International Journal of Botany Studies
ISSN: 2455-541X; Impact Factor: RJIF 5.12
Received: 08-04-2020; Accepted: 10-05-2020: Published: 10-05-2020
www.botanyjournals.com
Volume 5; Issue 3; 2020; Page No. 103-107

Secondary metabolite profiling of *Cymbopogon citratus*

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Abstract
Cymbopogon citratus (lemon grass) is an important species of Poaceae family. Lemongrass constitute various organic compounds and were reported to possess high medicinal potential. It has been used as traditional medicine for treatment of several diseases such as fever, sore throats, cough, laryngitis, bronchitis, oral candiasis, body ache, head ache, digestive problems etc. This study aimed to extract *Cymbopogon citratus* leaf using water as solvent with view to determine the phytochemical constituents followed by qualitative analysis by Thin Layer Chromatography. Phytochemical analysis revealed the presence of several bioactive compounds such as flavonoids, phenols, tannins, alkaloids etc. And TLC analysis further confirmed the presence of those secondary metabolites. The study revealed the presence of bioactive molecules in water extract in lemongrass.

Keywords: *Cymbopogon citratus*, Lemongrass, phytochemical constituents

1. Introduction

Plant based drugs are used for treatment of various diseases since ancient times. This is mainly due to various bioactive components present in it. These bioactive components have immense therapeutic value and also herbal medicines have the advantages such as easy availability and less toxicity and no adverse effect [1].

Lemongrass is the common name for herbaceous plants of Poaceae family and origin being tropical Asia. Binomial nomenclature of lemongrass is *Cymbopogan citratus.* It is a tall, monocotyledonous aromatic perennial plant with slender sharpedge green leaves with pointed apex. The name lemongrass is due to typical lemon-like odour of the essential oil present in the shoot. The Cymbopogon genus members also known as aromatic grasses since they produce volatile oils [4].

Leaves of the *Cymbopogon citratus* plant used for food, cosmetic as well as pharmaceutical applications. Lemongrass is one of the important medicinal plant and it has various applications in traditional medicines. Also, it can be used for treatment of HIV complications, especially secondary bacterial infections [7].

Plants synthesize a vast variety of chemical compounds classified as primary and secondary metabolites. Primary metabolites are involved directly in growth and development whereas secondary metabolites have several medicinal importance. There are wide range of secondary metabolites such as alkaloids, flavonoids, saponins, tannins, terpenoids, cardiac glycosides etc. Each of these have specific functions and health benefits. Hence they are used as raw materials for pharmaceutical and cosmetic industries [5]. India has tremendous variety of plant sources and is origin of traditional system of medicines [6].

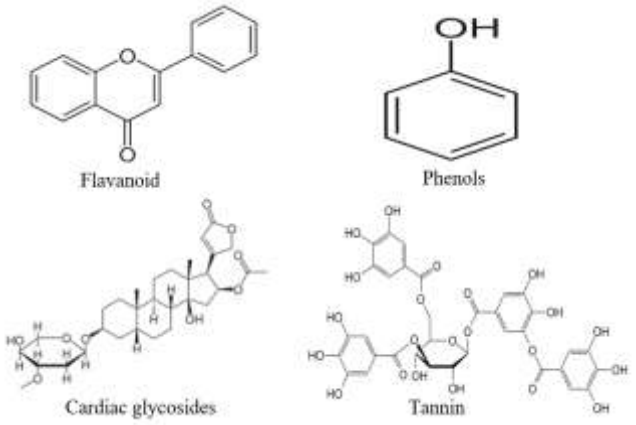
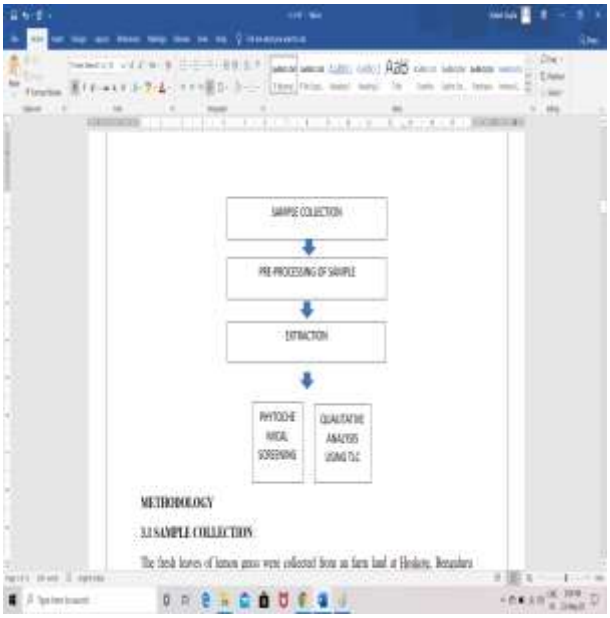


