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Research Article

PHYTOCHEMICAL INVESTIGATION AND EVALUATION OF *IN VITRO* ANTIOXIDANT ACTIVITY OF THE PLANT *CYPERUS TEGETUM* ROXB

ATANU CHATTERJEE[1]*, RITU KHANRA[2], PRANABESH CHAKRABORTY[1], HIMANGSHU SEKHAR MAJI[3]

[1]Department of Pharmaceutical Technology, Bengal School of Technology, Sugandha, West Bengal, India. [2]Department of Pharmaceutical Technology, NSHM Knowledge Campus, Kolkata, West Bengal, India. [3]Department of Pharmaceutical Technology, JIS University, Agarpara, West Bengal, India. Email: acchatterjee@yahoo.co.in

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ABSTRACT

Objective: The objective of the present study is to isolate the lead molecules and the antioxidant activity is also evaluated.

Method: *Cyperus tegetum* Roxb. (*Cyperaceae*) is found in the tribal area of West Midnapur district of West Bengal, India. It is commonly known as Madur Kathi. Different chromatographic techniques, namely, thin-layer chromatography, column chromatography, and high-performance liquid chromatography (HPLC) were used to isolate and identify the different secondary metabolites.

Results: The different spectral studies (nuclear magnetic resonance [NMR], infrared [IR], and ultraviolet [UV]) confirmed the presence of stigmasterol as an isolated compound from the extract of *C. tegetum* (ECT). HPLC analysis revealed the presence of flavonoids, namely, rutin (retention time [Rt]: 3.00), myricetin (Rt: 3.9), and quercetin (Rt: 5.6) and phenolic acids, namely, gallic acid (Rt: 4.0), caffeic acid (Rt: 5.4), chlorogenic acid (Rt: 7.3), and ferulic acid (Rt: 8.8) in ECT. ECT showed strong reducing power, diphenyl-2-picrylhydrazyl hydrate radical, superoxide anion scavenging, and hydrogen peroxide scavenging activities when compared to standard compounds.

Conclusion: From this study, several flavonoid and phenolic compounds were identified by RP-HPLC analysis. Flavonoids are rutin, quercetin, and myricetin and phenolic compounds are gallic acid, ferulic acid, chlorogenic acid, and caffeic acid, respectively. The different spectral studies (NMR, IR, and UV) confirmed the presence of stigmasterol as an isolated compound from ECT.

Keywords: Stigmasterol, *Cyperus tegetum*, Antioxidant.

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INTRODUCTION

In the field of health care, medicinal plants have been playing an important role in the treatment of various diseases from ancient times. The present research in drug discovery from medicinal plants engages a versatile approach including botanical, phytochemical, biological, and molecular techniques. There are large verities of phytochemicals (i.e., steroids, terpenoids, carotenoids, flavonoids, alkaloids, tannins, glycosides, etc.) which are non-nutrient secondary metabolites of plants [1]. Phytochemicals can be classified based on their chemical structures, phytochemical nature, and the biosynthetic pathway of production [2]. Every phytochemical has its pharmacological properties where signaling molecules work by utilizing different pathways, namely, redox signal, polyol pathway, Akt pathway, etc. [3]. Secondary metabolites are mainly derived from different biosynthetic pathways, namely, phenylpropanoid, isoprenoid, and alkaloid pathways. Recently, researchers have recognized that the secondary metabolites exhibit important roles in defense mechanisms (free radical scavenging, ultraviolet (UV) light absorbing, antiproliferative, antimicrobial, etc.) of the plant and help the plants to accommodate to different environments [4]. These secondary metabolites can be explored in various ways to utilize their potential in the modern development of drugs. Bioassay-guided fractionation may lead to standardized extract/fractions or isolated bioactive compounds as the new drugs [5].

