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Physicochemical Kinetic Evaluation and Biological Activities of Wild Horsetail Extract

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Abstract

The objective of this study is to evaluate the physicochemical, kinetic and bioactive characteristics of the alcoholic extract of Wild Horsetail. The total amount of phenolic and flavonoid was calculated and the antioxidant capacity was evaluated with the help of DPPH and ABTS tests. Potential for antidiabetic ability was also studied through the inhibition of α-amylase as well as α-glucosidase. In addition, the kinetics of trapping DPPH free radicals were studied. The chemical analysis revealed the presence of a variety of bioactive compounds, the major one being scoparone (26.45%) followed by phytol (7.43%) and methyleugenol (6.52%). Also, the total phenolic and flavonoid contents exhibited high amounts, specifically, 186.43 ± 4.18 mg gallic acid equivalent/g extract and 97.87 ± 2.66 mg quercetin equivalent/g extract, respectively. The extract displayed antioxidant capacity with IC50 of 71.37 ± 2.16 and 58.42 ± 1.87 µg/ml in DPPH and ABTS test respectively. It also showed significant inhibition of α-amylase and α-glucosidase enzymes with IC50 values of 92.78 ± 2.34 and 68.54 ± 1.92 µg/ml respectively. Based on the kinetic study, it was found that the DPPH free radical scavenging activity was described by a pseudo-first-order kinetic model. As a confirmation of the wild horsetail's phenolic and flavonoid content, ethanolic extract has a high concentration. These compounds have antioxidant ability and modulating effect on enzymes related to carbohydrate metabolism which can be incorporated into natural formulations. Such preparations can be used in pharmaceuticals and as dietetic supplements to prevent oxidative stress and assist in the treatment of diabetes.

Keywords: Wild Horsetail; Antioxidant Activity; DPPH Kinetics; ABTS Assay; Alpha-Amylase Inhibition.

INTRODUCTION

It is well-known that herbal remedies are an important source of biologically active substances that are used for medicinal and nutritional purposes. This is due to the presence of the phenolic compounds, flavonoids, and terpenoids in addition to other substances which exhibit a variety of biological effects. For instance, they have antioxidant, antidiabetic and anti-inflammatory effects, among others. Recently, the scientific community has turned its attention to the relatively safe alternatives that natural plant extracts can offer the food and drug industries in view of the harmful effects associated with oxidative damage and an increase in free radicals within cells (Dai and Mumper, 2010).

Oxidative stress is linked to many chronic diseases, including diabetes, cardiovascular disease, and cancer. It is caused by a buildup of free radicals and damage to cellular structures. The importance of plant antioxidants has increased due to their ability to prevent oxidation and donate electrons or hydrogen atoms to free radicals. The antioxidant capacity of plant extracts is usually determined through DPPH and ABTS tests. These tests are noted for their accuracy and simplicity and are used to quantify activities that scavenge free radicals (Brand-Williams *et al*., 1995; Re *et al*., 1999).

Diabetes mellitus is a chronic disease and one of the most common metabolic disorders. It occurs when the pancreas does not produce enough insulin, or when the body is unable to use the insulin it produces, leading to high blood sugar levels (McCue & Shetty, 2004). Many studies have focused on finding natural inhibitors of the alpha-amylase and alpha-glucosidase enzymes to reduce sugar absorption and lower blood glucose levels after eating (Sok Yen *et al*., 2021; Ćorković *et al*., 2022). Promising results have been observed in inhibiting these enzymes, stimulating reactions at their active sites, and reducing their catalytic activity when using plant extracts rich in phenolic compounds and flavonoids (Kim *et a*l., 2005; Sok Yen *et al*., 2021).

On the other hand, the gas chromatography-mass spectrometry (GC-MS) method is one of the most important techniques used to identify active chemical components in plant extracts. The technique provides precise information on the chemical composition of volatile and semi-volatile compounds, and it helps in

correlating chemical composition with the biological activity of plant extracts (Adams, 2007).

Lately, researchers have become increasingly interested in physicochemical studies of antioxidants to better understand the interaction pathways between antioxidants and free radicals and to determine the antioxidant dynamic properties. The use of kinetic frameworks, such as pseudo-first-order dynamics, is to describe the nature of the interaction of plant constituent and free radical and to evaluate the reaction rate and efficiency of free radical scavenging over time (Brand-Williams et al., 1995). As such, the research intends to carry out evaluation based on physicochemical kinetics as well as to study the biological properties of the wild horsetail plant. The chemical constituents were analyzed by GC–MS the total phenol and flavonoid contents were determined and its antioxidant potential was evaluated by the DPPH and ABTS assays. The study further explored its antidiabetic potential by studying it as an inhibitor of the enzymes α-Amylase and α-Glucosidase and examining the DPPH free radical scavenging kinetics of the extract.

