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Chemical compositions of ethanol extracts of *Mimosa pudica* root and *Anacardium occidentale* stem bark

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Abstract

The ongoing requirement to consistently supplement the existing global medicine supply has persisted in driving the exploration for bioactive substances derived from natural sources, such as plants. The objective of this study is to evaluate the chemical contents of ethanol extracts obtained from the roots of *Mimosa pudica* and the stem bark of *Anacardium occidentale*. The phytochemical examination was conducted using gas chromatography combined with a mass spectrophotometer. The amino acid profile was analyzed using high performance liquid chromatography (HPLC), while the presence of bioactive substances was screened using a Fourier transform infrared (FTIR) spectrophotometer. The assessment of its ability to scavenge free radicals was conducted by assessing its inhibitory impact on ferric reducing antioxidant potential (FRAP), Nitric oxide (NO), superoxide scavenging radical, and 2,2-diphenyl-2-picrylhydrazyl (DPPH) radicals. *M. pudica* showed the highest level of statistically significant ($P<0.05$) Nitric oxide radical scavenging activity. The findings indicate that the ethanol extracts of *M. pudica* roots and *Anacardium occidentale* stem bark contain a substantial number of beneficial phytochemicals, which possess notable antioxidant capabilities and the potential to eliminate free radicals. The proximate and mineral composition of the plants was determined using standard methods. The moisture content was found to be 3.65 ± 0.05% and 4.45 ± 0.07%, the fibre content was 3.55 ± 0.09% and 1.90 ± 0.05%, the ash content was 5.49 ± 0.12% and 3.94 ± 0.09%, the fat content was 0.89 ± 0.02% and 4.31 ± 0.07%, the protein content was 8.40 ± 0.10% and 9.90 ± 0.13%, and the carbohydrate content was 78.03 ± 1.18% and 75.52 ± 1.15%. In terms of minerals, the iron content was 3.98 ± 0.03% and 2.47 ± 0.07%, the copper content was 0.14 ± 0.01% and 0.10 ± 0.01%, and the zinc content was 0.34 ± 0.03% and 0.21 ± 0.02%. The composition of the sample is as follows: Magnesium (6.49 ± 0.86%), and (4.89 ± 0.93%), Potassium (6.46 ± 0.64%), and (6.04 ± 0.43%). The findings indicate that the bioactive chemicals found in the root of *M. pudica* and the stem bark of *Anacardium occidentale* have significant potential as a nutritional supplement and for the development of natural drugs.

Keywords: *Mimosa pudica; Anacardium Occidentale*; Proximate Compositions; Phytochemicals; Free Radical Scavenging Activity**.**

1. Introduction

Plants have gradually provided alternate sources of medicinal chemicals that are commonly utilized in the pharmaceutical sector. The field of herbal therapy is seeing rapid growth, and these therapies are becoming increasingly popular in both developing and developed countries due to their natural origins.

Presently, the use of medicinal plants is mostly directed towards the preservation of health and the provision of treatment alternatives for specific disorders in both contemporary and traditional medicinal methodologies (Ahn, 2017). Medicinal plants are the fundamental basis of traditional medicine. The integration of herbal products into the health-care system, along with the proliferation of herbal clinics, has become widespread in several countries, and is backed by national legislation.

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Boots *et al.* (2008) state that certain plant species have advantageous phytochemicals that can serve as natural antioxidants, fulfilling the requirements of the human body. Multiple studies have demonstrated that pharmaceutical chemicals originating from plants have been utilized for therapeutic purposes and as natural inhibitors for various diseases. This is attributed to the abundance of valuable secondary metabolites such as alkaloids, flavonoids, saponins, and terpenoids (Ncube *et al.,* 2008).

Safari and Ahmady (2019) found that certain plant extracts with free radical scavenging activity have both antibacterial and antimicrobial effects. This suggests a connection between the antioxidant and antibacterial characteristics of these plant species.

Mimosa pudica Linn is a member of the Fabaceae family. The English term for this plant is "sensitive plant". In the majority of regions in Nigeria, this plant is commonly referred to as "touch and die." The Igbo language typically refers to this plant species as kpakonukwu. The plant is a partially upright undershrub native to tropical regions of America and Australia. *Mimosa pudica* is recognised for its sedative, emetic, and tonic characteristics. The extract is frequently used to treat symptoms related to diabetes, diarrhoea, dysentery, tumour, and several urogenital diseases. Fattepur and Gawade (2007) reported that in Southern Nigeria, the root of this plant is used for pharmacological purposes, including antivenom, antifertility, and antioxidant effects. Carl Linnaeus originally documented it in his work "Species Plantarum" in 1753. It is utilised for several therapeutic applications, such as conducting phytochemical investigations. According to Johnson *et al.* (2014), multiple investigations on *M. pudica* have shown the existence of alkaloids, flavonoids, cardiac glycosides, steroids, terpenoids, tannins, and fatty acids.

Mimosa pudica is a shrub with a maximum height of 0.5 meters and possesses shiny green leaves. It thrives in tropical places with warm climates worldwide. *Mimosa pudica* is a low-growing plant that blooms either annually or perennially. It is frequently grown for its unique and intriguing qualities. Upon contact or agitation, the responsive foliage of the complex promptly retracts and descends, only to resume its original position after a brief interval.

Mimosa pudica has undergone extensive research and has shown potential effectiveness in various areas such as being an anti-venom, antifertility, antidiuretic, wound healing agent, antinociceptive, hyperglycaemic, anti-hepatotoxic, and anti-inflammatory (Karthikeyan and Deepa, 2010; Vikram *et al.,* 2012).

Anacardium occidentale is a tropical plant found in Northeastern Brazil. The tree is a perennial plant with long, penetrating roots, cultivated for its edible fruits, which are commonly referred to as nuts. The *A. occidentale* tree exhibits a main trunk that branches out and is accompanied by a distinct down crown. The *A. occidentale* trees can attain a vertical measurement of 12m (39.4 ft) and possess an economic lifespan of 25 years.

Anacardium occidentale is a tropical perennial tree indigenous to South America. *Anacardium occidentale* is responsible for the production of cashew seeds and apple fruits. The stem bark of *Anacardium occidentale* refers to the external layer of the tree's stem. The bark may seem inconsequential, akin to other components of trees that are destined to be used for timber. However, this is not the case with this particular species.

