

Invitro Study of Antibacterial Activity of *Cymbopogon Citratus*

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II. MATERIALS AND METHODS

Collection of Plant Material

The plant material were collected from Kolli Hill of Tamil Nadu and used for the study.

Extraction of Leaf Extracts:

The fresh leaves lemon grass was carefully washed under tap water and cut into tiny pieces. Aqueous extract was prepared as follows: 1kg whole leaves of the plant was blended in one litre of distilled water in a waring blender and sieved through a Buchner funnel to remove debris. Similarly the methanol extract was prepared by blending 1kg of the plant leaves with methanol. The extract was sieved through a Buchner funnel also to remove debris. The aqueous phase was concentrated on a rotatory evaporator to a small volume and then freeze-dried and used for further study.

Preparation of Stock Solutions

The stock solutions of methanol and aqueous were made by dissolving 1mg of the plant extract in 1ml of methanol and 1ml of distilled water. From the stock solutions serial dilutions were made to obtain the test solutions of different concentration of 1/1, 1/5, 1/10 and 1/20 respectively.

Antibacterial Assay

Antibacterial activity was done by the disc diffusion method, which is normally used as a preliminary check and to select between efficient concentrations of the extract. It was performed using an 18 h culture at 37°C.

The plant extract disc was prepared from Whatman filter paper by punching with a cork borer of 6mm diameter. The disc was autoclaved at 121°C for 15mins. The plant extract disc was dried in an oven and stored in refrigerator until required for use.

The test organism ie four gram-negative bacteria *Escherichia coli*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Proteus vulgaris*) and two grampositive bacteria *Bacillus subtilis* and *Staphylococcus aureus*) collected from National Chemical Laboratory, Pune were cultured on Nutrient Agar plates prepared by dissolving 28g of Nutrient Agar in one liter of water. The media was autoclaved at 121°C for 15mins. 9ml of this media was poured in plates and left to solidify. The plant extract discs were placed in the triplicate cultured plates (disc-diffusion method) using a sterile forceps. The discs were placed far from each other to avoid overlap of zone of inhibition. The culture was incubated in an incubator for 24hrs at 37°C. After 24hrs, the zone of inhibition of plant extracts was observed and measured. The zone of inhibition was measured and expressed in millimeter. Antibacterial activity was recorded if the zone of inhibition was greater than 6mm [2],[3] Sterile water disc was used as negative control and antibiotic disc used as positive control. For each microbial species, negative control was maintained where 100µl of methanol and distilled water without the drug was used for methanol extract

Abstract—Alcohol and water extracts of *Cymbopogon citratus* was investigated for anti-bacterial properties and phytochemical constituents. The extract was screened against four gram-negative bacteria *Escherichia coli*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Proteus vulgaris*) and two grampositive bacteria *Bacillus subtilis* and *Staphylococcus aureus* at four different concentrations (1:1, 1:5, 1:10 and 1:20) using disc diffusion method. The antibacterial examination was by disc diffusion techniques, while the photochemical constituents were investigated using standard chemical methods. Results showed that the extracts inhibited the growth of standard and local strains of the organisms used. The treatments were significantly different ($P = 0.05$). The minimum inhibitory concentration of the extracts against the tested microorganisms ranged between 150mg/ml and 50mg/ml. The alcohol extracts were found to be generally more effective than the water extract. The photochemical analysis revealed the presence of alkaloids and phenol but absence of cardiac and cyanogenic glycosides. The presence of alkaloid and phenols were inferred as being responsible for the anti-bacterial properties of the extracts.

Keywords—*Cymbopogon citratus*; gram negative and gram positive

I. INTRODUCTION

Cymbopogon citratus (DC) Stapf. (lemon grass), Poaceae – Gramineae, commonly known as lemon grass as well as oil grass is a native herb from India and is also cultivated in other tropical and subtropical countries. It is a tall perennial grass. It is consumed as an aromatic drink and used in traditional cuisine for its lemon flavor. Its leaves are used to make tea which can relieve stomach and gut problems. In many countries it is used to treat feverish conditions and as a relaxant and sleeping aid. It helps with emotional states and it is an antidepressant agent *Cymbopogon citratus* contains active ingredients like myrcene, an antibacterial and pain reliever, citronellal, citronellol and geraniol. Medicinal plants are the only affordable and accessible source of primary health care for them, especially in the absence of access to modern medicine facilities. Studies reveal that there are more traditional medicine providers than the allopathic providers especially in the rural areas WHO (2002-2005). The spread of drug-resistant pathogens is one of the most serious threats to successful treatment of microbial diseases. Throughout the ages essential oils and other extracts of plants have evoked interest as sources of natural products. They have been screened for their potential uses as alternative remedies for the treatment of many infectious diseases. The World Health Organization (WHO) noted that the majority of the world's population depends on traditional medicine for primary healthcare. Medicinal and aromatic plants are widely used as medicine and constitute a major source of natural organic compounds.

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and aqueous extract. Also, conventional drugs were used for positive controls. The results were recorded by measuring the diameter of the zones of growth inhibition surrounding the wells (cylinders).