MATERIALS AND METHODS

Plant Sample Collection and Alcoholic Extract Preparation

A wild horsetail plant was harvested in the Najaf desert, 170 kilometers southwest of Najaf Governorate's centre, and on the way to the Saudi Arabia border. Once collected, any foreign matter and soil were removed by washing with distilled water. As noted previously, the plant ingredients that were made available were dried at ambient temperature in shade. An electric grinding machine was utilized on the leaves to convert the cleaned plant material into a finely sifted powder. The method to isolate chemical components follows Dai and Mumper (2010) standard method. First, the fine powder was immersed in a 70% ethanol solution for 72 hours with constant stirring. Thereafter raw extract was obtained by filtering the mixture through Whatman No. 1 filter paper. The solvent was evaporated under reduced pressure in a rotary evaporator to complete the extraction. The resulting solution was stored at 4°C in the refrigerator.

Chemical Analysis of the Plant Extract Using GC-MS

Using a process suggested by Adams (2007), the compounds in the ethanolic extract were detected using GC-MS. To achieve the best separation of the constituents, the temperature of the oven was raised systematically. The compounds were identified by matching their obtained mass spectra with those from the NIST database. Retention time and the similarity ratio were largely indicative of confirmation.

Total Phenolic Content (TPC) Estimation

The alcoholic extract was analysed for total phenolic content in accordance with Singleton et al. (1999) method. The plant extract was prepared by mixing 2.5 mL of dilute Folin-Cioalto reagent (10%) with 0.5 mL of the sample followed by adding 2 mL of 7.5% sodium carbonate solution. Incubation of the samples was carried out in the dark for 30 minutes at room temperature and later absorbance was measured at 765 nm.

Total Flavonoid Content (TFC) Estimation

The total flavonoid amount was assessed using the aluminum chloride colorimetric technique (Chang et al., 2002). The plant extract was mixed with an $AlCl_3$ solution and compared with quercetin as a standard. The absorbance was measured at 415 nm after incubation. The results were expressed in milligram QE/g extract.

Antioxidant Activity Test Using DPPH

There were differing measurements of alcohol with the 1ml of the DPPP test administered. Equal weightage of 25, 50, 100, 200, and 400 µg/ml of 1 ml of an alcohol extract was taken. Then the mixed compounds are left in the dark room at room temperature for 30minutes. After that, the absorption was measured at 517 nm, following the method used by Brand-Williams et al. (1995).

Antioxidant Activity Test Using ABTS

We reacted an ABTS solution with potassium persulfate to prepare the ABTS free radicals. We then mixed this solution with various concentrations of the plant extract and measured absorbance at 734 nm. Trolox served as a reference standard for comparison. Last but not least, inhibition ratios were calculated and IC50 values were determined as per the method outlined by Re et al (1999).

α-Amylase Inhibition Test

To check the effectiveness of the alcoholic extract in inhibiting α--amylase, the enzyme was mixed with different concentrations of the extract followed by mixing with starch solution. The reaction was inhibited with the DNSA reagent and the absorbance was checked at 540 nm. We used acarbose as an inhibition standard for comparison. The inhibition ratios and IC50 values were calculated as described by McCue and Shetty's (2004) method.

α-Glucosidase Inhibition Test

To evaluate the effectiveness of the plant extract α-glucosidase, varying concentrations of the plant extract were mixed with the enzyme and the substrate. A spectrometer was used to measure the light absorbance to determine the amount of slowdown the extract caused. We compared results of our analysis with standard acarbose. As described by Kim et al. (2005), inhibition ratios and IC50 values were calculated.

Kinetic Study of the DPPH Reaction

The kinetic study of the DPPH radical trapping process was accomplished by looking at the absorbance changes at 517 nm at various time intervals (0–30 min). The values of ln(A_0/At) were calculated in order to available kinetic profile of the reaction.A_0 is the initial absorbance and At is the absorbance at time t. The graph of ln(A_0/At) vs duration has been plotted to establish the kinetic pattern. The results proved the reaction to follow pseudo first order pattern. This method is based on the method developed by Brand-Williams et al. (1995).

