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Original Research Article

In Vitro Alpha-Amylase Inhibitory Activity, Antioxidant Activity and HPLC Analysis of *Eichhornia crassipes* (water hyacinth) Methanol Extracts

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ABSTRACT

The upsurge in the use of local herbs in the management of diabetes mellitus everywhere in the world is as a result of its year-by-year global alarming and mortality rate. *Eichhornia crassipes* (water hyacinth) is the world's worst and most prevalent invasive aquatic weed found in the tropical and subtropical parts, but with a traditional claim in the treatment of diabetes mellitus. This study was therefore designed to determine the *in-vitro* antioxidant capacity, α-amylase inhibitory activity, and high-performance liquid chromatography (HPLC) analysis of methanol extracts of *E. crassipes*.

The α-amylase inhibitory activity of *E. crassipes* methanol extracts was investigated using various concentrations (0.3125–5.0 mg/mL) of the extracts; antioxidant assays- reducing power activity and DPPH (2,2-Dipheny-1-picrylhydrazyl) free radical scavenging activity were also determined using the spectrophotometry method. The HPLC technique was used in accordance with the standard procedure. Preliminary phytochemical screening revealed the presence of alkaloids, flavonoids, tannins, phenols, steroids, and cardiac glycosides in all plant extracts. According to the quantitative HPLC analysis, the stem extract (111.0649 ppm) has the highest phenolic, alkaloid and, flavonoid content followed by leaf (70.5957 ppm) and root (58.0538 ppm) extracts. Leaf extracts of *E. crassipes* had the lowest IC_{50} (0.35 mg/ml) showing the strongest inhibitory activity of α-amylase. The highest reducing power and the lowest DPPH free radical scavenging activity (IC_{50} =131 mg/mL) were found in the root extracts, indicating that the root extract have the strongest antioxidant activity. Hence, it is possible that the antioxidant and α-amylase inhibitory activities of *E. crassipes* are due to their phenolic components.

Keywords: Diabetes mellitus, *Eichhornia crassipes*, α-amylase inhibition, Antioxidant, HPLC.

Introduction

Free radicals are produced naturally during aerobic cell metabolism and the human body have an antioxidant defense system to deal with the activity of these free radicals formed. However, the imbalance between the free radical production and the antioxidant defense occurs when the free radicals are overproduced in the body, resulting in oxidative stress.[1-3] In turn, oxidative stress causes macromolecular oxidative damage, which leads to the development of various diseases, such as diabetes, cardiovascular diseases, cancer, hypertension, and other complications.[4-7] In the quest to combat these free radicals, researchers have investigated several medicinal plants to assist, complement, and increase the body's antioxidant levels by utilizing scientifically proven green leafy plants that have been traditionally claimed.

Since nearly 25% of modern medicines are derived from plants, approximately 60% of the world's population and approximately 80% of developing countries rely almost entirely on phytomedicine for disease management and treatment.[8,9]

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Nigeria is endowed with a plethora of cosmic fauna and flora biodiversity that is used for therapeutic and nutritional purposes. A wide variety of plant species from Nigeria's flora in both tropical and subtropical areas have traditionally been used for the management and treatment of diabetes due to their dietary importance, little or no side effects, and rich source of secondary metabolites, vitamins, minerals, and fiber for local consumers, [10-13] and *Eichhornia crassipes* is one of these plants.

Eichhornia crassipes (water hyacinth), a member of the Pontederiaceae family, is the world's worst invasive aquatic weed, typically found in tropical and subtropical regions.[14] Asia, Africa, Australia, Europe, South America, and North America are its natural habitats.[15, 16] Phytochemical analyses of these plant parts revealed the presence of tannins, flavonoids, alkaloids, and saponins, which are associated with various biological activities such as antioxidant, antimicrobial, anticancer, wound healing, and anti-inflammatory activities.[17-24]

Pancreatic α-amylase (E.C 3.2.1.1) is a major enzyme in the human digestive system and mammals that hydrolyzes α-bonds of large α-linked polysaccharides, like starch and glycogen to produce glucose and maltose.[25, 26] Inhibiting α-amylase plays an important role in lowering postprandial hyperglycemia and is considered a strategy for the treatment of carbohydrate uptake disorders such as diabetes and obesity.[27] Several α-amylase inhibitors have been isolated from medicinal plants to serve as an alternative drug with higher potency and fewer side effects than existing synthetic drugs.[28-31] The purpose of the study was to determine the antioxidant capacity, α-amylase inhibitory activity, and HPLC analysis of Eichhornia crassipes methanol extracts.

