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**ORIGINAL ARTICLE**

# ANALGESIC, ANTIMICROBIAL AND CYTOTOXIC EFFECT OF *CYPERUS ROTUNDUS* ETHANOL EXTRACT

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**ABSTRACT**

In present study preliminary screening was carried on *Cyperus routunds* crude extract which showed the presence of tannins, sapanins, carbohydrate in colour reaction method while the alkaloids, protein and sterol were absent.

Tail flick method was used for the determination of analgesic activity. The temperature and duration were 51 ± 1C° and 0, 1, 2, 3 and 4 hours respectively. The test drug was used in the concentrations of 300 and 500mg/kg orally.

Antimicrobial activity were carried out on human pathogens bacteria such as *Morexilla catarhalis*, *Escherichia coli*, *klebsiella Pneumoniae*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *acineto bacter* and fungi *Candida albicans* and *Aspergillus niger*. Excellent, moderate low and no activity were found on these organism such as 133.3% in *K. Pneumoniae* as compared to standard drug amoxicillin 20µg/ml and 70% ethanol (as fungicide). In case of *A. niger* and *S. aureus* 90 and 70 % inhibition was observed respectively while the crude extract showed low inhabitation that is 46.66, 37.5 and 33.3% in *E coli*, *P. aeruginosa* and *M. catarhalis* respectively. No zone of inhibition was observed in *Acineto bacter* and *C. albican*.

Haemagglutination activity test is suitable for determining the toxic effect on different human blood group ( $A^+$, $B^+$, $AB^+$, $O^+$, $A^-$, $B^-$, $AB^-$ and $O^-$). This test was carried out in different concentration of crude extract that is 0.3125, 0.625, 1.25 and 5mg/ml. Significant result were obtained in all groups at higher concentrations.

A quick and low cost toxicity test (Brine shrimp bioassay) was used to find out toxic action of drug in comparison to Etoposide standard drug ($LD_{50}$ =7.4625). The drug showed non toxic significant effects at 10, 100, 1000 µg/ml concentrations

**Keywords**: *Cyperus rotundus*, Antimicrobial, analgesic, cytotoxicity.

## INTRODUCTION

This medicinal plant is reported to be effective as attenuate antidyspeptic, aromatic, nervine ionic; alternative, diuretic, astringent and diaphoretic with other synonyms in *C. rotundus* Linn. *C. tuberoses* (Rottb) Kirk and *Forma latimarginatus* Kirk. It belongs to family Cyperaceae. Usually the Hakims or Traditional Healers used its knotted tubers of black colour for the cure of fever, diarrhea, dysentery, dyspepsia, anorexia, loss of appetite, vomiting, cholera, liver dysfunctions and brain debility. The chemical investigation on this plant revealed a number of chemical constituents belonging to different classes such as fat, gum resins, carbohydrates, essential oils, alkaloids, saponins, flavonoids,

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albuminous matters, fibers and ash. The major constituents of the oil are glycerides of oleic, palmitic and linoleic acids with small quantities of essential oil (Kalsi *et al.,* 1969; Hson-Mon-Chang *et al.*, 1987; Kapadia *et al*. 1967; Hikino *et al*., 1969). 1,8 cineole, 4α, 5 α-oxidoeudesm-11-en-3α-ol, Alkaloids, α-cyperone, α-rotunol, β-cyperone, β-pinene, β-rotunol, β-selinene, Calcium, Camphene, Copaene, Cyperence, Cypcerenone.

The ethanol extract of nutgrass tubers (*C. rotundus*) also possessed analgesic activity, elevating the pain threshold of mice similar to that of acetylsalisylic acid (Hson-Mon-Chang, *et al*., 1987).

The purpose of this research was to verify its therapeutic effects and margin of safety for human body.