Cyperus tegetum Roxb. belonging to the family *Cyperaceae* is a glabrous robust plant having 1.5–2 m height with woody rhizomes. These plants mostly cultivated in the lowland area of India [6]. It is also cultivated in Sri Lanka, Bangladesh, Nepal, China, Japan, Thailand, Malaysia, Java, and Sumatra. Conventionally, it is used by the tribal people for the treatment of cachexia, atrophy, and snakebite. The rhizomes of plant *C. tegetum* contain alkaloids, triterpenoids, flavonoids, saponins, and reducing sugar [7]. Going through literature survey, it is observed that little work has been attempted on different pharmacological activities such as antidiabetic and antidiarrheal [8,9]. However, no evident work on isolation of phytochemicals has been observed yet. This investigation was undertaken to isolate the lead antioxidant molecules from *C. tegetum* Roxb., employing bioassay-guided fractionation. The antioxidant activity of the ribosome extract of the plant was also evaluated.

MATERIALS AND METHODS

Plant material

Rhizomes of the plant of *C. tegetum* were collected from West Midnapur, West Bengal, in the month of June–July 2014. Plant taxonomical identification was done by the Botanical Survey of India, Kolkata. The voucher specimen is No. CNH/I-I (197)/2007/Tech.II/161. The specimen has been preserved in our laboratory for further reference.

Proximate analysis

Proximate analysis of the samples is defined by moisture content, ash, crude protein, fat, and crude fiber were determined by the method of Association of Analytical Chemist (2000) [10].

Preparation of extract

The fresh rhizomes of *C. tegetum* were chopped in small pieces and shade dried. Then, dried material was powdered in a mixture grinder. The dried powdered rhizomes (5.0 kg) were macerated with methanol (MeOH) (4 l×2.5 l) with continuous agitation. The MeOH extract was evaporated using the rotary vacuum evaporator. The extract of *C. tegetum* (ECT) was suspended in water and defatted with n-hexane

followed by fractioned by dichloromethane. Dichloromethane fraction was enriched with steroids and triterpenoids. The dichloromethane fraction (yield 5 % w/w) was subjected for further study.

Phytochemical analysis

Preliminary phytochemical analysis revealed the presence of triterpenoids, steroids, flavonoids, and phenolic compounds in ECT. Based on the preliminary phytochemical studies, dichloromethane fraction was subjected to silica gel (60–120 mesh) column chromatographic separation using mixtures of n-hexane-EtOAc and EtOAc-MeOH of increasing polarity to yield fractions (A-F). Fraction A (6.02 g) was separated with n-hexane-dichloromethane and dichloromethane-MeOH, to yield compound 1 (yield 0.7% w/w). High-performance liquid chromatography (HPLC) analysis of ECT was performed to detect the presence of phenolics and flavonoids by comparing with reference phytochemical markers [11]. Chromatographic studies were performed by HPLC system (Dionex Ultimate 3000, Germany), equipped with a reverse-phase (RP) C-18 column (250 mm×4.6 mm, particle size 5 µ) and a UV detector was used for the identification of individual phenolic and flavonoid compounds. Briefly, the standard markers and ECT were dissolved in MeOH (HPLC grade) and filtered by cellulose nylon membrane filter (0.45 µm). The aliquots of the filtrate were eluted with isocratic solvent mixtures comprising methanol: acetonitrile:acetic acid: o-phosphoric acid: water (20:10:1:1:20) for flavonoids and methanol: water:acetic acid (75:24:1) for phenolics with a flow rate of 1 ml/min and detected at 340 and 254 nm, respectively.

***In vitro* antioxidant assay**

Total phenolic content

The total phenolic content of the ECT was performed by the method of Slinkard and Singleton [12]. 0.5 mL of ECT was mixed with 1 mL of diluted Folin–Ciocalteu reagent and 2 mL of 7.5% sodium carbonate solution. The mixture was stood for 90 min at room temperature. The absorbance of the mixture was measured using a UV–visible spectrophotometer (UV-1800, Shimadzu, Kyoto, Japan) at 765 nm [13]. Total phenolic contents in the extracts were determined from a standard curve prepared with ascorbic acid, butylated hydroxyl toluene (BHT), and butylated hydroxyl anisole (BHA).

Total flavonoids content

The total flavonoid content of the ECT was measured by the calorimetric assay method based on the procedure of Nurul and Rabeta [14]. 1 mL ECT was added to 4 mL of distilled water (dH_2O). Approximately 0.3 mL of 5% (w/v) sodium nitrite and 0.3 mL of 10% (w/v) aluminum chloride were added, respectively. 2 mL of 1 M sodium hydroxide solution was added on the above mixture. The total volume was adjusted to 10 mL with distilled water. The absorbance of the sample was measured at 415 nm. Total flavonoid contents in the extracts were determined from a standard curve prepared with ascorbic acid, BHT, and BHA.