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Materials and Methods

Collection and identification of plant samples

Eichhornia crassipes parts (leaf, stem, and root) were collected in August, 2019 from a river in Ketu, Lagos State, Nigeria, and a voucher sample (LSH019/007) was dropped and identified at the Herbarium of Botany Department, Lagos State University, Lagos State, Nigeria.

Preparation of plant extracts

The root, leaf, and stem of *Eichhornia crassipes* were harvested, washed under a clean tap running water, and air-dried. The plants were ground to powder using electric blender (Binatone, Nigeria), 150 g each mixed with 1500 mL of methanol, stirred, and covered with aluminum foil for 72 hours. The resulting extract was then filtered using Whatman grade 1 filter paper, concentrated to dryness using a rotary evaporator (Heidolph, Germany) at 50^0C, and kept before analysis.

Determination of phytochemical constituents

Phytochemical analysis for alkaloids, saponins, flavonoids, tannins, steroids, phenols, reducing sugars, cardiac glycosides, terpenoids and anthraquinones were performed on the plants following the procedure reported by Yadav and Agarwala [32] and Njoku and Obi. [33]

Determination of antioxidant activity

DPPH (2,2-Dipheny-1-picrylhydrazyl) free radical scavenging activity

The DPPH scavenging activity of the plant was determined according to the methods of Behlil, Samiullah[34]. DPPH solution (0.5 mM) in methanol was prepared and 1mL of this solution was mixed with 2 mL of the extract (2g/20mL). Ascorbic acid was used as positive control while the mixture of 1ml methanol and 2 mL DDPH served as the negative control. 3mL methanol was used as the blank. The absorbance of each extract was measured at 517 nm using a UV-Visible spectrophotometer, and the lower the absorbance of the reaction mixture the higher the free radical scavenging activity.

% Inhibition of DPPH free radical scavenging activity was calculated as follows:

$$\% \text{ DPPH activity} = \frac{Ac-As}{Ac} \times 100$$

Ac = Absorbance of Control

As = Absorbance of sample

Reducing power assay

The extract was mixed with phosphate buffer (2.5 mL, 0.2 M, pH 6.6) and potassium ferricyanide (2.5 mL, 1%). The resulting mixture was then incubated at 50°c for a period of 20 minutes. Trichloroacetic acid (1 mL of 10% solution) was added to stop the reaction, which was then centrifuged at 3000 rpm for 10 minutes. The upper layer of solution (1.5 mL) was mixed with distilled water (1.5 mL) and $FeCl_3$ (0.1 mL, 0.1%) after mixing, the contents were incubated for 10 minutes and the absorbance was measured at 700 nm. Increased absorbance of the reaction mixture indicated increased reducing power. Ascorbic acid was used as a positive control). [34]

Alpha-amylase inhibitory assay

The α-amylase inhibitory assay was determined according to the procedure of Kazeem *et al*.[27] A total of 250 µL of the extract was placed in a tube and 250 µL of 0.02 M sodium phosphate buffer (pH 6.9) containing α-amylase solution was added. This solution was pre-incubated at 25°C for 10 min, after which 250 µL of 1% starch solution in 0.02 M sodium phosphate buffer (pH 6.9) was added at 2 minutes timed intervals and then further incubated at 25°C for 10 min. The reaction was terminated by adding 500 µL of dinitrosalicylic acid (DNS) reagent. The tubes were then incubated in boiling water for 5 mins and cooled to room temperature. The resulting mixture was diluted with 5 mL distilled water and the absorbance measured at 540 nm with the use of a spectrophotometer (Spectrum lab S23A, Globe Medical England). A control was prepared using the same procedure replacing the extract with distilled water. The α-amylase inhibitory activity was calculated as percentage inhibition.

$$\% \text{ Inhibition} = \frac{Ac-As}{Ac} \times 100$$

Ac = Absorbance of Control

As = Absorbance of sample

Concentrations of extracts resulting in 50% inhibition of enzyme activity (IC_{50}) were determined graphically.

High performance liquid chromatography (HPLC) analysis

Phytochemical analysis using the HPLC technique was performed with standard procedure. 20 µL of the plant extracts was injected and measured at 280nm using 1260 VWD (Agilent 1260 Infinity Variable Wavelength Detector) and system operator.

