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A STUDY OF PHYTOCHEMICAL SCREENING,ANTIOXIDANT,ANTI-BACTERIAL ACTIVITY AND TLC PROFILING OF CYMBOPOGON CITRATUS (LEMON GRASS)

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ABSTRACT The present study was to investigate the Phytochemical constituents in medicinal plants and to analyse the antioxidant and antibacterial activity of the medicinal plants Lemon grass(Cymbopogon Citratus) using TLC Profiling.Plants are natural factories that produce a variety of phytochemicals that have specific physiological effects on living organisms. Medicinal plants are of great importance to the health of individuals. Its various extracts possess reported number of pharmacological activities. Cymbopogon citrates(lemongrass) is a coarse grass with a strong lemon taste.The plant showed many pharmacological effects including antioxidant,antimicrobial,antidepressant,antiseptic, astringent, nervine and sedative properties.Phytochemicals are natural compounds that are found in plants and are responsible for many of the health benefits associated with a plant-based diet. The methanolic extract of lemon grass shows the presence of high number of phytocompounds. It shows the presence of alkaloids, steroids, terpenoids, flavonoids, phenols, cardiac glycosides,Saponins,and Tannins. The in-vitro antibacterial activities in the methanolic extract of lemon grass where studied against Streptococcus pyogenes , proteus mirabilis, staphylococcus aureus, klebsilla pneumoniae.TLC Analysis was carried out in the methanolic extract of the sample was analysed by TLC Plate has showed many variations. In this the Rf values was calculated and the peak values of the plant constituents was obtained to confirm the qualitative characterization of phytochemical screening and to determine the Purity and Quality analysis of the sample .

KEYWORDS : Cymbopogon citrates(lemongrass),phytochemical , Tannins, Saponins, Flavonoids, Alkaloids, Quinones,Streptococcus pyogenes , proteus mirabilis, staphylococcus aureus, klebsilla pneumoniae

INTRODUCTION

Citronella grass or lemongrass is the common name for Cymbopogon citrates class. This species is a member of the Gramineae family, which includes about 500 genera and 8,000 plant species. In our search for novel medicines, studying the phytochemical and pharmacological characteristics of medicinal plant extracts is a sensible strategy. It is a medicinal and fragrant perennial tall grass with rhizomes and fibrous roots that are densely tufted. It is a member of the Poaceae family, which is known for producing a lot of oil. Cymbopogon citratus (DC.) Stapf, commonly known as lemongrass, is a coarse grass with a strong lemon taste. It is a perennial herb widely cultivated in the tropics and sub-tropics. Researchers have found that lemongrass holds antidepressant, antioxidant, antiseptic, astringent, nervine and sedative properties. Other compounds identified in lemongrass EO include limonene, citronellal, β-myrcene and geraniol, along with trace compounds. On the compound level, citral, the major component of lemongrass EO, is known to be antibacterial. Furthermore, an earlier study has also reported about the capacity of thyme EO, cinnamon EO and lemongrass EO to reduce viable spores, which could be an important feature of these EOs, as B. thuringiensis, one aetiological agent of pitted keratolysis, is a typical spore-forming bacterium species, and spore fomation can influence survival and therefore therapeutic success (Kumar S et,al 2000).

Fig 1: Lemon Grass

Taxonomy

Kingdom: Plantae vision: Magnoliophyta Class: Liliopsida
Order: Poales Family: Poaceae
Genus: Cymbopogon Spreng Species: citrates

Morphology

Cymbopogon citratus is part of the grass family,Poaceae.They contain simple, bluish-green leaves with entire margins and are linear in shape. The blades tend to be 18-36 inches long.

Phytochemistry

Phytochemicals are natural compounds that are found in plants and are responsible for many of the health benefits associated with a plant-based diet. These compounds are not considered essential nutrients, but they have been shown to have a positive impact on human health, including the prevention and treatment of chronic diseases such as cancer, heart disease, and diabetes (John T et,al ,2013).

