Original Article

Chemical Composition and Antioxidant Potential of Polyphenol Compounds of *Cyperus rotundus* L. Rhizomes

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ABSTRACT

The aim of the study is extracted and purity flavonoids from *Cyperus rotundus* rhizomes. Ethanolic extract obtained by removing the oily material from it by using soxhlet methods with of petroleum ether solvent and then wash with ethanol solvent in concentration 70%, the extract which confirms the presence of polyphenol and flavonoids. Purified flavonoids was obtained by gel filtration column (Sephadex LH-20).The ethanolic extract and purified flavonoids were examined by spectral diagnostic using Thin Layer Chromatography (TLC) and high performance liquid chromatography (HPLC) which showed the presence of flavonoids compound. The ethanolic extract and flavonoids were first subjected to phytochemical analysis followed by evaluation of their antioxidant potential by measuring the total phenolic content. The free radical scavenging activity of Rhizomes from *Cyperus rotundus* evaluated by 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay the results revealed that activities of (BHT) synthetic antioxidant and vitamin C as free radical scavenging increased when is compared with flavonoid purified and ethanolic extract rhizomes, the free radical scavenging activity of flavonoid purified is less than synthetic antioxidant and flavonoid purified is highest free radical scavenging to ethanolic extract *C. rotundus*. Total phenolic of *Cyperus rotundus* sample ethanolic extract 10 and 25 mg/ml were 1.1758 and 2.0969mg/ml, respectively, and pure flavonoid 10mg/ml and 25mg/ml were 1.0159 and 1.1861mg/ml respectively. These results established the antioxidative potency of *C. rotundus* Rhizomes, which may account for some of the medical claims attributed to this plant.

Keywords: *Cyperus rotundus* L. rhizomes, Antioxidant, Total phenolic, Flavonoid, Ethanolic extract.

INTRODUCTION

Cyperus rotundus, the Arabic common name is Saed, Sajal, Seil and in English commonly called nut grass, pruple nut sedge, Nagarmotha and in china called Xiang Fu. Aromatic herbs and spices have been used for a long time in alternative medicine, not only to improve or modify the flavor of foods, but also to avoid its deterioration. *Cyperus rotundus* L., is widely distributed in the Mediterranean basin areas. This plant grows naturally in tropical, subtropical and temperate regions such as in Iraq, Egypt, Tunisia, China and India[1]. The tuber part of *C. rotundus* is one of the oldest known medicinal plants used for the treatment of dysmenorrheal and menstrual irregularities[2]. The polyphenolic extract of *C. rotundus* is greater and more effectual extract in toxicity against cancer cells *in vitro*, and both volatile and crude extracts varies between each other in their potential toxic effects against cancer cells *in vitro*[3].

The literature contains numerous references to the use of this plant's roots for polyphenol and its seeds for food products. Tuber extracts may reduce nausea and act as a muscle relaxant. A number of pharmacological and biological activities including anti-*Candida*, anti-inflammatory, antidiabetic, antidiarrhoeal, cytoprotective, antimutagenic, antibacterial, and antioxidant, activities have been reported for this plant. The phytochemical investigation of *C rotundus* had been revealed the presence of flavanol, glycoside, saponin, phenol, terpenoids cardiac glycosides[4]. The hydroalcoholic extract of C. rotundus (CRE) was evaluated by various antioxidant assays, including the antioxidant capacity of the phosphomolybdenum method, total antioxidant activity in linoleic acid emulsion systems, 1, 1-diphenyl-2-picrylhydrazyl (DPPH), superoxide, hydroxyl radicals, and nitric oxide (NO) scavenging[5]. The extract exhibited high reduction capability and powerful free radical scavenging, especially against DPPH and superoxide anions as well as a moderate effect on NO. CRE[6].

The rhizomes of *Cyperus rotundus* which are used as traditional folk medicines for the treatment of stomach, bowel disorders and inflammatory diseases. *Cyperus rotundus* contains essential oils, terpenes, flavonoids, b-sitosterol, and ascorbic acid. The main terpenes in *Cyperus rotundus* are cyperenes, which include sesquiterpene hydrocarbons[7].

MATERIALS & METHODS

The tubers of *Cyperus rotundus* rhizomes, its obtained from the local Baghdad market and farms Basrah has been diagnosed by the College of Science / Baghdad University, cleaned it after that broke and ground it by using electric grinder.

