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Phytochemical screening, extraction and thin layer chromatography of *Cymbopogon citratus*

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Abstract
The current study's objectives are to explore the medicinally active compounds found in the methanolic extract made from *Cymbopogon citratus* plant leaves and to develop phytochemical parameters. Alkaloids, terpenoids, steroids, flavanoids, tannins, polysaccharides, saponins, and phenolic chemicals were all found in the extracts after preliminary phytochemical screening. The study of plant material lends itself well to the use of TLC. The number of components in a crude extract can be determined using TLC, which is an easy, quick, and affordable process. TLC offers a number of benefits, including low cost, quick analysis, the potential for multiple detection, and particular derivatization on the same plate. For Thin Layer Chromatography, methanolic extract was chosen among the available extracts. For the optimum separation of the phytoconstituents in TLC, a novel solvent system was designed.

Keywords: *Cymbopogon citratus*, alkaloids, terpenoids, steroids, flavanoids, tannins, polysaccharides

Introduction
For ages, manufacturers of meals, cosmetics, cleaning supplies, scents, herbicides, and insecticides have used essential oils extracted from a wide variety of plants and herbs as a raw material. Aromatherapy, a field of complementary medicine that asserts that essential oils and other aromatic molecules have therapeutic effects, has recently drawn a lot of interest to plant essential oils. The global demand for essential oils is rising as a result of customers' increased concern about components from natural sources and their growing awareness of potentially dangerous synthetic spice additives. As a result, several studies are looking into an efficient extraction method in terms of cost, quality, and environmental friendliness to meet the demands of this high-demanding commodity. Hertog *et al* 1997; Dayrit *et al* 1990) [2, 4].

The genus Cymbopogon, sometimes known as lemongrass, contains roughly 55 species of grasses, the type species of which is Cymbopogon citratus, and it is indigenous to warm-climated tropical parts of the Old World and Oceania. Cymbopogon citratus, a tall perennial grass, contains 1 to 2% of its dry weight in essential oil. Citral, which is a major component of lemongrass (*Cymbopogon citratus)* essential oil (composed of neral and geranial isomers). The manufacture of ionone, vitamin A, and beta-carotene all employ citral as a basic ingredient (Carlson *et al*, 2001) [11]. Due to genetic variation, environment, and agronomic treatment of the crop, the chemical makeup of lemongrass (*Cymbopogon citratus*) essential oil varies greatly (Paviani *et al*, 2006) [12]. A common herb is lemongrass (*Cymbopogon citratus).* (Caceres *et al*.1992; Sultana and Ashraf. 2009) [5, 13] a result of its tangy lemon flavour, cuisines. Acne, athlete's foot, excessive sweating, flatulence, muscle aches, greasy skin, and scabies are just a few of the health ailments that have been treated using lemongrass essential oil (Brian, and Ikhlas, 2002) [14]. The antimicrobial, antifungal, antibacterial, and mosquito-repellent activities of various components of this essential oil have also been demonstrated by numerous bioactivity investigations. Due to these advantageous characteristics, lemongrass (Cymbopogon citratus) oil is highly valued and used in the agricultural industry, particularly for the preservation of stored agricultural items like the primary food crop, maize (Sultana and asraf 2009) [13].

Materials and Methods
The plant material for this study, Cymbopogon citratus, was gathered in the M.P. Satana District, which is located on the Vindhyachal Plateau. Between the Satpura and Vindhyachal ranges of hills is where the district is situated.

The northern boundary of the district is shared with the Banda district of Uttar Pradesh State, while the eastern, Rewa, and western, Panna and southern boundaries are shared with the Jabalpur, and Umaria districts. The district is situated between the latitudes of 23.58° and 25.12° North and the longitudes of 80.21° and 81.23° East. Around 305 metres above mean sea level, the district is located.

Extraction and Preliminary Phytochemical Screening

Preparation of Crude Methanol Extract: Using an electric grinder, dried *Cymbopogon citratus* plant leaves were pulverised. In a mixer grinder, finely grind the material, then precisely weigh it. For 48 hours, at room temperature, the powdered material underwent solvent extraction using a Soxhlet equipment and methanol. The resulting mixture was filtered and evaporated in a shaker water bath; temperature was maintained at 55-650C; the obtained dried crude extract was used for phytochemical analysis**. (**Trease 1996; Peach and tracey 1935) [8, 9].