Statistical Analysis

Analysis was performed using GraphPad Prism version 9.0 with three independent replicates for all the trials. The outcome was expressed as mean ± standard deviation (Mean ± SD). We determined IC50 values through linear regression analysis. A value of lower than 0.05 was established as significant.

RESULTS AND DISCUSSION

Phytochemical analysis by GC–MS

As observed from the table and the figure, scoparone is among the highest percentage of 26.45. Others include arsenous acid tris(trimethylsilyl) ester with a percentage of 10.62, phytol with 7.43, methyleugenol with 6.52 and several others. Thus, the presence of these bioactive compounds has great medicinal benefits. The presence of various phenolic compounds, lipid esters, and heterocyclic compounds at different levels indicated the presence of chemicals in the plant extract. According to Zhang et al., (2017), the antioxidant capacity and biological effectiveness of medicinal plants occur majorly due to phenolic compounds and plant terpenoids. These compounds injure free radicals and efficiently offer electro**.**

Table 1. Identification of prominent bioactive components through analysis of ethanolic extract using GC–MS.

No.	Compound	Retention Time (min)	Peak Area (%)
1	Scoparone	22.491	26.45
2	Arsenous acid, tris(trimethylsilyl) ester	11.419	10.62
3	Phytol	23.543	7.43
4	Methyleugenol	16.022	6.52
5	2(1H)-Pyridinone, 3,4-dihydro-6-phenyl-	19.273	3.02
6	Dibutyl phthalate	22.206	2.76
7	Phthalic acid, isobutyl undecyl ester	21.276	2.24
8	Oxime-, methoxy-phenyl-_	8.853	2.03
9	7,9-Di-tert-butyl-1-oxaspiro(4,5)deca-6,9-diene-2,8-dione	21.806	1.62
10	1,8-Naphthyridine	16.205	1.60
11	Trimethylsilyl fluoride	4.189	1.55
12	9-Octadecenoic acid, 12-hydroxy-, methyl ester	25.132	1.47
13	Hexadecanoic acid, ethyl ester	22.417	1.40
14	Bis(2-ethylhexyl) phthalate	27.169	1.39
15	Ethanone, 1-(6,6-dimethylbicyclo[3.1.0]hex-2-en-2-yl)-	15.044	1.25

Only major compounds with peak area ≥ 1% shown.

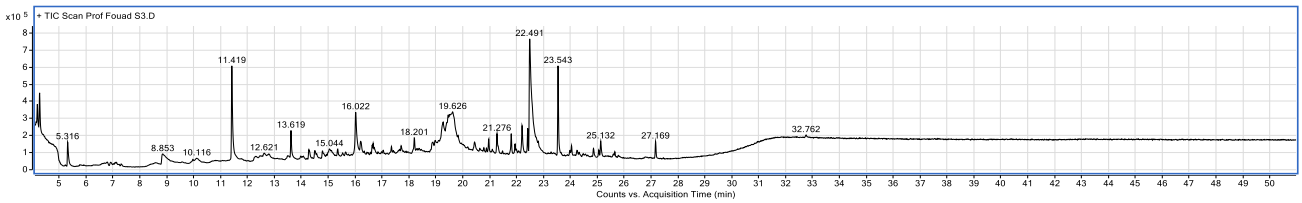

Figure 1 The chromatogram of the alcoholic extract showing major compounds with GC-MS techniques.

Phenolic and Flavonoid Composition

Results for total phenolic and flavonoid contents of the alcoholic extract were found to be quite high. The total phenolic content (TPC) was 186.43 ± 4.18 mg GAE/g extract and the total flavonoid content (TFC) was 97.87 ± 2.66 mg QE/g extract as shown in Table 2. This indicates the extract is rich in phenolic and flavonoid compounds which are known for their biological properties as well as free radical scavenging ability. The observations made by Dai and Mumper (2010) confirm that phenolics and flavonoids are one of the main plant compounds possessing antioxidant action due to their high capacity to donate electron and hydrogen.

Table 2. Total Phenolic and Total Flavonoid Contents.