Extraction and purification

The extraction of flavonoids from the rhizomes of *Cyperus rotundus* was in several methods as follows:-

The extract has been prepared according to the method used by Ozaki *et al.,*[8] *with some modification* .In this method used 50 gram of powder tubers of the rhizomes of *Cyperus rotundus* and put it in a bag of cloth then put it in the Soxhlet extractor with 350 ml of petroleum ether solvent in the flask of extraction and the extraction conducted by using Soxhlet extractor at the temperature 50°c for 6 hours in this method separated oily material from plant material where took the mixture in the flask of extraction after stopping the extraction operation and this mixture is the oil and petroleum ether solvent where got on the oily material after disposal of the solvent.

After that, took the second material after removing the oily material from it and put it in the volumetric flask and added to it a

certain size up to 350 ml of the ethanol solvent in concentration 70% and left it at room temperature for 48 hours, the solution has been filtered through a filter paper and evaporated to dryness under vacuum at 40°C, the dried extract (which called ethanolic extracted) has been weighed and stored at 4 °C.

Gel-filtration sephadex LH-20 column

A- Sephadex LH-20 gel was prepared with mixed with ethanol 99.9% solution left for a time and wash the gel 3 times with the same solution, then the slurry was degaussing under reduced pressure in order to remove air from the gel. The gel was allowed to equilibrate for 4-5hrs at room temperature and then poured carefully into a glass column.

B- According to Al-Jumaily *et al*.,[9] the flavonoid compound was separated from ethanolic extracted had been preceded using glass column (1.75 x 49) cm filled with Sephadex LH-20 (A) which prepared by mixed with ethanol 99.9% solution. The ethanolic extracted of the rhizomes of *Cyperus rotundus* was subjected to column and eluted with ethanol solution. The sample volume was 5ml, and the flow rate regulated to be 60 ml/min.

The elusions had been collected in large tubes for each mobile phase used and numbered as fractions; all fractions had been tested by ferric chloride solution 1%. Fractions containing flavonoid compound were pooled, concentrated to the required volume.

Tests conducted on material extracted

Thin layer chromatography (TLC)

This method was described by Sinisa *et al* .,[10] it involved using glass plated -58- A attention: (20 x 20 cm) coated with silica gel with thickness of 0.2 millimeter type (Silica gel 60 F 254 (Merck KGaA). The sample (10µl) was spotted on TLC plate the chromatogram was developed with standardized separation solution (mobile phase) consisted of (n-butanol: acetic acid: water) (62.5:36:1.5).The developed chromatogram was observed under Ultraviolet light.

High-performance liquid chromatography (HPLC)

The phenol compounds from *Cyperus rotundus* was identities by (HPLC) according to Redaelli*etal*.[11], using octyldodecyl silica ODS- reverse phase column and an elution system under the following conditions (Table 1).

Chemical detection for tannins and phenolic compounds

Ferric chloride test

According to Harborne[12], 1 ml of the plant extract was added to 1ml of ferric chloride solution 1%; the appearance of intense green, purple, blue or black colors indicated the presence of phenolic compound.

Lead acetate test

It was prepared by dissolving 1gm of lead acetate in 100ml D.W, and directly 1ml of solution was added to 1ml of extract. The positive result is white jelly residue[13].

1. Detection of tannins: To 1ml of extract added strong potassium dichromate solution 10%, a yellow color precipitate indicates the presence of tannins and phenolic compound.
2. Keep 1 ml of extract in a test tube and add drops of sulfuric acid Conc. Indicates the emergence of a reddish brown color to positive detection[14].
3. The extract is treated with sodium hydroxide; formation of yellow color indicates the presence of flavones[15].

Evaluation of antioxidant activity

DPPH radical scavenging assay

The antioxidant activity of the extracts was measured on the basis of the scavenging

activity of the stable 2, 2-diphenyl-2-picrylhydrazyl (DPPH) free radical according to the method described by Huang *et al*., [16] slight modifications.5ml of a freshly prepared 0.004% of DPPH in method was mixed with 50 ml of different concentration of pure and partial pure of total phenol compounds (5, 10, 15, 25, 35 and 50 mg/ml). Corresponding Blank. The sample was prepared and L-Ascorbic acid and Butylated hydroxytoluene (BHT) were used as positive control. Mixer of 1ml methanol and 1ml DPPH was used as a control. The reaction was carried out in triplicate and the decrease in absorbance was measured at 517 nm after 30 minutes in dark using UV-Vis spectrophotometer.The inhibition % was calculated as:

% Reduction = (Abs DPPH – Abs Dil.)/
Abs DPPH x 100.

