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Study of phytochemistry and antimicrobial activity of leaf extract of *Cymbopogon citratus*

• **Subah Nau Shahzadi** • **Ruby Kumari**
• **Urvashi Sinha**

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Corresponding Author : Urvashi Sinha

Abstract : *The leaf extracts of Cymbopogon citratus were subjected to phytochemical and antimicrobial analysis. Chloroform extract showed the best results. Presence of tannin, favonoid, phenol, carbohydrate and volatile oil were observed in the chloroform extract which showed the maximum activity against the pathogenic bacterial strains Escherechia coli, Staphylococcus aureus, Salmonella typhi and fungal strain Candida albicans with 7mm, 1mm, 3mm and 4mm of inhibition zone respectively compared to hexane and methanolic extract which showed the least activity. The dilution susceptibility test method was used to determine the Minimum Inhibition Concentration (MIC) of the chloroform extract. MIC value was determined as22.5mg/ml. The present data provides the basis that leaf extract of Cymbopogon citratus can also be used for therapeutic purposes against common pathogenic microbes.*

Key Words : *Antimicrobial activity, Cymbopogon citratus, MIC, zone of inhibition.*

Subah Nau Shahzadi
B.Sc. III year, Botany (Hons.),
Session : 2011-2014, Patna Women's College,
Patna University, Patna, Bihar, India

Ruby Kumari
B.Sc. III year, Botany (Hons.),
Session : 2011-2014, Patna Women's College,
Patna University, Patna, Bihar, India

Urvashi Sinha
Assistant Professor, Deptt. of Botany,
Patna Women's College, Bailey Road,
Patna – 800 001, Bihar, India.
E-mail : .urvashi_vrm@yahoo.co.in

Introduction :

Cymbopogon citratus (Lemongrass) belongs to the section of Andropogon called *Cymbopogon* of the family Gramineae. *Cymbopogon* is a genus of about 55 species, which are endogenous in tropical and semitropical areas of Asia and are cultivated in South and Central America, Africa and other tropical countries. These are tufted perennial C4 grasses with numerous stiff stems arising from a short, rhizomatous root stock (Kumar e*t al*., 2000). Leaves are very large and long, numerous erect lower ones sometimes reduced to their sheaths. Spikelet are very small, arranged in couples, one stalked, containing one male flower, and the other sessile with one hermaphrodite and often one barren flower, (Burger *et al.,* 1986).

Literature suggest the presence of Alkaloids, tannins, sugars, flavonoids, phenols in the leaf extracts of *Cymbopogon citratus* (Nehra *et al*., 2013). It has also been suggested that the antimicrobial activity is mainly due to the presence of alkaloids and other polyphenolic compounds or due to free hydroxyl groups (Simon *et al*., 1984).

Infectious diseases are the world's leading cause of premature deaths, killing almost, 50,000

people every day due to secondary infections (Nehra *et al*., 2013). Lemongrass has been reported to have innumerable therapeutic and other health benefits. Widely used to alleviate certain respiratory conditions including Laryngitis and sore throats. Called Fever grass, the vapour is inhaled, leading to increased perspiration and eventually the complete removal of fever (Kolodziej *et al*., 2005)

Materials and Methods:

Plant material: The plants of *Cymbopogon citratus* were collected from the Nursery near Patna Women's College, Bailey Road, Patna. The fresh leaves were cleaned and then were dried in hot air oven at 45^0C.

Preparation of extract: Dried leaves were ground to a course powder in a blender. To 200mg of coarse powder 500ml of each Hexane, Chloroform and Methanol were added and kept for 24 hours. The solvent from each extract was filtered for phytochemical and anti microbial screening.

Phytochemical Screening: Freshly prepared extracts of the leaves were subjected to phytochemical analysis to find out the presence of the following phyto constituent like sterols, tannins, sugar, alkaloids, flavonoids, saponins, terpenoids and glycosides by standard methods (Trese and Evan 1983; Kokate *et al.,* 1997).