Statistical analysis

Statistical analysis was performed using the Microsoft Excel Package (version 2016). All the results were in triplicate determinations and were expressed as Mean±SE at a significance level of $p<0.05$.

Results and Discussion

The percent yield of the methanol extract of *Eichhornia crassipes* roots, stems, and leaves is shown in Table 1. When compared to other extracts, the root extract had the highest percentage yield (4.7 percent). A screening of the phytochemical composition, as shown in Table 2, revealed the presence of alkaloids, flavonoids, phenols, tannins, steroids, and cardiac glycosides in all of the extracts, but no saponins or reducing sugars. Terpenoids were only found in the root extract, while anthraquinones were only found in the stem extract.

The results of the α-amylase inhibitory activities of the plant's methanol root, stem, and leaf extracts were shown in Figure 1. The inhibition of α-amylase increased with concentration. *Eichhornia crassipes* leaf extracts had the lowest IC_{50} (0.35 mg/mL) and the strongest inhibitory activity of α-amylase (Table 3).

DPPH scavenging and reducing power assay was used to determine each extract's antioxidant ability to inhibit, scavenge, and quench free radicals. Table 4 shows the IC_{50} values of each extract in the DPPH scavenging assay, with the root extract having the lowest IC_{50} (131 mg/mL). The DPPH free radical scavenging activity of each extract is shown in Figure 2, with the root extract having the lowest DPPH scavenging activity. The concentration increased the activity.

Table 1: Percentage yield of each of the extracts of *Eichhornia crassipes*

Extracts	Initial weight (g)	Final weight (g)	% yield
Root	50	2.6	1.2
Stem	50	6.7	2.9
Leaf	50	9.3	4.7

Table 2: Phytochemical analysis of methanol extracts of *Eichhornia crassipes*

Phytochemicals	Root	Stem	Leaf
Alkaloids	+	+	+
Saponins	-	-	-
Flavonoids	+	+	+
Tannins	+	+	+
Steroids	+	+	+
Phenols	+	+	+
Reducing sugars	-	-	-
Cardiac glycosides	+	+	+
Terpenoids	+	-	-
Anthraquinones	-	+	-

Key: + signifies presence, – signifies not detected

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Table 3: IC_{50} Value of various extracts of *Eichhornia crassipes* against α- amylase

Extracts	IC_{50} Value (mg/mL)
Root	2.91 ± 0.32
Stem	1.70 ± 0.24
Leaf	0.35 ± 0.20

Values are Mean ± SE of three determinations

Table 4: IC_{50} Values of DPPH free radical scavenging activity of various extracts of *Eichhornia crassipes*

