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Research Article

# *IN VITRO* FREE RADICAL SCAVENGING ACTIVITY OF METHANOL EXTRACT OF RHIZOME OF *CYPERUS TEGETUM* ROXB. (CYPERACEAE)

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**ABSTRACT**

In this study, the methanol extract of rhizome of *Cyperus tegetum* Roxb, belonging to the family Cyperaceae was prepared by successive extraction procedure (petroleum ether, chloroform, methanol) in Soxhlet apparatus and subsequently, the extract was evaluated for its phytoconstituents and pharmacological activity. Comparatively, higher yield (5.4%) of methanol extract was obtained. Phytochemical tests revealed the presence of alkaloid, glycosides, proteins, amino acids, phenolic compounds, flavonoids, tannins and saponins in the extract. The methanol extract was evaluated for its i*n vitro* antioxidant property by 1, 1-diphenyl-2-picryl hydrazyl (DPPH) radical assay; sodium nitroprusside generated nitric oxide (NO), and hydroxyl free radical scavenging methods. In addition, the reducing power of the extract was determined using potassium ferricyanide. The extract showed $IC_{50}$ values of 16.0 µg/ml, 65.0 µg/ml, and 3.0 µg/ml for DPPH free radical, nitric oxide (NO), and hydroxyl radical scavenging method, respectively. Therefore, it can be concluded that the methanol extract of rhizome of *Cyperus tegetum* possesses a significant antioxidant activity.

**Keywords:** *Cyperus tegetum*, Free radicals, Antioxidant, Acute toxicity, DPPH

## INTRODUCTION

Antioxidants are a group of substances, which inhibit or delay oxidative processes. In recent years there has been an increased interest in the application of antioxidants in medicine. Most of the potentially harmful effects of oxygen are due to the formation and activity of a number of chemical compounds, known as reactive oxygen species (ROS), which have a tendency to donate oxygen to other substances. Many such reactive species are free radicals and have a surplus of one or more free-floating electrons rather than having matched pairs and are, therefore, unstable and highly reactive. Types of free radicals include the hydroxyl radical (OH.), the superoxide radical ($O._2$), the nitric oxide radical (NO.) and the lipid peroxyl radical (LOO.). Free radicals are responsible for more than one hundred disorders in humans including atherosclerosis, arthritis, central nervous system injury, gastritis, cancer and AIDS[1, 2]. Free radicals due to environmental pollutants, radiation, chemicals, toxins, deep fried and spicy foods as well as physical stress, cause depletion of immune system antioxidants, change in gene expression and induce abnormal proteins. Due to these problems natural antioxidants as free radical scavengers may be necessary[2-4]. Recently there has been an increased interest in the therapeutic potentials of medicinal plants as antioxidants in reducing such free radical induced tissue injury. Although many plant species have been investigated in the search for novel antioxidants but generally there is still a demand to find more information concerning the antioxidant potential of plant species. It has been mentioned the antioxidant activity of plants might be due to their phenolic compounds[2]. Flavonoids are a group of polyphenolic compounds with known properties which include free radical scavenging, inhibition of hydrolytic and oxidative enzymes and anti-inflammatory action[5]. The biological actions of these compounds are related to their antioxidant activity[6]. An easy, rapid and sensitive method for the antioxidant screening of plant extracts is free radical scavenging assay using 1,1-diphenyl-2-picryl hydrazyl (DPPH) stable radical spectrophotometrically. In the presence of an antioxidant, DPPH radical obtains one more electron and the absorbance decreases[7]. In present study, we carried out a systematic record of the relative free radical scavenging activity the plant *Cyperus tegetum* Roxb. belonging to the family Cyperaceae is a glabrous and robust perineal sedge found throughout India up to an altitude 1800m[8]. The plant is commonly known as mat stick, madur kathi(Bengali) and cultivated as an economic crops in Paschim Midinipur district of West Bengal and traditionally used by the tribal people for the treatment of cachexia, atrophy and snake bite. Going through the literature survey, although activities like anticonvulsant[9], sedative [10], antimalarial[11], antidiarrhoeal[12], antidiabetic[13] etc. have been reported by several research workers on the other plants belong to Cyperaceae family, however there is no scientific report on the plant *Cyperus tegetum* Roxb of same family. Therefore the objective of the present investigation was to explore its phytoconstituents and probable pharmacological activity.

