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Studies on antioxidant, anti-diabetic and GC-MS analyses of methanol extract of *Aristolochia bracteolata* root bark

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Abstract: *Aristolochia bracteolata*is utilized in confronting multiple and complicated disease conditions such as cancer, lung inflammation, dysentery, syphilis, gonorrhea, arthritis, skindiseases, snake bite and oxidative stress relating to humans due to their acceptability, affordability and proximity. This investigation seeks to determine the antioxidant and anti-diabetic effects of methanol extract of *A. bracteolate* root bark *in vitro.* The phytochemical screening, antioxidant, and enzymes inhibitory (alpha-amylase and alpha-glucosidase) properties of root bark extract were evaluated by standard procedures. The methanol extract indicated the presence of diverse phytochemicals (tannins, saponins, flavonoids, alkaloids, phenols, glycosides, and terpenoids) and contained a remarkable amount of saponins (8.20±0.03%), phenols (6.82±0.01%), alkaloids (4.71±0.03%), and flavonoids (3.50±0.12%). The extract showed not only strong antioxidant properties against DPPH, FRAP, and TBARS radicals with IC_{50} value of 57.87, 54.64 and 47.54 mg/ml, respectively but also anti-diabetic activity by inhibiting alpha-amylase (IC_{50} =53.70 mg/ml) and alpha-glucosidase (IC_{50} =49.18 mg/ml). GC-MS chromatogram identified a diverse array of active metabolites in the methanol extract of *A. bracteolate* root bark. This study suggested that the methanol extract of *A. bracteolate* root bark possessed anti-oxidative and anti-diabetic activities.

Keywords: Antioxidant, phytochemicals, *Aristolochia bracteolata,* root bark extract, diabetes mellitus.

INTRODUCTION

Diabetes mellitus is a debilitating metabolic ailment portrayed by prolonged hyperglycemia, glucose intolerance, insulin resistance and relative insulin deficiency (Rudrapal *et al*., 2021). The prevalence of this ailment is growing rapidly and the number of diabetes cases is projected to increase upto 629 million by 2045 (IDF, 2017). Documented evidence has proven that the oxidative stress is an immediate effect of the hyperglycemia which has facilitated the initiation and development of diabetic complexities like retinopathy, nephropathy and neuropathy (Sayah *et al*., 2017; Sharifi-Rad *et al*., 2020; Ceja Garcia *et al*., 2022).

Hyperglycemia activates free radical production through protein kinase C, advanced glycationend products and polyol pathways, which play an energetic functionin the development of diabetic complexities (Elekofehinti, 2015; Chipiti*et al*., 2015; Vijaykrishnaraj and Wang, 2021). Ameliorating hyperglycemia and oxidative stress will help in the treatment option and control of free radicals-related disorders accompanied by diabetes mellitus. This could be attained via the application of antioxidants and by restricting postprandial hyperglycemia from carbohydrates consumption by inhibiting primary carbohydrate digestive enzymes (alpha-amylase and alpha-glucosidase) (Saraswathi *et al*., 2021). These two key enzymes causes postprandial hyperglycemia by facilitating the digestion of complex dietary carbohydrates into simple glucose, after which it gets absorbed and enters into the blood circulation (Bhatia *et al*., 2019). The elevated concentration of simple sugars in the blood, particularly glucose causes postprandial hyperglycemia which is a key factor for the coronary heart disease, oxidative stress, low-level inflammatory outcomes and deleterious effects on beta-cells which hinders insulin sensitivity (Ngozi and Olatunbosun, 2016). Prolonged postprandial hyperglycemia initiates type 2 diabetes complexities (Reed *et al*., 2021). Recently, inhibitors of alpha-amylase and alpha-glucosidase have attracted much interest in terms of anti-diabetic activity by retarding the digestion and absorption of carbohydrates (Prasathkumar *et al*., 2021). This action would lead to a decrease in the postprandial blood glucose levels and thus protects the pancreas, which will restore regular insulin production from the beta-cells (You *et al*., 2012).