Extracts	IC_{50} Value (mg/mL)
Root	131.0
Stem	137.0
Leaf	165.4

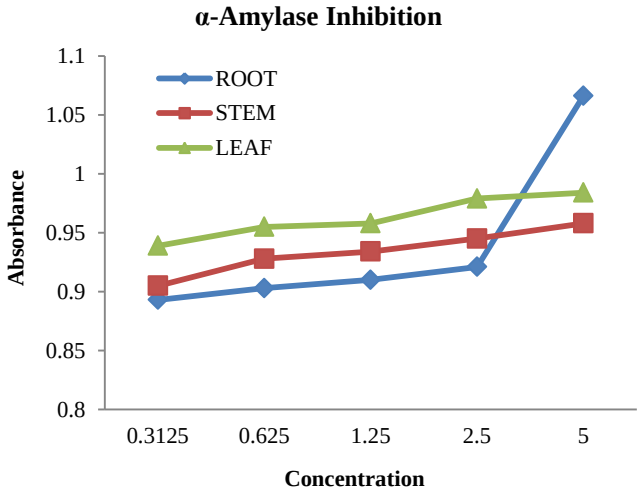

Figure 1: α-amylase inhibition of the extracts of *Eichhornia crassipes*

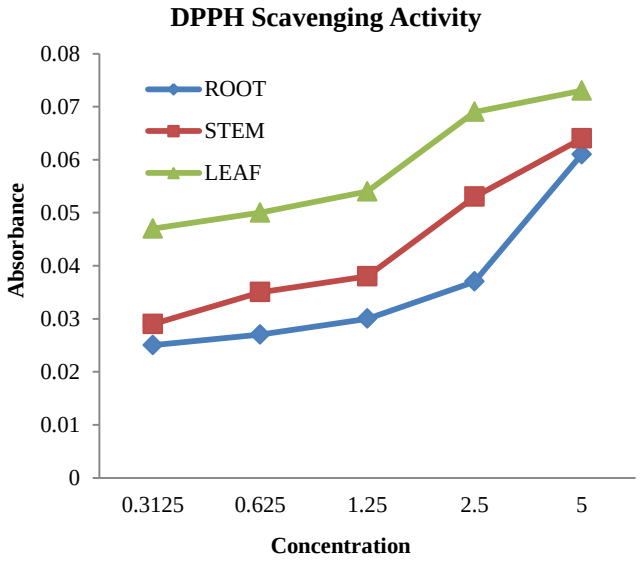

Figure 2: DPPH scavenging activity of the extracts of *Eichhornia crassipes*

The highest reducing power was also found in root extract, and the higher the reducing power, the higher the antioxidant activity (Figure 3).

The HPLC was programmed to separate at a wavelength of 280 nm. The compounds were detected in accordance with their retention times. The chromatogram shows distinct peaks with an analysis time ranging from 0 to 40 minutes. There were nine phytochemical components detected, including gallic acid, catechin, p-coumaric acid, ferulic acid, rutin, apigenin, quercetin, kaempferol, and theobromine, with the most abundant amount identified at 11.206 ppm, and caffeine was not detected in all of the extracts (Table 5).

The chromatograms of the calibration standard (pink lines) and the leaf extracts are shown in Figure 4 (blue lines). The peaks of each of the specific compounds were detected at different retention times, as shown in Figure 4. Catechin, p-coumaric acid, and ferulic acid were detected as phenolic acids constituents in the leaf sample, with ferulic acid having the highest ppm at retention time 21.138 valley to valley. Rutin, apigenin, quercetin, and kaempferol were detected as flavonoids in the leaf extracts, with quercetin having the highest ppm at retention time 36.610 valley to valley and apigenin having the lowest ppm at retention time 29.937 valley to baseline. The presence of phenolic acids was discovered before the presence of flavonoids.

The chromatograms of the calibration standard (pink lines) and the root extracts are shown in Figure 5. (blue lines). The peaks of each of the specific compounds were detected at different retention times, as shown in Figure 5. Catechin, p-coumaric acid, and ferulic acid were detected as phenolic acids in the leaf sample, with p-coumaric acid having the highest ppm at retention time 18.687 valley to baseline. Rutin, apigenin, quercetin, and kaempferol were found in the root extracts, with kaempferol having the highest ppm at retention time 38.318 valley to baseline and quercetin having the lowest ppm at retention time 36.658 valley to baseline. The presence of phenolic acids was discovered before the presence of flavonoids.

The chromatograms of the calibration standard (pink lines) and the stem sample are shown in Figure 6 (blue lines). The peaks of each of the specific compounds were detected at different retention times, as shown in Figure 6. Catechin, p-coumaric acid, and ferulic acid were detected as phenolic acids in the stem extracts, with p-coumaric acid having the highest ppm at retention time 18.403 baseline to baseline and ferulic acid having the lowest ppm at retention time 21.139 valley to valley. Rutin, apigenin, quercetin, and kaempferol were found in the stem extracts, with quercetin having the highest ppm at retention time 37.034 valley to valley and kaempferol having the lowest ppm at retention time 38.286 valley to baseline. The presence of phenolic acids was discovered before the presence of flavonoids.

Postprandial high blood glucose, related to type 2 diabetes is one of the earliest methods of glucose homeostasis.[35] The usual pharmacological approach to this condition has been the use of therapeutic agents such as medicinal plants consisting of important potential phytochemicals sources used for the treatment and management of Type 2 Diabetes Mellitus. Several reports showed that this therapeutic approach can decrease postprandial hyperglycaemia by inhibiting carbohydrate digesting enzymes (e.g α-amylase) resulting in a delay of carbohydrate digestion to absorbable monosaccharides.[27] In this research work, we evaluate the α-amylase inhibitory activities of different extracts of *E. Crassipes* to clarify its traditional claim and use for diabetes treatment and management. Our obtained result of *in vitro* analysis of the activity of α-amylase inhibition, slightly found in a dose-dependent manner confirms that the high amount of bioactive compounds (flavonoids, phenolic acids, and alkaloids) in the plant extracts at different concentrations may be responsible for the inhibitory activity of α-amylase.[36] Several phytochemical analyses have reported that extracts rich in polyphenolic components such as flavonoids have an α-amylase inhibitory activity that depends on phenolic profile. [37-39] More so, the leaf, stem and root extracts of *E. crassipes* showed α-amylase inhibitory properties at all the concentrations, suggesting that all the parts of the plant may be useful in the management of post-prandial glucose in diabetes mellitus.[40]

