

## FROM MEDICINAL PLANTS TO PHARMACEUTICALS: CORRELATING PHENOLIC COMPOUNDS WITH ANTIOXIDANT ACTIVITY IN CYPERUS ROTUNDUS L

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### ABSTRACT

Phenolic compounds are among the most important plant secondary metabolites responsible for antioxidant activity. This study aimed to evaluate the relationship between total phenolic content (TPC) and antioxidant capacity of rhizome extracts of *Cyperus rotundus* L. Total phenolic content was measured using Folin reagent, and flavonoid content was measured using the aluminum chloride method. Antioxidant capacity was determined by DPPH free radical scavenging. HPLC analysis was performed to detect flavonoids. The study revealed a strong correlation between phenolic concentration and antioxidant capacity. The phenolic concentration was 62.8 mg of standard gallic acid. The extract exhibited an antioxidant capacity of EC<sub>50</sub> at 0.014µg/ml. The study showed a linear correlation with a correlation coefficient (R<sup>2</sup>) of 0.69, indicating that 69% of the antioxidant capacity is attributed to the phenolic compounds of this plant. In addition, HPLC chromatographic analysis confirmed the presence of the

following flavonoids in the plant extract: Rosmarinic, Rutin, Hesperidin Quercetin, Naringin, Kampferol and Apegnine. From this study, we conclude that this plant is an important source of antioxidants, and further research is needed to investigate its biological activity.

**KEYWORDS:** Antioxidant capacity; *Cyperus rotundus* L, HPLC analysis, phenolic compounds.

## I. INTRODUCTION

Medicinal plants play a crucial role in healthcare systems worldwide, particularly in developing countries where they are used as primary therapeutic agents (Fukumoto, et al., (2000). These plants are rich in phytochemicals such as phenolics, flavonoids, alkaloids, and terpenoids, which are responsible for their biological activities. (Cory et al., 2018, Harnafi H. and S. Amrani (2007)). Oxidative stress caused by free radicals is associated with various chronic diseases, including cancer, diabetes, and cardiovascular disorders. Natural antioxidants derived from plants are increasingly preferred due to their safety and biological efficacy (Engwa, G. et al., 2022).

*Cyperus rotundus* L., commonly known as nut grass, is a medicinal plant widely used in traditional medicine for its anti-inflammatory, antimicrobial, and antioxidant properties. Phytochemical investigations reveal that its rhizomes contain phenolic acids, flavonoids, and other polyphenolic compounds(Kamala, et al., 2018).

Phenolic compounds act as primary antioxidants by scavenging free radicals and inhibiting oxidative processes(Vuolo,. et al., 2019). Previous studies have demonstrated that antioxidant activity in plants is strongly linked to phenolic content.

Therefore, this study aims to: Quantify phenolic compounds in *C. rotundus* rhizomes, Evaluate antioxidant capacity and Determine the correlation between phenolics and antioxidant activity.

## 2. MATERIALS AND METHODS

### 2.1. Collection of Plant Material

Rhizomes of *Cyperus rotundus* were collected from Kosti, White Nile State, Sudan. The plant was authenticated, and a voucher specimen was deposited at the Herbarium of the Medicinal and Aromatic Plants Research Institute (MAPRI), Khartoum.

### 2.2. Preparation of Extract

Rhizomes of *Cyperus rotundus* were collected, cleaned, shade-dried, and powdered. 25 grams of coarsely powdered plant were taken by maceration using ethanol (70%) in a conical flask

for 72 hours, filtered and evaporated by a rotary evaporator at 60 °C. The extract yielded, dried weighed and placed into an amber glass container and kept in a refrigerator until use.

### 2.3. Determination of Total Phenolic Content (TPC)

The total phenol content in the ethanolic extract of the surveyed plant was determined with Folin Ciocalteu reagent by the method described by Chinedu *et al.*, (2011). Each crude extract (50 mg) was mixed with Folin Ciocalteu reagent (1ml) and deionized water (7.5 ml). The mixture was kept at room temperature for 5 minutes and then 10 ml of 7% sodium carbonate were added to the mixture and then incubated for 90 minutes at room temperature. After incubation the absorbances against the reagent blank were determined at 760 nm using UV/visible spectrophotometer. The total phenolic content of each plant was expressed as mg/g Gallic acid equivalent. Samples were analyzed in triplicates.