Phytochemicals can be found in a variety of plant foods such as fruits, vegetables, grains, legumes, nuts, and herbs. There are thousands of different types of phytochemicals, and they are often classified based on their chemical structure (Tchen T.T1965).Some common types of phytochemicals include carotenoids,flavonoids, phenolic acids, and glycosylates. Carotenoids, for example,they are responsible for the bright colors of many fruits and vegetables and are important for eye health.

Antioxidant Activity

Antioxidants can protect against the cell damage that free radicals cause, known as oxidative stress.Activities and processes that can lead to oxidative stress include Trusted Source,mitochondrial activity excessive exercise tissue trauma, due to inflammation and injury ischemia and reperfusion damage consumption of certain foods, especially refined and processed foods, trans fats, artificial sweeteners, and certain dyes and additives smoking environmental pollution radiation exposure to chemicals, such as pesticides and drugs, including chemotherapy industrial solvents ozone. Such activities and exposures can result in cell damage (Hamid AA ,et,al 2010).The damage caused by oxidative stress has been linked to cancer, atherosclerosis, and vision loss. It is thought that the free radicals cause changes in the cells that lead to these and possibly other conditions. An intake of antioxidants is believed to reduce these risks. (Teresa MM , et,al 2011).

Antibacterial Activity

Antibacterial activity refers to the ability of a substance or agent to inhibit or kill bacteria. This can refer to natural or synthetic compounds, such as antibiotics, antiseptics, or disinfectants, which are used to treat or prevent bacterial infections.

Streptococcus Pyogenes

Streptococcus pyogenes is a species of Gram-positive, aerotolerant bacteria in the genus Streptococcus. These bacteria are extracellular, and made up of non-motile and non-sporing cocci (round cells) that tend to link in chains. They are clinically important for humans, as they are an infrequent, but usually pathogenic, part of the skin microbiota that can cause Group A streptococcal infection.

Proteus Mirabilis

Proteus mirabilis is a Gram-negative, facultatively anaerobic, rod-shaped bacterium. It shows swarming motility and urease activity. P. mirabilis causes 90% of all Proteus infections in humans. It is widely distributed in soil and water.Proteus mirabilis can migrate across the surface of solid media or devices using a type of cooperative group motility called swarming. Proteus mirabilis is most frequently associated with infections of the urinary tract, especially in complicated or catheter-associated urinary tract infections.

Staphylococcus Aureus

Staphylococcus aureus is a Gram-positive spherically shaped bacterium, a member of the Bacillota, and is a usual member of the microbiota of the body, frequently found in the upper respiratory tract and on the skin. Although S. aureus usually acts as a commensal of the human microbiota, it can also become an opportunistic pathogen, being a common cause of skin infections including abscesses, respiratory infections such as sinusitis, and food poisoning.Pathogenic strains often promote infections by producing virulence factors such as potent protein toxins, and the expression of a cell-surface protein that binds and inactivates antibodies.

Klebsiella Pneumoniae

Klebsiella pneumoniae is a Gram-negative, non-motile, encapsulated, lactose-fermenting, facultative anaerobic, rod-shaped bacterium. It appears as a mucoid lactose fermenter on Mac Conkey agar. Although found in the normal flora of the mouth, skin, and intestines, it can cause destructive changes to human and animal lungs if aspirated, specifically to the alveoli resulting in bloody, brownish colored jelly-like sputum.

Thin Layer Chromatography

Thin layer chromatography (TLC) is an affinity-based method used to separate compounds in a mixture. TLC is a highly versatile separation method that is widely used for both Qualitative and quantitative sample analysis. TLC can be used to analyze virtually any substance class, including pesticides, steroids, alkaloids, lipids, nucleotides, glycosides, carbohydrates, and fatty acids.In TLC, the stationary phase is a thin adsorbent material layer, usually silica gel or aluminum oxide, coated onto an inert plate surface, typically glass, plastic, or aluminum. The sample is spotted onto one end of the TLC plate and placed vertically into a closed chamber with an organic solvent (mobile phase).