The impact of bioactive extracts to stem the progress and treatment of diabetes mellitus has continued to receive

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commendation, especially in developing countries like Africa where most people do not have access to modern treatment. This is due to their potential to alter the increased blood glucose level via inhibition of two prominent enzymes i.e. α-amylase and α-glucosidase. In Africa, the use of medicinal plants as a therapeutic modality for diabetes is a common practice. Several studies have shown that extracts from medicinal plants, such as *Blighia sapida, Piper nigrum, Carica papaya, P. guineense, Punica granatum* possessed antidiabetic potential by targeting alpha-amylase and alpha-glucosidase enzymes (Kazeem *et al*., 2013; Oboh *et al*., 2013; Oboh *et al*., 2014; Ngozi and Olatunbosun, 2016; Mayasankaravalli *et al*., 2020; Sulaimon *et al.*, 2020). In this way, the life-threatening effects of hyperglycemia will be minimized.

Aristolochia bracteolata, known as worm killer belongs to the family of Aristolochiaceae and grows in tropical regions of Africa. It is a wild species used in Nigeria against several diseases since ancient times; especially the roots prepared in water are used against worms, skin disease, cancer, lung inflammation, dysentery, snake bites, and malarial fever (Gbadamosi and Egunyomi, 2011). The root is used to alleviate scorpion stings and sometimes in Northern Nigeria, the flowers are prepared and worn as a charm against scorpion stings and snake bite (Gbadamosi and Egunyomi, 2011). The root part of *A. bracteolate* was considered to be a source for most of the pharmacologically active metabolites viz. flavonoids, saponins, triterpenoids, alkaloids, steroids, glycosides, aristolochic acid-D and phenolic compounds (Devi *et al*., 2011; Thirumal *et al*., 2012; Nandhini *et al*., 2017; El Omari *et al*., 2019). Promising anti-diabetic and hypolipidemic activity of methanol extract of *A. bracteolata* have been reported (Raju and Reddy, 2017). To date, acarbose, miglitol and voglibose (inhibitors) known to inhibit α-amylase and α-glucosidase have improved the performance in the progress and treatment of type 2 diabetes. However, frequent use of these synthetic inhibitors comes with side effects (Chipiti *et al*., 2015). Therefore, this study was investigated to document the potential therapeutic property of *A. bracteolate* root bark extract by evaluating its *in vitro* antioxidant activity as well as α-amylase and α-glucosidase inhibitory effects.

MATERIALS AND METHODS

Plant preparation and extraction

A. bracteolata was collected from dry land in the Girei area of Adamawa State, and was identified by a Plant Taxonomist, Department of Plant Sciences, Moddibo Adama University Yola, Nigeria. The root barks of this plant were incised, flushed under the running faucet water, dried at room temperature and later ground into the powdered sample using a grinder. Two hundred grams of powdered *A. bracteolate* root bark were soaked in 1000 ml of 90% methanol (Sigma-Aldrich, Lagos, Nigeria) for 24 h, filtered through Whatman filter paper and the filtrate was evaporated in a water bath to obtain the methanol extract (Ngozi and Olatunbosun, 2016). The weight of the dried methanol extract was measured and expressed in percentage yield.

Preliminary phytochemical analysis

The methanol extract was assessed for the presence of tannins, alkaloids, cardiac glycosides, terpenoids, flavonoids and saponins by Harborne (1998), Sofowora (1993). Trease and Evans (2002), while the quantitative phytochemicals composition of saponins, terpenoids, phenols, flavonoids, alkaloids and tannins were estimated using the standard procedure (Harborne, 1998; Venkatadri *et al*., 2017, Sagbo *et al*., 2017).

Antioxidant activities

Estimation of DPPH scavenging activity

The assay described by Sayah *et al.* (2017) was used to estimate the DPPH free radical scavenging activity of the methanol extract of *A. bracteolate* root bark. Precisely, 10 mg/100ml (100μg/ml) of a stock solution of methanol extract and ascorbic acid (standard) were prepared. Then, 20-100mg/ml of methanol extract at different concentrations were mixed with 2 ml of freshly prepared DPPH solution (0.004% w/v). The mixture was vigorously vortexed and incubated for 15 min. The absorbance was recorded at 523 nm against the blank. The antioxidant activity was estimated and the IC_{50} value was deduced.

$$\text{Percentage of DPPH scavenged} = \frac{\text{Blank - Test}}{\text{Blank}} \times 100 \qquad (1)$$