Test for Alkaloid: Three ml of extracts were stirred with 3 ml of 1% HCl on steam bath. Mayer and Wagner's reagent was then added to the mixture. Brown red precipitate was taken as an evidence for the presence of alkaloid.

Test for Tannin: Two ml of extracts were stirred with 2 ml of $FeCl_3$ solution. Formation of green colour indicated the presence of tannin.

Test for Saponin: Five ml of extracts were shaken vigorously with 5 ml of distilled water in a test tube and warmed. The formation of stable foam was taken as an indication of the presence of saponin.

Test for Flavonoid: Two ml of extracts were treated with 2 ml of 10% lead acetate solution. Formation of a yellow precipitate was taken as a positive test for flavonoid.

Test for Terpenoid: Two ml of extracts were treated with 2 ml chloroform and evaporated to dryness. 2 ml of concentrated H_2SO_4 was added. Development of a greyish colour indicated the presence of terpenoid.

Test for Glycoside: Two ml of extracts were treated with 2 ml of acetic acid. The solutions were cooled in ice. H_2SO_4 was then added carefully. A colour change from violet to blue to green indicated the presence of glycoside.

Test for Steroid: Two ml of leaf extracts were dissolved in 2 ml of chloroform. 2 ml concentrated H_2SO_4 was added in it. The upper layer turned red and H_2SO_4 layer showed green colour. This indicated the presence of steroid.

Test for Phenol: Few drops of 5% ferric chloride were added to 2ml of extracts. Appearance of black colour indicated the presence of phenol.

Test for Volatile Oils: The extracts were distilled with water by steam distillation and the distillates were collected in a graduated tube. The aqueous portion which separated automatically was returned to the distillation flask. The formation of emulsion which floated on top of the aqueous phase owing to its low density was indicative of the presence of volatile oils.

Test for Carbohydrate: Two ml of leaf extracts were taken and few drops of iodine were added to it, purple colour indicated the presence of carbohydrates.

Anti microbial analysis:

Test microorganism: Pure culture of *Staphylococcus aureus, Salmonella typhi, Escherichia coli and Candida albicans* were

obtained from the Microbiology department, PMCH, Patna. All the strains used for the study was sub-cultured in the laboratory of the department of Microbiology Patna Women's College, Patna.

Media used:

For bacterial strains: In 1000ml of distilled water 28g Nutrient Agar was dissolved. Plates of the media were sterilized for 24 hours 37°C.

For fungal strain: In 1000ml of distilled water 65g Sabouraud's Dextrose Agar was dissolved. Plates of the media were sterilized for 24 hours 37°C.

Disc diffusion method: 100 Discs of uniform shape and size were sterilized. The discs were impregnated with the plant extracts and with control Chloramphenicol and Fluconazole for bacterial and fungal strain respectively. The discs were kept in opaque vials in refrigerator till use.

Antimicrobial analysis: Loop full of each bacterial isolates was mixed with 5ml of sterilized saline water. With the help of micropipette 100 micro liters of each isolates were spread over the surface of the media with the help of a sterile glass spreader. All the plates were incubated at 37°C for 15-20 minute. Three discs (1 of each solvent) were loaded on 3 plates (1 plate for each bacterium) strictly under aseptic condition. One disc of positive control with Chloromphenicol was loaded on all the three plates as control measure. The plates were incubated at 30°C for 24 hrs. The diameters of the zones of complete inhibition were measured and these apparent zones of inhibition indicated the antibacterial activity.

Similarly plates for fungal isolate were also prepared. The positive control used for fungus was Fluconazole. The zones of inhibition indicated the antifungal activity.

Concentration screened: Concentrations ranging from 2**.5, 5, 7.5,** 10, 12.5, 15, 17.5, 20, 22.5 and 25mg/ml concentrations were used to measure the Minimum Inhibition Concentration (MIC) by dilution susceptibility test.