MATERIALS AND METHODS

Preparation Of The Lemon Grass Powder

Fig 2: Dried Lemon Grass

Fig 3: Powdered Lemon Grass

Preparation Of The Sample

The leaves of lemongrass were obtained from the company local herbal shop,Chennai, Tamilnadu. The infusion was prepared using 5.0 g of dried lemongrass leaves and adding 50 ml methanol. The plant materials were keep in shaker for 48 hrs.

Soxhlet Extractiion

The dried powdered sample were packed in filter paper and placed in Soxhlet apparatus and extracted against methanol for 6 hrs. The crude stored in air tight container for further analysis.Soxhlet Extraction has been used widely for extracting valuable bioactive compounds from various natural sources.

Phytochemical Analysis

The preliminary qualitative analysis of plant extracts were performed using standard procedures(Harborne.et.al.,1999).

1. Test For Alkaloids

For this test procedure, few drops of Hager's reagent (saturated picric acid solution) added to 2 ml of the respective plant extract. Bright yellow precipitate formation indicated the existence of alkaloids

2. Test For Flavonoids

The plant extract was mixed with 10 ml of ethanol and filtrated.2ml of the filtrate, concentrated HCl, and magnesium ribbon were mixed. The formation of a pink or red color indicates the presence of flavonoids.

3. Test For Cardiac Glycosides

To 1 ml of acetic acid and 2 drops of ferric chloride were added to 2 ml of extract, then 2 ml of sulfuric acid (concentrated) was added and the color change was observed. Reddish-brown color formation was deemed to be a positive test for cardiac glycosides.

4. Test For Terpenoids

Salkowski test was used to detect terpenoids. Extract (5ml) was mixed with chloroform (2ml), and concentrated sulphuric acid (3ml) was carefully added to form a layer. A reddish brown coloration of the inter face was formed to show positive results for the presence of terpenoids.

5. Test For Phenols

A small amount of the ethanolic extract was taken with 1 mL of water in a test tube and 1 to 2 drops of Iron III chloride (FeC13) was added. A blue, green, red or purple color is a positive test.

6. Test For Tannins

Ferric chloride test: A quantity (1 ml) of the filtrate was diluted with distilled water and added 2 drops of ferric chloride. A transient greenish to black color indicates the presence of tannins.

7. Test For Steroids

To 2ml of Distilled water, add pinch of sample, 2ml of chloroform followed by 2ml of Sulphuric acid was added. Formation of Red colour appearance indicates the presence of Steroids.

8. Test For Saponins

1ml solution of extract was diluted with distilled water to 20 ml and shaken in a graduated cylinder for 15 minutes. Development of stable foam suggests the presence of saponins.

Thin Layer Chromatography

Thin layer chromatography (TLC) is a type of planar chromatography. TLC is a convenient method for separation of many compounds based on polarity, binding tightly to the adsorbent and migrating less than others. TLC is a chromatographic technique which is applied for the separation of mixture of compounds.

Solvent System (Mobile Phase)

For each extracts, different solvent systems were developed to run the TLC. The solvent system was made from methanol: chloroform the ratio of 1:1.2 (v/v) for methanol extract.

Preparation Of Tlc Plate

The sterile silica coated plates with the size range of 2cm x 6cm were prepared and the bottom of the sheet is marked with 1cm margin for the application of sample. The lemon grass was spotted on the sheets separately with the help of capillary tubes and they were allowed to dry before introducing them into the running chamber where the respective mobile phase is present. The glass jars marked with specific label are filled with respective mobile phase upto 1cm height from the bottom and the chambers were closed to prevent the evaporation of solvents.With the help of sterile forceps, TLC plates were prepared and kept inside the saturated TLC chamber with the tops leaning against the chamber walls. The plates were removed from chamber with forceps after the solvent reached the top of the plates and the solvent front was marked and dried. The TLC plates were visualized for band formation with a portable UV lamp and the Rf values were calculated by measuring the distance moved by the solvent and distance moved by solute using a scale.

$$\mathbf{RF} = \frac{\text{Distance moved by spot front}}{\text{Distance moved by compounds}}$$

Antioxidant Activity Dpph Assay

Dpph Assay (2, 2- Diphenyl 1- Picrylhydrazyl)