Determination of reducing power

The reducing power of ECT was determined with the help of the Oyaizu method [15]. Different concentrations (50, 100, 150, 200, 250, and 300 µg/ml) of ECT were prepared with distilled water and each of the concentrations was mixed with 2.5 ml phosphate buffer (0.2 M, pH 6.6) and 2.5 ml of 1% potassium ferricyanide. The mixture was incubated at 50°C for 20 min. 2.5 ml of trichloroacetic acid (10%) was added to this mixture, which was then centrifuged at 3000 g for 10 min. The upper layer of the solution (2.5 ml) was mixed with 2.5 ml of distilled water and 0.5 ml of 0.1% FeCl3, and the absorbance was measured at 700 nm. All the tests were performed in triplicate. BHA, BHT, and ascorbic acid were used as a reference standard. The gradual enhancement of absorbance with concentration indicates the reducing power of the sample.

Determination of 1-diphenyl-2-picrylhydrazyl hydrate (DPPH) radical scavenging activity

The spectrophotometric method was utilized to measure free radical scavenging activity of ECT against stable DPPH (Sigma-Aldrich Chemie, Germany). In that reaction, DPPH gets reduced by an antioxidant compound, which can donate hydrogen. The color changes from deep violet to light yellow and the absorbance was measured at 515 nm [16]. 1 ml of 0.1 mM solution of DPPH in ethanol was added to 3 ml of various concentrations (5–100 µg/ml) of ECT. After 30 min, absorbance was measured at 517 nm. The percentage of inhibition was calculated by comparing the absorbance values of the control and test samples using BHA, BHT, and ascorbic acid as a reference compound all the tests were performed in triplicate. The free radical scavenging activity against DPPH radical was calculated using the following equation:

$$\% \text{ DPPH scavenging activity} = (A_{control} - A_{test})/A_{control}$$

Where, $A_{control}$ is the absorbance of the control reaction and A_{test} is the absorbance in the presence of ECT. The antioxidant activity of the catechin was expressed as IC_{50}. The IC_{50} value is defined as the concentration (in µg/ml) of extract that inhibits the formation of DPPH radicals by 50%.

Determination of hydrogen peroxide (H_2O_2) radical scavenging activity

Ruch *et al*. [17] method was utilized to measure the scavenging activity of ECT against H_2O_2. A 40 mM solution of H_2O_2 was prepared in phosphate buffer (pH 7.4). Different concentrations of ECT (50, 100, 150, 200, 250, and 300 µg/ml) were prepared in distilled water and H_2O_2 solution was mixed to each solution (0.6 ml, 40 mM). Absorbance was determined after 10 min at 230 nm. Phosphate buffer without H_2O_2 was considered as blank and BHA, BHT, and ascorbic acid were treated as reference antioxidants.

$$\% \ H_2O_2 \text{ radical Scavenging activity} = (A_{Control} - A_{test})/A_{Control}$$

Where, $A_{Control}$: The absorbance of the control reaction
A_{test}: The absorbance in the presence of the sample of ECT.

Superoxide anion scavenging activity

Measurement of superoxide anion scavenging activity of ECT was done based on the method described by Liu *et al*. and Ye *et al*. [18,19] with slight modification. 0.1 ml of sample ECT was treated with 1 ml of nitroblue tetrazolium (NBT) solution (156 mmol/L NBT) and 1 ml nicotinamide adenine dinucleotide (NADH) solution (468 mmol/L). 100 ml of phenazine methosulfate solution (60 mmol/L) was mixed to the above solution to start the reaction. The incubation temperature was 25°C for 5 min, and the absorbance was measured at 560 nm. Decreased absorbance of the reaction mixture indicated increased superoxide anion scavenging activity. The percentage inhibition of superoxide anion generation was calculated using the following formula:

$$\% \text{ inhibition} = (A_{Control} - A_{test})/A_{Control}$$

Where, $A_{Control}$: The absorbance of the control reaction
A_{test}: The absorbance in the presence of the sample of ECT.