Parameter	Result
Total Phenolic Content (TPC)	186.43 ± 4.18 mg GAE/g extract
Total Flavonoid Content (TFC)	97.87 ± 2.66 mg QE/g extract

Antioxidant activity assay by DPPH

The result of DPPH antioxidant test demonstrated an increasing trend in free radical inhibition as the concentration of alcoholic extract increased as shown in Table 3 and Figure 2. The inhibition increased from 31.5 ± 1.3 % at 25 µg/mL to 91.3 ± 1.9 % at 400 µg/mL. The extract had an IC50 value of 71.37 ± 2.16 µg/mL, while ascorbic acid had an IC50 value of 39.82 ± 1.27 µg/mL indicating that the extract was capable of neutralizing free radicals effectively.

Phenolic and flavonoid components in the extract are responsible for this potential, which can donate electrons or hydrogen atoms to free radicals (Belcher and Murray, 1996). The findings are consistent with those noted by Prior et al. (2005) who noted that the phenolic compounds of plants have a good behavior in DPPH tests because of their high capacity for preventing oxidation.

Table 3. DPPH Radical Scavenging Activity.

Concentration (µg/mL)	DPPH Inhibition (%)	Ascorbic Acid (%)
25	31.5 ± 1.3	42.9 ± 1.2
50	48.7 ± 1.6	61.5 ± 1.4
100	66.9 ± 1.9	79.3 ± 1.6
200	81.6 ± 2.2	92.5 ± 1.4
400	91.3 ± 1.9	96.9 ± 1.3

IC50: Ethanolic extract = 71.37 ± 2.16 µg/mL; Ascorbic acid = 39.82 ± 1.27 µg/mL

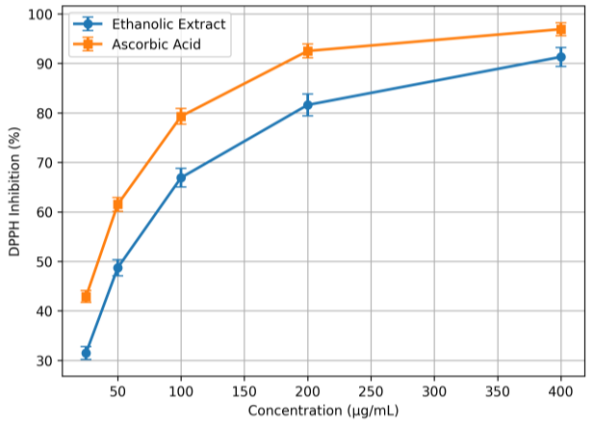

Figure 2. DPPH Radical Scavenging Activity.

ABTS Free Radical Scavenging Capacity

The results of the ABTS assay elucidated that the free radical scavenging ability of the alcoholic extract was significantly increased with the concentration increase as presented in Table 4 and Figure 3. The inhibition percentage rose from 35.9 ± 1.5 % at 25 µg/mL to 93.8 ± 1.8 % at 400 µg/mL. The extract's IC50 value was established at 58.42 ± 1.87 µg/mL against the standard Trolox at 31.26 ± 1.04 µg/mL indicating good antioxidant potential.

This is because the phenolic and flavonoid compounds can give up electrons and effectively neutralize free radicals. The findings here corroborate those by Re et al. (1999) who mentioned that the ABTS test is a sensitive method for assessing the antioxidant capacity of phenolic-rich plant materials.

Table 4. ABTS Radical Scavenging Activity.

Concentration (µg/mL)	ABTS Inhibition (%)	Trolox (%)
25	35.9 ± 1.5	48.2 ± 1.3
50	54.7 ± 1.7	67.4 ± 1.6
100	72.6 ± 1.9	84.7 ± 1.5
200	86.4 ± 2.1	94.2 ± 1.2
400	93.8 ± 1.8	98.3 ± 0.9

IC50: Ethanolic extract = 58.42 ± 1.87 µg/mL; Trolox = 31.26 ± 1.04 µg/mL.

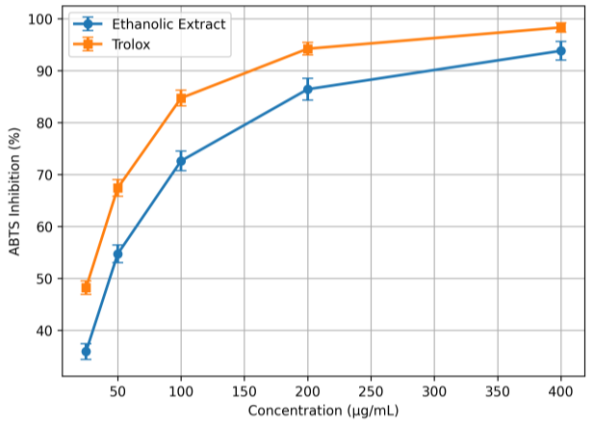

Figure 3. ABTS Radical Scavenging Activity.

