Volume : 5 | Issue : 3 | March 2016 • ISSN No 2277 - 8179 | IF : 3.508 | IC Value : 69.48

Research Paper

Biochemical Analysis and Antibacterial Effect of Cymbopogan Citratus Against Selected Pathogens

Biosciences

KEYWORDS : C.citratus, Antibacterial activity, Biochemical analysis, Pathogens.

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ABSTRACT

The present study is based on the biochemical analysis and investigation of antibacterial activity of aqueous and ethanolic extracts of Cymbopogan citratus. The microorganisms used in the study are pathogens viz Staphylococcus aureus, Proteus vulgaris and Escherichia coli.

The susceptibility of bacterial strains against the two extracts was determined using disk diffusion method. The most susceptible microorganism was S. aureus while the least susceptible organisms were E.coli. Highest antibacterial activity was observed with ethanolic extract of Cymbopogan citratus against S.aureus (12mm) while minimum activity was observed with aqueous extract against E.coli (5mm).

Ethanolic extract of the sample is more effective in comparison to the aqueous extracts in inhibiting the growth of pathogens under study. They were less potent when compared with ciprofloxacin used as positive control.

Biochemical analysis shows the presence of alkaloid, flavanoids, and tannins in both the extracts of Cymbopogan citrates while steroids were found only in the aqueous extracts of the plant.

INTRODUCTION

For ages nature has gifted us plenty of herbs and plants which form the main source of traditional medicines used to help in relief from illness and are still widely used all over the world. Herbal treatment is still used for many health problems. Herbs are safe, less toxic, economical and a reliable key natural resource of drugs all over the world. Medicinal plants are the only affordable and accessible source of primary health care, especially in the absence of access to modern medicine facilities. 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 compound .The medicinal value of plants lies in some chemical substances that produce a definite physiological action in human body. The most important of these bioactive constituents are alkaloids, tannins, flavonoids, phlobotannins, saponins and cardiac glycoside. [1]

Cymbopogon citratus (lemon grass) is an aromatic perennial tall grass with rhizomes and tufted fibrous roots. It is a native herb from India and also cultivated in other tropical and sub-tropical countries. It is used as a traditional folk medicine in the treatment of malaria, typhoid fever, nervous gastro-intestinal disturbances fever, hypertension, stomachache. It has applications in soap, perfumery industries and cosmetics.

The aim of our study is to examine the antibacterial activity and biochemical analysis of ethanol and crude extracts from leaves of *Cymbopogon citratus*.

MATERIAL and METHODS

Collection of plant material----Plant *Cymbopogon citratus* was collected from a local nursery in the month of September. Further whole plant material containing leaves and stems were planted, cultivated and harvested in the department.

Pretreatment of the samples----The leaves of the plant were collected and washed with clean water for 3-4 times and further with distilled water for 2 times. It was chopped and then spread on dried clean blotting paper under the room temperature for 15 days.

Extraction--- The dried samples were grinded and sieved. The powdery sample were then partitioned in two parts:

ETHANOLIC EXTRACT- (12 gms) of powdered sample were extracted with 100ml of ethanol. The powdered sample was soaked in the solvent for 24 hours and plant extract was prepared using reflux and steam distillation method. In this method, plant material is immersed in a solvent in the round- bottomed flask, which is connected to a condenser. The solvent is heated up to its boiling point. As the vapors are condensed, the solvent is recycled into the flask.

AQUEOUS EXTRACT- (12 gms) of powdered sample were extracted with distilled water .The powdered sample was soaked in 100 ml water for 24 hours and plant extract was prepared using the same above mentioned method.

Bacterial Strains – The test organisms used for the study were *E.coli, P.vulgaries, S.aureus.* These organisms were collected from Choithram Hospital and Research Institute, Indore. The organisms were sub-cultured and maintained at 4° C.

Antibacterial Activity— Antibacterial activity of aqueous and ethanolic extracts of the plant was performed by agar disc diffusion method. The plates were prepared by spread plate method by using Muller Hinton agar medium. Plates were inoculated with 0.1 ml of the inoculums of test organisms used in the study. The Hi Media sterile test discs were used. These discs were saturated with 100 µl of test compounds, allowed to dry and were seeded on the inoculated agar plates .The plate were incubated overnight at 37 ^{0}C in incubator. The zone of inhibition of plant extracts was observed and measured. The results were recorded by measuring the diameter (mm) of the zone of growth inhibition surrounding the disc. Sterile water disc was used as negative control and antibiotic ciprofloxacin disc was used as positive control.[2],[3]

Biochemical analysis of extracts—Biochemical analysis for the major phyto-constituents of the plant extracts was done using standard qualitative methods[4]

A) ***Steroids*** - 10mg of extract was dissolved in chloroform. Few drops of acetic anhydride were added followed by 1 ml of conc. Sulphuric acid. Blue color in chloform layer which changes to green shows the presence of steroids, whereas the appearance of pink color 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. Mag-

Volume : 5 | Issue : 3 | March 2016 • ISSN No 2277 - 8179 | IF : 3.508 | IC Value : 69.48

nesium turnings were added into this followed by con HCL. A magenta color shows the presence of Flavonoids.

