

Phytochemical observation and antibacterial activity of *Cyperus esculentus* L.

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Abstract: In the present study various extracts of *Cyperus esculentus* L. such as acetone, 50% ethanol, chloroform and petroleum ether were evaluated for their antibacterial activity against several human pathogens such as *Escherichia coli*, *Staphylococcus aureus*, *Salmonella sp*, *Klebsiella pneumoniae*, *Proteus vulgaris*, *Pseudomonas aeruginosa* and *Citrobacter freundii*, by using disc diffusion method. The activity of the samples were compared with that of standard antibiotics. The qualitative phytochemical analysis was also carried out with all the extracts of the sample. Of all the extracts 50% ethanol and acetone extract were found to be rich in phytochemicals such as alkaloids, flavonoids, phenols, tannins, steroids, terpenoids and glycosides. Acetone extract showed highest inhibitory activity against *S.aureus*, *K.pneumoniae* and *P.vulgaris*. 50% ethanolic extract showed maximum activity against *E.coli*, *S.aureus* and *Salmonella sp*. Chloroform extract maximally inhibited the growth of *S.aureus* whereas, petroleum ether extract showed positive results against *Salmonella sp* respectively. All extracts were sensitive to *C.freundii*.

Keywords: Antibacterial activity, *Cyperus esculentus*, Phytochemicals, Alkaloids, antibiotics.

INTRODUCTION

India leads to the world, as the source for plant derived drugs, in its wide variety of flora. In the present day world 80% of medical care is still taken care of by traditional systems and home care. The therapies so followed include a major part of plant extracts and their active principle¹. Infectious diseases are world's leading cause of premature death, killing almost 50,000 people everyday².

Resistance to antimicrobial agent is emerging in a wide variety of pathogens and multiple drug resistance (MDR) is becoming common in diverse organisms such as *Staphylococcus aureus*, *S.epidermidis*, *Salmonella typhi* and *S.parathyphi*³. Over the past 20 years, there has been a lot of investigation of plants as sources of new antimicrobial agents. But still there is an immediate need to identify novel substances active towards pathogens with high resistance^{4,5}. Recent studies have shown that several alcoholic extracts of various medicinal plants exhibit antimicrobial activity⁶.

During the last few decades there has been an increasing interest in the study of medicinal plants and their traditional use in different parts of the world⁷. Today according to the World Health Organization (WHO), as many as 80% of the

world's people depend on traditional medicine for their primary health care needs. There are considerable economic benefits in the development of indigenous medicines and in the use of medicinal plants for the treatment of various diseases⁸.

Cyperus esculentus L. or tiger nutsedge also known as *Bhadra musta* in sanskrit; is an annual or perennial herb growing up to 90cm long with solitary stems growing from a tuber. According to ayurveda, the tuber is cooling acrid, galactogue to the bowels, aphrodisiac; sharpens the appetite and improves the taste; useful in eye troubles, burning sensations and leprosy; disturbs both heat production and heat regulation in the body⁹.

The present study investigates the qualitative phytochemical analysis and antibacterial activity of various extracts (acetone, 50% ethanol, chloroform and petroleum ether) of the tubers of *Cyperus esculentus* L. against the selected human bacterial pathogens.

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MATERIAL AND METHODS

Collection of the plant material

The plant material used in the study namely *Cyperus esculentus* L. was collected from the local areas of Coimbatore district, Tamilnadu, India. The plant was confirmed by the Botanist, PSG College of Arts and Science, Coimbatore. The tubers of the plant were used for the assay.

Test microorganisms

The test microbes for determining antibacterial activity such as *Escherichia coli*, *Klebsiella pneumoniae*, *Proteus vulgaris*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Salmonella sp.*, and *Citrobacter freundii* were obtained from PSG Institute of Medical Sciences and Research, Coimbatore. The cultures were carefully cultured in agar slants and maintained at 4°C.

Preparation of various extracts of the plant

The collected plant tubers were shade dried, powdered and 50g of the tubers is mixed with the different solvents such as acetone, 50% ethanol, chloroform and petroleum ether separately in a round bottomed flask and kept air tight for 72 hours and was shaken frequently for uniform mixing and distribution of the powdered sample. Then the solution is filtered through a Whatmann No: 1 filter paper and the solvents present in the filtrate were evaporated to dryness. Finally crude forms of the crystals were obtained which were stored in a refrigerator until further use.

Chemicals used: All the chemicals used were of analytical grade (AR) and were obtained from Hi Media Chemicals, Mumbai, India.

Qualitative phytochemical analysis^{10,11}

The various extracts of the plant tubers were screened for the presence of various secondary metabolites (phytochemicals) such as alkaloids, flavonoids, tannins, phenols, saponins, glycosides, terpenoids and steroids.

In vitro Antibacterial assay:

Disc diffusion method¹²

Muller Hinten agar was prepared in the plates as the media for test organisms. Sterile filter paper discs (Whatmann No: 1) were prepared. The microbial culture was spread evenly onto the surface of the agar plates using a sterile cotton bud

before the discs were positioned on the inoculated agar surface. After the inoculation of the culture the sterile discs were carefully placed onto the agar plates. 20µl of the extracts (corresponding to 100µg of the sample) were then added in the sterile discs. The antibiotics such as chloromphenicol (for *E.coli* and *S.aureus*) and tetracycline (for *P.vulgaris*, *Pseudomonas aeruginosa*, *K.pneumoniae*, *Salmonella sp* and *Citrobacter freundii*) were used as the positive controls. All the plates were incubated at 48 hours at 37°C. After the incubation time, the anti microbial activity was interpreted from the size of the diameter of zone of inhibition measured to the nearest millimeter (mm) as observed from the clear zones surrounding the discs. Zone of inhibition from 15-20mm indicates the maximum antibacterial activity, zone of inhibition from 10-15mm indicates minimum antibacterial activity and zone less than 10mm indicates moderate or no antibacterial activity.