Phytochemical Screening of Extracts

The phytochemical screening of the extracts was done using standard procedure as described by [3]. The following qualitative tests were carried out as follows.

A) *Steroids and Terpenoids*

10mg of extract was dissolved in chloroform. Few drops of acetic anhydride were added followed by 1 ml of con Sulphuric acid. Blue colour in chloroform layer which changes to green shows the presence of steroids, whereas the appearance of pink colour in chloroform layer shows the presence of terpenoids.

B) *Alkaloids*

10mg of extract is dissolved in con HCL and filtered. A few drops of solution is poured into the centre of watch glass. Mayer reagent is added in the sides of the watch glass with the help of a glass rod. Formation of a gelatinous white precipitate at the junction of the two liquid shows the presence of alkaloids.

C) *Flavonoids*

10mg of extract was dissolved in methanol. Magnesium turnings were added into this followed by con HCL. A magenta colour shows the presence of Flavonoids.

D) *Coumarins*

10mg of extract is dissolved in methanol and alcoholic KOH was added. Appearance of yellow colour which decolorizes while adding con HCL shows the presence of Coumarin

E) *Saponins*

Extract was dissolved in water and shaken well. Froth which last for a long time shows the presence of saponins

F) *Tannins*

10 mg of extract was boiled with 1 ml water for 30 min. the extract is filtered clear and to this 0.5 ml 2% gelatin was added. A curdy white precipitate indicates the presence of tannin.

G) *Phenolic compounds*

Extract was dissolved in alcohol and 1 drop of neutral ferric chloride was added to this. The intense colour indicates the presence of phenolic compound.

H) *Anthraquinone*

To the extract Magnesium Acetate solution was added the pink colour developed indicates the presence of Anthraquinone.

I) *Quinone*

Few mg of the substrate in alcohol is treated with sulphuric acid. The colour developed indicates the presence of Quinone.

J) *Catechin*

Few mg of the substrate in alcohol is treated with few drops of Ehrlich reagent and few drops of concentrated HCl. The pink colour developed indicates the presence of catechin.

Test for cardiac glycosides

Five milliliter of each aqueous extract was treated with 2ml of glacial acetic acid which contains one drop of ferric chloride solution. One milliliter of H₂SO₄ was later added. The formation of a brown ring indicates the presence of cardenoids.

Quantitative Determination

To determine the quantity of each of the constituents, 2g of each powdered sample was first defatted with 100ml of diethyl ether for 2h using a soxhlet apparatus.

Determination of Total Phenol

This was determined by spectrophotometric methods. Each fat-free sample of the plants was boiled with 50ml of ether for 15min. 5ml of the extract is added to 10ml of distilled water. Two milliliter of ammonium hydroxide solution and 5ml concentrated amyl alcohol were also added. The mixture was left for 30mins after which it was observed for colour development. The optical density was read at a wavelength of 505nm.

III. RESULTS

The result showed that the extract screened against four gram-negative bacteria *Escherichia coli*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Proteus vulgaris*) and two gram-positive bacteria *Bacillus subtilis* and *Staphylococcus aureus* at four different concentrations (1:1, 1:5, 1:10 and 1:20) using disc diffusion method shows that lemon grass possessed bactericidal. The methanol and aqueous extracts of *C. citratus* inhibited all the test organism but however The percentage inhibition of varied with the type of leaf extracts, extract concentration, as well as the type of bacteria. The results showed that inhibition of microbial growth was greater at high concentrations of the methanol extracts and, less inhibition was observed as the concentration was lowered. Though the zone of inhibition was seen in water extract but it was less when compared with methanol extract. This implies that the inhibitory compound of the plant extracts are either more efficacious, or exist in higher concentrations and also the inhibitory compound is more soluble in alcohol than water. This shows that the effectiveness of the extracts is directly related to the concentration of the extracts. The crude leaf extracts from methanol significantly ($P \leq 0.05$) inhibited growth of all the bacteria more than their aqueous extracts (Table 1). The control did not inhibit growth of all the bacteria. Phytochemical studies of the plants extracts showed the presence of tannins, saponins, alkaloids, phenol and steroids in both the water and alcohol extracts. All extracts showed the presence of glycosides, but none showed the presence of O and C glycosides. (Table 2).

TABLE I ANTIMICROBIAL ACTIVITY OF C. CITRATUS AGAINST, AND E. COLI, K. PNEUMONIAE, P. AERUGINOSA, B. SUBTILIS AND S. AUREUS USING DISC DIFFUSION METHOD

| Pathogen | Methanol Extract | | | | Water Extract | | | |
|-------------------------------|------------------|------|------|------|---------------|------|------|------|
| | Concentration | | | | Concentration | | | |
| | 1:1 | 1:5 | 1:10 | 1:20 | 1:1 | 1:5 | 1:10 | 1:20 |
| <i>Escherichia coli</i> | 14.3 | 11.6 | 7.8 | --- | 12.8 | 10.5 | --- | --- |
| <i>Klebsiella pneumoniae</i> | 17.2 | 14.3 | 9.4 | --- | 13.7 | 11.7 | --- | --- |
| <i>Pseudomonas aeruginosa</i> | 26.5 | 19.8 | 12.5 | 9.6 | 17.2 | 14.6 | 8.1 | --- |
| <i>Proteus vulgaris</i> | 22.2 | 20.3 | 19.4 | 12.2 | 19.8 | 16.9 | 14.8 | 8.2 |
| <i>Bacillus subtilis</i> | 22.6 | 18.4 | 14.2 | 8.5 | 18.5 | 15.5 | 10.5 | --- |
| <i>Staphylococcus aureus</i> | 24.8 | 20.5 | 19.4 | 12.1 | 19.4 | 14.7 | 10.3 | --- |



Fig. 1 Preliminary Phytochemical Screening of Plant extract of C. citratus.