Standard curve was prepared by 100, 200, 300, 400 and 500 µg/ml solutions of galic acid in methanol: H<sub>2</sub>O (50:50v/v) total phenol values are expressed in terms of galic acid equivalent mg g<sup>-1</sup> of dry mass which in a common reference compounds.

### 2.4. Determination of Total Flavonoid Content (TFC)

The flavonoidal content in the ethanolic extract of surveyed plant was determined as described by Chinedu *et al.*, (2011). Extract of plant material (1 ml containing 100 µg/ml was diluted with 4 ml water in 10 ml volumetric flask. Initially 5% Sodium nitrite (0.3ml) was added and after 5 minutes 10% Aluminium chloride (0.3ml) was added followed by addition of 2 ml 1 M Sodium hydroxide after 6 minutes. Water (2.4 ml) was then added to the reaction flask and mixed well. Absorbances of the reaction mixture were read at 510 nm using UV/visible spectrophotometer. The flavonoidal content of the plant was expressed as mg/g Quercetin equivalent. sample were analyzed in triplicates.

### 2.5. Antioxidant activity of plant ethanolic extract

This method was carried out according to that described by Mensor *et al.*, (2011). Sample stock solution (0.1g/100ml) was diluted to final concentrations of 250, 125, 50, 10 and 5 µg/ml in methanol. 1.0 ml of a 0.3 mM 2, 2 diphenyl-2-picryl hydrazyl (DPPH) in methanol solution was added to a 2.5 ml solution of the different concentrations of the extract and allowed to react at room temperature for 30 minutes. The absorbance of the resulting mixture was measured at 518 nm and converted to percentage antioxidant activity (AA %), using the formula below

$$AA\% = \frac{\text{Absorbance of control} - \text{Absorbance of sample}}{\text{Absorbance of control}} \times 100$$

Methanol (1.0 ml) plus plant extract solutions (2.5 ml) was used as a blank. DPPH solution (1.0 ml; 0.3 mM) plus methanol (2.5 ml) was used as control. Stock solution (1 mg/ml) of Quercetin was diluted to final concentrations of 250, 125, 50, 10 and 5 µg/ml in methanol used as positive control.

## 2.7. HPLC Analysis

HPLC analysis of plant ethanolic extracts was conducted as adopted by Mattila *et al.* (2000). The method was carried out on HPLC chromatograph (Hewlett Packard<sup>®</sup>, USA (series 1050); C18 column 250 × 4.6 mm, UV detection at 330 nm and quarter HP pump (series 1050). The column temperature was maintained at 35 °C. Gradient separation was carried out with methanol and acetonitrile as a mobile phase at flow rate of 1 ml/min. The standards rosmarinic acid, rutin, hesperidin, quercitrin, quercetin, naringenin, kaempferol, apigenin and the plant extract (1 µg/ml) were dissolved in 1ml mobile phase and 20 µl were then injected. Retention times and peak areas were used to determine identity and respectively the concentrations.

## 2.8. Statistical Analysis

data are presented as mean ± standard deviation (SD). comparisons among multiple groups were conducted using one-way ANOVA. Kaplan–Meier survival analysis was performed using the log-rank test. pearson's correlation coefficient was used for correlation analysis. Statistical analyses were performed using Graphpad prism 9.0 (Graphpad Software, San Diego, CA, USA) and R version 4.2.0 (R Foundation for Statistical Computing, Vienna, Austria). A p value < 0.05 was considered statistically significant. All experiments were performed in triplicate. Results were expressed as mean ± standard deviation (SD). Correlation analysis was conducted using linear regression, and significance was considered at p < 0.05. "Correlation analysis was conducted using linear regression": A statistical test was used to determine the strength and direction of the relationship between two variables.

