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Phytochemical screening and qualitative analysis of *Cymbopogon citratus*

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Abstract
Cymbopogon citratus, commonly known as Lemongrass is a small herbaceous plant of Poaceae family. It is 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 deals with the preliminary phytochemical screening of ethyl acetate extract of lemon grass leaves followed by qualitative analysis by Thin Layer Chromatography. Antimicrobial activity of the extract was also been tested to highlight the medicinal values. 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 ethyl acetate extract showed significant antibacterial activity against *Bacillus subtilis*. The study revealed the medicinal potential of lemongrass.

Keywords: Phytochemical screening, qualitative analysis, *Cymbopogon citratus*

1. Introduction
Plants are important source of medicinal agents as they possess numerous active constituents of immense therapeutic value [1]. Since ancient times, plants and herbs have been given a unique place in all the civilizations throughout the world [2]. Plant-based drugs are used worldwide for the treatment of various diseases because of their easy availability and less toxic effect to recipient compared to that of synthetic drugs [1]. The use of herbal drugs increasing rapidly and it represents a substantial part world drug market [3]. More than 75% of the world population depends upon medicinal plants for their basic health needs. Plant-based medicine has become a popular alternative for synthetic medicine because it does not cause any adverse effect [4].

1.1 Secondary metabolites
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].

1.2 Lemongrass
Cymbopogon citratus, commonly known as Lemongrass belongs to the poaceae family and genus *Cymbopogon* is a tall, monocotyledonous aromatic perennial plant with slender sharpedge green leaves with pointed apex. The origin of the plant is tropical Asia.
Taxonomic details of the lemongrass:

Kingdom	:	Plantae
Division	:	Magnoliophyta
Class	:	Liliopsida
Order	:	Poales
Family	:	Poaceae
Genus	:	*Cymbopogon*
Species	:	citrates

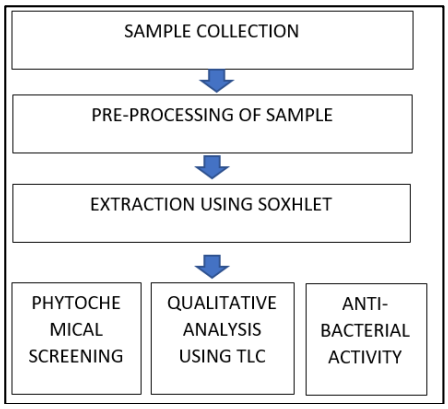
The name lemongrass is due to typical lemon-like odour of the essential oil present in the shoot. The *Cymbopogon* genus members also known as aromatic grasses since they produce volatile oils [4].

1.3 Medicinal Properties of Lemongrass

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].

Lemon grass has been traditionally used to treat various medical conditions due to various secondary metabolites present in it. It has been also used to treat fever, cough, elephantiasis flu, leprosy, malaria and other digestive problems. Antimicrobial activity of lemongrass against various bacteria, fungi, protozoa has also been reported. The scientific investigations and information on the therapeutic potentials of lemongrass is limited. The lack of scientific knowledge has restricted the use of lemongrass for clinical applications [4]. This study focusses on qualitative analysis of the phytochemistry of lemongrass (*Cymbopogon citratus*), and determination of the antimicrobial effect of the leaf extracts on some selected microbial isolates.

2. Methodology



Methodology Flow

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 27-30 degree 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 1: Sample processing

2.3 Extraction Using Soxhlet Unit

The extraction process using the Soxhlet unit was followed as per the description given by Williams *et al*. 2007 [10]. The processed sample powder was subjected to the extraction in the Soxhlet unit using the ethyl acetate as solvent.

Here 20grams of powder sample was taken for the process of extraction with 230ml of ethyl acetate as solvent. The boiling temperate of the Soxhlet unit was maintained to 67-70 degree Celsius. The flask containing the extraction solvent was heated to reflux. The process of extraction was carried out for 3 days (15-20 cycles). After the process of extraction, the sample was collected from the distillation flask and subjected to the process of filtration using Whatman filter paper and followed by the evaporation using the water bath to allow the solvent to get evaporated and to obtain the concentrated sample which can be used for the further experimental process [11].

