Introduction

*Cyperus rotundus* L., (Cyperaceae family) is a traditional medicinal plant, which is widespread in many tropical and sub-tropical regions of the world[1]. The medicinal plant was mainly cultivated in India, Sri Lanka, Southern Asia, Australia, South & Central America and Southern United States. *Cyperus rotundus* is one of the oldest known medicinal plants used in folk medicine as a sedative, carminative, stimulant, tonic, aphrodisiac, diuretic, stomachic, anthelmintic, colic remedy and to remove renal calculi[1]. Additionally, they are used as hypertensive, estrogenic, antiemetic, remedy for dysentery and women’s diseases. Infusion of this herb has been used in pain, fever, diarrhoea, dysentery, vomiting and other intestinal problems. It was found to have a number of pharmacological and biological activities such as anti-inflammatory, antidiabetic[2], antidiarrhoeal[3], antiradical, antimutagenic, anti-malarial, antibacterial, antioxidant, free radical scavenging, cytotoxic and apoptotic[4], anti-pyretic and analgesic activities and wound healing property were reported from this plant extracted and characterized compounds. Previously the chemical constituents of the *Cyperus rotundus* plant revealed the presence of major and minor chemical compounds of herbs such as essential oils[5], alkaloids[6], flavonoids[7], steroids, tannins, starch, glycosides, furochromones etc are reported in this plant part. Also many novel mono- and sesquiterpenoids[8,9] derivative compounds has isolated from this plant. Other chemical compounds like phenylpropanoids, phenolic acids, saponin[10] and nitrogenous substances were also been isolated in the plant. We have identified too many more number of compounds are may be present in this plant. But, in the past years few numbers of phytochemical compounds were isolated and structurally identified. In the present study, we extract and preliminary phytochemical analyses from the aerial part of *Cyperus rotundus* flower extract and investigated into *in-vitro* antibacterial activity against gram positive and gram negative bacteria done by agar well diffusion method.

Experimental methods

Plant materials

Fresh aerial part of flowers *Cyperus rotundus* L., was collected from Padmanabhapuram, Kanyakumari District, Tamil Nadu, India in December 2010. The species of this plant was authenticated by taxonomist, and the voucher specimen was deposited to our department.

Extraction/Isolation method

Freshly collected and air-dried aerial parts of the flowers *Cyperus rotundus* (650gm) were powdered in a high speed mixi. The powdered flowers were soaked/extracted in methanol at room temperature for two days. After the methanol extracts was filtered through
Whatmann # 1 filter paper, and then concentrated to obtained the yield 8% (approximate the yield w/w). The crude paste was eluted with silica gel (230–400mesh, Merck) flash column chromatography method. Initially, the paste was eluted and defatted to using petroleum ether and followed by the paste has separated as increasing order of polarity solvent such as chloroform, ethyl acetate and methanol. Chromatographically separated the each fraction has concentrated thus obtained the yields as 0.5, 1.0 and 3.0% (w/w). Each fraction was initially analyzed to the preliminary phytochemical screening test. Further, the phytochemically identified these compounds has been used in the ethnopharmacology studies of anti-bacterial activity.

**Antibacterial activity**

The chromatographic separated and preliminary phytochemical tested compounds were investigated in the gram positive and negative antibacterial activity in the agar well diffusion method. The tested microorganism strains against four different bacteria viz., *Bacillus subtilis*, *Staphylococcus aureus*, *Escherichia coli* and *Pseudomonas aeruginosa* were obtained from the Center for Advanced Studies in Botany, University of Madras, Chennai, India and the biological screening of the compounds were performed there itself. All the bacterial strains were maintained on nutrient agar medium in petriplates at room temperature (30±2°C). From the tested fractions were prepared by dissolved in 10% DMSO and applied range from 5, 10, 25 and 50μg/mL and 10% DMSO has control to added in separated well in the bacteria. The observed results on the antibacterial activity of the compounds and control drugs (tetracycline as a commercial antibiotic) are given in the form of MIC. The MIC value was taken as the lowest concentration of compound that showed prominent inhibition of bacterial growth after 24 hrs of incubation at 37°C.

**Results and discussion**

Chromatographically separated compounds have investigated to preliminary phytochemical functional group analyses and in-vitro antibacterial activity by disc diffusion well method. The chloroform fraction proves the positive phytochemical test results for the presence of steroids when treated with sulphuric acid of Salkowski test, Libermann-Burchard test for triterpenoinds, Wagner’s test from alkaloids, methanol and aqueous sodium hydroxide test for the glycosides and the yellow precipitate obtained for the ferric chloride test in the phenolic compounds are identified in this fraction.