## MATERIALS AND METHODS

### Plant material

The plant *Cyperus tegetum* Roxb (Family:Cyperaceae) was collected from the cultivated land of Paschim Medinipur, West Bengal in the month of June-July. Botanical Survey of India taxonomically identified the plant. A voucher specimen (CNH/I-I (198)/2007/Tech.II/162) has been preserved in our laboratory for further references. The rhizomes were washed, dried at room temperature under shed and then grounded in a mill to a coarse powder.

### Extraction of plant materials

The powdered rhizomes were subjected to successive Soxhlet extraction using a series of solvents of increasing polarity starting from petroleum ether, chloroform, and methanol respectively. The extracts were vacuum dried and the percentage yields of the extracts were 2.1%, 3.0%, and 5.4%, respectively.

### Preliminary phytochemical analysis

The phytochemical tests were performed using various reagents as described in Table 1. The extract was tested for the presence or absence of alkaloid, glycosides, tannins, steroids, reducing sugars, proteins and amino acids, phenolic compounds and flavonoids (Table 1).

### Acute toxicity study

The acute toxicity of methanol (MeOH) extract of *Cyperus tegetum* Roxb was studied on Swiss albino mice (20-25 gm) following Karber's method. The Institutional Animal Ethical Committee permitted the use of the animals for this purpose. After fasting condition for overnight, the animals divided into six groups (four in

each group), were administered a dose of 100, 200, 400, 800, 1600 and 3000 mg/ kg BW intraperitoneally. No animals were found died after 24h.

**Evaluation of Antioxidant activity of methanol (MeOH) extract**

**DPPH radical scavenging activity MeOH extract**

The stable 1, 1-diphenyl-2-picryl hydrazyl radical (DPPH) was used for determination of free radical scavenging activity of the MeOH extract[8] (Koleva *et al.* 2002). The MeOH extract was added at different concentrations (5-100 µg/ml) with an equal volume, to methanol solution of DPPH (100 µM). After incubation for 15 min at room temperature, the absorbance was recorded at 517 nm(Table 2). The experiment was carried out in triplicate. The $IC_{50}$ value i.e the concentration of sample required to scavenge 50% of stable DPPH free radicals was determined from the % inhibition vs. concentration of MeOH extract curve(Fig.1) by comparing the absorbance values of control ($A_0$) and test compounds ($A_t$).

$$\%Inhibition = [(A_t - A_0)/A_0] \times 100$$

**Table 1**: **Preliminary phytochemical screening of MeOH extract**

| Phytoconstituents | Test performed/reagents used | Presence or absence |
|---|---|---|
| Alkaloid | Mayers test | + |
| | Dragendorffs test | + |
| | Hagers test | + |
| Steroid | Libermann-Burchard test | - |
| Flavonoids | Shinoda test | + |
| Tannins | Ferric chloride | + |
| | Lead acetate | + |
| Saponin | Test for stable foam | + |
| Glycoside | Borntager test | - |
| Protein and amino acid | Ninhydrin test | + |
| Reducing suger | Fehlings test | + |
| | Benedict test | + |

**Table 2**: **DPPH free radical scavenging of MeOH extract**

| Concentration of MeOH extract (µg/ml) | Absorbance at 517 nm (Mean ± sd) | % inhibition of DPPH free radicals (Mean ± sd) |
|---|---|---|
| 0 | 0.1207 ± 0.0046 | 0 |
| 5 | 0.0977 ± 0.0006 | 18.9633 ±3.5045 |
| 10 | 0.0780 ± 0.0052 | 35.2400 ±5.9219 |
| 20 | 0.0453 ± 0.0042 | 62.3633 ±4.1215 |
| 30 | 0.0237 ± 0.0093 | 80.4400 ±7.5669 |
| 40 | 0.0207 ± 0.0093 | 82.9267 ±7.5957 |
| 50 | 0.0263 ± 0.0100 | 78.2600 ±7.7815 |
| 60 | 0.0270 ± 0.0104 | 77.7433 ±8.2595 |
| 70 | 0.0280 ± 0.0115 | 76.9033±8.9879 |
| 80 | 0.0310 ± 0.0115 | 74.3169±8.9879 |
| 100 | 0.0323 ± 0.0066 | 73.1900±5.5322 |

Values are the average of triplicate experiments and values expressed as mean ± sd