Reducing power assay

The reducing ability of methanol extract was ascertained following ferric reducing antioxidant power (FRAP) protocols as described by Sayah *et al.* (2017). The reacting solution contained 1ml of methanol extract at different concentrations (20, 40, 60, and 100 mg/ml), 1ml of 0.2M sodium phosphate buffer (pH 6.6) and 1ml of 1% $C_6FeK_4N_6$ (potassium ferricyanide). They were thoroughly mixed and then placed in a regulated water bath for 20 min at 50°C. Subsequently, 1ml of 10% $C_2HCl_3O_2$ (TCA) was immediately added, and the mixture was centrifuged for 10min at 3000 rpm. One ml of supernatant was measured and added into 1 ml of distilled water and 200μl of ferric chloride (0.1% $FeCl_3$). The absorbance was recorded at 700 nm against blank and the percentage reducing power was determined from the equation as mentioned below:

$$\%\ \text{FRAP} = \frac{\text{Blank - Test}}{\text{Blank}} \times 100 \qquad (2)$$

TBARS (Thiobarbituric reactive substance) assay

The assay was set up by adding an equal volume of 20% trichloroacetic acid, 0.67% thiobarbituric acid (TBA) and

2ml of the mixtures containing 20, 40, 60 and 100mg/ml of the methanol extract in 4ml of 99.5% methanol (final concentration 0.02%). The resulting mixture was incubated in a water bath (100°C) for 10 min and allowed to cool. Thereafter, it was centrifuged at 3000 rpm for 20 min and the absorbance of the reaction was recorded at 532 nm (Sayah*et al.,* 2017). The percentage of TBARS antioxidant activity was deduced using the following equation:

$$\text{Percentage of TBARS scavenged} = \frac{\text{Blank - Test}}{\text{Blank}} \times 100 \qquad (3)$$

In vitro anti-diabetic activity

Alpha-amylase inhibition test

The α-amylase inhibitory effect of methanol extract of *A. bracteolate* root bark (20-100mg/ml) was assessed using the protocol of McCue and Shetty (2004). The IC_{50} value of the methanol extract was determined using a standard linear regression curve.

Alpha-glucosidase inhibition test

The alpha-glucosidase inhibitory effect of methanol extract of *A. bracteolata*root bark (20-100mg/ml) was investigated according to the protocol of Kim *et al.* (2005). The IC_{50} value of the methanol extract was determined using a standard linear regression curve.

Gas chromatography-mass spectrometry (GC-MS) analysis of methanol extract

The presence of bioactive metabolites in the methanol extract of root bark was identified utilizing GC-MS (SHIMADZU QP2010) as described by Venkatadri *et al.* (2017). The GC specification encompasses transporter gas-helium (99.999%) purity at a column velocity rate of 1 ml per min in the split ratio (10:1) v/v. Injection of 8µl of methanol extract was introduced into the column at 250°C inlet temperature. The initial column oven temperature commenced at 70°C (maintained for 5min). After that it was increased at a pace of 10°C per minute to 280°C (without hold). Holding was taken into consideration for 6 mins at a controlled rate of 5°C per minute. The temperature of ion sources was kept at 200°C. Different MS operating parameters were observed and compounds identification was completed employing spectrum comparison.

STATISTICAL ANALYSIS

Statistical Package for Social Sciences (SPSS, version 28.0)with t-testwas deployed statically to implement the difference between mean and results stated as mean ± standard error of the mean (SEM). The level of significance was regarded at ($P<0.05$). IC_{50} values were calculated through a simple linear regression curve.

RESULTS

Phytochemical analysis of A. bracteolata extract

In this study, the crude methanol extract was dark brownish in colour with a total yield of 25.08%. table 1 displays the diverse array of phytochemical components present in the methanol extract of *A. bracteolate* root bark. The study revealed positive results for tannins, flavonoids, saponins, alkaloids, glycosides, terpenoids, and phenols in the methanol extract. The quantitative phytochemical composition of tannins, saponins, flavonoids, alkaloids, phenols, and terpenoids are shown in table 2. The methanol extract was found to be rich in the highest amount of saponins content (8.20±0.03%), followed by phenols (6.82±0.01%), alkaloids (4.71±0.03%) and flavonoids (3.50±0.12%).