Whereby:

Abs DPPH = average absorption of the DPPH solution

Abs Dil. = average absorption of the three absorption values of each dilution.

With the obtained values, a graphic was done using Microsoft Excel. The EC50 of each extract (concentration of extract or compound at which 50%of DPPH is reduced) was taken from the graphic.

Estimation of total phenolic contents

The procedure used was based on the methods outlined by Folin- Ciocalteau[17]. A calibration curve was built using standard aqueous solutions of gallic acid (0-0.05mg/ml) (Figure 1). One mL of each solution was added to 250 µ L of sodium carbonate and 25 µ L of the Folin–Ciocalteau reagent in a test tube, homogenized and allowed to react for 30 minutes at a temperature of 20 °C. Absorbance was measured at 710 nm with a spectrophotometer and the calibration curve calculated by the minimal squares method. The dry extracts of *Cyperus rotundus* were dissolved in absolute alcohol to a concentration of 20% (w/v), one mL of this ethanol solution was further diluted in 1000 mL of distilled water and homogenized. One mL of this final solution was prepared and analyzed in the same way as the standards.

RESULTS AND DISCUSSION

Rhizomes of *Cyperus rotundus* were used in the extraction, because all the chemical constituents of that herb were present in these parts[2]. The extract was 70% ethanolic extract and fifty gram of the *Rhizomes of Cyperus rotundus* gave three grams of curd ethanolic extract. The yield was varied from extracts to another due to the part and the type of extraction this can be in many plant extracts. Ethanolic extract of Rhizomes of *Cyperus rotundus* when was dryness giving resinous brown product and was indicated by sulfuric acid was the emergence of less reddish color these explain that ethanolic extract could not contain just flavonoid. The dark brown color may be due to the presence of large amounts of polyphenolic compounds and flavonoids[18,19]. But contain mainly polyphenol compound diagnose and determine the purity of the compound flavonoids used TLC, liquid chromatography, high efficiency to determine the purity of this compound so resulted showed of these examination presences flavonoid compounds. Liquid chromatography media designed for molecular sizing of natural products such as steroids, terpenoids, lipids and low molecular weight peptides (up to 35 amino acid residues depending on the chosen solvents. Flavonoids were eluted as a symmetrical peak on Sephadex LH-20 confirming the suitability of this matrix for the superior separation rather than silica gel. The used methods, was very good in its yield and it protected the extract from rising temperature or alkalinity during the extraction process, because these two factors may damage the active compounds during extraction[20].

Thin layer chromatography (TLC)

C. rotundus roots have the highest amount of quercetin. Myricetin and kaempferol are also present in good quantities[12]. TLC technique is considered as a good technique for the isolation and purification to get a yield of purified components in grams amount; therefore, different samples of ethanolic extract (crude), purified flavonoid have been applied to the TLC plate using n- butanol –acetic acid-water (62.5:36:1.5), the results show that all samples of the ethanolic extract, purified flavonoid contain myricetin, quercetin, kaempferol and Apgenin which have been appeared as a brown spots in the upper third of the plate R*f* (0.84,0.43,0.64,0.89)[11] (Figure 2).

High performance liquid chromatography

All flavonoid aglycones contain at least one aromatic ring and, consequently, efficiently absorb UV light it is evident that phenolics absorb well in the UV range and UV detection is therefore a convenient method to localize a phenol in the effluent of a column. However, no single wavelength is sufficient for their simultaneous monitoring in various natural plant extracts. Detection at 280 nm is most generally used for the simultaneous separation of mixtures of phenolic acids, although for dual monitoring 254 and 280 nm, or 280 and 320 nm, can be ideal wavelengths used liquid chromatography technology, high efficiency to determine the purity of this compound, the results showed the time of detention in Figures (3 and 4) for each of the Flavonoid purified and Ethanolic extract (crude).

Chemical detection of phenolic compounds

The phytochemical detection of the ethanolic extract shows the presence of flavonoids, tannins, phenols as shown in (Table 2).