Results and Discussion:

The results of the investigation indicate the presence of five active phytochemicals in the Chloroform extract. Methanol extract contains three whereas the Hexane extract contained only two secondary metabolites as shown in Table 1. All the extracts showed variable degree of antimicrobial activity against selected test microorganisms. Chloroform extract was observed as most effective crude sample against the selected bacterial strain *E.coli*. Hexane extract was found to be more effective against the fungal strain *Candida albicans.* The results of the antimicrobial analysis indicate that chloroform extract had more inhibitory effect on *Escherichia coli* than *Staphylococcus aureus* which showed inhibition zone of 7mm and 1mm respectively. Methanol extract was ineffective against any test microorganism. The results are tabulated in Table 2. The Minimum Inhibition Concentration (MIC) value indicated that high concentration of extract was effective against the pathogenic microorganisms.

Table 1. Phytochemical screening of leaf extract of lemon grass in different solvents

Phytochemical tested	Different Leaf extract in different solvent		
	Chloroform	Hexane	Methanol
Tannin	+	–	+
Flavanoid	+	+	+
Saponin	–	–	–
Steroid	–	–	–
Volatile oil	+	+	–
Terpinoid	–	–	–
Glycoside	–	–	–
Phenol	+	–	–
Carbohydrate	+	–	+

(Present +, absent –)

Table 2. Antimicrobial screening of leaf exract of *Cymbopogon citratus*

Micro-organisms tested	Diameter of zone of inhibition (mm)			Control (+ve)
	Chloroform	Hexane	Methanol	
Staphylococcus aureus	1	–	–	5
Escherichia coli	7	–	–	4
Salmonella typhi	3	4	–	10
Fungi				
Candida albicans	4	5	–	7

Chloramphenicol(bacteria)was used as standared antimicrobial agent. Fluconazole (fungi) was used as standard antifungal agent.

Extraction and phytochemical screening of bioactive agents from medicinal plants permits the demonstration of their physiological activities. The present study on leaf extract of *Cymbopogon citratus* shows the presence of tannins, flavonoids, carbohydrates and phenol. The results are similar to the earlier work of Puatanachokchai *et al*. (2002) which also showed the presence of these phytocompounds. Phytochemical screening of *Cymbopogon citratus* also revealed the presence of volatile oils, called essential oils. According to (IUPAC, 1995), the leaves of the plant posses volatile oil that gives plant their specific aromas which is confirmed by the aroma produced by the sample material during the extraction procedure. According to Kolodziej *et al*. (2005), tannins and phenol compounds have been found to inhibit bacterial and fungal growth and also capable of protecting certain plants against infection. The present study showed that Chloroform extract was most effective against the bacterial strains and it contained most of the phytochemicals. *Staphylococcus aureus* shows minimum inhibition zone and *E.coli* shows maximum inhibition. The Hexane extract showed the maximum inhibitory effect against the fungal strain. The isolation of volatile oils in *Cymbopogon citratus* confirmed the activity showed against the test organisms by this plant and also in part confirmed the report of (Babayi *et al*., 2004) of the oils isolated from same plant by distillation to exhibit great antibacterial activity and also confirmed the potency of this particular plant against skin cancer prevention as reported by (Nakamura *et al*., 2003).

Conclusion:

The results of this study suggest that the leaves of *Cymbopogon citratus* are rich in phytochemicals which have intermediate antimicrobial activity against the pathogenic microbes *Staphylococcus aureus, Salmonella typhi* and *Escherichia.coli. Candida albicans* is markedly resistant to Lemongrass. The MIC of leaf extracts of *Cymbopogon citratus* reveals that a higher dose of the plant extract is required to bring about a significant activity in the body. Four active ingredients were identified in the plant leaf which include flavonoids, tannins, carbohydrates and volatile oils. Further studies can be made to identify the chemical nature of the antimicrobial properties present in the leaf extract of *Cymbopogon citrates*

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