α-Amylase Inhibitory Activity

The α-amylase inhibition assay results show an increasing percentage of inhibition with increasing concentrations of alcoholic extract as provided in Table 5 and Figure 4. The percentage of inhibition increased from 24.9 ± 1.3% at a concentration of 25 µg/mL to 86.6 ± 1.9% at a concentration of 400 µg/mL. The extract also shows the IC50 value of 92.78 ± 2.34 µg/mL as compared to standard acarbose drug with IC50 value of 43.61 ± 1.28 µg/mL which indicates that extract has a strong potential for enzyme inhibition.

This capability is probably due to the presence of phenolic and flavonoid species that can bind to the active site of the enzyme and inhibit its catalytic activity. As per McCue and Shetty (2004) plant extracts with a rich phenolic content can inhibit the action of carbohydrate digestion enzymes which can ultimately lower postprandial glucose levels.

Table 5. α-Amylase Inhibitory Activity.

Concentration (µg/mL)	Extract Inhibition (%)	Acarbose (%)
25	24.9 ± 1.3	38.8 ± 1.2
50	41.5 ± 1.7	56.9 ± 1.4
100	58.9 ± 1.9	73.7 ± 1.6
200	74.3 ± 2.1	88.2 ± 1.7
400	86.6 ± 1.9	95.4 ± 1.3

IC50: Ethanolic extract = 92.78 ± 2.34 µg/mL; Acarbose = 43.61 ± 1.28 µg/mL

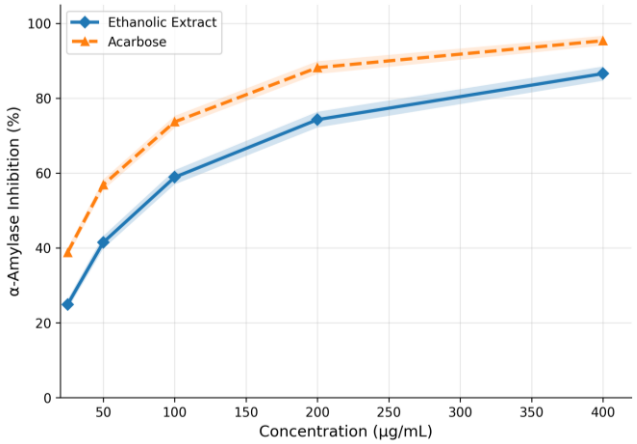

Figure 4. α-Amylase Inhibitory Activity

α-Glucosidase Inhibitory Activity

Findings of α-glucosidase inhibition assay indicate that the inhibition increased with the increase of concentration of alcoholic extract (table 6 and figure 5). Inhibition increased from 29.6 ± 1.3% at 25 µg/mL to 91.9 ± 1.7% at 400 µg/mL. The IC50 of the extract was 68.54 ± 1.92 µg/mL, while of the acarbose the standard was 34.73 ± 1.12 µg/mL indicating a strong inhibition by the extract.

It can be attributed to extract's phenolic and flavonoid substances, which inhibit carbohydrates breakdown by blocking α-glucosidase, resulting in postprandial lower blood sugar up to certain range. According to Kim et al. (2005), plant constituents high in phenols can inhibit sugar metabolizing enzymes and display promising anti-diabetic property. This results are in agreement with these authors.

Table 6. α-Glucosidase Inhibitory Activity.

Concentration (µg/mL)	Extract Inhibition (%)	Acarbose (%)
25	29.6 ± 1.3	44.8 ± 1.2
50	47.9 ± 1.5	63.3 ± 1.3
100	65.8 ± 1.8	80.5 ± 1.6
200	82.7 ± 1.9	92.8 ± 1.4
400	91.9 ± 1.7	97.5 ± 1.1