Fig 1: Basic structures of some pharmacologically important plant derived phytochemicals.

The use of lemongrass for the treatment of various medical conditions such as fever, cough, elephantiasis flu, leprosy, malaria and other digestive problems has been reported. The lack of scientific knowledge has restricted the use of lemongrass for clinical applications [4]. Hence the study focuses on qualitative analysis of the phytochemistry of lemongrass.



2. Methodology Flow

Methodology

2.1 Sample Collection

The fresh leaves of lemon grass were collected from an farm land at Hoskote, Bengaluru Karnataka, on 23rd February 2019.

2.2 Sample Preparation

The collected sample was washed with running tap water to remove the dust particles and other contaminants present on the sample. The clean sample was once again washed with distilled water to avoid any cross contamination of the sample and the sample was air dried under the sun for 8-10 days at 27-30degree Celsius and then subjected to electrical grinder to obtain fine powder. The powder was then subjected to sieving to obtain fine powder so that the extraction can be carried out at larger surface area [8, 9].

Fig 2: sample processing

2.3 Extraction at Room Temperature

15 grams of powdered sample was weighed and dissolved in 150ml of distilled water and incubated for 72 hours at room temperature. After the incubation period the sample was filtered using the muslin cloth to remove the large insoluble particles. The sample was once again filtered using the Whatman filter paper to remove the smallest insoluble particle to obtain a clear fluid. The filtered sample was subjected to the process of evaporation in water bath at 100degree Celsius to allow the sample to evaporate and to obtain a concentrated solution which can be used for the further experimental process [10].

c

Fig 3: Filtration of the sample using muslin cloth & Whatman filter paper after 72hrs of incubation in distilled water

Fig 4: Evaporation of the solvent using water bath at 100 degree Celsius

Fig 5: Concentrated sample after evaporation

Phytochemical Screening

Phytochemicals are the chemical compounds which are produced by the plants. They are produced as a result of primary and secondary metabolism in plants. These phytochemicals are usually considered as the research compounds because of the biological activity of the compounds are still under the scientific and experimental study towards the health effects.

Thereby the phytochemical analysis of lemon grass extract was carried out using the standard protocol method [11, 12].

Test for alkaloids

Mayer's test: 1o 1 ml of extract add 1 ml of conc. Hcl followed by few drops of Mayer's reagent, formation of white or green precipitate indicate the presence of alkaloids.

Test for phenols

Ferric chloride test: To 1ml of extract add 1ml of 5 % ferric chloride solution, formation of reddish-brown precipitate indicates the presence of phenols.

Test for flavonoids

Lead acetate test: To 1 ml of the extract add 1 ml of 10% lead acetate solution, formation of yellow precipitate indicates the presence of flavonoids.

Test for tannins

Braymer's test: To 0.5 ml of extract add 1 ml of distilled water followed by 1 ml of 5 % ferric chloride solution, formation of blue-green colour indicates the presence of tannins.