Besides exploring the new pharmacological and biological activities of *Cyperus rotundus* one of the aspects of present research is to reproduce the standard method of preliminary screening of medicinal plants. This is achieved through the extracting of mixture of pharmacological active compounds and also through their fractions. The fractions are made in different solvent to separate the compounds and then performed different activities on various parameters. Extract of the plant and fractions were correlated with the effectiveness in term of percent of response and presented graphically. The graphs represent the comparative best pharmacological and biological activities.

The worldwide increasing demand for medicines from natural sources has motivated search for drugs with potential hypotensive activity as well.

## MATERIAL AND METHODS

The plant material was purchased from local market and after identification it was deposited in the herbarium of Research Institute of Pharmaceutical Sciences (Voucher specimen number A-2003-1). The rhizome was ground and extract with ethanol. After filtration, the extract was reduced by rotary evaporator to get a crude extract in thick mass (Ahmad *et al*., 2011).

### *Test Animals*

Animals (albino mice of either sex; weight 25-30 gm) were obtained from HEJ animal house. They were kept at 35-37°C and acclimatized for at least one week.

### *Analgesic Activity*

The analgesic activity was evaluated by tail-flick method (Di Stasi *el al.,* 1988). Mice of either sex were selected and divided into four groups of five animals in each group. Animal of group 1 and 11 received 0.9% saline and Diclofenac Na respectively while group 111 and IV received drug to be tested in 300 and 500 mg/kg. The time of reaction to pain stimulus was taken to with draw its tail from hot water bath heated at 51°C ± 1 by the tail immersion method at 0, 1, 2, and 3 and 3.5 hours respectively. The drug was prepared by dissolving crude extract in 0.9% saline solution prior to use and given orally to the animals. Reading was taken after 30 min.

### *Antimicrobial Assay*

The antibacterial and antifungal activity was evaluated by the agar-well diffusion method (Kavanagh *et al*., 1963 and Mehjabeen *et al.,* 2011).

### *Haem-Agglutinating activity*

*Dilution Preparation:* Different dilutions were prepared for each plant extract by dissolving 0.1 gram crude extract in 20 ml of a mixture of distilled water and a series of dilutions i.e. 1:2, 1:4, 1:8 and 1:16 were prepared for experiments. For dilution preparation the classical method was used (Muhammad 2006).

*Preparation of Buffer:* Phosphate buffer (pH 7) was prepared by mixing of 70 ml solution of $Na_2HP0_4$ (52 g/L or 5.2 g/200 ml) in 130 ml solution of anhydrous $NaH_2PO_4$ (36 g/L or 3.6 g/ml) (Muhammad 2006).

*Preparation of RBCS*: Samples of $Rh^+$ human blood (group $A^+$, $B^+$, $AB^+$ and $O^+$) were obtained from Husaini Blood Bank, Karachi. RBCs (erythrocytes) were obtained by simple centrifugation of the blood samples and 2% RBCs suspension was prepared in phosphate buffer (pH 7) i.e. in 1 ml RBCs and 49 ml buffer.

Haem-agglutination activity was investigated in a series of dilutions of each plant extract against all four blood groups. For this purpose 1 ml of each was added to 1 ml of 2% suspension of RBCs in a small test tube followed by incubation in a water bath at 25°C. Blank samples were also run simultaneously as control. Smooth button like formation (due to the sedimentation of erythrocytes) at the bottom of the test tube indicated the negative activity of the extract whereas a rough granular deposition (due to agglutination of erythrocytes) showed a positive action. During experimentation very weak or trace, weak, moderate and strong agglutinations were determined on the basis of the extent of granule deposition (Muhammad 2006).

***Brine Shrimp Bioassay***

The six crude plant materials extracts were prepared for the estimation of LD50 activity in brine shrimps. The procedure described by Meyer *el al.* (1982) and Arnason *et al.* (1989) was adopted for this work.