2.4 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 [12].

2.4.1 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.

2.4.2 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.

2.4.3 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.

2.4.4 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.

2.4.5 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.

2.4.6 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.

2.4.7 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.

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

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

2.5 Qualitative Analysis Using TLC
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

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

2.6 Anti-Microbial Activity
The potency of the plant extract in inhibiting the growth of the microbe was studied by carrying out the disc diffusion method.

2.6.1 Anti-Bacterial Activity: The ability of the plant extract to inhibit the growth of the bacteria was studied by performing the disc diffusion method, the study was carried out using the *Bacillus subtilis*. To prepare 150 ml of LB media, 3grams of LB & 1.8 grams of agar was dissolved in 150 ml of distilled water and then autoclaved at 121 degree Celsius at 100kPa above atm pressure for 15mins [17].
The sample stock of 100mg/ml was made and from that the 20ul, 25ul & 30ul of sample was taken to observe the anti-microbial activity

3. Result & Discussion
3.1 Results of Phytochemical Analysis

Table 1: 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, tannins, alkaloids, cardiac glycosides & coumarins).

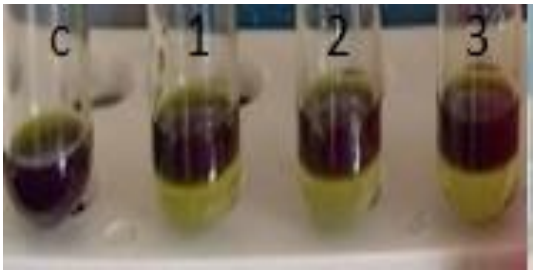


Fig 2: Results of flavonoids

Fig 3: Results of phenols

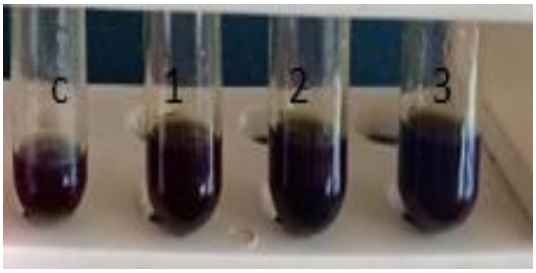


Fig 4: Results of tannins

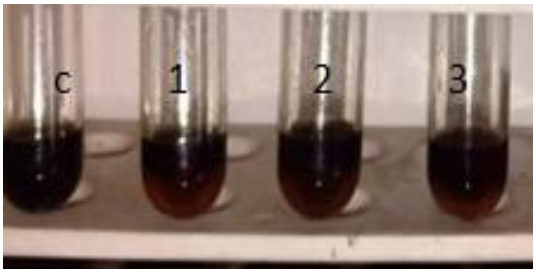


Fig 5: Results of saponins

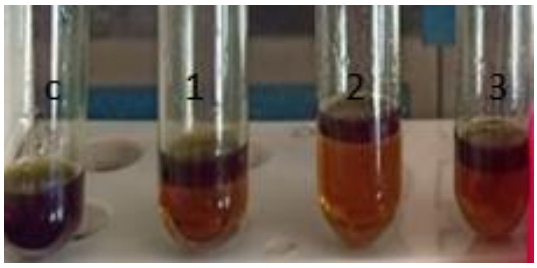


Fig 6: Results of cardiac glycosides

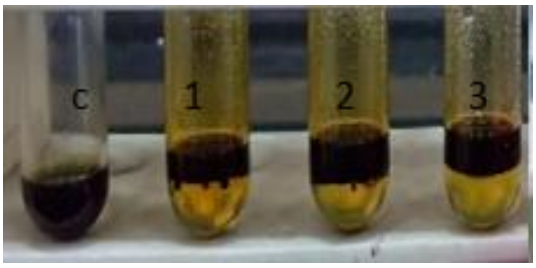


Fig 7: Results of alkaloids

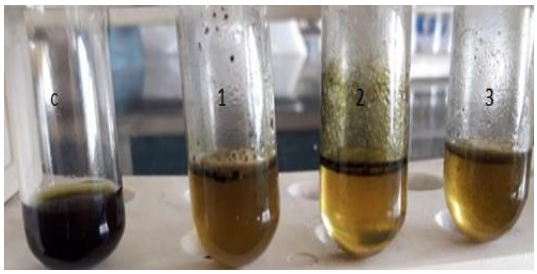


Fig 9: Results of Terpenoids

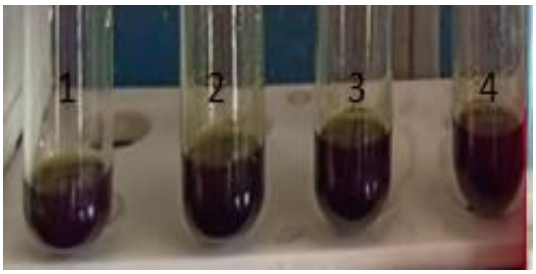


Fig 10: Results of quinones test

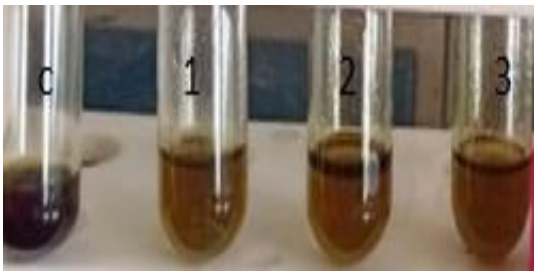


Fig 11: Results of coumarins test
(c is negative control& 1,2,3 test tubes are three trials of the test)

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 2: Results of TLC

Solvent system	Ratio	Rf	Inference
Methanol: distilled water	18:2	0.81	Flavonoids
Ethyl acetate: chloroform: distilled water	5: 3:1	0.86	Flavonoids
n-butanol: ethyl acetate: distilled water	5:10:15	0.88	Tannins
Chloroform: distilled water	6: 4	0.92	Tannins
Hexane: ethyl acetate	6:3	0.92 0.61 0.56 0.49 0.35	Tannins Alkaloids Alkaloids Terpenoids Terpenoids