Table 1: Phytochemical components detected in methanol extract of *A. bracteolate* root bark

Phytochemicals	Methanol extract
Carbohydrates	_
Tannins	+
Saponins	+
Flavonoids	+
Alkaloids	+
Quinones	_
Glycosides	+
Terpenoids	+
Phenols	+
Steroids	_
Anthraquinone	_

'+' = present, '-' = absent

Table 2: Percentage composition of some phytochemical components in the methanol extract of *A. bracteolate* root bark

Phytochemicals	Methanol extract
Saponins	8.20 ± 0.03
Terpenoids	0.51 ± 0.02
Flavonoids	3.50 ± 0.12
Phenols	6.82 ± 0.01
Alkaloids	4.71 ± 0.03
Tannins	0.42 ± 0.01

Values are mean ± SEM

Antioxidant activity of A. bracteolata extract

Table 3 depicts the DPPH radical scavenging property of the methanol extract of *A. bracteolate* root bark and its IC_{50} value. The methanol extract demonstrated significant scavenging of DPPH (35.00±1.03-84.00±0.05%) at various concentrations (40-100 mg/ml), as compared to ascorbic acid (27.34±0.12-69.71±3.00%). It was disclosed that the extract activity (IC_{50}= 57.87 mg/ml) was higher than ascorbic acid (IC_{50} = 68.80 mg/ml). *A. bracteolata* extract having a lower IC_{50} value suggested a better scavenging property of DPPH radical when compared to ascorbic acid.

Table 4 shows the FRAP of the methanol extract of *A. bracteolate* root bark and its IC_{50} value. As concentration increased, the FRAP of the methanol extract also increased proportionately. The extract significantly ($P<0.05$) produced a higher inhibition property (92.19±1.25%) at maximum concentration (100 mg/ml) as compared to ascorbic acid (75.00±0.01%), appraising higher antioxidant property.

Table 3: DPPH radical scavenging activity of methanol extract of *A. bracteolate* root bark.

Concentration (mg/ml)	Methanol extract	Ascorbic acid
20	18.20 ± 0.10	15.13 ± 1.04
40	35.00 ± 1.03*	27.34 ± 0.12
60	56.19 ± 4.30*	48.20 ± 2.08
80	68.40 ± 0.22*	59.11 ± 0.03
100	84.00 ± 0.05*	69.71 ± 3.00
IC_{50} (mg/ml)	57.87	68.80

Values are mean ± SEM (n = 3). *Significantly different ($P<0.05$) from standard

Table 5 indicates the TBARS antioxidant property of the methanol extract of *A. bracteolate* root bark. The extract was found to exhibit a significant inhibition property of 45.76±1.03-68.12±0.02% at tested concentrations (40-60 mg/ml) as compared to ascorbic acid (37.28±3.15-63.12±2.01%). However, at 20, 80, and 100 mg/ml concentrations, there was no significant difference in the percentage inhibition. The IC_{50} result of methanol extract (47.54 mg/ml) was estimated to be lowered compared to ascorbic acid (50.20 mg/ml).

Table 4: FRAP of methanol extract of *A. bracteolate* root bark.

Concentration (mg/ml)	Methanol extract	Ascorbic acid
20	17.02 ± 0.06*	12.95 ± 5.03
40	39.00 ± 1.19*	32.17 ± 0.02
60	48.57 ± 1.22	53.11 ± 1.19
80	76.20 ± 0.16*	63.38 ± 3.01
100	92.19 ± 1.25*	75.00 ± 0.01
IC_{50} (mg/ml)	54.64	63.57

Table 5: TBARS antioxidant property of methanol extract of *A. bracteolate* root bark

Concentration (mg/ml)	Methanol extract	Ascorbic acid
20	21.33 ± 2.28	19.23 ± 1.07
40	45.76 ± 1.03*	37.28 ± 3.15
60	68.12 ± 0.02*	63.12 ± 2.01
80	87.23 ± 3.56	84.00 ± 0.06
100	98.14 ± 1.45	95.32 ± 1.02
IC_{50} (mg/ml)	47.54	50.20