The stem bark of *Anacardium occidentale* is utilised in the production of herbal medicines because of its purported health advantages, as it is believed to possess antifungal, antimicrobial, and antibacterial characteristics, as well as antioxidants (Mercheeti, 2014).

According to a survey by the World Health Organisation (WHO), around 69% of the world's population incorporates the use of plants as therapeutic substances in their healthcare practices (Manas *et al.,* 2012).

2. Material and methods

2.1. Collection and authentication of the plant samples

The fresh roots of *Mimosas pudica* and the stem bark of *Anacardium occidentale* were collected from the premises of the Federal University of Technology, Owerri (FUTO). They were then identified, confirmed, and assigned voucher numbers 0000022657 and F-52 by Dr. C. P. Onoh, a plant taxonomist from the Department of Crop Science and Technology at the Federal University of Technology, Owerri.

2.2. Preparation of Plant Materials

The fresh roots and stem bark were rinsed with tap water to eliminate dirt particles and attached debris, and subsequently rinsed with distilled water. The samples were divided and left to dry in the air for a period of 10 days. Subsequently, the milling machine was employed to pulverise them into a coarse powder, which was then stored in hermetically sealed containers until it was utilised for subsequent tasks.

2.3. Extraction of plant samples

The samples were uniformly prepared using specific procedures to get an aqueous extraction. The 250-gram ground plant samples were dissolved in distilled water using a 2.5 Litre volumetric flask. Subsequently, the solution was adjusted to the desired level and then separated by decantation and filtration. The filtrates were accurately labelled.

2.4. Phytochemistry

2.4.1. Gas Chromatography and Mass Spectrophotometry GC-MS

One gramme of the pulverised sample was put into a test tube, followed by the addition of 25 ml of ethanol. The test tube underwent a reaction on a hot plate for a duration of 90 minutes. Following the completion of the reaction, the resulting product in the test tube was subsequently transferred to a separation funnel. The tube was effectively cleansed using 20 ml of ethanol, followed by 10 ml of cold water, 10 ml of hot water, and 3 ml of hexane, all of which were then transferred to the funnel. The extracts were consolidated and rinsed three times with a 10% v/v ethanol aqueous solution. The solution was dehydrated using anhydrous sodium sulphate and the solvent was removed through evaporation. The sample was dissolved in 1000 µL of pyridine, and then 200 µL of the solution was transferred to a vial for analysis.

The bioactive components of various plant extracts were analysed using Agilent Technologies GC systems, specifically the GC-220 model. (Varian, Santa Clara, CA, USA), which was equipped with an HP-5ms column measuring 40m in length, 250 Nm in diameter, and 0.25 Nm in thickness of film. The initial temperature was set to 50°C, but it rose to 150°C at a pace of 10°C each minute. The temperature was raised to 300°C at a rate of 10°C per minute. The carrier gas employed was helium, with a flow rate of 1 m/min. The Spit voltage was 1/0.1, while the Ionisation voltage was 70e V.

2.5. Proximate analysis.

The proximate compositions of the roots of *Mimosa pudica* and the stem bark of *Anacardium occidentale* were determined using the AOAC technique from 1990.

2.6. Moisture content

The Petri dishes were cleaned, dried in the oven, and then weighed. Two grammes of pulverised samples were individually placed in an oven and subjected to heating at a temperature of 105°C for a duration of 2 hours. During this period, the weight measurements were recorded and the

sample was further heated for an additional hour until stable weights were achieved. The moisture content of the analysed samples was determined by subtracting the final stable weight from the starting stable weight of the samples.

The formula to calculate the moisture content is expressed as a percentage and is given by dividing the difference between the initial weight (W1) and the final weight (W2) of the sample by the weight of the sample, and then multiplying the result by 100.

$$\%\ moisture\ content\ = \frac{W1-W2}{weight\ of\ sample} \times 100 \text{.....Eqn. 2.1}$$

Where,

- W1 = Weight of petri dish and sample before drying
- W2 = Weight of petri dish and sample after drying.

2.7. Ash content

The platinum crucibles were cleansed, dried, and measured without any contents. Two grammes of ground roots and stem bark were individually measured and placed into crucibles. The crucibles were then subjected to a muffle furnace

at a temperature of 550°C for a duration of 3 hours. The ash content percentage was calculated using the following formula.

The expression (W3 - W1)/(W2 - W1)×100/1…….....Eqn. 2.2

2.8. Crude Fibre

The pulverised roots of *Mimosa pudica* and the stem bark of *Anacardium occidentale* were each weighed and then treated with petroleum ether to remove the fat content. The defatted samples were subjected to reflux boiling for 30 minutes with H2SO4 and subsequently placed into a beaker. Sodium hydroxide in a carbonated form was introduced and separated through filtration. The remaining substances were dried in an oven, then cooled and measured for weight.

= Weight of fibre x 100 $Weight\ of\ sample$

$$\%\ Crude\ Fibre\ = \frac{Weight\ of\ Fibre}{weight\ of\ sample} \times 100 \quad \text{…… Eqn. 2.3}$$

2.9. Crude Fat

This analysis was conducted using the Soxhlet fat extraction method. The roots of *Mimosa pudica* and the stem bark of *Anacardium occidentale* were extracted using 300 ml of petroleum ether in a pre-weighed boiling flask. The boiling flasks were moved into an oven, allowed to cool, and then weighed again. The crude fat content is determined by calculating the difference between the between the boiling flask and the boiling flask with extract. The percentage of fat is calculated by dividing the weight of the flask by the weight of the sample, then multiplying by 100.

$$\%\ fat = weight\ of\ flask + oil = \frac{weight\ of\ flask}{weight\ of\ sample} \times 100 \quad \text{…….. Eqn. 2.4}$$

2.10. Crude Proteins

To determine protein content, the macro Kjeidah method was utilized and protocol adopted from AOAC, (1990).

The samples (0.5 g) were weighed into a 30 ml Kjeidah flask. Then,0.5g of the Kjeidah catalyst mixture was added. The mixture was heated cautiously in a digestion rack under heat until a clear solution appeared.

% Nitrogen = Titre value x 0.01 x 14 x 4…….. Eqn. 2.5

Total nitrogen = (100 x (vA- VB)

(0.0140) /10 x W) x 100

Where

- VA =Volume (ml) of HCL used in the samples titration.
- VB = volume b1(ML) of Hck used in the blank titration.
- N =Normality of HCL
- W = weight of sample (g).