Samples and discs of 3 different concentrations 10, 100 and 1000 µg/ml were prepared according to the direction as given in the literature (Arnason 1989). Brine shrimp (*Artemia salina)* nauplii were hatched in a specific tank. Ten shrimps were transferred to each sample vial and then seawater was added to make the volume 5 ml. Later on dry yeast suspension was added as food to each vial including control. The vials were kept for 24 hours, thereafter the active nauplii were counted and death percentage was calculated at each dose and analyzed the data with Finney computer program in order to determine $LD_{50}$ values.

***Analgesic activity***

Acetic acid induced writhing test and tail flick was performed according to the modified method of Koster *et al*., 1959, Turner 1971 and Ahmad *et al*., 2011 respectively.

## STATISTICAL ANALYSIS

The results were expressed as mean±S.E.M. All statistical comparisons were made by means of Student's *t*-test and a *P* value smaller than 0.05 was regarded as significant.

## RESULTS AND DISCUSSION

The crude extract of *Cyperus rotundus* was used for identification of chemical constituents by colour reaction (Table 1).

The analgesic activity was evaluated by tail-flick method. The reaction time to pain stimulus was increased after crude extract administration (Table 2).

Antimicrobial activity was carried out against *Staphylococcus aureus, Pseudomonas aeruginosa, Klebsiella pneumoniae, Moraxella catarrhalis Acineto bacter, Candida albicans, Aspergillus niger* and *Escherichia coli* (Table 3, Figure 2). The dose was given in a single concentration (1 mg/ml). *Acineto bacter* and *C. albicans* showed no zone of inhibition against crude extract.

The haemagglutination test was carried out on $A^+$, $B^+$, $AB^+$, $O^+$, $A^-$, $B^-$, $AB^-$, and $O^-$ groups. The crude extract of *C. rotundus* was used in 0.3125, 0.625, 1.25, 2.5 & 5 mg/ml in each blood group (Table 4).

Brine Shrimp Bioassay was performed to evaluate the toxicity of medicinal drug. The crude extract of *C. rotundus* was used in 10, 100 and 1000 µg/ml dose and the lethality was observed in term of death of larvae along with standard drug Etoposide (Table 5).

*Cyperus rotundus* has various important constituents, which are useful for the treatment

of several diseases. It may be a good remedy for indigestion in the light of constituents present in it, for example, there are many enzymes for carbohydrates and minerals which act as catalyst for various biochemical reactions and helps in digestion. It is also useful for dietary management of psychotic diseases and metabolic disorders (The Wealth of India, 1950).

The preliminary screening was carried out according to the WHO recommendations, our sample of *C. rotundus* showed the presence of tannins, saponins, carbohydrates in colour reaction method and the absence of alkaloids, protein and sterols in this sample. Jeong *et al.* (2000) reported the occurrence of 3 novel sesquiterpene alkaloids. Rotundine A, B and C. from rhizome of it.Basic identification test of fluorescence in three different lights i.e. ordinary light, 254 nm infrared light and 366 nm ultraviolet light in different chemical solutions These results can serve as standards for future researches (Table 1).

Analgesic activity test is one of the most important tests in those drugs used for relieving flu, fever, pain etc. In this evaluation tail flick method was used. The crude extract of *C. rotundus* showed highly significant results (5±0.45 seconds reaction time) at 300 mg/kg oral dose in comparison with control and standard drug (Dioclofenic sodium) (Table 2). Mohsin *et al.* (1989) reported that ethanol

**Table 1**
Identification of chemical constituents and fluorescence analysis of *C. rotundus*

| Chemical constituents | Results | Fluorescence analysis treatment | Observation under UV | | |
|---|---|---|---|---|---|
| | | | Ordinary light | 254nm UV | 366 nm UV |
| Tannins | ++ | Dry powder as such | Light brown | Light brown | Light brown |
| Saponins | + | Powder treated with 1N NaOH in methanol | Black | Black | Greenish Black |
| Alkaloids | + | Powder treated with 1N HCl | Brown with Black spot | Brown with black spot | Brownish black |
| Carbohydrates | + | Powder treated with 50% $HNO_3$ | Brown with black spots | Brown with black spots | Brown with black spots |
| Proteins | - | Powder with 50% $H_2SO_4$ | Brownish black | Dark brown | Dark brown |
| Sterols | - | Powder treated with $FeCl_3$ | Light yellowish colour | Yellowish brown | Mustard colour |