### 3. RESULTS AND DISCUSSION

#### 3.1. Total Phenolic Content

high phenolic content was detected in *Cyperus rotundus* estimated as 162 mg Gallic acid/g dry source of poly phenolic, flavonoids and phenolic acid notably Rosmarinic, Quercetin, Naringin, Kampferol, Apegnine.

#### 3.2 Total Flavonoid Content

flavonoidal content was estimated in *Cyperus rotundus* 86.0 mg plant is a good source of antioxidant as they are rich in phenolic compounds.

Identification of flavonoids constituents: different flavonoidal compounds normally have specific chromatographic behavior (retention time tR) and uv spectral characteristic.

#### 3.3: Correlation between total phenolic content and antioxidant capacity of plant extract

Antioxidant Activity  $IC_{50} = 69.16, \mu\text{g/mL}^1 / EC_{50} = 0.014$  and total phenolic = 19 Indicates strong antioxidant activity.

#### 3.4 Antioxidant Capacity of plant extracts

**Table 1: DPPH scavenging activity of plant extract.**

| Plant extract    | Concentration        | Scavenging activity |
|------------------|----------------------|---------------------|
| Cyperus rotundus | 250 $\mu\text{g/ml}$ | 84.48%              |
|                  | 125 $\mu\text{g/ml}$ | 77.21%              |
|                  | 50 $\mu\text{g/ml}$  | 43.23%              |
|                  | 10 $\mu\text{g/ml}$  | 25.6%               |
|                  | 5 $\mu\text{g/ml}$   | 10.77%              |

#### 3.4 Correlation Analysis

$R^2 = 0.69$  Indicates a moderate positive correlation between phenolics and antioxidant activity.

#### 3.5. HPLC Analysis

Identification of the major flavonoids compound in was carried out using HPLC. The flavonoids (Rosmarinic, Rutin, Hesperidin, Quercetin, Naringin, Hesperetin, Kampterol and Apegnine (fig. 2) shows a typical HPLC chromatogram of flavonoids standards in this study.

**Table 2: HPLC analysis of *Cyperus rotundus* plant extract.**

| Compound   | Test results mg/100g |
|------------|----------------------|
| Rosmarinic | 765.80               |
| Rutin      | 545.99               |
| Hesperdin  | 211.78               |
| Quercetin  | 104.05               |
| Naringin   | 233.4                |
| Kaempferol | 351.44               |
| Apegnine   | 107.22               |

#### 4. DISCUSSION

Many assay methods for antioxidant activity *in vitro* and *in vivo* have been developed, but only a few rapid and reliable methods applicable to antioxidant activity assay for a huge number of plant extract samples exist (Miller *et al.*, 1995).

Total antioxidant capacity assay, such as ABTS and DPPH methods, is more common for antioxidant activity for large scale examination.

The DPPH methods, was successfully used in this study to systematically assess the total antioxidant capacity of plant extract being simple, fast, inexpensive, this effective and efficient method can be used for systematic screening of medicinal herbs and dietary plant for their relative antioxidant content. Previous studies found that there was a direct relationship between antioxidant activity and total phenolic content in selected herb and this plant. Phenolic compounds has a major contribution to antioxidant activity (Velloso; *et al.*, 1998, Sun; *et al.*, 2002). The results demonstrate that *Cyperus rotundus* rhizomes contain high levels of phenolic and flavonoid compounds, which significantly contribute to their antioxidant activity. The strong antioxidant effect (low IC<sub>50</sub> value) can be attributed to the presence of compounds such as rosmarinic acid, rutin, and kaempferol, which are known for their free radical scavenging abilities.<sup>[7]</sup> Singh, R., *et al.* (2008).

The correlation coefficient ( $R^2 = 0.69$ ) suggests that phenolic compounds play a major role in antioxidant activity, although other phytochemicals may also contribute.

Compared to previous studies, (Wang, Y., *et al.* (2022)). the phenolic content and antioxidant activity observed in this study are relatively high, which may be due to environmental factors, extraction method, or plant origin.



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