% Protein = % Nitrogen x 6.25……Eqn 2.6

The protein content was determined using the macro Kjeldahl technique, using the procedure taken from AOAC (1990).

The specimens, weighing 0.5 grammes each, were carefully measured and placed into a 30 millilitre Kjeidah flask. Subsequently, 0.5 grammes of the Kjeidah catalyst mixture was introduced. The mixture was carefully cooked on a digesting rack until a transparent solution formed.

2.11. Carbohydrate Determination

The carbohydrate content was determined using the differential technique. The formula to calculate the percentage of carbohydrates in a substance = 100 - %moisture - %protein - %lipid - %ash - %fibre .....Eqn. 2.7

2.12. Free radical scavenging activity

2.12.1. Nitric oxide scavenging activity

A 10 nM solution of sodium nitroprusside (SNP) was produced in 0.5 ml of phosphate buffer at a pH of 7.4. Subsequently, 0.5 ml of the previously prepared extract, which had a concentration of 50 mg/ml, was added. The mixture was incubated at a temperature of 25°C for 30 minutes. Subsequently, 0.5 ml of Griess reagent was introduced with stirring, and the absorbance was instantly recorded at a wavelength of 542 nm. The equation below was utilised to

$$\frac{Abs\ control - Abs\ sample}{Abs\ control} \times 100 \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots Eqn.\ 2.8$$

Abs control

Where,

- Abs control = Absorbance of control.
- Abs sample = Absorbance of sample.
  The analysis was conducted with the method by Green et al.,(1992).

2.12.2. Ferric Reducing Antioxidant Potential (FRAP)

The Pulido *et al.* (2000) approach was utilised to ascertain the reducing power of the plant extract. 0.25 millilitres of each sample were mixed with 200 millilitres of sodium phosphate buffer (0.25 ml at pH 6.6) and 1.0 ml of potassium ferrocyanide. The combination was subjected to incubation at a temperature of 50°C for a duration of 20 minutes. Subsequently, 0.25 mL of a trichloroacetic acid solution at a concentration of 100 mg/L (10% w/v) was added. The mixture was combined with 1.0 ml of distilled water and 0.2 ml of ferric chloride solution. A spectrophotometric study was performed to measure the absorbance of the reaction mixture at a wavelength of 700 nm.

$FRAP = A/A_o\ x\ M/M_o$ …….. Eqn. 2.9

Where:

- A = Absorbance of sample
- A_o = mean absorbance of the standard
- M_o= mass of standard
- M = mass of sample

2.12.3. 2-,2-Diphenyl-2-picry hydroxyl (DPPH) Scavenging Activity

The evaluation approach was implemented using the methodology described by Mensor *et al.* (2001). A 2 ml aliquot of extract solution with varying concentrations was combined with 0.1 mm of DPPH solution. Afterward, the samples underwent a 30-minute incubation period, and the optical density was determined at a wavelength of 518 using a spectrophotometer (Genesys 10-s, USA).

% DPPH scavenging activity.

$$\frac{= Abs\ control - Abs\ sample}{Abs\ control} \times 100 \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots Eqn.\ 2.10$$

Where:

- Abs control = Absorbance of control.
- Abs sample = Absorbance of sample.

2.12.4. Superoxide Scavenging Activity

% superoxide scavenging activity = (Ao - A1) x 100 …….. Eqn. 2.11

Where;

- Ao - Absorbance before illumination
- A1 - Absorbance after illumination.

The method used to measure superoxide scavenging activity was conducted according to the protocol outlined by Winterboum *et al.* (1975). Superoxide anions were produced in samples consisting of 3.0 ml of the extracts (20mg), 0.2ml of EDTA, 0.1 ml of napthylethylene, 0.05 ml of riboflavin, and 2.64 ml of phosphate buffer. The control tubes were arranged. Subsequently, all tubes were vigorously mixed and the initial optical density was assessed at 560 nm using a spectrophotometer (Genesys,10- s, USA). The tubes were lit with a fluorescent bulb for duration of 30 minutes. A second measurement of absorbance was taken at a wavelength of 560nm. The disparity in absorbance prior to and subsequent to illumination was suggestive of the activity of scavenging superoxide anions.

3. Results

3.1. Phytochemical analysis using Gas chromatography -Mass Spectrophotometry (GC-MS)

The findings of the phytochemical examination of the two plant extracts are displayed in the table 1 – 2. Twenty-two compounds were isolated from *M. pudica* and twenty for *Anacardium occidentale*. The table 1 shows that most predominant phytochemicals identified in *M. pudica* ethanol extracts include (R+, R+)-5-Hydroy-4-methyl-3-heptanone (22.87%), Cis-vaccenic acid (12.69%), n-Hexadecanoic acid (12.12%), Ether 6-methyl hepty vinyl (6.31%), Oleic acid (5.64%), Decanoic acid, 3-methyl (5.45%). Conversely, Butoxyacetic acid (0.96%), 1-octenylsuccinic anhydride (0.66%) and Octasiloxane, 1,1,3,3,5,5,7,7,9,9,11,11,13,13,15,15-hexadecamethyl- were found to be the least abundant bioactive phytochemicals present in *M. pudica* extract. The prevailing bioactive phytochemicals identified in the ethanol extracts of *A. occidentale* according to table 2 include, Linoelatic acid (24.92%), Oleic acid (12.97%), n-Hexadecanoic acid (7.12%),6-Octadecenoic acid (Z) (10.94%), and Octadecenoic acid (7.73%) while the least abundant compounds were Pyrimidine-4, 6(3H,5H),-dione, 2-butythio-(0.98%). Heptadecanoic acid, 16-methy-, methyl ester (0.88%), Trichloroacetic acid, Pentadecyl ester (0.81%), Ethyl Oleate (0.72%) and Carbonic acid, Propyl 2,2,2- trichloromethyl ester (0.66%).