Phytochemical evaluation

Testing several classes of compounds using industry-recognized methodologies14 is used to evaluate the phytochemistry of the plant and identify the substance shown in Table 1.

With the help of the methanolic extract of *Cymbopogon citratus* Lam, early phytochemical studies were conducted. Using a normal standard methodology, plant leaves are used to identify phytochemical components qualitatively. Analytical grade was used for all of the chemicals and reagents used.

Tests for Alkaloids

Mayer's Reagent Test: 100 ml of water required the dissolution of 1.36 g of mercuric chloride and 3 g of potassium iodide. A small amount of each extract was diluted in a watch glass with hydrochloric acid, and a few drops of the reagent were added. The formation of a cream-colored precipitate indicated the presence of alkaloid.

Hager's Reagent Test: It is a picric acid-in-water saturated solution. A yellow precipitate indicating the presence of alkaloids was produced after the test filtrate was treated with this reagent.

Wagner's Reagent Test: It is a solution of potassium triiodide in water that was made by combining 1.3 grammes of iodine with a 100 millilitre solution of potassium iodide. Alkaloids are present in the extract if brown precipitate forms when this reagent is added.

Test for Flavonoids

Shinoda Test: Conc. hydrochloric acid was added drop by drop after the crude extract had been combined with a few fragments of magnesium ribbons. Within a few minutes, pink and scarlet colours start to develop, signifying the presence of flavonoids.

Zinc Hydrochloride Test: Zinc dust and concentrated hydrochloric acid should be added to the test solution. After a short while, it turns red, indicating flavonoids.

Tests for Carbohydrates

Molish test -A concentrated 2 ml of sulphuric acid was gently poured down the side of the test tube, forming a heavy layer at the bottom, after dissolving around 0.1 g of the sample in 2 ml of water and adding 2-3 drops of 1% ethanolic solution of alpha napthol. If there are carbs present, a strong violet colour results.

Fehling's Test: Fehling's A and B solutions in a volume of 1 ml each were combined, and the mixture was heated for 1 minute. After adding an equal volume of sample, it was cooked for 5-10 minutes in a pot of boiling water. Carbohydrates are seen as first a yellow colour and subsequently a brick red tone.

Test for Saponins

Foam Test -In a water bath, 2 g of the powdered sample were cooked in 20 ml of distilled water before being filtered. After vigorously shaking, 10 ml of the filtrate was combined with 5 ml of distilled water. The presence of saponins was suggested by persistent foam.

Test for Tannins: Samples were taken separately, warmed up, and filtered in water. The filtrate was used in tests with the following reagents.

$FeCl_3$ Solution Test -Ferric chloride was produced as a 5% w/v solution in 90% alcohol. A small amount of the filtrate from above was mixed with a few drops of this solution. Colors like deep blue or dark green indicate the presence of tannins.

Lead acetate Test: The test filtrate was mixed with a basic lead acetate 10% w/v solution in distilled water. Tannins are indicated by precipitation.

Test for Amino acids

Ninhydrin test: 3 drops of 5% ninhydrin were added to 3 ml of the crude sample, and the mixture was cooked in a boiling water bath for 10 minutes. Amino acids were denoted by a purple or bluish colour.

Test for anthraquinone glycosides

Borntrager's Test

Sulphuric acid that had been diluted was added to the 3ml of the sample, heated, and fluttered. A similar volume of chloroform was added and stirred into the filtrate. Ammonia was injected following the separation of the organic layer. The presence of these glycosides is shown by the ammoniacal layer turning pink.

Tests for sterol/steroids

Salkowaski Test: A few milligrammes of the material were dissolved in 2 ml of strong sulphuric acid and 2 ml of chloroform, then shaken. When the chloroform layer turns red, sterols or steroids are present, which is why.

Test for Terpenoids

Liebermann–Burchard Test: In 1 ml of chloroform and a few drops of acetic anhydride, a few milligrammes of the sample were dissolved. By the side of the test tube, concentrated sulfuric acid was added. Triterpenoids and sterols are both indicated by the production of purple and blue-green colours, respectively.