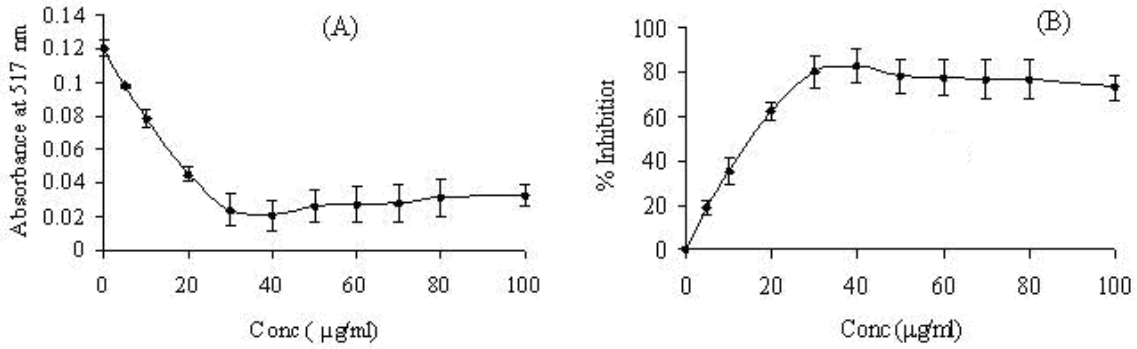


**Fig. 1**: **DPPH scavenging activity of MeOH extract. (A) Conc. Vs Absorbance; (B) Conc. Vs % inhibition**

**Nitric oxide scavenging activity of MeOH extract**

Nitric oxide scavenging activity was measured following the method reported earlier[14,15]. The reaction mixture containing 1.5ml sodium nitropruside(10mM) in phosphate buffer salineand 1.5ml of different concentration (5-80 µg/ml) methanol extract was incubated at 25°C for 150 min. Following incubation, 0.5ml of reaction mixture was mixed with 0.5 ml of Griess reagent (1% sulphanilamide, 2% $H_3PO_4$ and 0.1% naphthyl ethelene diamine

dihydrochloride). The percentage inhibition of nitric oxide generated from sodium nitroprusside was measured by comparing the absorbance values of control ($A_o$) and test compounds ($A_t$) at 546 nm(Taable 3). The following equation was used:

$$\%Inhibition = \left[(A_t - A_0)/A_0\right] \times 100$$

**Scavenging Activity of Hydrogen peroxide radical**

The hydrogen peroxide scavenging of methanol extract was determined according to the method reported elsewhere[16]. A solution of hydrogen peroxide (40 mM) was prepared in phosphate buffer (pH 7.4) and concentration was determined spectrophotometrically at 230 nm. Different concentration of extracts (1, 2, 3, 4, and 6 µg/ml) in distilled water was added to a hydrogen peroxide solution (0.6 ml, 40 mM) and the absorbance of hydrogen peroxide at 230 nm was determined after 20 min against a blank solution in phosphate buffer without hydrogen peroxide (Table 4). The percentage scavenging of hydrogen peroxide was calculated using the following equation:

$$\%\ Scavenged\left[H_2O_2\right] = \left|(A_{control} - A_{sample})/A_{control}\right| \times 100$$

The concentration of the extract was plotted against the % inhibition (Fig.3) and $IC_{50}$ value was determined.

**Reducing power of MeOH extract of *C tegetum* Roxb**

Reduction capability of methanol extract was measured by using the method of Oyaizu *et al* 1986.[17] Different concentrations (200, 300 and 400 µg/ml) of the extract in 1 ml of distilled water was mixed with 2.5 ml phosphate buffer (0.2 M, pH 6.6) and 2.5ml 1% (w/v) potassium ferricyanide. The mixture was incubated at 50° C for 20 min by adding a 2.5 ml of 10 % trichloroacetic acid. Then the mixture was centrifuged for 10 min and upper layer of solution (2.5 ml) was mixed with distilled water (2.5 ml) and ferric chloride (0.5 ml, 0.1%), and the absorbance was measured at 700 nm by a spectrophotometer(Table 5). Higher absorbance of the reaction mixture indicated greater reducing power. The reductive capability was compared with butylated hydroxy toluene (BHT). Increased absorbance of the reaction mixture indicated increasing reducing power (Fig.4).