IC50: Ethanolic extract = 68.54 ± 1.92 µg/mL; Acarbose = 34.73 ± 1.12 µg/mL

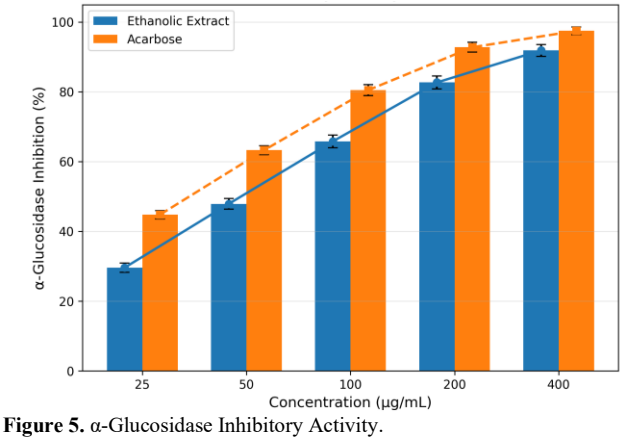

Figure 5. α-Glucosidase Inhibitory Activity.

Kinetic Analysis of DPPH Radical Inhibition

The kinetic study of DPPH reaction reveals a decrease in absorbance values with time, with a marked increase in the values of ln (A_0/At) as listed in Table 7 and Figure 6. The ln(A_0/At) values increased from 0.000 to 0.990 ± 0.019 after 30 minutes. This shows the efficiency of alcoholic extract of Senna tora in scavenging free radical increases with time. The kinetic representation also showed a clear linear relationship between ln (A0/A1) and time which means the DPPH radical-scavenging reaction followed a pseudo-first-kinetic model. This is due to the phenolic and flavonoids in the extract that can steadily and consistently donate electrons or hydrogen atoms.

The results are similar to those of Brand-Williams et al., which show that the antioxidant activities of phenol-rich plant extracts often have duration-dependent kinetic behaviour due to direct interaction with free radicals.

Table 7. Kinetic Study of DPPH Radical Scavenging Activity.

Time (min)	Absorbance (517 nm)	ln(A_0/At)
0	0.813 ± 0.014	0.000 ± 0.000
5	0.686 ± 0.017	0.170 ± 0.009
10	0.564 ± 0.015	0.366 ± 0.012
15	0.472 ± 0.013	0.543 ± 0.014
20	0.393 ± 0.012	0.727 ± 0.016
30	0.302 ± 0.011	0.990 ± 0.019

Values are expressed as mean ± SD (n = 3).

The data for ln(A_0At) as a function of the reaction time was fitted to the equation y = 0.0337x + 0.0168 with an R2 value of R2=0.9947, which represents its linear regression. The R^2 value of data is high, indicating a high degree of linearity in data.

Table 8. Linear Regression Parameters of the Pseudo-First-Order Kinetic Model.

Parameter	Value
Regression equation	y = 0.0337x + 0.0168
Rate constant (k)	0.0337 min^{-1}
R^2	0.9947
Kinetic model	Pseudo-first-order

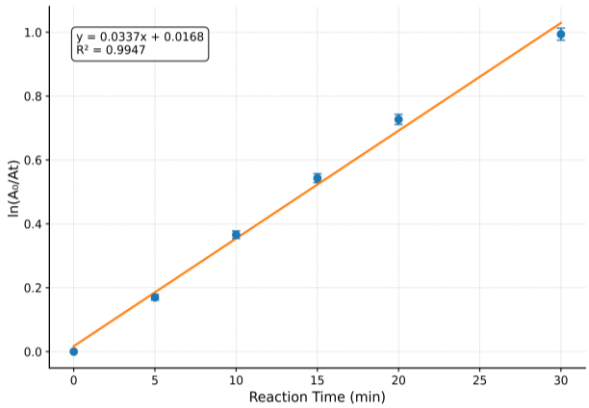

Figure 6. Figure 6. Pseudo-first-order kinetic diagram illustrating the rise in ln(A_0/At) values with reaction duration through the elimination of DPPH radicals by the alcoholic extract

CONCLUSION

The investigations have shown that the alcoholic infusion of wild horsetail contains a range of biologically active substances determined using GC-MS, the most abundant of which was scoparone. The results also showed a considerable amount of total phenol and flavonoid, which is well reflected in the antioxidant capacity in both the DPPH and ABTS assays. The study findings also indicated that the extract has the ability to inhibit alpha-amylase and alpha-glucosidase, indicating its potential use as a natural anti-diabetic agent. In addition, the kinetic evaluation indicated that the free radical scavenging activity by DPPH obeyed pseudo-first order kinetics, thus highlighting the physicochemical significance of the plant extract for future pharmaceuticals and nutraceutical properties.

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Competing Interests: The authors declare that there is no conflict of interest.

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