Antidiabatic activity of A. bracteolata extract

Tables 6 and 7 show the α-amylase and α-glucosidase inhibitory properties of methanol extract and IC_{50} value of *A. bracteolate* root bark. Table 6 portrays that extract at all tested concentrations significantly restricted the activity of α-amylase, producing 16.20±0.70-88.19 ±1.14% inhibition at 20-100 mg/ml. This inhibitory effect was correlated to that acarbose which showed 11.36±0.10-59.30±0.33% of α-amylase inhibition with an IC_{50} value of 76.55 mg/ml with respect to the extract (53.70mg/ml). In α-glucosidase inhibition, the extract significantly exhibited 39.10±1.17-92.00±1.36% of inhibitory activity as compared to acarbose (31.70±0.03-69.81±1.08%) (table 7). The extract showed lower IC_{50} value of 49.18 mg/ml than acarbose (63.63 mg/ml).

Table 6: Alpha-amylase inhibitory property of methanol extract of *A. bracteolate* root bark

Concentrations (mg/ml)	Methanol extract	Acarbose
20	16.20±0.70*	11.36 ± 0.10
40	41.22 ±3.13*	35.20 ± 1.05
60	55.64 ±0.56*	45.19 ± 2.08
80	79.42 ±1.12*	51.18 ± 0.14
100	88.19 ±1.14*	59.30 ± 0.33
IC_{50} (mg/ml)	53.70	76.55

Table 7: Alpha-glucosidase inhibitory property of methanol extract of *A. bracteolate* root bark

Concentrations (mg/ml)	Methanol extract	Acarbose
20	27.30 ± 1.00	24.22 ± 2.04
40	39.10 ± 1.17*	31.70 ± 0.03
60	65.40 ± 0.02*	53.04 ± 3.12
80	89.17 ± 0.18*	67.14 ± 0.01
100	92.00 ± 1.36*	69.81 ± 1.08
IC_{50} (mg/ml)	49.18	63.63

GC-MS analysis of A. bracteolata root bark

The GC-MS chromatogram for the methanol extract of *A. bracteolate* root bark is shown in fig 1. Table 8 illustrated the presence of seventeen chemical constituents in the methanol extract of *A. bracteolate* root bark. The major bioactive compounds identified in the extract of *A. bracteolate* in terms of their relative abundance were 9,12-Octadecadienoic acid (Z,Z) (27.38%), resorcinol (15.37%), 3-O-Methyl-D-glucose (9.50%),6-Ethoxy-6-methyl-2-cyclohexenone (8.79%), 9,12-Octadecadienoyl chloride, (Z,Z) (7.85%), and Isooctane, (ethenyloxy) (6.19%).

DISCUSSION

Diabetes is a heterogeneous disease with multiple complications (Elksnis *et al*., 2019). New therapeutic modality is anticipated to prevent and eradicate the damaging effects of hyperglycemia. The use of methanol