**Table 3**: **Nitric oxide (NO) scavenging of MeOH extract**

| Concentration of MeOH extract (µg/ml) | Absorbance (Mean ± sd) | % Inhibition of DPPH free radicals (Mean ± sd) |
|---|---|---|
| 0 | 1.6700 ± 0.0410 | 0 |
| 5 | 1.5873 ± 0.0783 | 4.9852 ± 2.4299 |
| 10 | 1.4253 ± 0.0403 | 14.6077 ± 0.5082 |
| 20 | 1.3769 ± 0.0583 | 17.5569 ± 1.7941 |
| 30 | 1.3874 ± 0.0348 | 16.9079 ± 0.2143 |
| 40 | 1.2875 ± 0.0506 | 22.9103 ±1.1921 |
| 50 | 1.1913 ± 0.0384 | 28.6546 ± 0.7838 |
| 60 | 1.0333 ± 0.0271 | 38.1196 ± 0.1284 |
| 70 | 0.6964 ± 0.0249 | 58.2504 ± 2.4865 |
| 80 | 1.0901 ± 0.0202 | 34.6126 ± 1.2287 |

Values are the average of triplicate experiments and values expressed as mean ± sd

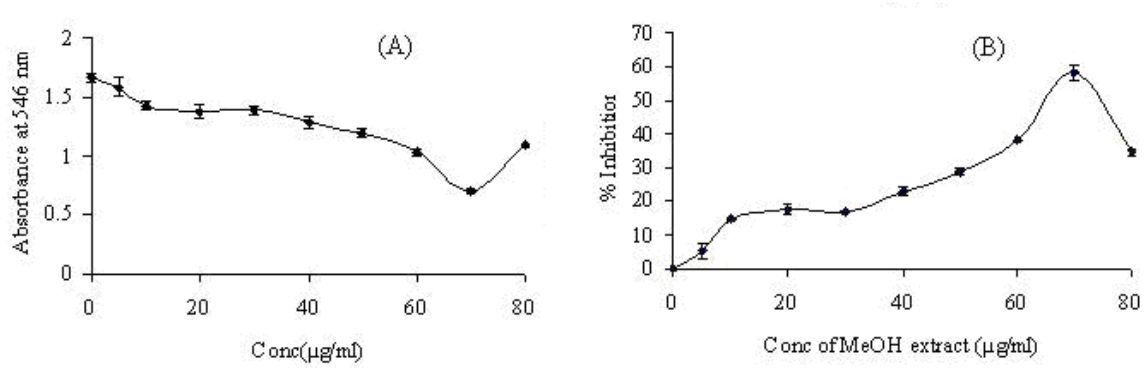


**Fig. 2**: **Nitric oxide (NO) scavenging activity of MeOH extract. (A) Conc. Vs Absorbance; (B) Conc. Vs % inhibition**

**Table 4**: **Hydrogen peroxide($H_2O_2$) scavenging activity of MeOH extract. Values are the average of triplicate experiments and values expressed as mean ± sd**

| Concentration of MeOH extract (µg/ml) | Absorbance (Mean ± sd) | % inhibition of DPPH free radicals (Mean ± sd) |
|---|---|---|
| 0 | 0.0230 ±0.0009 | 0 |
| 1 | 0.0193 ± 0.0015 | 16.1478 ± 3.2119 |
| 2 | 0.0153 ± 0.0015 | 33.5336 ± 3.8956 |
| 3 | 0.0117 ± 0.0005 | 49.0019 ± 2.9318 |
| 4 | 0.0093 ± 0.0015 | 59.6124 ± 4.9386 |
| 6 | 0.0087 ± 0.0006 | 62.3311 ± 2.9743 |