Evaluation of antioxidant activity

DPPH radical scavenging assay

Free radicals are produced in normal and or pathological cell metabolism. Oxidation is essential to many living organisms for the production of energy to fuel biological processes. Exogenous chemical and endogenous metabolic processes in the human body or in the digestive system might produce highly reactive free radicals, especially oxygen derived radicals, which are capable of oxidizing biomolecules, resulting in cell death and tissue damage[21].

Figure (5) illustrates the concentration of DPPH radical due to the scavenging ability of the flavonoid, ethanolic extract and standard BHA and vit C were used as references. The radical scavenging capacity (EC_{50}) of flavonoid purified and ethanolic extract was found to be 21 and 26 µg/ml which is the concentration that decreases the initial DPPH radical concentration by 50%. On the other hand the (EC_{50}) of vit C and BHT were 4.0 and 2.5 µg/ml respectively.

Effectiveness of antioxidant properties is inversely correlated with EC_{50} values. DPPH radical was used as a stable free radical to determine antioxidant activity of natural compounds. The antioxidant activity of plant extracts containing polyphenol components is due to their capacity to be donors of hydrogen atoms or electrons and to capture the free radicals [22].

Cyperus rotundus at different extract demonstrated significant DPPH scavenging activity indicating their abilities to act as radical scavengers.

Saleh *et al.,*[23] determined *in vitro* antioxidant activity of 248 essential oils belonging to 18 botanical families of medicinal, herbal and wild flora as well as two mammalian essential oils by using DPPH radical scavenging, at does 5, 25, 100 mg essential oil /mL. Seven percent of the tested

essential oils were found to have very high antioxidant activity.

Total antioxidant activity of aqueous rhizome extract of *H. rubrum*, *H. coronarium* and *H. spicatum* in terms of ascorbic acid equivalent (AAE) was 207.3, 157.5 and 102.6 µg/ml of extract and DPPH assay *H. rubrum*(32.3%) showed the highest free radical scavenging activity followed by *H. coronarium* (21%) and lowest activity in *H. Spicatum* (5.76%)[24].

Estimation of total phenolic content

The Folin-Ciocalteu method is a rapid and widely-used assay investigating the total phenolic content, but it is known that different phenolic compounds gave different responses with this method[25]. The data present in table (3) that showed the total phenolic contents of *Cyperus rotundus* sample (Gallic acid equivalents, mg/ml) ethanolic extract 10 and 25 mg/ml were 1.1758 and 2.0969mg/ml, respectively, and purified flavonoid 10 and 25mg/ml were 1.0159 and 2.0969mg/ml respectively. These results agreed with the study by Al-Hilli *et al*.,[26] about *Cyperus rotundus* contained higher total phenolics which ethanol extracted by Soxhlet and maceration different days.

CONCLUSION

We can conclude from the results that the pure flavonoid and ethanolic extraction from *Cyperus rotundus* Rhizomes, which may account for some of the medical claims attributed to this plant and can be used as a source of antioxidant for pharmacological prepared.

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Table 1. Conditions on HPLC on phenol compound[11]

Column	ODS
Column length	25 cm
Flow rate	1 mL /min
Wave length	275 nm
Mobile phase	Acetic acid, deionized, water, acetonitrile (0.1: 40: 60)
Retention time	1 mL /min

Table 2. General phenolic compound tests

Test	Result
1% Ferric chloride solution	Green colour (+)
10% Potassium dichromate solution	Yellow color precipitate
1%Lead acetate solution	White color precipitate
Sulfuric acid Conc.	Brown color (+)

Table 3. Total phenolic content of *Cyperus rotundus*

Sample	Concentration (mg/ml)	Total phenol (mg/ml)
Flavonoid Purified	10	1.0159
	25	1.1861
Ethanolic extract (crude)	10	1.1758
	25	2.0969