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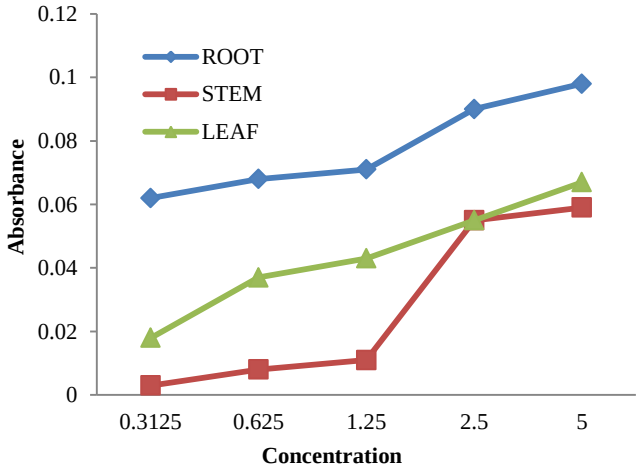

Figure 3: Reducing power activity of the extracts of *Eichhornia crassipes*

Table 5: HPLC Analysis of Methanol extracts of *Eichhornia crassipes*

Compounds detected	Root extract (ppm)	Stem extracts (ppm)	Leaf extracts (ppm)
Catechin	1.15835	0.97321	1.64336
P-coumaric acid	0.71370	1.07527	0.92715
Ferrulic acid	0.92927	1.85666	3.42699
Rutin	4.56262	5.11084	7.76510
Apigenin	1.50925	3.36672	1.03519
Quercetin	1.26413	12.61280	12.67254
Kampferol	5.06470	1.52827	2.55906
Theobromine	-	3.11436	1.06263
Gallic Acid	6.18546	-	-
Caffeine	-	-	-