**Table 2**
Analgesic effect of crude extract of *Cyperus rotundus* in mice (Tail flick method)

| Time in sec | 0 | 1 | 2 | 3 | 4 |
|---|---|---|---|---|---|
| Control | 2.98 ±0.44 | 2.4±0.24 | 2.2±0.2 | 2.2±0.2 | 2.4±0.24 |
| Diclofenac Na 50 mg | 1.97±0.33 | 3.6±0.25* | 4.1±0.10* | 3.3±0.19 | 2.1±0.10 |
| *C. rotundus* 300 mg/kg | 2.4±0.71 | 3±0.06 | 4.5±0.50** | 5±0.45** | 4.5±0.2** |
| *C. rotundus* 300 mg/kg | 1.8±0.02 | 3.5±0.01* | 4.4±0.02** | 4.9±0.04** | 3.9±0.04* |

Values are expressed with Mean ± SEM (student t test), at $P < 0.05$, $N = 5$; * Significant and ** highly Significant

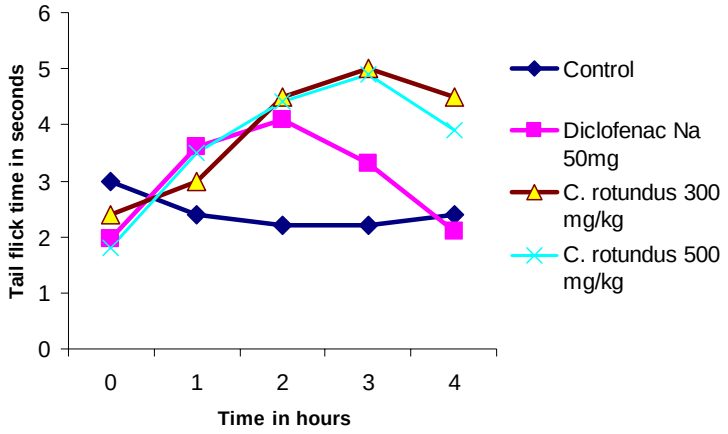


Figure 1: Analgesic effect of *C. rotundus* crude extract

**Table 3**
Antimicrobial activities of crude extract of *Cyperus rotundus*

| Micro-organisms | | Zone of inhibition of crude extract in mm | Amoxicillin 20µg/ml | Ethanol 70% |
|---|---|---|---|---|
| Gram +ve cocci | *M. catarrhalis* | 5(+), 33.33% | 15 | NA |
| Gram-ve bacilli | *E. coli* | 7(++), 46.66% | 15 | NA |
| Gram-ve bacilli | *K. pneumoniae* | 8(++++), 133.3 | 06 | NA |
| Gram-ve bacilli | *P. aeruginosa* | 6(++), 37.5 | 16 | NA |
| Gram +ve cocci | *S. aureus* | 7(+++), 70% | 10 | NA |
| Gram-ve bacilli | *Acineto bacter* | 0 (-) | 09 | NA |
| Candida sp. | *C. albicans* | 0 (-) | 0 | 09 |
| Aspergillus sp. | *A. niger* | 9(+++), 90% | 0 | 10 |

NA= Not applied, Zero (-)= No activity, + = 01-05 mm, ++ = 05-10 mm, +++ = 10-15

(95%) extract of entire plant (cultivated in Saudi Arabia), administered to mice at a dose of 500 mg/kg by gastric intubation, and was found inactive against hot plate method. Chow *et al* (1979) reported that ethanol (95%) and hot water extracts of dried rhizome, at a dose of 12.7g/kg administered intraperitoneally to mice, were inactive in hot plate method. Hot water extract, administered orally at a dose 12.7 g/kg, was also inactive against acetic acid writhing inhibition test.