Table 1 Bioactive Compounds Present in *Mimosa pudica* Ethanol Roots Extracts by GC-MS

S/N	Retention Time	Bioactive Components	Area (%)
1	11.877	(R*, R*)-5-Hydroxy-4-methyl-3-heptanone	22.87
2	12.460	Decanoic acid, 3-methyl-	5.45
3	12.699	Butoxyacetic acid	0.96
4	14.435	Ether, 6-methylheptyl vinyl	6.31
5	14.527	Docosyl octyl ether	1.70
6	14.703	3-Deoxy-d-mannoic lactone	2.49
7	15.474	Oxazole, 5-hexyl-2,4-dimethyl-	2.39
8	16.982	Hexadecanoic acid, methyl ester	2.94
9	17.486	1,2-Benzenedicarboxylic acid, butyl 2-ethylhexyl ester	1.17
10	17.621	n-Hexadecanoic acid	12.12
11	18.208	1-Octenylsuccinic anhydride	0.66
12	18.800	cis-13-Octadecenoic acid, methyl ester	5.41
13	19.102	1H-Cycloprop[e]azulene, decahydro- 1,1,4,7-tetramethyl- [1aR-(1a.alpha.,4.beta.,4a.beta.,7.beta.,7a.beta., 7b.alpha.)]-	6.18
14	19.425	cis-Vaccenic acid	12.69
15	19.604	Oleic acid	5.64

16	19.915	Oleic acid	1.63
17	20.268	7-Pentadecyne	2.33
18	20.564	2-Propenoic acid, 2-methyl-, 3-[tris(2-methoxyethoxy)silyl]propyl ester	0.75
19	23.602	6-Octadecenoic acid	2.60
20	23.931	4-Thiazolidinone, 3-ethyl-5-[(phenylamino)methylene]-2-thioxo-	1.54
21	26.110	Glyceric acid (ISP-TFA)	1.24
22	29.714	Octasiloxane, 1,1,3,3,5,5,7,7,9,9,11,11,13,13,15,15-hexadecamethyl-	0.95

Table 2 Bioactive Compounds Present in *Anacardium occidentale* Ethanol Stem Bark Extracts by GC-MS

S/N	Retention Time	Bioactive Components	% Report
1	9.840	5-Octadecene, (E)-	1.07
2	12.828	Pyrimidine-4,6(3H,5H)-dione, 2-butylthio-	0.98
3	12.893	Trichloroacetic acid, pentadecyl ester	0.81
4	13.113	Dodecanoic acid	2.69
5	15.408	Tetradecanoic acid	2.11
6	16.880	Carbonic acid, propyl 2,2,2-trichloroethyl ester	0.66
7	16.952	Hexadecanoic acid, methyl ester	1.44
8	17.410	n-Hexadecanoic acid	2.14
9	17.538	n-Hexadecanoic acid	7.12
10	18.743	10-Octadecenoic acid, methyl ester	6.63
11	18.980	Heptadecanoic acid, 16-methyl-, methyl ester	0.88
12	19.301	Linoelaidic acid	24.92
13	19.497	Octadecanoic acid	7.73
14	19.753	9,12-Octadecadienoic acid (Z,Z)-	2.98
15	19.869	9,12-Octadecadienoic acid (Z,Z)-	4.56
16	19.999	9,12-Octadecadienoic acid (Z,Z)-	2.91
17	20.137	Linoelaidic acid	5.76
18	20.900	6-Octadecenoic acid, (Z)-	10.94
19	30.931	Oleic acid	12.97
20	31.429	Ethyl Oleate	0.72

3.2. Functional groups of extracts from plant samples

Table 3 and 4 shows the findings of functional groups investigation of the ethanol root extracts of *Mimosa pudica* and *Anacardium occidentale*. The functional groups found in *M. pudica* include, aromatic amine, alkanes, conjugated alkene, alkene, isothocyante, thiocyanate, phosphine, thiol, carboxylic acid, alkane, alcohol, aliphatic primary amine and alcohol. Twelve vibrational groups were confirmed in the stem bark extracts, which are aromatic amine, conjugated alkene, alkene, alkynes, nitrile, phosphine, thiol, alcohol, intra molecular bonded alcohol, alkanes, alkene, and aliphatic primary amine.

Table 3 FTIR Peak Values and Functional Groups of Ethanol Extract of *Mimosa pudica* Roots

Wave number	Bond	Compound
1271.626	C-N stretching	Aromatic amine
1429.161	C-H stretching	Alkanes
1619.454	C=C stretching	Conjugated alkene
1859.913	C=C asymmetric stretching	Alkene
2025.331	N=C=S stretching	Isothiocyanate
2199.526	S-C≡N stretching	Thiocyanate
2455.92	P-H stretching	Phosphine
2577.837	S-H stretching	Thiol
2753.635	O-H stretching	Carboxylic acid
2983.821	C-H stretching	Akane
3168.677	O-H stretching	Alcohol
3394.73	N-H stretching	Aliphatic primary amine
3786.138	O-H stretching	Alcohol

Table 4 FTIR peak values and functional groups of ethanol extract of *Anacardium occidentale* stem bark

Wave number	Bond	compound
1301.67	C-N stretching	Aromatic amine
1616.976	C=C stretching	Conjugated alkene
1848.272	C=C asymmetric stretching	Alkene
2195.068	C≡C stretching	Alkynes
2203.099	C≡N stretching	Nitrile
2452.184	P-H stretching	Phosphine
2605.649	S-H stretching	Thiol
2691.843	O-H stretching	Alcohol
2817.247	O-H stretching	Intra molecular bonded Alcohol
2966.044	C-H stretching	Alkanes
3193.33	C-H stretching	Alkene
3499.946	N-H stretching	Aliphatic primary amine

3.3. Proximate compositions of the plants

Table 5 shows the proximate compositions of the plants. All the parts of the two plants were found to contain nutrient compositions in vary levels. Moisture (3.65 ± 0.05%), (4.45 ± 0.07%) Fibre (3.55 ± 0.09%), (1.90 ± 0.05%) Ash (5.49 ± 0.12%), (3.94 ± 0.09%), Fat (0.89 ± 0.02%), (4.31 ± 0.07%), Protein (8.40 ± 0.10%), (9.90 ± 0.13%), Carbohydrate (78.03 ± 1.18%), (75.52 ± 1.15%).

Table 5 Proximate composition (%) of the *Mimosa pudica* root and *Anacardium occidentale* stem bark

Parameter (%)	Root extract	Stem bark
Moisture	3.65 ± 0.05	4.45 ± 0.07
Fibre	3.55 ± 0.09	1.90 ± 0.05
Ash	5.49 ± 0.12	3.94 ± 0.09
Fat	0.89 ± 0.02	4.31 ± 0.07
Protein	8.40 ± 0.10	9,90 ± 0.13
Carbohydrate	78.03 ± 1.18	75.52 ± 1.15

Values are mean ± standard deviation of triplicate determinations

3.4. Mineral composition (%) of *Mimosa pudica* root and *Anacardium occidentale* stem bark extracts

Table 6 shows mineral content. They include Iron (3.98 ± 0.03%), (2.47 ± 0.07%), Copper (0.14 ± 0.01%), (0.10 ± 0.01%), Zinc (0.34 ± 0 03%), (0.21 ± 0.0 2%) Magnesium (6.49 ± 0.86%), (4.8 9 ± 0.93%), Potassium (6.46 ± 0.64%), (6.04 ± 0.43%).