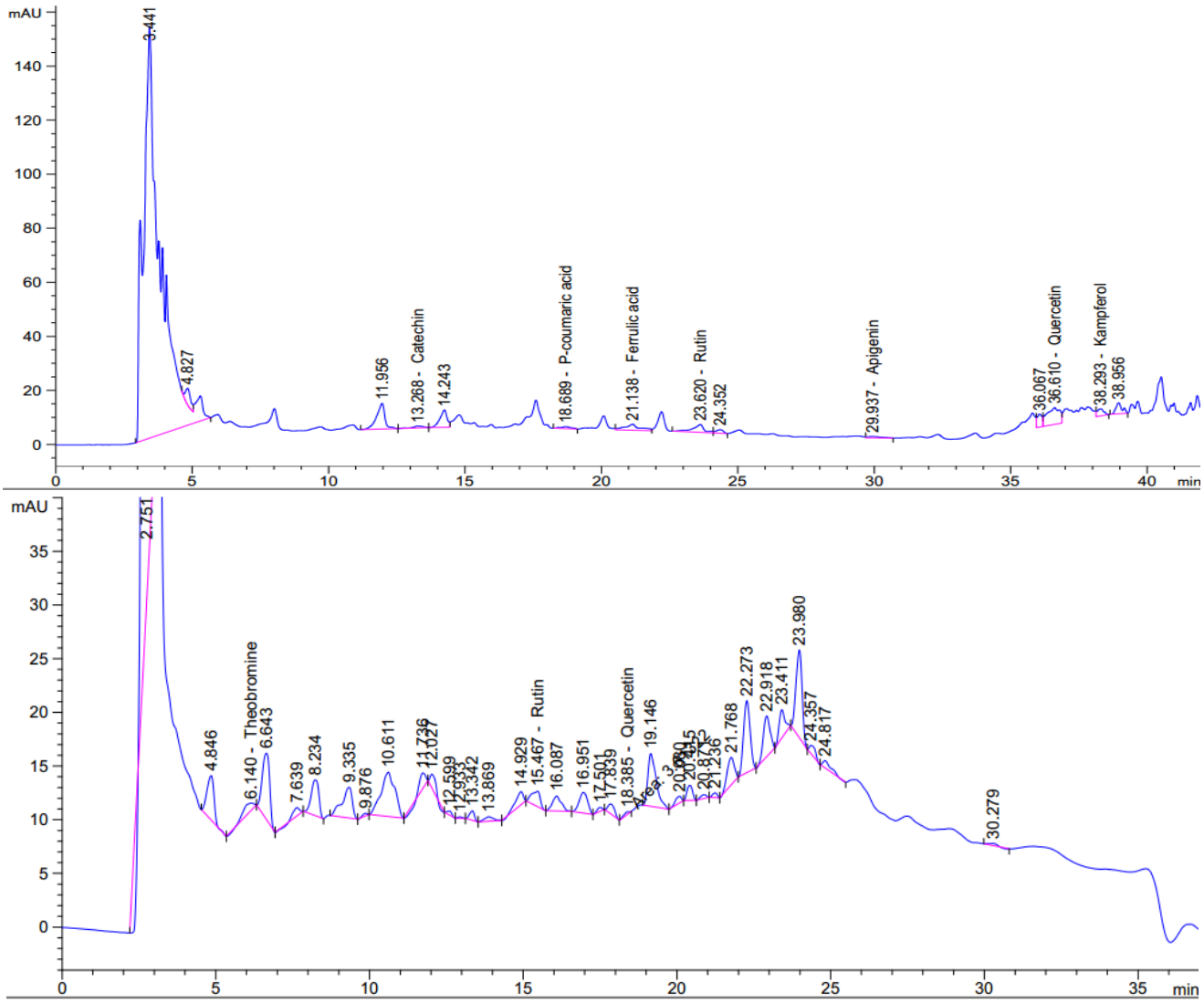

Figure 4: HPLC Chromatogram of Flavonoids, Phenolic acids, and Alkaloids profile for *E. crassipes* leaf extract.

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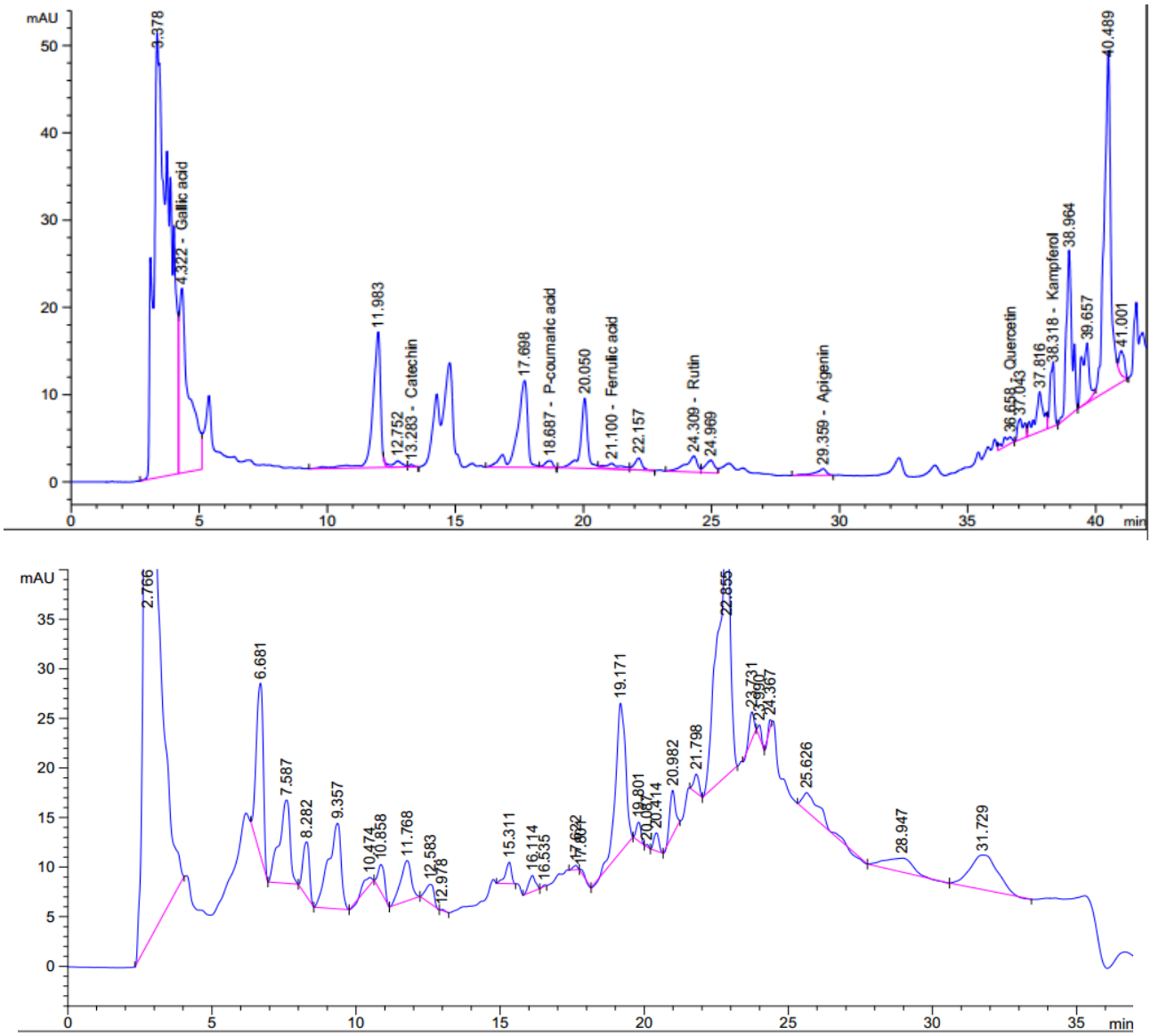