Test for Proteins

Biuret Test: A small amount of 1% CuSO4 and 4% NaOH

were added to the sample. The presence of proteins is indicated by violet or pink colour

Xanthoproteic Test: Sample was combined with 1 ml of concentrated sulfuric acid; the appearance of a precipitate indicates a successful test.

Millon's Test: The presence of proteins was determined by mixing 3 ml of the sample with Millon's reagent.

Separation of chemical constituents

The TLC method was used to check each eluted sample's purity. It is a method used to separate a variety of chemicals with biological significance. It can be used for preliminary, qualitative, and quantitative work [Stahl, 1965] [10]. About 0.1-0.2 ml of conc. of the petroleum ether extract was submitted to thin layer chromatography. Using a capillary tube, methanolic extract was put onto the plate. Spotted plates were used for elution while being gently dried. Initial tests were conducted on a variety of solvents, including benzene, pet ether, ethanol, and chloroform. Later, various solvent combinations were investigated based on polarity. Three spots appeared on the plate after the spotting was done in the plate's centre. Drying the spotted plate with care.

Development of chromatogram

The eluted spotted plates were dried at room temperature before being put in iodine chambers for chromatogram development. The Rf values of the cleaned sport were computed, and the appropriate solvent system was found. The results are reported in Table 2. Chromatography in a column in 10 ml of benzene, 50 ml of concentrated petroleum ether were dissolved. To progressively incorporate the pet ether extract into the benzene solution, add activated silica gel H. We permit the chromatograms to mature. After the entire band development, elution was initiated and regulated to 12–15 drops per millimetre. A tidy 50 ml container with a volume of about 10 ml of the eluted solvent was used, and it was labelled with the number 6.18.

Table 1: Preliminary Phytochemical screening of Extract of leaves of *C. citratus.*

S. No.	Tests	Plant type	Observation for extracts	
			Petroleum Ether	Methanol
1	**Carbohydrates *C. citratus.***			
1.1	Molish test		Negative	Positive
1.2	Fehling	*C. citratus.*	Negative	Negative
1.3	Barfoed's Test	*C. citratus.*	Negative	Positive
2.	**Proteins and amino acids**			
2.1	Biuret's test	*C. citratus.*	Negative	Negative
2.2	Ninhydrin test	*C. citratus.*	Negative	Negative
3	**Glycosides**			
3.1	Legal's test	*C. citratus.*	Positive	Positive
3.2	Keller-Killani test	*C. citratus.*		Positive
4	**Saponins**			
4.1	Froth test	*C. citratus.*	Negative	Positive
5	**Alkaloids**			
5.1	Mayer's test	*C. citratus.*	Negative	Positive
5.2	Hager's test	*C. citratus.*	Negative	Positive
5.3	Wagner's test	*C. citratus.*		Positive
6	**Flavonoids**			
6.1	Lead acetate test	*C. citratus.*	Positive	Positive
6.2	Alkaline reagent test	*C. citratus.*	Positive	Positive
7	**Triterpenoids and Steroids**			
7.1	Libermann- burchard, s test	*C. citratus.*	Positive	Negative
7.2	Salkowski, s test	*C. citratus.*		Positive
8	**Tannins and Phenols**			
8.1	Ferric chloride test	*C. citratus.*	Positive	Positive
8.2	Lead acetate test	*C. citratus.*	Positive	Positive
8.3	Gelatin test	*C. citratus.*		Positive

Table 2: Showing *Rf* values of methanolic extract of *Cymbopogon citratus* L.

Solvent System	Spot No.	R*f* Values	Colors of Peaks
Toulene: Aceteic acid: Formic acid (5:3.5:0.5)	1	0.08	Blue
	2	0.39	Yellow
	3	0.44	Brown
	4	0.7	Green
	5	0.73	Green

Stationary Phase: Silica gel. 60-120 mesh size (Merk).