Test for saponins

Foam test: To 1 ml of extract add 1ml of distilled water and shake vigorously, formation of the foam indicates the presence of saponins.

Test for cardiac glycosides

Keller- killiani test: To 1 ml of sample add 2 ml of glacial acetic acid followed by 2ml of glacial acetic acid, add 1 ml of 5% ferric chloride solution along with 1 ml of dilute Hcl, formation of brown ring at the interface indicate the presence of cardiac glycosides.

Test for terpenoids

Ferric chloride test: To 1 ml of extract add 2 ml of water followed by 1ml of 10% ferric chloride solution, formation of intense colour indicates the presence of terpenoids.

Test for quinones

To 1 ml of extract add 0.5 ml of con Hcl, formation of yellow precipitate indicates the presence of quinones.

Test for coumarins

To 1ml of extract add 1.5 ml of 10% NaOH, formation of yellow colour indicates the presence of coumarins.

2.4 Qualitative Analysis using TLC

Thin Layer Chromatography

The process of TLC was carried out in order to isolate the components that were present in the plant extract.

Different solvent systems with different ratio was prepared to carry out the thin layer chromatography studies and to identify the best solvent system that is capable of showing better resolution [13, 14, 15, 16].

The precoated silica TLC plates were taken and cut into the required dimension. The sample was loaded on the TLC plate using the capillary tube. The sample loaded TLC plate were then placed in mobile phase and allowed the capillary action of the solvent to take place. The TLC plates were removed once the solvent reaches 3/4th of the TLC plate. The TLC plate was then air dried using the hot air oven for one min and then observed under the UV-TLC reader.

Rf = Distance moved by sample/distance moved by solvent (Rf: retention factor)

Solvent Systems Used

Table 1: Different solvent system used for TLC

Solvent System	Ratio
Hexane: ethyl acetate	18: 2
Ethyl acetate: chloroform: distilled water	5: 3: 1
n-butanol: ethyl acetate: distilled water	5: 10:15
Chloroform: distilled water	6: 4
Methanol: distilled water	6: 3

3. Result & Discussion

3.1 Results of Phytochemical Analysis

Table 2: Results of phytochemical screening

TEST	Inference	Ethyl acetate extract
Test for flavonoids	Formation of white precipitate	+ve
Test for phenol	Formation of dark blue/intense color	+ve
Test for saponins	Formation of persistence foam.	-ve
Test for tannins	Formation of blue greenish color.	+ve
Test for cardiac glycosides	Brown ring at the interface.	+ve
Test for alkaloids	Presence of green color or white precipitate.	+ve
Test for terpenoids	Formation of intense color.	+ve
Test for quinones	Yellow precipitate	-ve
Test for coumarins	Formation of yellow color.	+ve

The results of this study showed the presence of the phytochemicals namely (flavonoids, phenols, saponins, tannins, alkaloids, terpenoids, cardiac glycosides & coumarins).