The antioxidant assay was estimated using DPPH as a free radical. (Brand William et al., 1995). 2, 2- diphenyl 1- picrylhydrazyl is a stable free radical and synthesized nitrogen centered radical with three benzene rings. The paired function was lost by unpaired electron of centered nitrogen. The scavenging reaction between antioxidant (H-A) and DPPH was written as, (DPPH) + (H-A) and DPPH-H + (A)

Reagents Required

1. 0.1mM DPPH solution- Dissolved 4mg of DPPH in 100ml of ethanol

2. DMSO
3. Ethanol

DPPH Assay

DPPH assay is commonly used to evaluate the antioxidant activity of plant extract. In the present study, the free radical scavenging activities of different extracts of lemon grass was evaluated using DDPH assay. The result presented in Table no shows the free radical scavenging activities using DPPH assay for methanol extracts of lemon grass. The free radical scavenging activities has been carried out with 2µg/ml, 4 µg/ml, 6µg/ml, 8µg/ml, 10 µg/ml concentrations with all the five extracts.The various concentration of plant extracts were taken from 2 µg/ml to 20 µg/ml and make upto 40 ul with DMSO. Then, 2.96ml of DPPH solution (0.1mM) was added to the mixture and kept for incubation at dark room for 20min. After incubation, the optical densities were read at 517nm.For control, 3ml of DPPH was taken for the reaction.

Calculation: The free radical scavenging activity was calculated using the below formula

$$\text{Radical scavenging activity (\%)} = \frac{\text{Abs control} - \text{Abs sample} \times 100}{\text{Abs control}}$$

Antibacterial Activity

Collection Of Bacterial Strains:

The pure cultures of bacterial strains were used in the present study namely Klebsiella pneumonia, Enterococcus faecalis, Pseudomonas fluorescens, Streptococcus pyogenes were collected.Thirty four grams of Muller Hinton agar was dissolved in 1000ml of distilled water and boiled till dissolved completely. The dissolved medium was autoclaved at 15lbs pressure at 121° for 15minutes. The medium were then poured to 100mm petridishes and allowed to solidify.

Nutrient Broth:

The liquid medium composed of Peptone - 5.0g
Beef extract - 3.0g NaCl- 5.0g Distilled water - 1000ml
PH - 7.3 ± 0.1 at 25°

Dissolved all the reagents in 1000ml of distilled water and autoclaved.

Standard Antibacterial Agent

0.9% Saline solution:

Dissolved 9g of NaCl in 700ml distilled water and made upto 1000ml.

Mcfarland Standard Preparation

0.5 McFarland standard was prepared using 0.05ml 0f 1.175% barium chloride dehydrate ($BaCl_2.H_2O$) and 9.95ml of 1% sulphuric acid (H_2SO4). The two chemicals are mixed well to form a barium sulphate precipitate which causes turbidity.

Antibacterial Assay

Agar- Disk Diffusion In Vitro Method

The zone of inhibition assay using agar disk diffusion method was performed by the standard operating procedure

Procedure:

The prepared solidified Muller Hinton agar plates were streaked evenly on the surface of the medium using sterile glass L-rod. After 5 minutes, sterile Whatmann No.1 filter paper discs with 6mm in diameter were impregnated with different concentration (25mg/ml, 50mg/ml and 100mg/ml) of five extracts were dried at room temperature. Then the discs were placed on the medium surface and gently press down for proper adherence using sterile forceps. The readily available standard antibiotics discs (30µg/ml) were placed on the surface of the medium. These plates were then incubated at 37℃ for 24h. After the incubation period, the diameter of the zone of inhibition was measured with the help of a ruler. The test was performed thrice and the results were substantially expressed as mean ± standard error (SE).