Table 6 Minerals composition (%) of *Mimosa pudica* root and *Anacardium occidentale* stem bark

Parameter (ppm)	Provisional Maximum Tolerable Daily Intake (mg/kg body weight/day)	Root extract	Stem bark
Iron	0.8	3.98 ± 0.03	2.47 ± 0.07
Copper	2.0	0.14 ± 0.01	0.10 ± 0.01
Zinc	1.0	0.34 ± 0.03	0.21 ± 0.02
Magnesium	2.0	6.49 ± 0.86	4.89 ± 0.93
Potassium	0.3	6.46 ± 0.64	6.04 ± 0.43

Maximum daily intake (WHO, 2007); Values are mean ± standard deviation of triplicate determinations.

3.5. Amino acids compositions (%) of *Mimosa pudica* root and *Anacardium occidentale* stem bark extracts

Table 7 shows the amino acids compositions. Eighteen amino acids were detected in the plants' extracts which include, Glycine (Gly), Alanine (Ala), Serine (Ser), Proline (Pro), Valine (Val), Threonine (Thr) Isoleucine (Iso), Leucine (Leu), Aspartate (Asp) and Lysine (Lys). Phenylalanine (Phe), Methionine ((Met), Histidine (His), Tryptophan (Try) Glutamate (Glu) Arginine (Arg) Cystine (Cys) Serine (Ser). The bark showed higher amino acids concentration (96.06 ± 3.55%) than the root extract (90.91 ± 3.42%).

Table 7 Amino acid profile composition (%) of *Mimosa pudica* root and *Anacardium occidentale* stem bark

Amino acid (g/100g protein)	Root extract	Stem bark
Glycine	4.11 ± 0.01	4.21 ± 0.04
Alanine	5.75 ± 0.05	6.29 ± 0.08
Serine	4.39 ± 0.06	4.33 ± 0.02
Proline	2.85 ± 0.01	3.03 ± 0.02
Valine	4.37 ± 0.09	4.65 ± 0.04
Threonine	4.15 ± 0.05	4.34 ± 0.09
Isoleucine	4.20 ± 0.02	4.60 ± 0.04
Leucine	8.00 ± 0.06	8.98 ± 0.10
Aspartate	10.35 ± 0.10	10.65 ± 0.09
Lysine	8.89 ± 0.06	9.72 ± 0.07
Methionine	1.37 ± 0.02	1.50 ± 0.03
Glutamate	14.34 ± 0.09	14.46 ± 0.10
Phenylalanine	3.95 ± 0.01	3.91 ± 0.09
Histidine	2.99 ± 0.02	3.37 ± 0.04
Arginine	5.69 ± 0.03	6.55 ± 0.07
Tyrosine	3.00 ± 0.09	2.92 ± 0.02
Tryptophan	1.14 ± 0.03	1.12 ± 0.01
Cystine	1.35 ± 0.04	1.42 ± 0.02
TOTAL	90.91 ± 3.42	96.06 ± 3.56

Values are mean ± standard deviation of duplicate determinations

3.6. Free Radical Scavenging activity

Free Radical Scavenging Activity of Analysis of invitro antioxidant activity shows that the plant extracts possessed significant 2,2-diphenyl-1-picrylhyrazyl (Figure 1) comparable to the standard (BHT). *Mimosa pudica* extract showed significantly ($p<0.0.5$) highest ferric reducing antioxidant potential (79.74 ± 1.78%) when compared with *A. occidentale* counterparts (Figure 2). However, the standard reference compound, Ascorbic acid gave a better ferric reducing antioxidant potential (FRAP) than the two extracts. Figure 4 showed that the Nitric Oxide scavenging activity of *M. pudica* at concentration of 5 to 10Mg/ml was lower than that of *A. occidentale* including the standard (Ascorbic acid). The Super Oxide scavenging radical activity of ethanol extract of *A. occidentale* (85.75 ± 1.45%) was significantly higher ($P<0.05$) than of *M. pudica* (61.70 ± 1.12%) compare to the standard ascorbic acid (82.98 ± 0.79%).