Determination of 2, 2′-azinobis-3 ethylbenzothiazoline-6-sulfonic acid (ABTS) radical cation decolorization assay

7 mM ABTS salt and 2.4 mM potassium persulfate were taken in equal volume. The mixture was kept in dark for 16 h at room temperature. The solution was considered as a stock solution. The resultant ABTS solution was diluted with methanol until an absorbance of about 0.70±0.01 at 734 nm was reached. Different concentrations of ECT (1 ml) were treated with 1 ml of the ABTS solution and the absorbance was measured at 734 nm within 3–7 min using the spectrophotometer. The ABTS scavenging capacity of ECT was compared with BHA, BHT, and ascorbic acid and the percentage inhibition calculated as follows:

$$\text{ABTS radical scavenging activity (\%)} = ([Abs_{control} - Abs_{sample}]/[Abs_{control}]) \times 10.$$

Statistical analysis

The data were analyzed by one-way ANOVA followed by Dennett's *t*-test GraphPad Prism and GraphPad InStat software. The data were expressed as mean±SD. The significance was considered when $p<0.05$.

RESULTS

The phytochemical investigation reveals that the ETC contains several polyphenolic and triterpenoid compounds. Chromatographic and spectroscopic analysis (UV, nuclear magnetic resonance [NMR], and MS) produce the proof of the presence of stigmasterol as the lead compound. The phenolic and flavonoid compounds are very important plant constituents due to their scavenging ability due to their hydroxyl groups [20]. Some authors found a correlation between phenolic content, flavonoid content, and antioxidant activity [21]. The phenolic and flavonoid compounds may contribute directly to antioxidant activity [22]. It is suggested that polyphenolic and flavonoid compounds have inhibitory effects on mutagenesis and carcinogenesis in humans, when up to 1.0 g daily ingested from a diet rich in fruits and vegetables.

Proximate analysis

The moisture content of the fresh rhizome was 39.02±0.25%. The removal of water and other organic matters present in rhizomes by heating produces an inorganic residue is known as ash. Ash content is an index of the total mineral element. Ash content of the fresh *C. tegetum* rhizome was 2.03±0.06%. Mineral content is directly proportional to drying. Hence, the value of ash content in the fresh sample is low due to less surface area and low reaction rate. Nitrogen content determination in food samples is based on its conversion into simple nitrogen derivatives. Table 1 shows that the protein content of the fresh rhizome of *C. tegetum* ranged from 1.31% to 1.89% and the average protein content was 1.60%.

Phytochemical analysis

Phytochemical analysis was done using the techniques of column chromatography and HPLC. Silica gel column chromatographic separation coupled analysis of the dichloromethane fraction yielded one lead molecule compound 1 (yield 0.7% w/w). The structure of lead was characterized by physicochemical and spectroscopic analysis (infrared [IR], NMR, and MS). Compound 1 was identified as stigmasterol (Table 2).

Phytochemical profiling through HPLC of ECT identified flavonoids and phenolic acids by comparing retention time (Rt) and UV spectra with standards. HPLC analysis revealed the presence of flavonoids (Figs. 1 and 2), namely, rutin (Rt: 3.00), myricetin (Rt: 3.9), and quercetin (Rt: 5.6) and phenolic acids (Figs. 1 and 2), namely, gallic acid (Rt: 4.0), caffeic acid (Rt: 5.4), chlorogenic acid (Rt: 7.3), and ferulic acid (Rt: 8.8) in ECT.

***In vitro* antioxidant assay**

Total phenolic and total flavonoids content

Total phenolic and flavonoids content can be correlated with the scavenging activity of the plant extract. Table 3 summarizes that total phenolic and flavonoid contents of ECT were 20.68 mg/g and 361.01 mg/g in terms of gallic acid and quercetin equivalent, respectively.