RESULTS

Phytochemical Analysis:

The phytochemical components were analysed: phenolic compounds ,Flavonoids, Alkaloids, Terpenoids, Tannins, saponins, triterpenoids and cardiac glycosides

Table 1: Qualitative Analysis Of Phytochemicals Present In Methanolic Extract

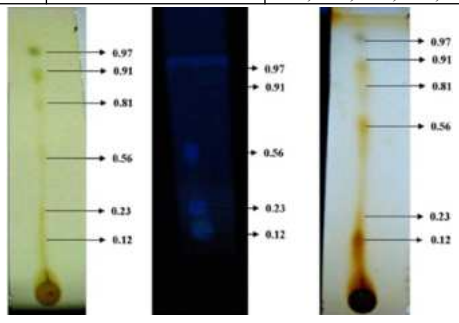
S. no	Phytochemicals	Results
1	Alkaliods	(++)
2	Flavonoids	(+++)
3	Tannin	(+)
4	Glycosides	(+)
5	Cumarine	(+)
6	Terpenoids	(++)

- Not detected
+ Mild Positive
++ Moderate Positive
+++ Strong Positive

Thin Layer Chromatography

Rf value of the fractions separated from plant using methanol solvents

Extract	Solvent system	Rf Values
Methanol	Methanol: Chloroform	0.97,0.91,0.81,0.56,0.23,0.12



Antioxidant Activity Test

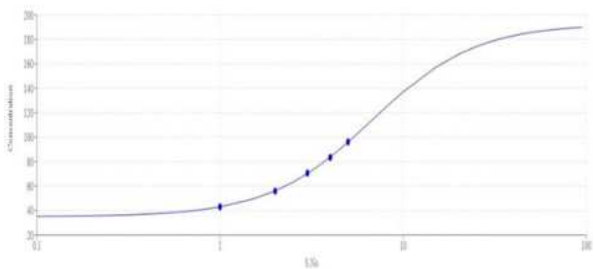
Dpph Test (2,2-Diphenyl Picryl Hydrazyl)

S. No	Concentration	Percentage of inhibition
1	20 mg/ml	65.95
2	40 mg/ml	71.62
3	60 mg/ml	81.25
4	80 mg/ml	86.75
5	100 mg/ml	92.24
6	10 mg/ml (Control)	98.52

$$Y = \text{Min} + \frac{\text{Max} - \text{Min}}{1 + \left(\frac{X}{IC50}\right)^{\text{Hill coefficient}}}$$

$$Y = 35.0021 + \frac{192.5976 - 35.0021}{1 + \left(\frac{X}{6.7395}\right)^{-1.54}}$$

$^{IC}50$	6.7395



Antibacterial Activity

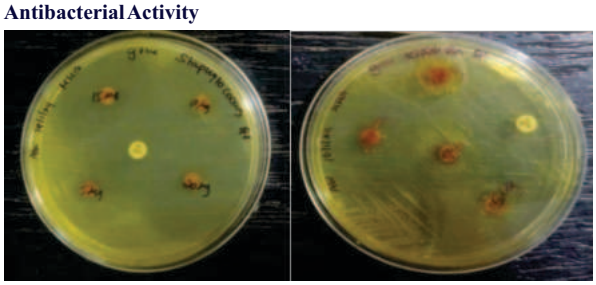


Fig 4: Staphylococcus Aureus **Fig 5:** Klebsella Pneumonia

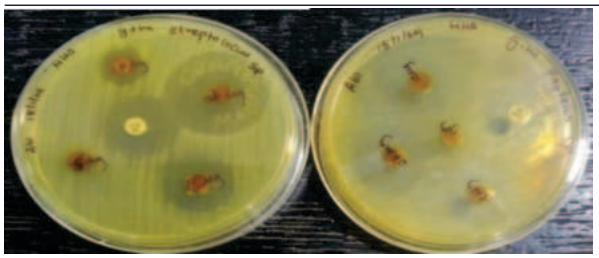


Fig 6: Streptococcus Pyrogenes **Fig 7:** Proteus Mirabilis

STANDARD DISKS	CONCENTRATION OF LEMON GRASS			
	5µg	**10µg**	**15µg**	**20µg**
Ceftriaxone	8mm	12mm	19mm	27mm
Ceftriaxone	13mm	20mm	22mm	28mm
Ceftriaxone	12mm	17mm	34mm	40mm
Ceftriaxone	16mm	27mm	30mm	34mm

DISCUSSION

Plants have been used for medicinal purpose long before prehistoric period these medicinal plants are also used as food, flavonoids, medicine side effects of several synthetic drugs and development of resistance to currently used drugs for infectious diseases have led to increased emphasis on the use of plant material as a source of medicines for a wide variety of human ailments.The qualitative phytochemical analysis of the lemon grass is summarized in Quantification of important phytocompounds. The methanolic extract of lemon grass shows the presence of high number of phytocompounds. It shows the presence of alkaloids, steroids, terpenoids, flavonoids, phenol, cardio glycosides,, Saponins, and Tannins.