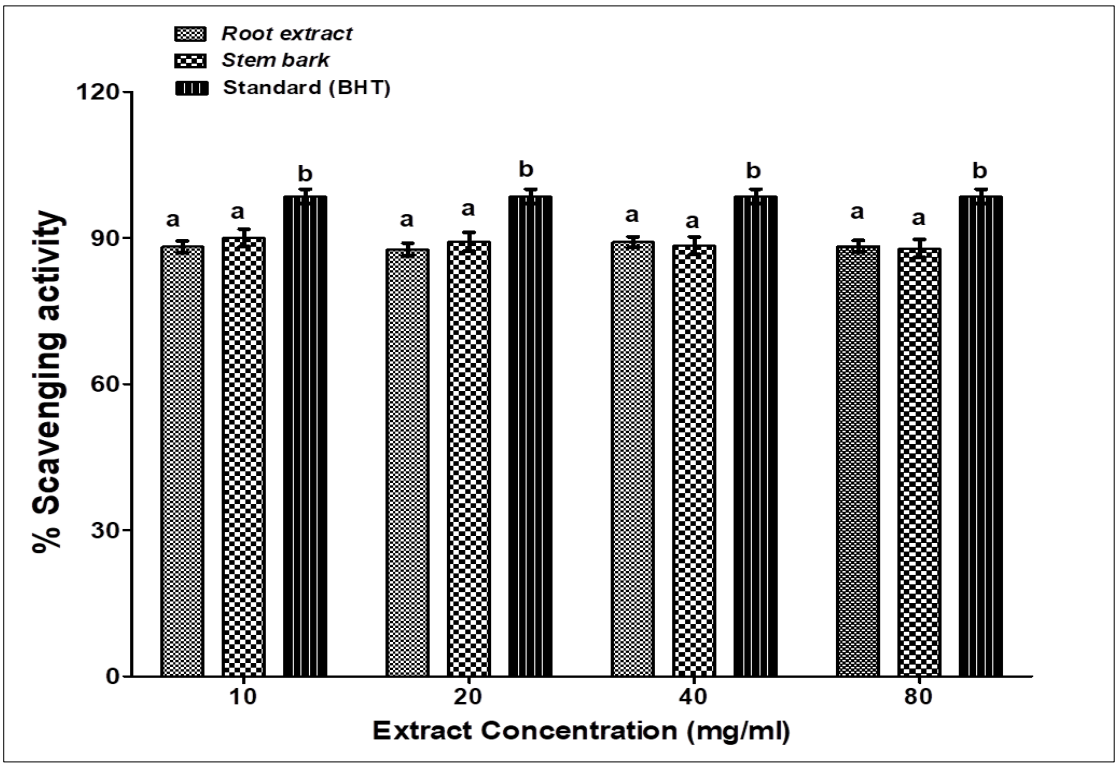

Figure 1 DPPH radical scavenging activity (%) of *Mimosa pudica* root and *Anacardium occidentale* stem bark extracts. Bars are mean ± standard deviations of triplicate determination. Bars bearing different alphabet letters per extract concentration are statistically significant (P<0.05)

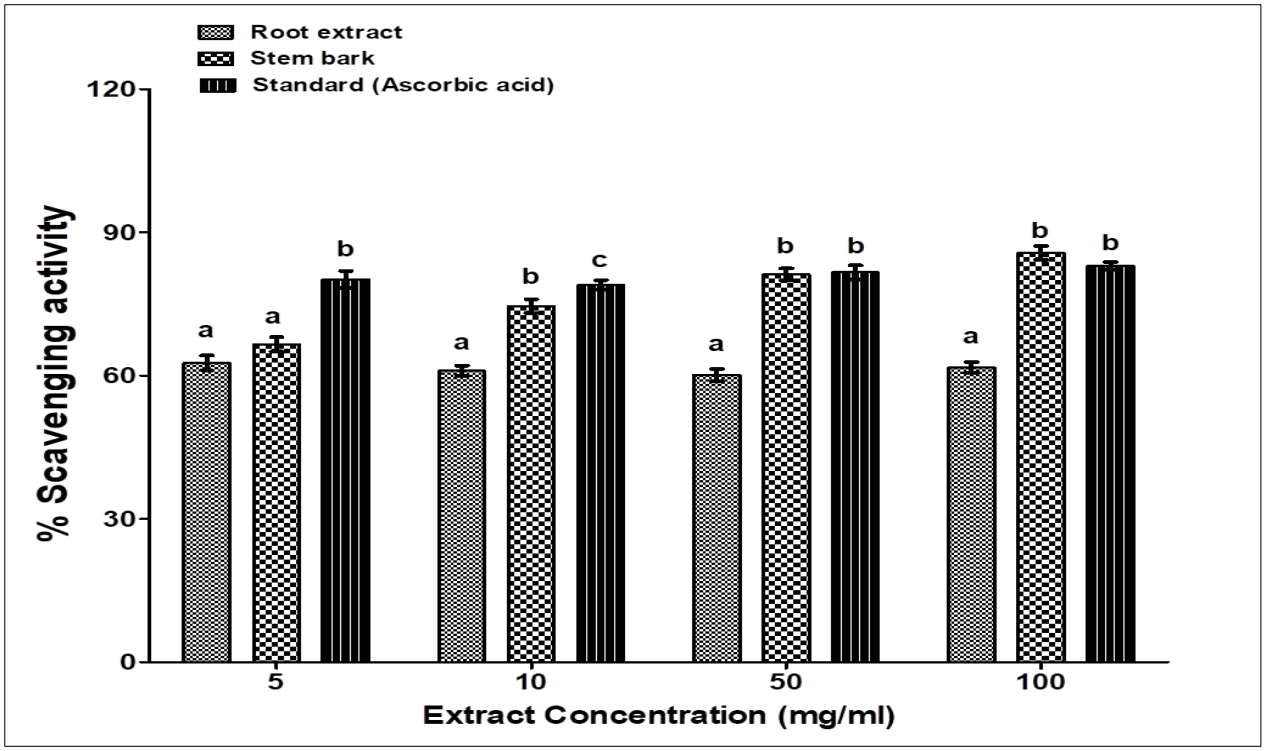

Figure 2 Superoxide scavenging activity (%) of *Mimosa pudica* root and *Anacardium occidentale* stem bark extracts. Bars are mean ± standard deviations of triplicate determination. Bars bearing different alphabet letters per extract concentration are statistically significant (P<0.05)