Results and Discussion

Phytoconstituents such as terpenoids, tannins, saponins, and steroids were abundant in the leaf extract of *Cymbopogon citratus* Lam, according to Table No. 1 of the findings of preliminary phytochemical screening of the leaves in methanol and petroleum ether. To separate and identify the bioactive components found in the leaves of the methanolic extract of *Cymbopogon citratus* Lam, petroleum ether extract was treated to TLC. The largest discriminating power TLC plates shown in the fluorescence light under UV at 254-365 nm wave length and find these active spots in TLC plate with the following Rf values were found to be the most suitable TCL system for analysis in the current research work, with alkaloids, carbohydrates, glycosides, terpenoids, saponins, tannins, and steroids (0.08, 0.39, 0.44, 0.7 and 0.73).

Conclusion

The *Celastrus paniculata*. of *Cymbopogon citratus* Lam plant's methanolic leaf extracts, obtained through solvent extraction by Soxhlet apparatus, have been used as raw material for the synthesis of many drugs in the current situation and thus continue to be a significant source of new therapeutic agents. Children with mental retardation have been proven to benefit from *Cymbopogon citratus* Lam's positive effects on learning and memory. The methanolic extract made from the plant *Cymbopogon citratus* Lam. The ethnopharmacological uses of the plant in Indian folk medicines have been demonstrated through serial solvent extraction. *Cymbopogon citratus* Lam was examined for phytochemicals. Preliminary and significant feature. The facts lead to the conclusion that *Cymbopogon citratus* Lam. leaves had a vital function in medicinal chemistry for the creation of life-saving medications.

References

1. Al-Bari M, Sayeed MA, Rahman MS, Mossadik MA. Characterization and antimicrobial activities of a phenolic acid derivative produced by Streptomyces bangladeshiensis; a novel species collected in Bangladesh. Res J Med Sci. 2006;1:77-81.
2. Hertog MG, Sweetnam PM, Fehily AM, Elwood PC, Kromhout D. Antioxidant flavonols and ischemic heart disease in a Welsh population of men: the Caerphilly Study. Am J Clin Nutr. 1997;65:1489-94.
3. Soobrattee MA, Neergheen VS, Luximon-Ramma A, Aruoma OI, Bahorun T. Phenolics as potential antioxidant therapeutic agents: mechanism and actions. Mutat Res Fundam Mol Mech Mutagen. 2005;579:200-213.
4. Dayrit FM, Alcantar AD, Villasenor IM. Studies on *Cymbopogon citratus* leaves. The antibiotic compound and its deactivation in aqueous solution. Philipp J Sci. 1990;119:23-33.
5. Caceres A, Saravia A, Rizzo S, Zabala L, De Leon E, Nave F. Pharmacologic properties of Moringa oleifera. 2: Screening for antispasmodic, anti-inflammatory and diuretic activity. J Ethnopharmacol. 1992;36:233-237.
6. Sreelatha S, Padma PR. Antioxidant activity and total phenolic content of Moringa oleifera leaves in two stages of maturity. Plant Foods Hum Nutr. 2009;64:303-11.
7. Singh BN, Singh B, Singh R, Prakash D, Dhakarey R, Upadhyay G, Singh H. Oxidative DNA damage protective activity, antioxidant and anti-quorum sensing potentials of Moringa oleifera. Food Chem Toxicol. 2009;47:1109-1116.
8. Trease GE, Evans WC. Pharmacognosy. 14th ed. London: ELSB, Baillière Tindall; c1996.
9. Peach K, Tracey MV. Modern Methods of Plant Analysis. Berlin: Springer-Verlag; c1935.
10. Stahl WR. Algorithmically unsolvable problems for a cell automaton. Journal of Theoretical Biology. 1965 May 1;8(3):371-94.
11. Carlson L, Marcu D. Discourse tagging reference manual. ISI Technical Report ISI-TR-545. 2001 Sep;54(2001):56.
12. Paviani L, Pergher SB, Dariva C. Application of molecular sieves in the fractionation of lemongrass oil from high-pressure carbon dioxide extraction. Brazilian Journal of Chemical Engineering. 2006;23:219-25.
13. Sultana B, Anwar F, Ashraf M. Effect of extraction solvent/technique on the antioxidant activity of selected medicinal plant extracts. Molecules. 2009 Jun 15;14(6):2167-80.
14. Brian TS, Ikhlas AK. Comparison of Extraction Methods for Marker Compounds in the Essential Oils of Lemongrass and Eucalyptus Leaves"; GC, J Agric. Food Chemicals; c2002.