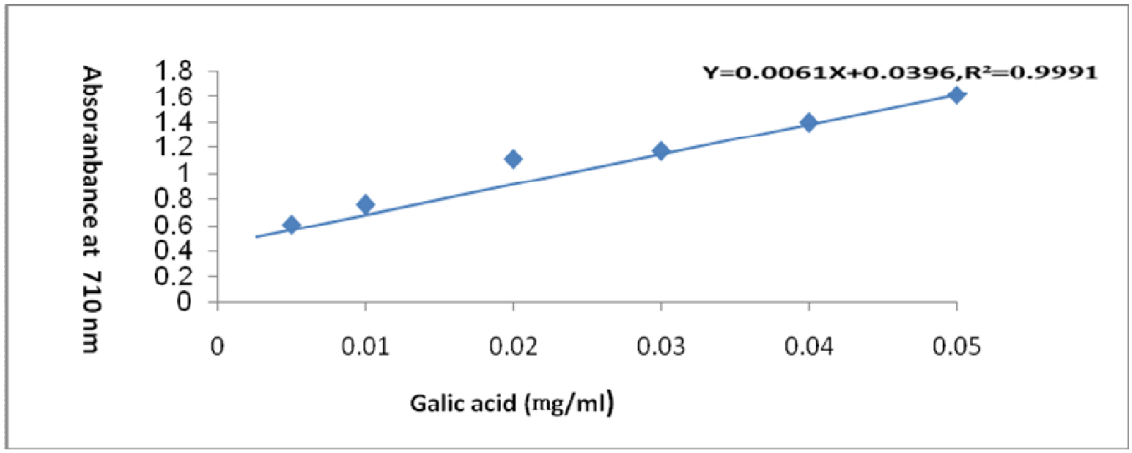

Figure 1. Standard curve of gallic acid

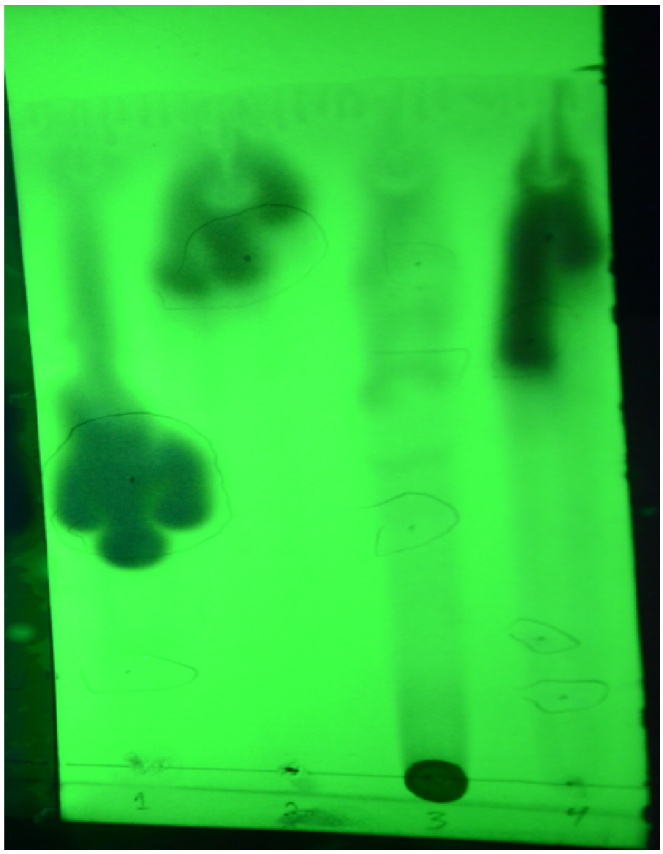

Figure 2. TLC of the *cyperus rotund* in butanol– Acetic acid-water (62.5:36:1.5) the brown zone indicate the presence of flavonoid compound