Reducing power

Fig. 3 shows the reductive capabilities of ECT compared to BHA, BHT, and ascorbic acid. For the measurements of the reductive ability, it was investigated that the Fe3+ to Fe2+ transformation in the presence of ECT samples using the method of Oyaizu (1986). The reducing capacity of a compound may serve as a significant indicator of its potential antioxidant activity [23]. However, the antioxidant activity of antioxidants has been attributed to various mechanisms, among which prevention of chain initiation, binding of transition metal ion catalysts, decomposition of peroxides, prevention of continued hydrogen abstraction, reductive capacity, and radical scavenging are there [24,25]. The reducing power of ECT increased with an increasing amount of sample. All of the amounts of ECT showed higher activities than control and these differences were statistically very significant ($p<0.01$). Reducing the power of ECT and standard compounds followed the order: BHA>BHT>ECT>ascorbic acid.

Table 1: Proximate composition of the matured leaves of *Cyperus tegetum*

S. No.	Proximate analysis	%
1.	Moisture	39.02±0.25
2.	Ash	2.03±0.06
3.	Protein	1.60±0.03

Table 2: Physicochemical and spectral data of compound 1: Stigmasterol

Physicochemical and spectroscopic technique	Data
Melting point	165°C
Physical appearance	White crystal
UV (λ_{max})	256 nm
IR (KBr cm^{-1})	3445, 2956, 2854, 1676, 1461, 1324, 1240, 1191, 1050, 969, 883
^{1}H-NMR ($CDCl_3$)	δ_H 5.35 (1H, m, H-6), 5.15 (1H, dd, H-22), 5.02 (1H, dd, H-23), 3.55 (1H, m, H-3), 2.28 (1H, m, H-4a), 1.99 (1H, m, H-7b), 1.02 (3H, s, H-19), 0.97 (3H, s, H-24), 0.86 (3H, d, H-26), 0.78 (3H, d, H-27), 0.67 (3H, s, H-18)
^{13}C-NMR ($CDCl_3$)	δ 140.7 (C-5), 138.2 (C-22), 129.3 (C-3), 121.7 (C-6), 71.8 (C-3), 56.08 (C-17), 51.2 (C-24), 50.1 (C-9), 45.8 (C-25), 42.3 (C-13), 39.7 (C-12), 36.1 (C-10), 31.6 (C-9), 29.1 (C-16), 24.3 (C-15), 21.0 (C-21), 19.0 (C-19), 12.0 (C-29), 11.8 (C-18)

Table 3: Total phenolic and total flavonoid content of the extract of plant *Cyperus tegetum*

Experiment	Result
1. Total phenolic content (gallic acid equivalent mg/g)	20.68
2. Total flavonoid content (quercetin equivalent mg/g)	361.01

Determination of DPPH radical scavenging activity

DPPH is a stable free radical and accepts an electron or hydrogen radical to become a stable diamagnetic molecule [26]. The reduction capability of DPPH radicals was determined by the decrease in its absorbance at 517 nm induced by antioxidants. Hence, DPPH is usually used as a substrate to evaluate the antioxidative activity of antioxidants. Fig. 4a illustrates a significant ($p<0.01$) decrease the concentration of DPPH radical due to the scavenging ability of soluble solids in the ECT and standards. We used BHA, BHT, and ascorbic acid as standards. The scavenging effect of ECT and standards on the DPPH radical decreased in the order of ascorbic acid >BHA>ECT>BHT. These results indicated that the ECT has a noticeable effect on scavenging free radicals. Free radical scavenging activity also increased with increasing concentration.

Determination of hydrogen peroxide (H_2O_2) radical scavenging activity

The ability of the ECT to scavenge hydrogen peroxide was determined according to the method of Ruch *et al*. [17]. The scavenging ability of ECT on hydrogen peroxide is shown in Fig. 4b and compared with BHA, BHT, and ascorbic acid as standards. The ECT was capable of scavenging hydrogen peroxide in an amount dependent manner. Fig. 4b shows that the ECT had strong hydrogen peroxide scavenging activity. Those values close to BHA, but lower than that BHT and ascorbic acid. There was