RESULTS

Qualitative phytochemical analysis

The qualitative phytochemical analysis of various extracts of *C.esculentus* has been shown in **Table no: 1**. The qualitative phytochemical analysis revealed the presence of important secondary metabolites such as alkaloids, flavonoids, tannins, steroids, terpenoids and glycosides in the various extracts of the sample. Presence of higher amount of the phytochemicals is indicated by +++ sign, moderate level by ++ sign, low level by + sign and absence by - sign. 50% ethanolic extract and acetone extract of the sample showed the presence of higher levels of phytochemicals when compared to the other extracts. This may be due to the high solubility of the phytochemical compounds in the respective solvent medium.

Antibacterial Activity

The zone of inhibition of the various extracts of the sample against the tested bacterial species has been presented in **Table no: 2**

Maximum antibacterial activity:

50%ethanolic extract of the tubers showed maximum antibacterial activity against *E.coli*, *S.aureus*, and *Salmonella sp*. Acetone extract showed maximum antibacterial activity against *S.aureus*, *K.pneumoniae* and *Proteus vulgaris*.

TABLE: 1 QUALITATIVE PHYTOCHEMICAL ANALYSIS

Test	Acetone	50% Ethanol	Chloroform	Pet. Ether
ALKALOIDS	+++	+++	++	++
FLAVONOIDS	+++	+++	++	++
SAPONINS	-	-	-	-
PHENOLS	++	+++	+	+
STEROIDS	+++	++	+	+
TANNINS	++	+++	+	+
PHLOBATANNINS	-	++	-	-
GLYCOSIDES	++	+++	+	-
TERPENOIDS	++	+++	+	-

Chloroform extract of the sample showed maximum antibacterial activity against *S.aureus*. Petroleum ether extract of the sample showed maximum antibacterial activity against *K.pneumoniae*.

Medium antibacterial activity:

50%ethanolic extract of the tubers showed medium antibacterial activity against *Proteus vulgaris*. Acetone extract showed medium antibacterial activity against *Salmonella sp.*, and *Pseudomonas aeruginosa*. Chloroform extract of the sample showed medium antibacterial activity against *Salmonella sp.*, *K.pneumoniae* and *Proteus vulgaris*. Petroleum ether extract of the sample showed medium antibacterial activity against *E.coli*, *S.aureus*, *K.pneumoniae*, *Proteus vulgaris* and *P.aeruginosa*.

Moderate antibacterial activity:

50%ethanolic extract of the tubers showed moderate antibacterial activity against *Citrobacter freundii*, *Pseudomonas aeruginosa* and *K.pneumoniae*. Acetone extract showed moderate antibacterial activity against *Citrobacter freundii* and *E.coli*. Chloroform extract of the sample showed moderate antibacterial activity against *Citrobacter freundii*,

Pseudomonas aeruginosa and *E.coli*. Petroleum ether extract of the sample showed moderate antibacterial activity against *Citrobacter freundii*.

DISCUSSION:

The Flavonoids exert antimicrobial activity in the wound healing and in the treatment of skin diseases¹³. Preliminary photochemical analysis showed that phenolics and certain secondary metabolites showed antimicrobial activity¹⁴.

Tannins complex compound with astringent and bitter taste indicated that tannins exert antimicrobial activity¹⁵. The antimicrobial activity of the medicinal plants may be related to the presence of natural glycosides and saponins¹⁶.

From the phytochemical analysis it is shown that all extracts of the selected plant, *C.esculentus* L. are rich in various secondary metabolites such as alkaloids, flavonoids, phenolics, and tannins. These might effectively contribute for the antimicrobial activity of the plant. Moderate or less antimicrobial activity of certain extracts against the tested organisms suggests that those microbes are resistant to the extracts and might possess complex chemical protection.

TABLE: 2
ANTIBACTERIAL ACTIVITY OF VARIOUS EXTRACTS OF
C.esculentus L. tubers

Organisms	Zone of inhibition (mm)				
	Standard	50% ethanol	Acetone	Chloroform	Pet. ether
<i>E.coli</i>	20	20	4	4	8
<i>S.aureus</i>	60	20	18	15	10
<i>Salmonella sp</i>	50	18	10	8	15
<i>K.pneumoniae</i>	30	2	20	8	12
<i>Proteus</i>	15	6	15	8	8
<i>P. aeruginosa</i>	20	2	10	2	12
<i>C .freundii</i>	20	2	2	2	2

Zone 15-20mm = maximum inhibition
 Zone 10-15mm= minimum inhibition
 Zone below 10mm = moderate or no inhibition

CONCLUSION

From the present study it could be concluded that all the extracts especially acetone and 50% ethanolic extract were effective against the selected organisms. The same extracts were found to be rich in various phytochemicals which could contribute to the medicinal property of the plant. Further isolation and analysis of the active principles of the plant and their purification and characterization might be still effective in the process of drug development.

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