1.2.3-Flavonoid purified, 4-Ethanolic extract

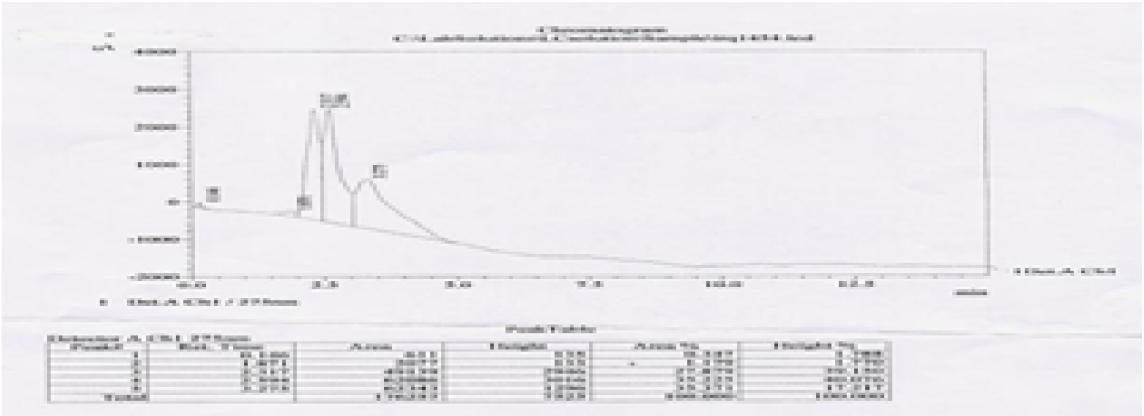

Figure 3. Chromatography high efficiency liquid flavonoid compound

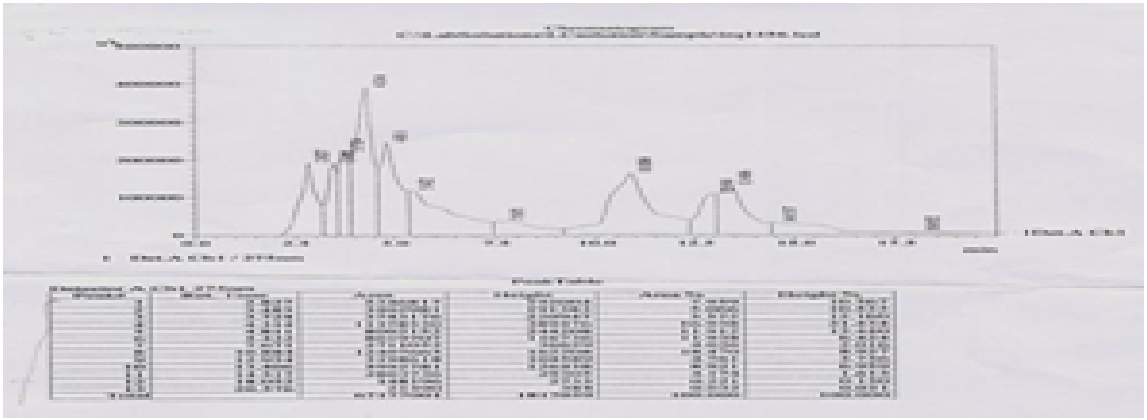

Figure 4. Chromatography high efficiency liquid Ethanolic extract

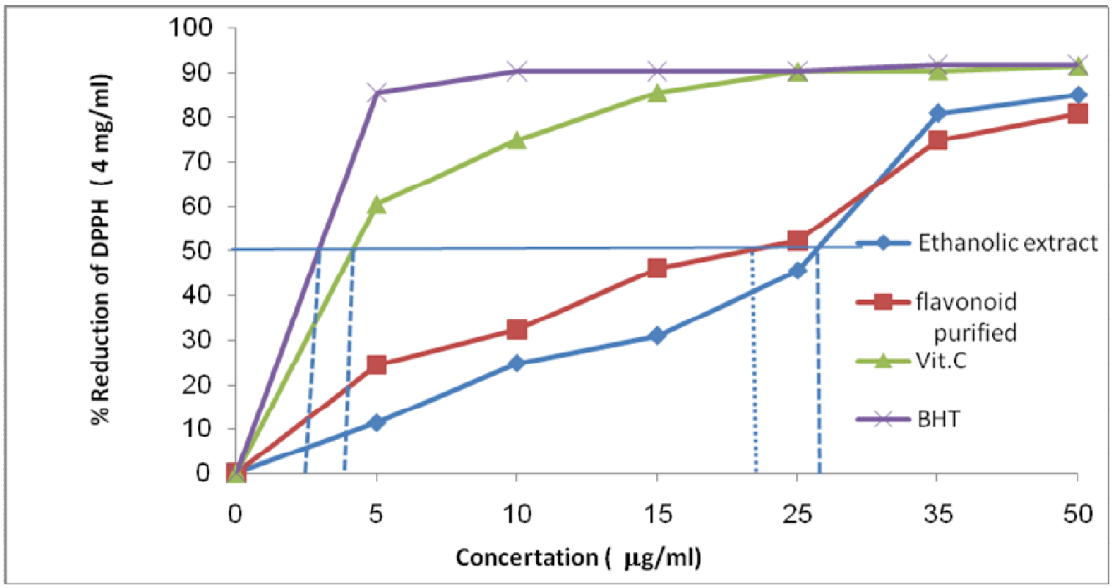

Figure 5. Percentage of DPPH reduction using *Cyperus rotund*us (flavonoid pure and ethanolic extract) and appropriate controls after 30 min of exposure. The corresponding EC50 are also outlined