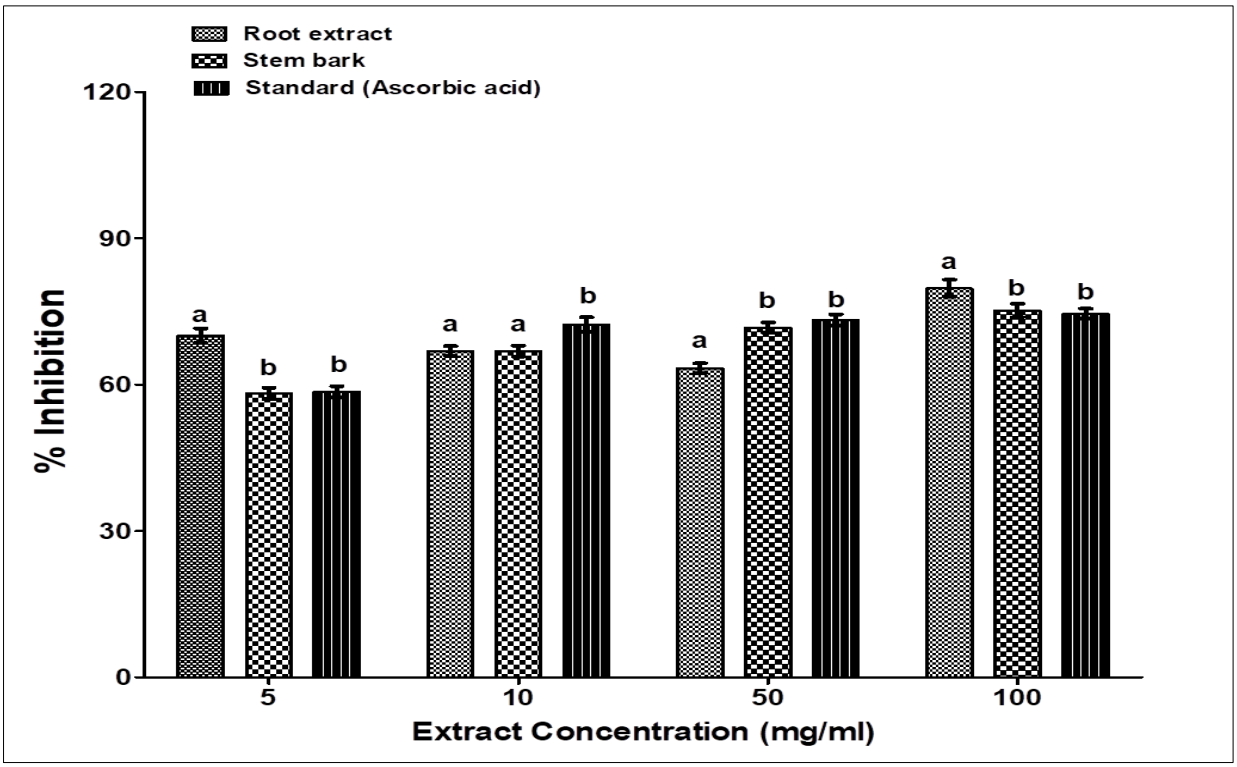

Figure 3 Ferric Reducing Antioxidant Potential (FRAP) scavenging activity (%) of *Mimosa pudica* root and *Anacardium occidentale* stem bark extracts. Bars are mean ± standard deviations of triplicate determination. Bars bearing different alphabet letters per extract concentration are statistically significant (P<0.05)

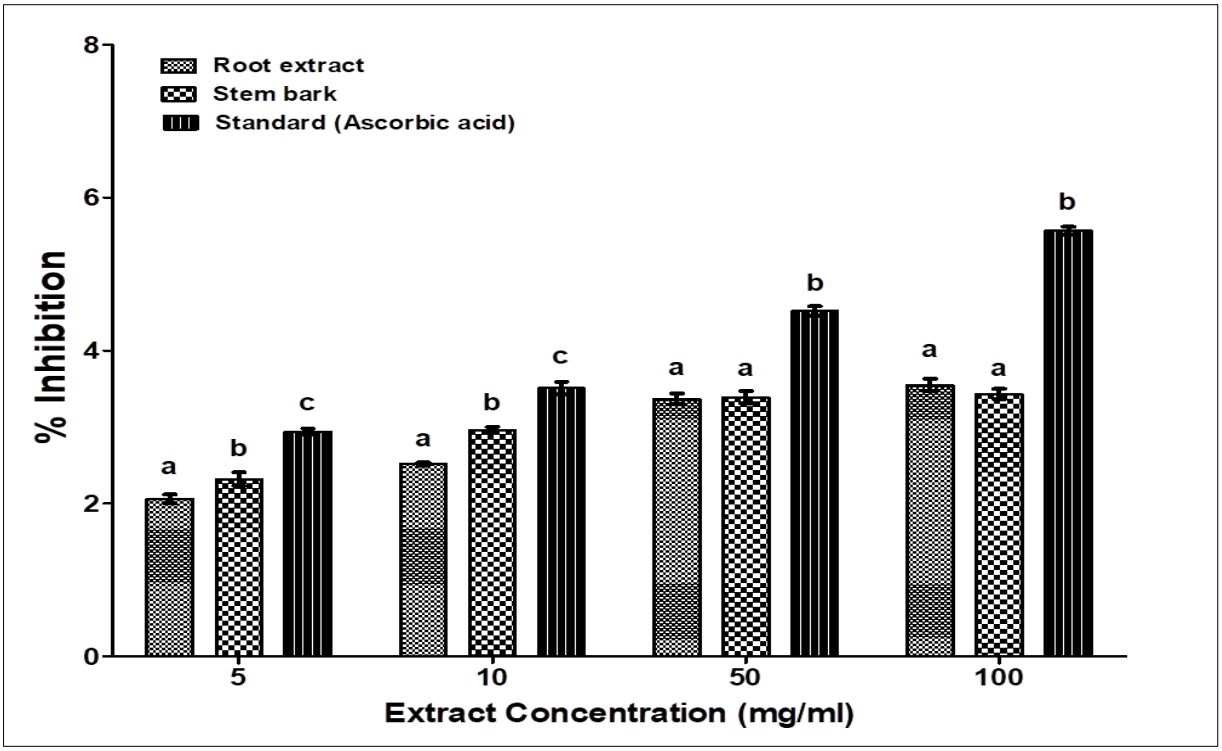

Figure 4 Nitric oxide scavenging activity (%) of *Mimosa pudica* root and *Anacardium occidentale* stem bark extracts. Bars are mean ± standard deviations of triplicate determination. Bars bearing different alphabet letters per extract concentration are statistically significant (P<0.05)

4. Discussion

Plants have served as a significant reservoir of natural substances that contribute to the preservation of human well-being. The utilisation of plants as agents for traditional medicine is increasingly becoming popular on a global scale. The GC-MS analysis of *M. pudica* and *A. occidentale* showed the presence of several secondary metabolites. The *A. occidentale* contains a higher percentage of chemicals compared to *M. pudica* (R+, R+).The examined plant extracts contained 5-Hydroxy-4-methyl-3-heptanone, n-Hexadecanoic acid, Cis-vaccenic acid, and Octadecenoic acid, which have previously been found to possess antivenom, antibacterial, and therapeutic effects (Chao and Lin, 2010). The cashew nut (*Anacardium occidentale*) contains tetradecanoic acid, which exhibits antibacterial, antibiotic, anticoagulant, and anticholesterol effects (Oguoma, Igwe, Nwaoguikpe & Reginal, 2023). Carboxylic chemicals are utilised as solvents. Shang-T Zan, Huan-You, and Tzu-Chang (2018) found that certain fatty acids, including Oleic acid, n-Hexadecanoic acid, Dodecanoic acid, and Cis-vaccenic acid, have antimicrobial, antifungal, and antibacterial effects. These properties were previously observed in plants by Akinpelu (2001) and Kang *et al.* (2004). Furthermore, the plant's extracts contain Alkene compounds, including 5-Octadecene (E)-, which possess pharmacological features such as antibacterial and antifungal effects (Kang *et al.,* 2004). The presence of glycosides, specifically 9,12-octadecenoic (Z, Z) glycosides, was observed in the plants. They are a constituent of pharmaceuticals utilised in the management of associated ailments. Their pharmacological activities encompass antipyretic and anti-inflammatory actions (Azam *et al.,* 2015). The Fourier transform infrared (FTIR) is a highly regarded vibrational spectroscopic technique that is capable of precisely identifying significant functional groups present in plant extracts, biological substances, and synthetic chemicals. Table 4 and 5 display the FTIR functional groups, which exhibit 12 and 13 peaks respectively, indicating a total of twenty-five functional groups.