Phytochemicals such as Saponins, terpenoids, and alkaloids have hypoglycemic activities. The Lemon grass show positive result for Flavonoids which can act as antioxidants. Phytochemicals have highest therapeutic efficiency in pharmaceutical field; it helps to undertake further studies on isolation and identification of specific phytocomponents for pharmacological studies.The in-vitro antibacterial activities in methanolic extract of lemon grass where studied against Streptococcus pyogenes , proteus mirabilis, staphylococcus aureus, klebsilla pneumoniae. The antibacterial activity of the methanol extracts tested was mainly effective against Gram-negative bacteria, which have a hydrophilic outer membrane owing to their lipopolysaccharides molecular structure. Thus, small hydrophilic molecules pass through the outer membrane. This outer membrane also has properties that allow it to by pass the lipophilic compounds and macromolecules and permeating the outer membrane of the micro-organisms is prerequisite condition for any solute that exhibits antibacterial activity. The methanolic extracts have limited solubility in water it penetrates the outer membrane of Gram-negative bacteria and disturbs their cellular function, metabolism, and cellular constituents. This study shows that lemon grass methanolic extract had the antioxidant Activity and Thin layer chromatography is done in the lemon grass to verify the presence of Flavonoids.

CONCLUSION

The present research work was aimed to do phytochemical screening Antioxidant and Antibacterial activity and TLC Profiling on the methanolic extract of lemon grass. The qualitative analysis showed the presence of Alkaloids, Flavonoids, Tannins, Cardiac Glycosides, steroids, saponins and Terpenoids .In quantitative analysis the lemon grass shows highest Flavonoids content. The antibacterial activities were tested against the Streptococcus pyogenes , proteus mirabilis, staphylococcus aureus, klebsilla pneumoniae by invitro agar well diffusion assay. The zone of inhibition was increased with the concentration of methanolic extracts, hindering bacterial growth.The lemon grass has antibacterial agents in the treatment of infectious organism, lemon grass has its unique therapeutic efficacy the plant could be utilized as an alternative source of useful antimicrobial drugs, Since many of the existing synthetic drugs cause various side effects.

REFERENCES

1. Hamid AA, Aiyelaagbe OO, Usman LA, Ameen OM, Lawal A (2010) Antioxidants: its medicinal and pharmacological applications.
2. Harborne, Jeffrey B: Baxter. Herbert: Moss, Gerard P., eds. (1999). General Introduction;. Phytochemical dictionary a handbook of bioactive compounds from plants (2nd ed.). London.
3. John T. Arnason; Rachel Mata; John T. Romeo (2013-11-11). Phytochemistry of Medicinal Plants.
4. J. Nguefack, O. Tamgue, J. Dongmo, C. Dakolea, V. Leth, H. Vismer, P. Zollo, A. Nkengfack Synergistic action between fractions of essential oils from Cymbopogon citratus,Ocimum gratissimum and Thymus vulgaris against Penicillium expansum Food Control23 (2012)
5. Kumar S, Dwivedi S, Kukreja AK, Sharma JR, Bagchi GD, editors. Cymbopogon: The Aromatic Grass Monograph. Lucknow, India: Central Institute of Medicinal and Aromatic Plants; 2000.
6. Tchen, T. T. (1965). "Reviewed Work: The Biosynthesis of Steroids, Terpenes & Acetogenins
7. Teresa MM, Magdalena W, Andrzej KA (2011) Systematic review of the effect of vitamin C infu-sion and vitamin E-coated membrane on hemodialysis-induced oxidative stress.