*D) **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.

RESULTS

1) Crude ethanolic and aqueous extracts of C.citratus were isolated and phytochemical analysis was performed (Table 1). It has been observed that most of the secondary metabolites were identified in the polar extracts. Biochemical analysis showed the presence of alkaloids, flavonoids and tannins in both the extracts of Cympobogan citratus while the presence of steroids was found only in the aqueous extract.

Qualitative analysis of Ethanolic and Aqueous Extracts of C. citratus

Table 1

Biochemical constituents	Ethanol	Aqueous
Steroids	-	+
Alkaloids	+	+
Flavonoids	+	+
Tannins	+	+

The antibacterial activity of Cympobogan citratus showed varied results with solvents used for extraction (Table 2). The test organisms showed inhibition activity with the zone range of 5-15mm. The zone of inhibition was found to be more in the ethanolic extracts in comparison with the aqueous extracts. The zones of inhibition for aqueous extract are 5mm, 9mm, 6mm for E.coli, S.aureus, P.vulgaries respectively. The zones of inhibition for ethanolic extract are 8mm, 12mm, 10mm for E.coli, S.aureus, P.vulgaries respectively. Hence in this study the most susceptible microorganism was S. aureus while the least susceptible organisms were E.coli. Ciprofloxacin was used as a positive control. Test organisms were more susceptible to ciprofloxacin as compared to both the extracts of C.citratus.

Analysis of Antibacterial activity of Ethanolic and Aqueous Extracts of C. citratus

Table 2

Pathogens	Aqueous extract	Ethanol Extract	Ciprofloxacin(control)
E.coli	5mm	8mm	10mm
S.aureus	9mm	12mm	15mm
P.vulgaries	6mm	10mm	13mm

DISCUSSION

Many crude preparations of herbal drugs are used recently in medicinal practices. Ethanol pharmacologists, Botanists, Microbiologists and Natural products chemists are combining the earth for phytochemicals, which could be developed for treatment of infectious diseases. Laboratories of the world have found literally thousands of phytochemicals having inhibitory effects on microorganisms. [5]

Plants are rich in a wide variety of secondary metabolites, such as, tannins, alkaloids, flavonoids, terpenoids and steroids, which have in-vitro antimicrobial activities. Indian traditional system of medicine that is Ayurveda had successfully employed plant derived products in the treatment of all types of ailments in human and animals. [6]

The World Health Organization (WHO) noted that majority of the world's population depends on traditional medicine for primary health care. Medicinal and aromatic plants are widely used as medicine and constitute a major source of natural organic compounds. Different types of plants extracts like crude , methanolic ethanolic, phenolic etc have been used for many thousands of years in food preservation[7] ,alternative medicines and natural medicines[8],[9]

The results showed that inhibition of microbial growth was greater with the ethanol extracts, though the zone of inhibition was seen in water extract but is was less when compared with ethanol extract. This implies that the inhibitory compound of the plant extracts is more soluble in alcohol than water. Plant extracts are potential sources of novel antimicrobial compounds [10] especially against bacterial pathogens. In vitro studies of C.K. Hindumathy, showed that the plant extracts inhibited bacterial growth but their effectiveness varied with the concentration [11]. The antimicrobial activity of many plant extracts has been previously reviewed and classified as strong, medium or weak [12]. In our study, alcohol extract exhibited strong activity against the selected bacterial strains. Several studies [13], [14] 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 show different results in correlation with phytochemical components involved in the inhibition of the bacteria.

Gopinath. S.M et al. studies observed the absence of steroids in both the aqueous as well as ethanolic extracts and presence of alkaloids, flavonoids and tannis in both the polar solvents [3]. In our study, we detected the absence of steroids in ethanolic extracts while presence of steroids in aqueous extracts. We found the presence of alkaloids, flavonoids and tannins in both the polar solvents. These active components usually interfere with growth and metabolism of microorganisms in a negative manner [6] .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.

Ciprofloxacin is the most widely used as the second-generation quinolone antibiotics that came into clinical use in the late 1980s and early 1990s. Since Ciprofloxacin is a broad spectrum antibiotic its bactericidal activity includes most strains of bacterial pathogens responsible for respiratory, urinary tract, gastrointestinal, and abdominal infections, including Gram negative and Gram positive organisms. Therefore it has been used as control in our study.

CONCLUSION

In conclusions of this study it is possible to state that C. citratus possess antibacterial activity. On comparing with the literature and present work indicates that, most diverse results can be obtained. Plants extracts have shown inhibitory effects on the growth of the test organisms studied. Further research is needed toward isolation and identification of active principles present in the extracts which could possibly be exploited for pharmaceutical use.

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