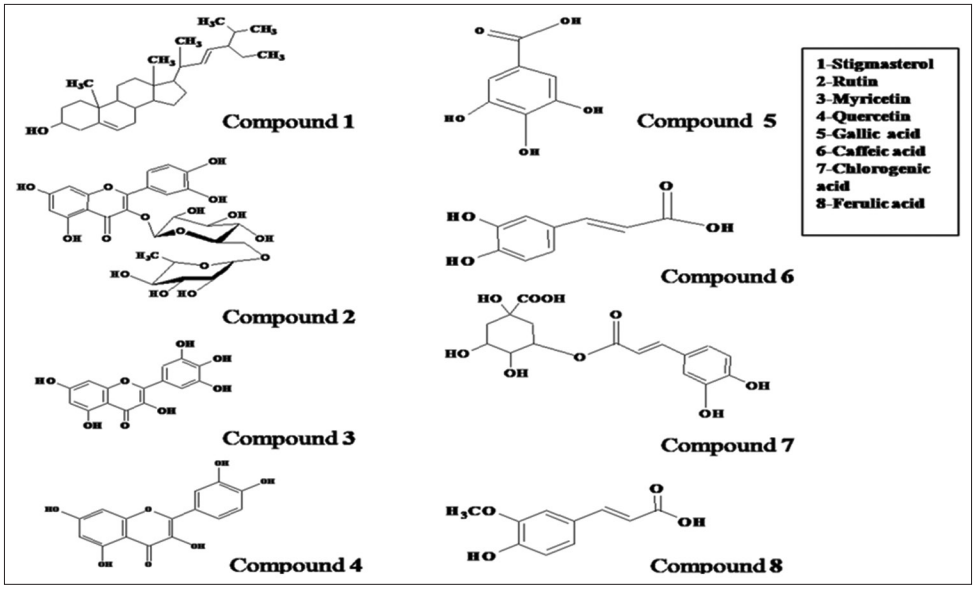

Fig. 1: Structure of isolated and identified compounds from *Cyperus tegetum*

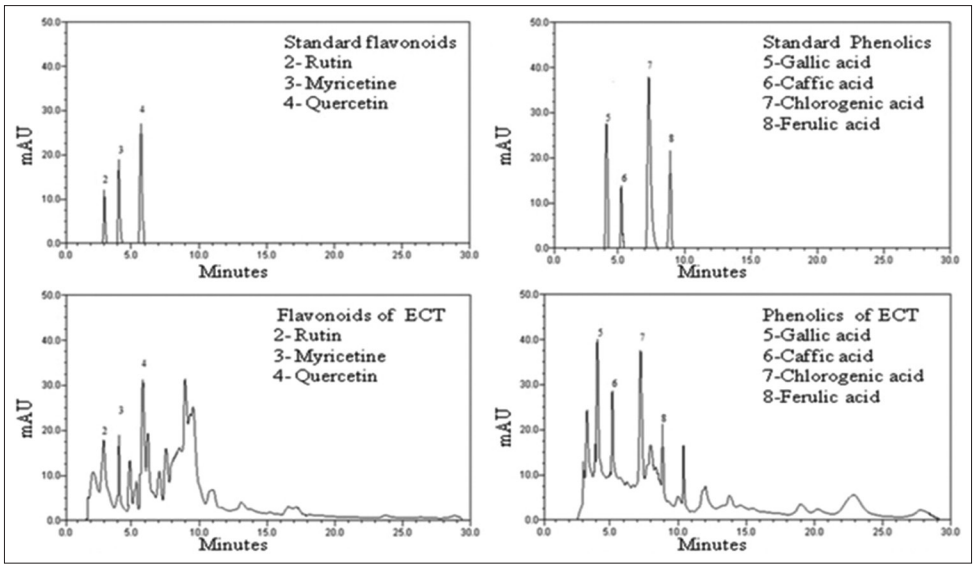

Fig. 2: High-performance liquid chromatography chromatograms of standard flavonoid, phenolic acid, and ECT where rutin (retention time [Rt]: 3.00), myricetin (Rt: 3.9), quercetin (Rt: 5.6), gallic acid (Rt: 4.0), caffeic acid (Rt: 5.4), chlorogenic acid (Rt: 7.3), and ferulic acid (Rt: 8.8)