The compounds listed include aromatic amines, alkanes, conjugated alkenes, alkenes, isothiocyanates, thiocyanates, phosphines, thiols, carboxylic acids, alcohols, aliphatic primary amines, alkynes, nitriles, and intramolecularly bonded alcohols. The therapeutic activities of *M. pudica* and *A. occidentale* may be attributed to the presence of these functional groups. The proximate compositions of both plant extracts revealed significant amounts of glucose, protein, fat, ash, and fibre. All highlighting the nutritional composition and energy content of these significant plants. Both plants had significant carbohydrate content, with values of 78.03 ± 1.18% and 75.52 ± 1.15% respectively. This indicates that they could potentially be a valuable energy source for cells, especially for the brain.

The moisture level of a plant is an indicator of its water activity and is utilised to assess the stability and vulnerability to microbial harm (Uyoh *et al.,* 2003). The low moisture content detected in both plants (3.65 ± 0.05%, 4.45 ± 0.07%) supports the apparent natural resistance to microbial deterioration, resulting in an extended shelf-life (Omoregie and Osagie, 2011).

The study found a significant quantity of protein in the extracts of both plants. The values are 8.40 ± 0.10% and 9.90 ± 0.13%. According to Igile *et al.* (2013), protein is a crucial element in the human diet that is necessary for the regeneration of damaged tissues and for providing energy and the necessary amino acids.

Lipids play a significant function in nutrition. They have been demonstrated to be significant sources of energy for humans. In addition, they are responsible for sustaining the integrity of the cell membrane, with a range of 0.89 ± 0.02% to 4.31 ± 0.07%. The low crude fat content found in *M. pudica* and *A. occidentale* indicates that they can be easily included in a weight-reducing diet. The *M. pudica* and *A. occidentale* had a low Ash content of 5.49 ± 0.12% and 3.94 ± 0.09%, respectively, suggesting a poor mineral content. The mineral elements serve as inorganic co-factors in metabolic processes (Igile *et al.,* 2013).

Crude fibre refers to the quantity of undigested carbohydrates found in plant extracts. Prior research has demonstrated that fibres contribute to the formation of bulk in the diet (Ayoola and Adeyeye, 2009). The significant levels of crude fibre found in both plant samples (3.55 ± 0.09%, 1.90 ± 0.05%) suggest their potential utility in managing colon cancer (Lacopetta *et al.,* 2017). Analysis of the mineral composition of *M. pudica* root and *A. occidentale* stem bark revealed that *M. pudica* exhibited significantly elevated amounts ($P < 0.05$) of iron, potassium, and magnesium. However, both plants exhibited comparable levels of Zinc and copper ($p < 0.05$). Zinc is considered an indispensable element for the production of proteins and nucleic acids, as well as for normal bodily development (Melaku, 2005). Potassium aids in maintaining body weight and regulating water and electrolyte balance in the blood and tissues (National Research Council, 1989). Magnesium is essential for maintaining the structural stability of nucleic acids and facilitating the absorption of electrolytes in the intestines (Igile *et al.,* 2013).

The analysis of the amino acid compositions of *M. pudica* and *A. occidentale* showed that they contain a significant amount of both essential and non-essential amino acids. Glutamate was found to be the most abundant amino acid,

making up 14.34 ± 0.09% of the total composition, while Tryptophan was the least abundant. Amino acids serve as the fundamental units of proteins, essential for the regeneration of deceased tissues. The concentrations of several indispensable amino acids are relatively in accordance with the World Health Organization's guidelines. Najafian and Bobs (2015) found that Leu, Val, Phe, Ala, Lys, Pro, and Try play a significant role in the overall high antioxidant capacity.

The extract of *Mimosa pudica* had a moderate inhibitory effect on nitric oxide free radicals, with a value of 3.55 ± 0.08%. This was followed by *A. occidentale*, which showed an inhibition value of 3.43 ± 0.07%, when compared to the standard. The ethanol plant's extracts exhibited a notable disparity in the Ferric Reducing Antioxidant Potential (FRAP). Nevertheless, *M. pudica* had the greatest FRAP activity, measuring 79.74 ± 1.76%, in comparison to *A. occidentale*. The DPPH free radical's scavenging ability is commonly employed to assess the antioxidant potential of naturally derived foods and plants. Based on the data presented in figure 1, it can be observed that all of the plant's extracts exhibited inhibitory activity against DPPH free radicals. The *M. pudica* and *A. occidentale* exhibited a moderate scavenging power of (88.34 ± 1.18%) = 0.10% and (87.86 ±1.86%) = 0.10% respectively in relation to DPPH. *M. pudica* had the highest scavenging activity and significantly ($P < 0.05$) inhibited the other evaluated extracts. Robak & Glyglenoski (1998) reported the activity of scavenging superoxide radicals. The investigation found that the superoxide radical characteristics of *A. occidentale* were higher (85.75 ± 1.54%) compared to *M. pudica* (61.70 ± 1.2%) ($P < 0.05$), but lower than the standard ascorbic acid (82.98 ± 0.79%).

The findings of this study provide initial confirmation that the roots of *M. pudica* and the stems of *A. occidentale* bark contain bioactive substances such as alkaloids, flavonoids, fatty acids, and diterpenes. These chemicals possess both nutritional and medicinal functions. The phytochemical substances that have been found are likely to be the cause of the plants' antivenom, antioxidant, antibacterial, antidiabetic, anticancer, and anti-inflammatory effects.

5. Conclusion

The results obtained in this study for the first confirmed that the presence of bioactive compounds such as alkaloids, flavonoids, fatty acids, and diterpenes in root of *M. pudica* and *A. occidentale* stem bark which are endowed with nutritional and therapeutic Functions. The identified phytochemical compounds may be responsible for the antivenom, antioxidant, antimicrobial, antidiabetic, anticancer and anti-inflammatory actions of the plants.

Compliance with ethical standards

Disclosure of conflict of interest

No conflict of interest to be disclosed.

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