Figure 5: HPLC Chromatogram of Flavonoids, Phenolic acids and Alkaloids profile for *E. crassipes* root extract.

The presence of alkaloids, flavonoids, phenols, tannins, steroids, and cardiac glycosides was detected in all *E. crassipes* extracts through qualitative phytochemical screening. In this study, the alkaloids, flavonoids, and phenols were confirmed using quantitative HPLC analysis. Flavonoid, one of these compounds, has been proposed to have the ability to control starch digestion, which may serve as a structural requirement in inhibiting human α-amylase.[28] Quercetin (a flavonoid) has also been reported to act as chain-breaking antioxidant, which may aid in prevention of degenerative diseases by preventing the oxidation of low-density lipoprotein via macrophages and metal ions such as copper.[41-43] This is consistent with our findings that methanol extracts of *E. crassipes* have antioxidant activity which may protect against and aid in the metabolic diseases such as diabetes mellitus. It is also possible that the combined phenolic components (p-coumaric acid, quercetin, catechin, gallic acid, rutin, theobromine, kaempferol, apigenin, and ferulic acid), which are types of alkaloids, flavonoids and phenols acids, as confirmed by HPLC analysis of *E crassipes*, may directly contribute to these α-amylase inhibitory and antioxidant activities of this plant's extracts. [36,44]

The extracts' antioxidant activity was determined by measuring their reducing power and DPPH free radical scavenging activity. The DPPH radical has been extensively used as a free radical to test extracts for their reductive ability of extracts as free radical scavengers or hydrogen donors, as well as to evaluate plant extract antioxidant capacity; [45, 46] the lower the IC_{50} value of the plant extract, the higher the antioxidant activity. Antioxidant compounds react with DPPH, reducing it to 1,1- diphenyl-2-hydrazine (DPPH-H) by providing electron or hydrogen atoms.[47] Our findings shows that the root extract has the lowest IC_{50} value as well as the highest reducing power (figure 3), implying that *Eichhornia crassipes* root extract contains bioactive components that can reduce, stabilize, or scavenge free radicals when compared to the stem and leaf. In this study, high liquid performance chromatography (HPLC) at a wavelength of 280nm revealed the result of the most abundant compound present in *Eichhornia crassipes* leaf, root, and stem extracts. There were nine (9) phytochemical components detected, including flavonoids, alkaloids, and phenols including gallic acid, catechin, p-coumaric acid**,** ferulic acid, rutin, apigenin, quercetin, kaempferol, and theobromine, with the most abundant being at 11.206ppm. These compounds are the purest components of the extracts and play an important role in their medicinal functions. [48-50] According to the quantitative HPLC analysis in this study, the stem extract (111.0649ppm) has the highest phenolic, alkaloid, and flavonoid content, followed by the leaf (70.5957ppm) and the root (58.0538ppm) extracts.