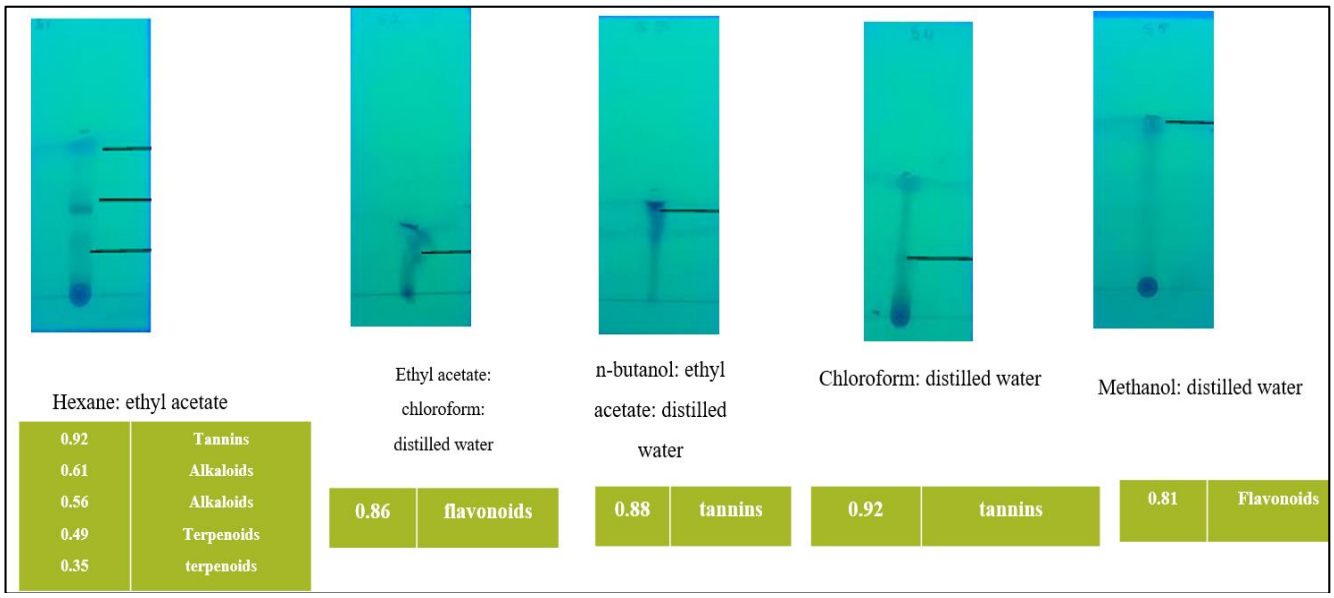


Fig 12: TLC plates observed under the UV light

From the study we can conclude that the solvent (hexane: ethyl acetate) showed the better resolution in separation of the phytochemicals, almost 6 phytochemicals was inferred by comparing the standard.

3.3 Anti-Bacterial Activity

The microbe was inoculated on the LB media and kept for incubation at 28 degree Celsius for 2 days and observed that there was zone of inhibition, from the above figure we can interpret that the ethyl acetate extract has inhibition on the growth of the bacteria.

Table 3: Zone of inhibition

S. No	Zone of Inhibiotion at 3rd Day	Zone of Inhibition at 6th Day	Zone of Inhibition at 9th Day
1	20ul: 0.8cm 25ul:1cm 30ul:1.1cm	20ul: 0.8cm 25ul:1cm 30ul:1.1cm	20ul: 0.8cm 25ul:1cm 30ul:1.1cm
2	20ul:0.9cm 25ul:1.1cm 30ul:1.3cm	20ul:0.9cm 25ul:1.1cm 30ul:1.3cm	20ul:0.9cm 25ul:1.1cm 30ul:1.3cm
3	20ul:0.8 cm 25ul:1.2cm 30ul:1.5cm	20ul:0.8 cm 25ul:1.2cm 30ul:1.5cm	20ul:0.8 cm 25ul:1.2cm 30ul:1.5cm

From this study we can observe that the 30ul (3mg/30ul) was the optimal concentration in inhibition the growth of the microbe, where as minimal zone of inhibition was observed even in 20ul &25ul of sample.

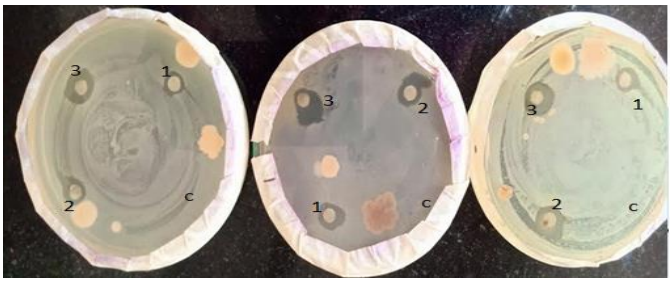


Fig 13: Zone of inhibition observed (c is control, 1 is 20ul, 2 is 25ul &3 is 30ul)

4. Conclusion

The study revealed presence of several phytochemicals present in the extract (flavonoids, alkaloids & coumarins). In TLC results it was observed that (hexane: ethyl acetate) is a better solvent for separation of compounds compared to the other solvents and antimicrobial activity was observed which inhibited the growth of the bacteria.

5. Conflict of Interest

The authors listed in this paper have no conflict of interest known best from our side. There was also no problem related to funding. All authors have contributed equally with their valuable comments which made the manuscript to this form.

6. Acknowledgment

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