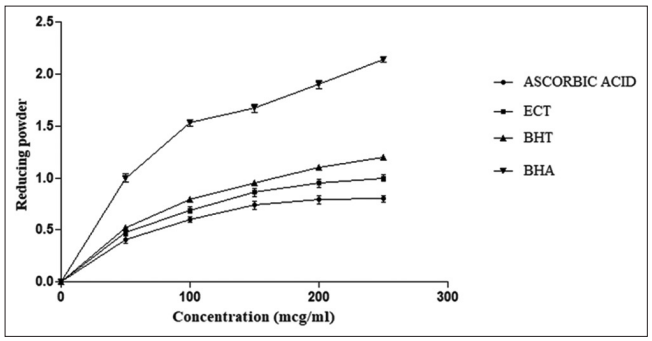

Fig. 3: Reducing power of the extract of *Cyperus tegetum* at various concentrations. The data were expressed as mean±SD. The significance was considered when p<0.05

a statistically significant correlation between those values and control (p<0.01). The hydrogen peroxide scavenging effect and standards decreased in the order of BHT>ascorbic acid>ECT>BHA. Hydrogen peroxide itself is not very reactive, but it can sometimes be toxic to cells because it may give rise to hydroxyl radicals in the cells [27]. Thus, the removing of H_2O_2 is very important for antioxidant defense in cells or food systems.

Superoxide anion scavenging activity

In the NADH-NBT system, superoxide anion derived from dissolved oxygen by the NADH coupling reaction reduces NBT. The decrease of absorbance at 560 nm with antioxidants thus indicates the consumption of superoxide anion in the reaction mixture. Fig. 4c shows the percentage inhibition of superoxide radical generation of 50–500 mg of ECT and comparison with the same doses of BHA, BHT, and ascorbic acid. The ECT has strong superoxide radical scavenging activity and exhibited higher superoxide radical scavenging activity than BHT and ascorbic acid. The results were found statistically significant (p<0.01). Superoxide radical scavenging activity of those samples followed the order: BHA>ECT>BHT>ascorbic acid.

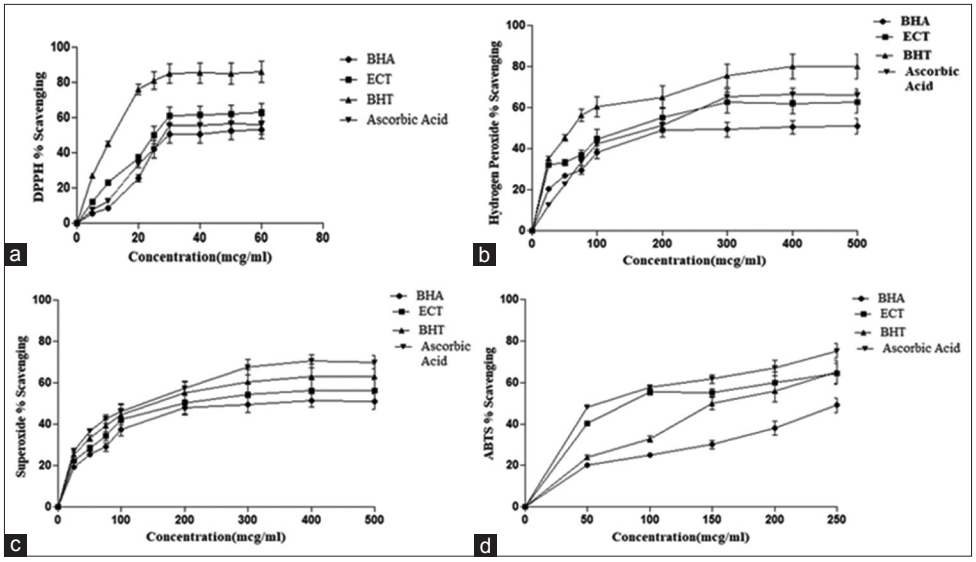

Fig. 4: Antioxidant activity of the extract of *Cyperus tegetum* at various concentrations. (a) 1-diphenyl-2-picrylhydrazyl hydrate radical scavenging activity, (b) hydrogen peroxide radical scavenging activity, (c) superoxide radical scavenging activity, (d) 2, 2′-azinobis-3-ethylbenzothiazoline-6-sulfonic acid radical scavenging activity. The data were expressed as mean±SD. The significance was considered when $p<0.05$

Evaluation of ABTS radical cation decolorization assay

Fig. 4d shows the scavenging ability of ECT on ABTS radical as compare to BHA, BHT, and ascorbic acid in a concentration-dependent way (25–250 µg/ml). The present results showed that ECT has high-quality ABTS radical scavenging ability and exhibited privileged ABTS radical scavenging activity than ascorbic acid. The ABTS radical scavenging ability of samples can be ranked as BHA>BHT>ECT>ascorbic acid exhibited prominent ABTS radical scavenging activities.