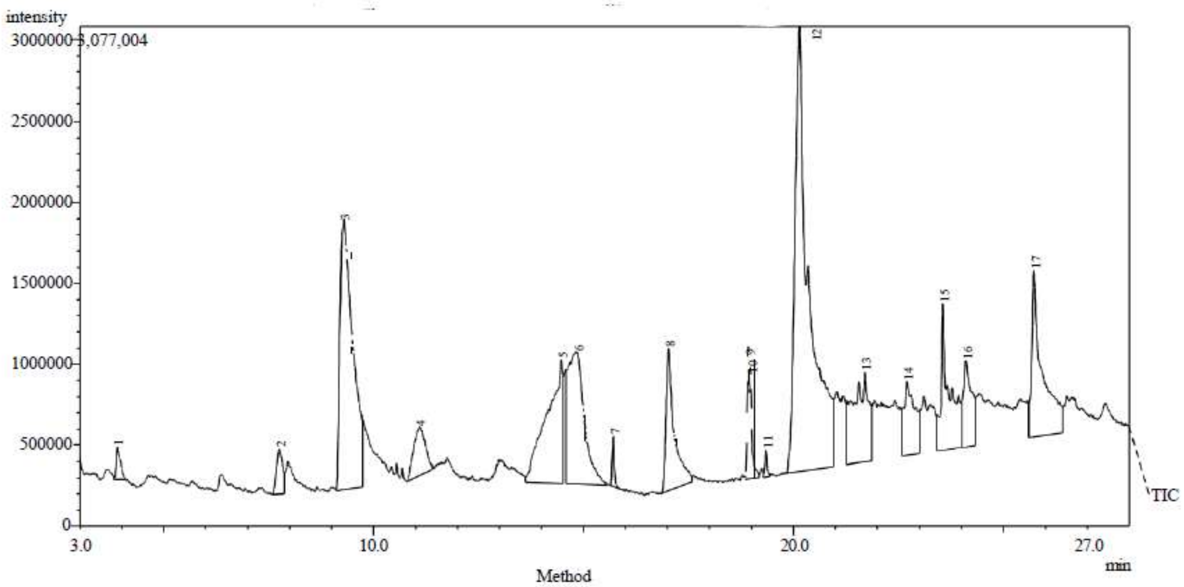

Fig. 1: GC-MS chromatogram of methanol extract of *A. bracteolata* root bark.

Table 8: Identified compounds in methanol extract of *A. bracteolate* root bark using GC-MS.

S/N	Retention time (s)	Area (%)	Molecular Formula	Molecular Weight (g/mol)	Compound name
1	3.894	0.60	C_8H_{16}	112	4-Octene, (Z)
2	7.758	1.18	C_8H_8O	120	Phthalan
3	9.291	15.37	$C_6H_6O_2$	110	Resorcinol
4	11.097	2.49	$C_{12}H_{22}O_{11}$	342	Alpha.-D-Glucopyranoside
5	14.469	8.79	$C_9H_{14}O_2$	154	6-Ethoxy-6-methyl-2-cyclohexenone
6	14.826	9.50	$C_7H_{14}O_6$	194	3-O-Methyl-D-glucose
7	15.705	0.46	$C_{17}H_{34}O_2$	270	Pentadecanoic acid, 14-methyl-, methyl ester
8	7.030	5.19	$C_{16}H_{32}O_2$	256	Palmitic acid
9	18.918	1.21	$C_{19}H_{34}O_2$	294	9,12-Octadecadienoic acid, methyl ester, (E,E)
10	18.974	1.15	$C_{19}H_{36}O_2$	296	11-Octadecenoic acid, methyl ester
11	19.340	0.26	$C_{11}H_{22}O_2$	186	Decanoic acid, methyl ester
12	20.144	27.38	$C_{18}H_{32}O_2$	280	9,12-Octadecadienoic acid (Z,Z)
13	21.706	6.19	$C_{10}H_{20}O$	156	Isooctane, (ethenyloxy)
14	22.709	3.62	$C_{22}H_{43}NO$	337	13-Docosenamide, (Z)
15	23.571	5.55	$C_{22}H_{42}O_2$	338	13-Docosenoic acid, (E)
16	24.113	3.18	$C_{19}H_{38}O_4$	330	Hexadecanoic acid, 2,3-dihydroxypropyl ester
17	25.731	7.85	$C_{18}H_{31}ClO$	298	9,12-Octadecadienoyl chloride, (Z,Z)

in the extraction of different parts of *Aristolochia bracteolata* has been reported (Das *et al*., 2016, Goji and Nadro, 2021; Gabriel *et al*., 2022).

This might impact the solubility of the chemical constituents and percentage yield.

The presence of distinctive phytochemicals in the methanol extract of *A. bracteolate* root bark can be considered a remarkable source of antioxidant and anti-diabetic compounds. This multitude of phytochemicals such as tannins, flavonoids, saponins, alkaloids, terpenoids, phenols and glycosides was envisaged to be accountable for therapeutic activities which have demonstrated to play an enormous role in the remedy of diseases globally (Sagbo *et al*., 2017; Saraswathi *et al*., 2020; Goji and Nadro, 2021). The methanol extract of *A. bracteolate* root bark integrated tannins, flavonoids, saponins, alkaloids, glycosides, phenols and terpenoids as their prominent phytochemical constituents.