Fig 6: Results of flavonoids

Fig 7: Results of phenols

Fig 8: Results of tannins

Fig 9: Results of saponins

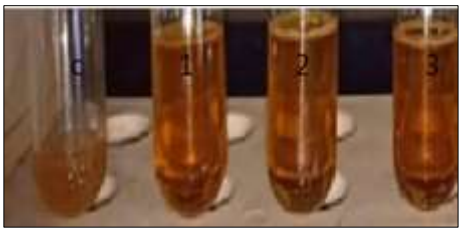


Fig 10: Results of cardiac glycosides

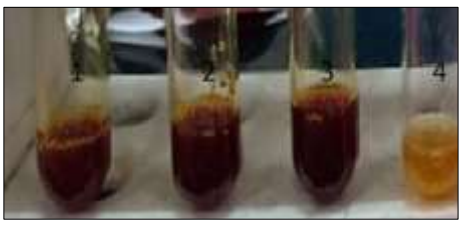


Fig 11: Results of alkaloids

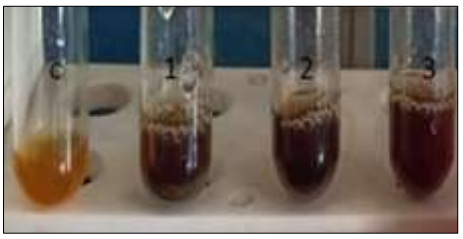


Fig 12: Results of terpenoids

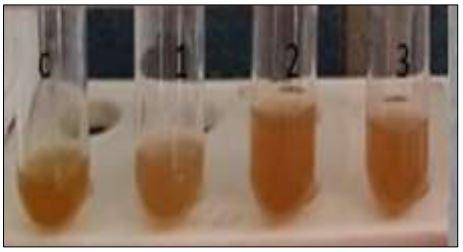


Fig 13: Results of quinones test

(C is negative control & 1, 2, 3 test tubes are three trials of the test)

Fig 14: Results of coumarins test

3.2 Results of Thin Layer Chromatography

The qualitative analysis ethyl acetate extract was carried out by performing the thin layer chromatography using the combination of various solvents and calculating the Rf value to identify the respective phytochemical by comparing with the standard chart.

Table 3: Results of TLC

Solvent System	Ratio	Rf	Inference
Methanol: distilled water	18: 2	0.88	phenol
Ethyl acetate: chloroform: distilled water	5: 3: 1	0.55	alkaloids
n-butanol: ethyl acetate: distilled water	5: 10:15	0.75	flavonoids
Chloroform: distilled water	6: 4	0.95	Tannins
Hexane: ethyl acetate	6: 3	0.89	flavonoids

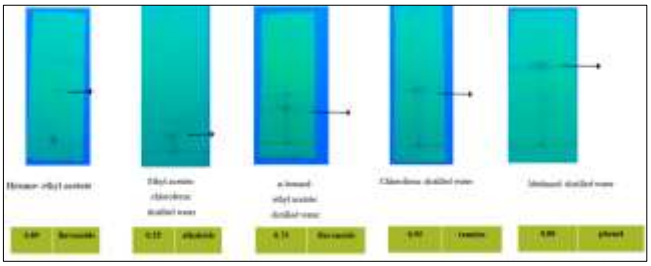


Fig 12: TLC plates observed under the UV light

From the study we can conclude that different solvent systems was used for separation of the phytochemicals, almost 5 phytochemicals was inferred by comparing the standard.

4. Conclusion

The study revealed presence of several pharmacologically important phytochemicals in the water extracts of lemongrass leaves viz. flavonoids, phenols, saponins, tannins, alkaloids, terpenoids, cardiac glycosides & coumarins). Qualitative phytochemical screening explored the scientific basis of medicinal potential of lemongrass. In TLC different solvent systems were used for separation of compounds of interest and the result indicated the presence of several phytochemicals (flavonoids, phenols, tannins, alkaloids, terpenoids & saponins) in the water extract of lemongrass. TLC profiling supported the claim of presence of the secondary metabolites. Hence the lemongrass can be used as an easily accessible source for pharmacological and food industry.

5. Future Scope

- This study can lead to further research in a way of isolation and identification of active compounds from lemongrass using chromatographic and spectroscopic techniques.
- The plant could be investigated further for more potent bioactive compounds of medicinal utilities. This research will lead to rational use of lemongrass plant in the modern system of healthcare.
- The process of extraction can be expanded using different solvents and the analysis can be carried out to find the appropriate solvent which is suitable for the extraction of phytochemicals.
- The sample can be further analyzed using the FTIR, spectroscopic and other advanced techniques.
- The process of quantification and optimization of the phytochemicals can be carried out.
- The anti-oxidant & scavenging activity can be carried out.
- The anti-microbial activity against various microbes can be carried out.

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