Mohsin *et al.* (1989) and Chow *et al.* (1979) found no analgesic activity in *C. rotundus* while Vu and Mai (1994) reported this activity in *C. stoloniferus*. Our sample also exhibited the same results as reported by Vu and Mai (1994).

Antimicrobial activity tests were carried out on human pathogens bacteria (gram negative and gm positive) and fungi viz. *C. albicans* and *A. niger*. The highest percentage of inhibiton was observed against *K. pneumoniae* (133.33%). Amoxicillin 20µg/ml and ethanol (as fungicide) 70% were used as positive control. Moderate inhibition was observed in case of *A. niger* and *S. aureus* (90 and 70% respectively). No zone of inhibition was observed in *Acinto bacter* and *Candida*

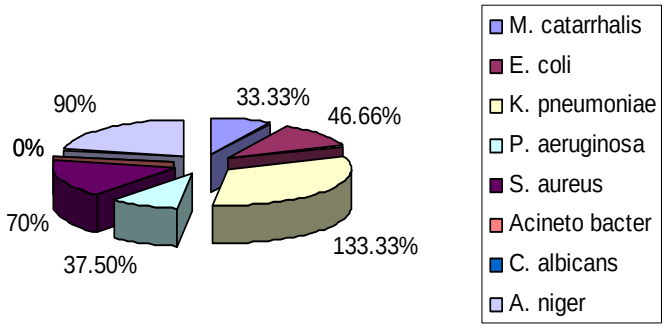


Figure 2: Percentage of Response of *C. rotundus* against different micro-organism

**Table 4**
Haem-agglutination activity of *Cyperus rotundus*

| Blood groups | Dose of Crude extract of *C. rotundus* (mg/ml) | | | | |
|---|---|---|---|---|---|
| | 5 | 2.5 | 1.25 | 0.625 | 0.3125 |
| $A^+$ | ++ | + | + | - | - |
| $B^+$ | ++ | + | + | - | - |
| $O^+$ | ++ | + | + | - | - |
| $AB^+$ | ++ | + | + | - | - |
| $A^-$ | + | + | + | - | - |
| $B^-$ | ++ | + | + | - | - |
| $O^-$ | ++ | + | + | - | - |
| $AB^-$ | + | + | + | - | - |

Each positive sign is an indication of 20% response of a drug, traces shows 10% response and –ve sign indicates no response, ++++ = high activity, +++ = medium activity, ++ = low activity, + = very low activity.

**Table 5**
Brine Shrimp lethality bioassay of crude extract of *C. rotundus*

| Dose µg/ml | No. of Shrimps | No. of Survivors |
|---|---|---|
| 1000 | 30 | 28 |
| 100 | 30 | 30 |
| 10 | 30 | 30 |
| $LD_{50}$ of crude extract of *C. rotundus* | Nill | |
| $LD_{50}$ of Standard drug Etoposide | 7.4625 | |

*albicans* (Table 3). The anti- bacterial activity is similar to published data but 90% activity was found against *Aspergillus niger* which has not been mentioned by any author prior to these studies.

Haemagglutination activity test is a suitable test for determining the toxic effect on different human blood groups ($A^+$, $B^+$, $AB^+$, $O^+$, $A^-$, $B^-$, $AB^-$, and $O^-$). This test was carried out in different concentrations of crude extract

i.e. 0.3125, 0.625, 1.25 and 5mg/ml and significant results were obtained in all groups at higher concentrations (Table 4). Previously no such activity was carried out on this plant. Similarly in Brine Shrimp Bioassay the crude extract at different concentration did not produced any toxicity.

The purpose of the research is to identify the phytomedicinal usage of the herbs that show great advantage for the human body. This research review both current and historical analysis as well as the hidden profile of *Cyperus rotundus*.

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