The preservation of the integrity of beta-cell function relating to diabetes reflects the beneficial effects of antioxidant action (Kifle and Enyew, 2020). The effectiveness of DPPH assay in the discovery of antioxidant properties of plant extracts has been reported (El Omari *et al*., 2019; Gabriel *et al*., 2022). The DPPH is a free radical which have hydrogen acceptor functionality to antioxidants. The extent of the reduction is an indication of the free radical scavenging ability of the extract (Sagbo *et al*., 2017). The antioxidant properties of this extract may be related to the presence of a higher composition of phytochemicals particularly saponins, phenols, alkaloids, and flavonoids. Saponins and phenols can donate the hydrogen atoms of their hydroxyl groups. These antioxidant phytochemicals have been appropriated to play an energetic role in ameliorating free radicals by way of limiting the rate of oxidation and protecting beta-cells from damage (El Omari *et al*., 2019, Agada *et al*., 2021).

The FRAP measures the extract capability to facilitate the reduction of Fe^{3+} to Fe^{2+}. The extract significantly produced greater antioxidant properties, indicating the ability to reduce oxidative stress (Saraswathi *et al*., 2020). The reducing power of methanol extract of *A. bracteolate* root bark correlates with the report of El Omari *et al*. (2019). However, some independent studies have reported that plant secondary metabolites such as saponins, phenols, flavonoids, and tannins in plant extracts have antioxidant activities against oxidative stress (Elekofehinti, 2015; Chipiti *et al*., 2015; Sagbo *et al*., 2017; Eftekhari *et al*., 2021).

TBARS method measures the extent of lipid peroxidation (Abeyrathne *et al*., 2021). In this assay, the extract scavenged TBARS radical at all the investigated doses. The TBARS scavenging activity observed in this study might be due to the bioactive compounds that exhibited antioxidant properties towards oxidative stress-related diseases. These results supported the earlier findings of Goji and Nadro (2021).

Digestive enzymes (alpha-amylase and alpha-glucosidase) are known to prompt the conversion of complex dietary carbohydrates to oligosaccharides and disaccharides which are finally converted into monosaccharides, which play a critical role in the pathogenesis of type 2 diabetic mellitus. Inhibitors of these enzymes have been identified as a therapeutic target for the modulation of postprandial hyperglycemia (Prasath kumar *et al*., 2021). The potential of methanol extract in this study to inhibit or suppress the action of alpha-amylase and alpha-glucosidase reflects its anti-diabetic properties. Medicinal plants provide the needed platform for safer and low-cost anti-diabetic drugs. Although numerous studies have documented a wide variety of therapeutic plant extracts with α-amylase and α-glucosidase inhibitory potentiality (El Omari *et al*., 2019; Kifle and Enyew, 2020; Prasath kumar *et al*., 2021), these effects were ascribed to the pharmacological action of secondary metabolites such as saponins, tannins, alkaloids, flavonoids and terpenoids (Asgar, 2013; Oboh*et al*., 2013; Elekofehinti, 2015; Sagbo *et al*., 2017). The inhibitory potential observed in this study may be due to the availability of saponins, alkaloids and phenols contents.

The identification of 9,12-Octadecadienoic acid (Z,Z), resorcinol, 3-O-Methyl-d-glucose, 6-Ethoxy-6-methyl-2-cyclohexenone, 9,12-Octadecadienoyl chloride, (Z,Z), and isooctane, (ethenyloxy) in methanol root extract could berelated to the observed activities. These prominent compounds have been reported to portray a vast array of pharmacological properties including antioxidant, anti-diabetic, anti-obesity and antimicrobial activities (Thilagam *et al*., 2013; Chipiti *et al*., 2015; Ezekwe, 2017; Abubakar *et al*., 2022). 9,12-Octadecadienoic acid Z,Z is a polyunsaturated fatty acid while resorcinol is a phenolic compound. The presence of these bioactive compounds might be responsible for the strong antioxidant, α-amylase, and α-glucosidase inhibition potentialities of methanol extract of *A. bracteolata* root bark.

CONCLUSION

Methanol extract of *A. bracteolate* root bark possessed remarkable *in vitro* antioxidant and anti-diabetic activities which were mediated through antioxidants and enzymes (α-amylase and α-glucosidase) inhibitory activity. The inhibitory activity was observed to be related to the diverse bioactive metabolites foundin this extract. Consequently, this study supports the use of *A. bracteolate* root bark extract in the control/treatment of diabetes mellitus in folk medicine.

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