The ethyl acetate residual part gives the positive Shinoda test which gives yellow colour precipitation when treated with Mg•HCl and thus confirms the presence of flavonoids compounds. Similarly, ferric chloride test for phenolic compounds, brown precipitate of the Wagner’s reagent test for alkaloids, Fehling’s, solution test and Molisch’s test for carbohydrate are present in this fractions. The final methanol fraction analyzed contains flavonoids, isoflavonoids, alkaloids, carbohydrates and amino acids are present in the methanol residual fractions. Phytochemically identified all the fractions has thoroughly investigated to *in-vitro* antibacterial activity in the microorganism stains of human pathogenic bacteria both gram positive and gram negative bacteria such as *Bacillus subtilis*, *Staphylococcus aureus*, *Escherichia coli* and *Pseudomonas aeruginosa* were studied under agar well diffusion method. The observed data on the antibacterial activity of the compounds and standard antibiotic tetracycline are given in table 1 and in the form of MIC.

All the three phytochemical tested fractions contains an active compounds are present. Specifically, the ethyl acetate fraction of the compounds was examined to more superior activity has compared to control and the commercial standard antibiotics tetracycline and other two residual fractions activities are shown in the figure 1. Generally more number of phytochemically isolated and characterized compounds has investigated in the antibacterial activity. But only the least number of compounds has best activity in any one of the gram positive or negative bacteria or both. In the case, our presently isolated/fractionated compounds were superior active in both types of the bacteria. The activity was related to methanol and butanol extract of other plant viz., *Lactuca sativa* and *Carpodotus edulis* for the antibacterial, antioxidant and antiviral activities. The Soxhlet apparatus methanol solvent extract of the *Bridelia ferruginea* leaves, stem bark and roots has very less antibacterial activity. But, the room temperature methanol extract of *Cyporus rodants* flower has found to highly antibacterial activity compounds are present. The ethyl acetate fractioned compound was found to a marvelous antibacterial activity when compared to control and standard commercial antibiotic tetracycline. However, the antibacterial activity of methanol residual part was found to be equal to that of standard antibiotic tetracycline on the tested human pathogenic bacteria.

**Fig.1.** Antibacterial potential of the fractions CHCl₃ (1), EtOAc (2) and MeOH (3): to apply the gram negative and positive bacteria viz., *Bacillus subtilis* (a); *Staphylococcus aureus* (b); *Escherichia coli* (c) and *Pseudomonas aeruginosa* (d); from the concentration i) = 5; ii) = 10; iii) = 25 and iv) = 50 μg/mL; c) = control (10% DMSO).
<table>
<thead>
<tr>
<th>Compounds/fractions</th>
<th>Positive bacteria</th>
<th>Antibacterial activity (MIC in μg/mL)</th>
<th>Negative bacteria</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>B. subtilis</td>
<td>S. aureus</td>
<td>E. coli</td>
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<tr>
<td>Chloroform</td>
<td>15</td>
<td>20</td>
<td>25</td>
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<tr>
<td>Ethyl acetate</td>
<td>5</td>
<td>5</td>
<td>10</td>
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<tr>
<td>Methanol</td>
<td>15</td>
<td>20</td>
<td>15</td>
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<tr>
<td>Tetracycline</td>
<td>10</td>
<td>15</td>
<td>10</td>
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<tr>
<td>Control</td>
<td>NI</td>
<td>NI</td>
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NI = No Inhibition, tetracycline is standard antibiotic compound.

**Conclusion**

In conclusion, the chromatographically separated chloroform, ethyl acetate and methanol residual part of the fractions has phytochemical tested contains alkaloids compounds are mainly present. The other compounds such as flavonoids and carbohydrates are present in the ethyl acetate and methanol fractions. All the separated compounds have investigated in the antibacterial activity. Particularly, the ethyl acetate fractionated compounds has splendid activity are present. The main reason is due to the presence of alkaloids flavonoids and carbohydrate compounds. These compounds has easily metabolized in the human pathogenic bacteria.

**Abbreviations**

Dimethyl sulphoxide (DMSO)

Minimum Inhibitory Concentration (MIC)

**References**


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