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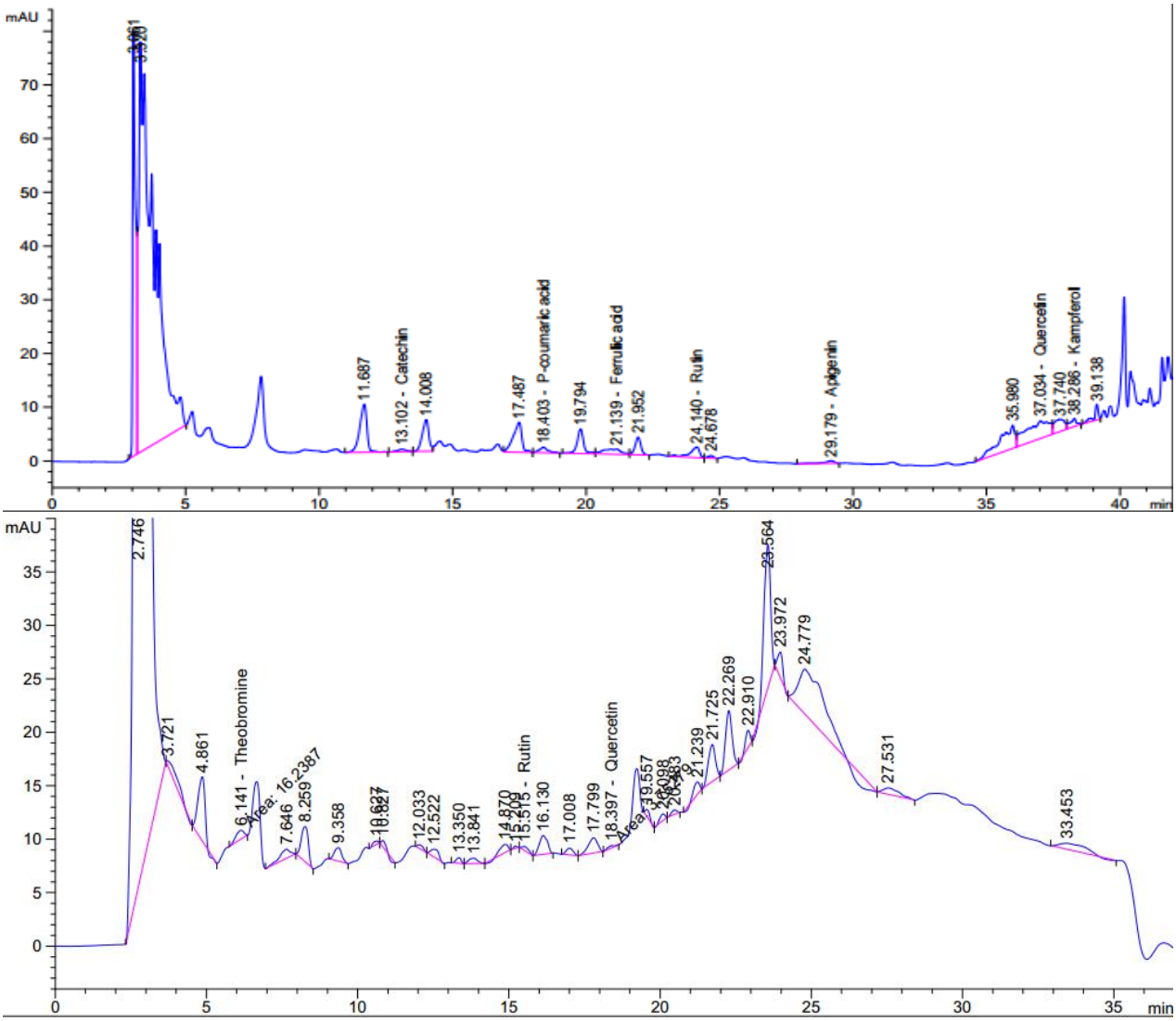

Figure 6: HPLC Chromatogram of Flavonoids, Phenolic acids and Alkaloids profile for *E. crassipes* stem extract.

Conclusion

This present study concludes that the presence of bioactive secondary metabolites in *Eichhornia crassipes* extracts, revealed by the preliminary phytochemical study and HPLC analysis, makes it an effective gift of nature with important antioxidant and α-amylase inhibitory activities and thus, is suggested to be effective in the management of diabetes.

Conflict of Interest

The authors declare no conflict of interest**.**

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

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