi* and *Staphylococcus aureus* (Umar et al, 2016). Oil and powder of Lemongrass shows efficiency in protecting stored seeds against bean worm, repels insects and effective against mosquitoes (Negrelle et al, 2007).

Lemongrass extracts have the pro-apoptotic activity in several hematopoitic cell lines (Ganjewala et al, 2009). It also has been found to posses cytotoxic activity against P388 leukemia cells (Balz, 1999)

In order to study various properties of lemongrass it is required to isolate the different components that make up the biochemistry of the herb. Each individual bio-molecule contributes to a specific medicinal property. In present time, many different chromatographic methods exist that effectively isolate and identifies different bio-molecules of a plant extract like HPLC, GLC, Ion-exchange Chromatography, Size exclusion Chromatography etc. These methods require highly sophisticated machinery, thus add to the cost of the study. However, these methods have their own importance and place in the field of biochemical studies. Thin Layer Chromatography or TLC is a cheap, easy and considerably effective technique to isolate and study individual biochemical pigments of tissue extract.

The current study aims to obtain maximum possible number of bands of biochemicals and pigments with a better resolution by generating a TLC profile of the alcohol and kenotic extract of the lemongrass leaves. The biochemicals were analyzed depending on their Rf value data.

Materials and Methods

Sample Collection: The lemon grass samples of young and

healthy leaves were obtained from the laboratory garden.

Sample processing: The leaf samples were cut into small pieces and processed for extraction using Acetone and Ethanol as extraction solvents into the Soxhlet Apparatus. The extracts obtained by the respective solvents were collected in a centrifuge tube and labeled properly. The extracts were kept for semi-drying into the desiccator.

Thin Layer Chromatography: The TLC plates were prepared by pouring the molten Silica Gel onto the glass TLC plates and kept in Oven for drying. The respective lemongrass extracts were loaded on the Silica Gel plates and kept for running in a pre-saturated TLC chamber. On one spot acetone extract was loaded, on second spot ethanol extract, and on the third spot a mixture of acetone extract and ethanol extract was loaded.

The TLC Chromatogram obtained, was analyzed by comparing the R_f values of the separated pigments. The Rf values of each band of pigments was calculated by the following formula:

$$Rf = \frac{\text{Distance travelled by solute (cm)}}{\text{Distance travelled by solvent (cm)}}$$

Results and Discussions: The distance travelled by different pigments of the acetone and ethanol extracts of lemongrass and the TLC running buffer was noted (Figure 1). R_f value of each pigment was calculated using the previously mentioned formula.

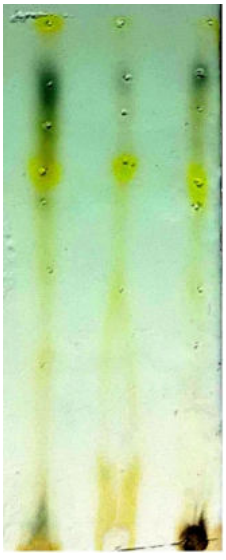

Figure 1: Thin Layer Chromatogram of Lemon grass extracts.

Distance travelled by TLC running buffer was noted to be 15.6 cm.

Table -1.

Distance travelled by pigments (in cm)		
Acetone	**Ethanol**	**Acetone + Ethanol**
15.6	15.5	15.6
14.0	13.9	14.1
12.8	13.0	13.1
11.5	11.6	11.0
10.5	_	10.4
8.5	7.8	7.7
5.8	_	6.0
1.0	_	1.0

Table -2.

Rf Values of different pigments		
Acetone	**Ethanol**	**Acetone + Ethanol**
1.0000	0.9936	1.0000
0.8974	0.8910	0.9038
0.8205	0.8333	0.8397
0.7371	0.7436	0.7051
0.6730	_	0.6666
0.5448	0.5000	0.4935
0.3717	_	0.3846
0.0641	_	0.0641

The chromatogram obtained clearly shows the difference in the banding pattern of the extracted components. The ethanol extract lacked three pigments while the acetone extract had three components more than the ethanol extract (Table 1 and 2). Thus, it can be concluded that in comparison with ethanol, acetone proves to be a better solvent for the extraction of lemongrass components by Soxhlet method. The extracted pigments or components can be further analyzed for their respective properties and their application in different industries.

Reference

1. Balz R and Rodolph (1999) The Healing power of Essential oils. Motilal Banarsidass Publishers Pvt Ltd, Delhi.
2. Behboud J, Amirreza E, Babak M A, Zarifeh H (2012) Antibacterial Activities of Lemon Grass Methanol Extract and Essence on Pathogenic Bacteria. American-Eurasian J. Agric. & Environ. Sci. 12 (8), 1042-1046.
3. Celso A, Rodrigues A C, Daniele O K, Valéria M L, André C G, Jorge C F, Mirtes C (2011) The GABAergic system contributes to the anxiolytic-like effect of essential oil from Cymbopogon citratus (lemongrass). Journal of Ethnopharmacology. 137, 828-836.
4. Chaisripipat, Lourith, Kanlayavattanakul (2015) Anti-dandruff Hair Tonic Containing Lemongrass (Cymbopogon flexuosus) Oil. Forsch Komplementmed. 22(4), 226-229.
5. Ganjewala D (2009) Cymbopogon essential oils: Chemical compositions and bioactivities. International Journal of Essential Oil Therapeutics. 3, 56-65.
6. Jose R L, Maria L V S, Eliana M, Katia A, Deborah S, Sergio T, Sergio K, Helena M C E, Carlini A (1986) Pharmacology of lemongrass (Cymbopogon citratus stapf). III. Assessment of eventual toxic, hypnotic and anxiolytic effects on humans.17, 75-83.
7. Joseä C, Cristina T, Jaime R, Guillermo S H (2005) Free Radical Scavengers and Antioxidants from Lemongrass (Cymbopogon citratus (DC.) Stapf.).
8. Vanisha S N and Hema M (2012) Potential Functions of Lemon Grass (Cymbopogon citratus) in Health and Disease. International Journal of Pharmaceutical & Biological Archives. 3(5),1035-1043.
9. Nair K K N and Jayakumar R (1999) Ethno botany of Hill-Pulaya tribe in the context of biodiversity rehabilitation at Chinnar wildlife sanctuary, Western Ghats of India. Journal of Economic and Taxonomic Botany. 23(2), 431-449.
10. Negrelle R R B and Gomes E C (2007) Cymbopogon citratus (DC.) Stapf : chemical composition and biological activities. Rev. Bras. Pl. Med., Botucatu. 9(1), 80-92.
11. Olorunnisola S K, Asiyanbi H T, Hammed A M, Simsek S (2014) Biological properties of lemongrass: An overview. International Food Research Journal. 21(2), 455-462.
12. Paranagama P A, Abeysekera K H T, Abeywickrama K, Nugaliyadde L (2003) Fungicidal and anti-aflatoxigenic effects of the essential oil of Cymbopogon citratus (DC.) Stapf. (lemongrass) against Aspergillus flavus Link. isolated from stored rice. Letters in Applied Microbiology. 37, 86–90.
13. Sonu K, Spondon C, Yadav S N (2014) Potential of lemongrass cultivation for soil conservation and oil production in NE Region. National Conference on Emerging Technology Trends in Agricultural Engineering. 498-501.