DISCUSSION

Plethora of polyphenolic compounds are found in nature. Most of them are excellent antioxidant which protects our body from oxidative stress caused by reactive oxygen species. Many synthetic antioxidants such as BHA, BHT, propyl gallate, and tert-butyl hydroxyl quinone, are available in market. BHA and BHT were also used as the standard antioxidant sample in our experiment. On the one hand, they protect our body against oxidative damage [28]; on the other hand, they have some adverse side effects and it leads to the mass campaign to search for natural food, herbs, and species that are rich in antioxidant properties [29].

In our experiment, ECT showed strong reducing power, DPPH radical, superoxide anion scavenging, and hydrogen peroxide scavenging activities when compared to standards such as BHA, BHT, and ascorbic acid. The IC_{50} value of the above test is available in Table 4. The results of this study show that the ECT can be used as an easily accessible source of natural antioxidants and as a possible food supplement or in the pharmaceutical industry. It can be used in stabilizing food against oxidative deterioration. However, the polyphenolic compounds or other components responsible for the antioxidant activity of ECT are already known.

After going through the experiment, it was observed that ECT contents various polyphenolic compounds and the presence of triterpenoid was also confirmed. The number of flavonoid and phenolic compounds is correlated with the free radical scavenging activity. Free radicals are often responsible for a wide range of pathological manifestations, from aging, inflammation, rheumatoid arthritis, diabetic, to various neoplastic diseases by producing free radical scavenging activity. Thus, ECT can play an important role in protection against those life-threading diseases. In this study, several flavonoid compounds were identified by RP-HPLC analysis. HPLC analysis reveals the presence of flavonoids mainly rutin, quercetin, and myricetin. The different spectral

Table 4: Radical scavenging activities of the extract of *Cyperus tegetum* at different concentrations

Plant extract/ chemicals	IC_{50} values (µg/ml) of radical scavenging			
	DPPH radical	Hydrogen peroxide	Superoxide radical	ABTS radical
ECT	26	155	133	96
BHA	30	60	190	200
BHT	12	200	126	150
Ascorbic acid	32	180	100	58

ECT: Extract of *Cyperus tegetum*, BHA: Butylated hydroxyl anisole, BHT: Butylated hydroxyl toluene, ABTS: 2, 2′-azinobis-3-ethylbenzothiazoline-6-sulfonic acid, DPPH: 1-diphenyl-2-picrylhydrazyl hydrate

studies (NMR, IR, and UV) confirmed the presence of stigmasterol as an isolated compound from ECT. HPLC also reveals the presence of phenolic compounds, namely, gallic acid, ferulic acid, chlorogenic acid, and caffeic acid. However, from literature survey, it can be observed that various species of *Cyperaceae* family have different pharmacological activities. The other species (*Cyperus rotundus, Cyperus articulatus,* and *Cyperus esculentus*) of *Cyperaceae* family are reported to have activities such as antioxidant, convulsion, and anthelmintic. The anticancer activity has been observed in the species of *Cyperus rotundus* [30,31]. It is reported that stigmasterol also has anticancer activity in a mouse model of 7,12-dimethylbenz(a)anthracene-induced skin carcinoma [32]. Therefore, it can be suggested that various works associated with free radical generation in the body such as anticancer activity could be performed on the different extracts of *Cyperus tegetum.*

CONCLUSION

Using different chromatography, stigmasterol is isolated and identified the compound; on another hand, gallic acid, ferulic acid, chlorogenic acid, caffeic acid, rutin, quercetin, and myricetin are identified from the extract of the plant *C. tegetum* exhibited antioxidant properties using UV spectroscopy.

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AUTHORS' CONTRIBUTIONS

AC made a significant contribution to the acquisition of data, analysis, and drafting of the manuscript. RK has made a substantial contribution to conception and design, interpretation of data for HPLC. PC and HSM participated in the design of the experiment.

CONFLICTS OF INTEREST

The authors declare that they have no conflicts of interest.

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