TABLE II THE RESULT OF THE QUALITATIVE ANALYSIS OF THE PHYTOCHEMICAL COMPONENTS OF THE PLANTS

| Sno. | Chemical Constituents | Results | |
|------|-----------------------|---------------|------------------|
| | | Water Extract | Methanol Extract |
| 1 | Steroids | +ve | -ve |
| 2 | Terpenoids | -ve | -ve |
| 3 | Alkaloids | +ve | +ve |
| 4 | Flavanoids | -ve | -ve |
| 5 | Coumorins | -ve | -ve |
| 6 | Tannins | +ve | +ve |
| 7 | Saponins | +ve | +ve |
| 8 | Phenolic Compounds | +ve | +ve |
| 9 | Anthra Quinone | -ve | -ve |
| 10 | Quinone | +ve | +ve |

IV. DISCUSSION

Plant extracts have been used for many thousands of years in food preservation [4], pharmaceuticals, alternative medicine and natural therapies [5],[6]. It is necessary to investigate those plants scientifically which have been used in traditional medicine to improve the quality of healthcare. Plant extracts are potential sources of novel antimicrobial compounds [7] especially against bacterial pathogens. *In vitro* studies in this work showed that the plant extract s inhibited bacterial growth but their effectiveness varied with the concentration. The antimicrobial activity of many plant extract has been previously reviewed and classified as strong, medium or weak [8]. In our study, alcohol extract exhibited strong activity against the selected bacterial strains. Several studies [9] ,[10] have shown that lemon grass had strong and consistent inhibitory effects against various pathogens. Even though earlier studies have reported better antimicrobial activity for lemon grass our study correlates with photochemical components involved in the inhibition of the bacteria. This study indicated that plant extract may possess antibacterial activity and can be exploited as an ideal treatment for future human disease management programs eliminating bacterial spread. Recently, there has been a considerable interest in extracts and essential oils from aromatic plants with antimicrobial activities for controlling pathogens and/or toxin producing microorganisms in foods [11], [12], [13], [14]. Essential oils are natural products extracted from vegetal materials, which because of their antibacterial, antifungal, antioxidant and anti-carcinogenic properties can be used as natural additives in many foods [15]. In general, the levels of medicinal plants and their compounds necessary to inhibit microbial growth are higher in foods than in culture media. This is due to interactions between phenolic compounds and the food matrix [16], [17] and should be considered for commercial applications. The plant extracts and/or essential oil, especially the oil for its citral content, presented positive antibacterial activity for *Escherichia coli* [18]. *Pseudomonas aeruginosa*, *Streptococcus pneumoniae*, *S. pyogenes*, *Neisseria gonorrhoeae*, *Clostridium perfringens* *Aeromonas veronii* biogroup *sober*, *Enterobacter faecalis*, *Klebsiella pneumoniae*, *Salmonella enterica* subsp. *Enterica* sorotipo *typhimurium*, *Serratia marcescens* *Proteus mirabilis*, *Shigella flexneri* and *Salmonella typhi* [18],[19],[20].

Antimicrobial properties of plants are desirable tools in the control of undesirable microorganisms especially in the treatment of infections diseases and in food spoilage. The active components usually interfere with growth and metabolism of microorganisms in a

negative manner [21]. Preliminary phytochemical screening showed that the aqueous extracts contain most of the phytochemicals (Table 5 and 6) like alkaloids, tannins, saponins, flavonoids, quinine and anthraquinone. Several phenolic compounds like tannins present in the cells of plants are potent inhibitors of many hydrolytic enzymes such as proteolytic macerating enzymes used by plant pathogens. Other preformed compounds like saponins also have antifungal properties Many plants contain non-toxic glycosides that can get hydrolyzed to release phenolics that are toxic to microbial pathogens [22]. Therefore, the compounds detected may be responsible for the antibacterial activity. Similarly various other compound present in several plants showed such antimicrobial activity against disease causing pathogens. [23], [24],[25].

In conclusions of this study it is possible to state that the lemon grass bear antimicrobial activity. Comparisons with pertinent data from literature indicate that, according to the methodology adopted in studies on antimicrobial activity, the most diverse results can be obtained. Plant extracts have shown inhibitory effect on the growth of the bacteria studied, although of distinct forms. It is therefore recommended that the nature and the number of the active antibacterial principles involved in each plant extract be studied in detail.

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