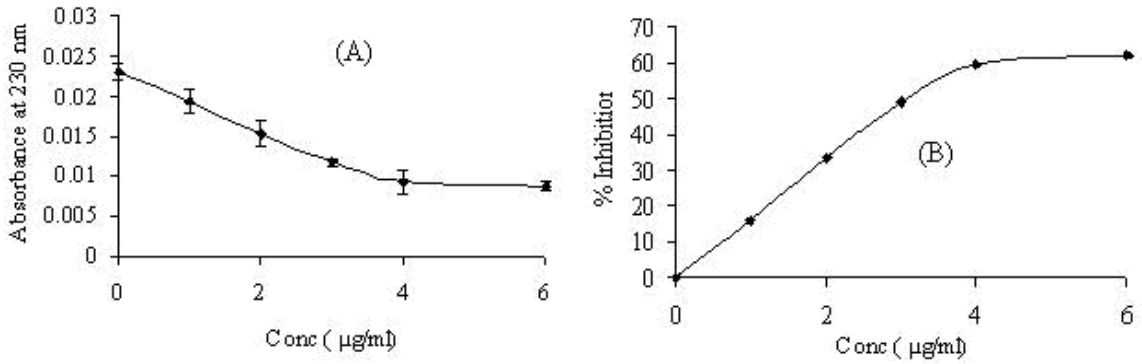


**Fig. 3**: **Hydrogen peroxide scavenging activity of MeOH extract. (A) Conc. Vs Absorbance; (B) Conc. Vs % inhibition.**

**Table 5**: **Reducing power of MeOH extract**

| Concentration of methanol extract ( µg/ml) | Absorbance |
|---|---|
| 200 | 0.311 |
| 300 | 0.4235 |
| 400 | 0.7195 |
| 200 (BHT) | 1.3425 |

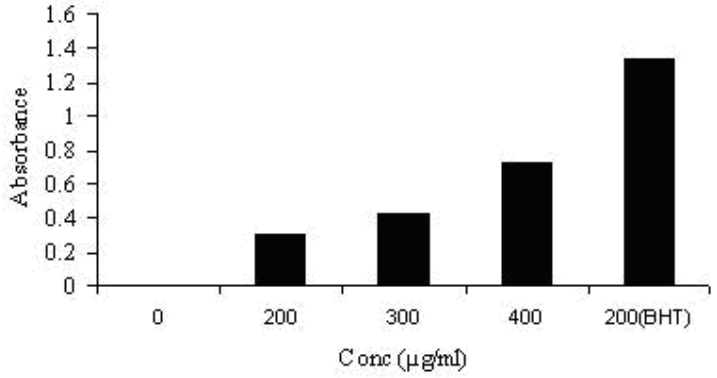


**Fig. 4**: **Reducing power of MeOH extract**

## RESULTS AND DISCUSSION

The acute toxicity study did not show any mortality of the experimental animals up to dose of 3000 mg/kg BW and hence, the methanol extract of the rhizome of *Cyperus tegetum* Roxb could be safely used up to that dose. The flavonoids, phenolic compounds, glycoside, reducing sugars, tannins, triterpenoids in the extract was identified by chemical tests (Table 1). A number of reports on flavonoids, triterpenoids, polyphenols indicated that they possess antioxidant and free radical scavenging activity[5]. These phytoconstituents may exert multiple biological effects against tumors, heart disease, AIDS, and different pathologies due to their free radical scavenging activities. Realizing these facts, this work was carried out to evaluate the antioxidant activity of the methanol extract of *C tegetum* Roxb. A significant decrease in the concentration of DPPH radical was observed due to the scavenging ability of the methanol extract (Table 2). Free radical scavenging activity increased with increasing concentration of the extract. The hydrogen atoms or electron donation capability of the methanol extract was measured from the bleaching of purple colored MeOH solutions of DPPH and it exhibited an $IC_{50}$ value (i.e the concentration of the extract that inhibit 50% free radicals) of 16 µg/ml (Fig.1). Whereas the reported $IC_{50}$ values for standard BHT and quarcetin were 54 and 10 µg/ml respectively[18]. The extract significantly inhibited nitric oxide in a dose dependent manner (Table 3) with the $IC_{50}$ being 65 µg/ml (Fig. 2). This nitric oxide scavenging activity could be attributed the presence of phenolic and polyphenolic compounds in the extract. As shown in Table 4, the extract also demonstrated hydrogen peroxide decomposition activity in a concentration dependent manner with an $IC_{50}$ of 3 µg/ml (Fig. 3). Similarly, the reducing power of the extract increased with increasing concentration (Table 5, Fig. 4). The hydrophilic polyphenolic compounds of the extract probably induced the greater reducing power.

## CONCLUSION

The present study suggested that *C. tegetum* rhizomes could be a potential source of natural antioxidant and thus could be useful as therapeutic agents in preventing or slowing the progress of aging, age-associated oxidative stresses-related degenerative diseases. Further studies are required to isolate and identify the components in methanol extract responsible for eliciting such pharmacological response.

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