

## Phytochemical, antioxidant and anti-diabetic potentials of the ethanol leave extracts of four medicinal plants

### Abstracts

For many years, medicinal plants have been a resource for healing in several local communities around the world and the phytochemicals in them such as flavonoids, alkaloids, phenolic, tannins, and terpenoids are attributed to their many medicinal values. *Vernonia amygdalina*, *Senna alata*, *Jatropha curcas*, and *Grewia pubescens* are important plants with immense value. In this study, phytochemical screening, antioxidant analysis and the potential anti-hyperglycemic properties of the plants was investigated *in-vitro*.

The ethanol leave extracts of the plants were subjected to qualitative phytochemical screening and tannin, flavonoids and phenol quantification. Ferric reducing antioxidant power and DPPH radical inhibition of the extracts was done by spectrophotometric method while the anti-diabetic potential was analyzed through the *in-vitro*  $\alpha$ -amylase and  $\alpha$ -glucosidase inhibition.

Phytochemicals detected in the ethanol leave extracts of the four plants are tannins, flavonoids, phenolics, terpenoids, steroids, alkaloids, cardiac glycosides, and saponins. Flavonoids, phenols, and tannin content were highest in *Senna alata* (0.27 $\pm$ 0.0002 mg: mg of rutin per g of extract, 10.63 $\pm$ 0.0017 mg: mg of gallic acid per g of extract, and 6.72 $\pm$ 0.06 mg/g respectively) followed by *V. amygdalina* (0.20 $\pm$ 0.0002 mg: mg of rutin per g of extract, 8.27 $\pm$ 0.0017 mg: mg of gallic acid per g of extract, and 7.98 $\pm$ 0.03 mg/g respectively). While the least content of all was found in the extracts of *Jatropha curcas*. Concentration dependent and statistically significant difference was observed in both the FRAP and DPPH radical inhibition of all the extracts. *Senna alata* showed the strongest reducing power followed by the *V. amygdalina*. Both *Senna alata* and *V. amygdalina* showed DPPH radical inhibition that is not significantly ( $p>0.05$ ) different from that of trolox.  $\alpha$ -amylase and  $\alpha$ -glucosidase inhibition was also demonstrated in a concentration dependent manner. In both the  $\alpha$ -amylase and  $\alpha$ -glucosidase inhibition, *V. amygdalina* and *S. alata* exhibited the most significant inhibitory properties among the plant extracts.

The overall result in this study suggested that *V. amygdalina*, *S. alata* with the highest content of the phytochemicals, and antioxidant activities are potential source of antioxidant constituents and might be useful for the management of diseases such as diabetes.

**Keywords:** medicinal plants, phytochemicals,  $\alpha$ -amylase,  $\alpha$ -glucosidase, diabetes

### Introduction

For many years, medicinal plants have been a resource for healing in several local communities around the world (Fitzgerald *et al.*, 2020). “Traditional or herbal medicines are referred because they are from plant origin and are generally regarded as safe at the concoction dosage, based on their historical usage in various cultures” (Ugboko *et al.*, 2020).

“Consequently, plants are the most abundant source of active drugs and are invaluable in the ethno-medicinal treatment of diverse ailments. Medicinal plants are generally sources of various phytochemicals, which are usually responsible for their biological activities” (Ugboko *et al.*, 2020). Most knowledge of medicinal plant uses originated from ethnobotanical and ethnopharmacological repositories (de Freitas and de Almeida, 2017) and this knowledge has ensured the preservation of the ethnomedicinal diversities.

“The antioxidant phytochemicals are often attributed to the therapeutic efficacy of several medicinal plants and the screening of plants with potential bioactive properties is on the increase as well as the concomitant isolation and characterization of these bioactive principles for prevention and combating a wide array of diseases” (Farombi and Owoeye, 2011; Njan, 2012).

“*Senna alata* (*Cassia alata*) Linn. Roxb (Leguminosae), known as Ringworm plant, winged senna, craw-craw plant or king of the forest, is important in traditional medical practice in many developing countries” (Adedayo *et al.*, 2001). “In western part of Nigeria, the plant is called ‘Asunwon oyinbo’ and ‘Nelkhi’ in the Western and Eastern regions respectively. Reports are available on the anti-bacterial, anti-fungal, and antiviral properties of the leaf” (Adedayo *et al.*, 2001; Woradulayapinij *et al.*, 2005) and also “on the laxative (Hennebelle *et al.*, 2009) and abortifacient properties in Wistar rats” (Yakubu *et al.*, 2010). “Of particular interest is the use in the management of sugar-related disorders such as diabetes mellitus” (Abo *et al.*, 2008; Woradulayapinij *et al.*, 2005; Palanichamy *et al.*, 1988).

“*Vernonia amygdalina* Del. on the other hand is called ‘African bitter leaf’. The plant is a leafy vegetable commonly consumed as food and in the traditional treatment of diseases, such as malaria, infertility, diabetes, gastrointestinal problems and sexually transmitted diseases, in tropical Africa” (Farombi and Owoeye, 2011). The plant has also attracted attention due to its usage in providing remedy for diabetes mellitus and hypertension (Atangwho *et al.*, 2014).

“*Grewia pubescens* (family Maliceae) is a shrub or sometimes, scandent tree distributed widely in many part of Nigeria. Many of the *Grewia* spp. finds application in folk medicine to cure stomach upset, skin, and intestinal infections while some of them have mild antibiotic properties” (Chopra *et al.*, 1956).

The Euphorbiaceae family is home to *Jatropha curcas*, a plant that is valued for its medicinal properties. The plant's seeds, leaves, and bark are all utilized in traditional and veterinary medicine in the form of fresh preparations or decoctions (Patil *et al.*, 2013).. *Jatropha curcas* oil is known for its potent laxative properties and is also used to alleviate skin ailments and rheumatism pain. In addition, the plant's leaves are brewed to treat coughs and serve as an antiseptic post-birth, while its branches are used as chewing sticks in Nigeria (Gupta *et al.*, 2010; Nayak and Patel, 2010). The majority of *Jatropha curcas*' components are used to treat a variety of human and animal illnesses, with traditional applications for diarrhea and dysentery. Furthermore, *Jatropha curcas* has been shown to have antibacterial properties (Patil *et al.*, 2013).

These medicinal plants are potential source of many remedies and could provide alternative means for the management of diabetes and other oxidative stress related diseases. In this study, phytochemical screening, antioxidant analysis and the potential anti-hyperglycemic properties of the plants was investigated in-vitro.

## **Materials and Method**

### **Reagents used for the Experiment**

Distilled water, Ethanol, Lead acetate, Sodium Hydroxide, Concentrated hydrochloric acid, Concentrated ammonia, Diluted ammonia solution, Ferric chloride solution, Glacial acetic

acid, Dilute hydrochloric acid, Chloroform , Concentrated Sulphuric acid, Sodium hydroxide, Folin-Ciocalteau reagent, Alpha-amylase, Alpha-glucosidase.

### **Sample collection and preparation of leave Extract**

Fresh leaves samples of *Vernonia amydalina*, *Senna alata*, *Jatropha curcas*, and *Grewia pubescens* were collected around Iya Ijado, Ilaro-yewa South, Ogun State, Nigeria on February 19, 2022. A Botanist did the authentication, and each plant was designated with voucher number: *V. amydalina*: LUH 9754, *S. alata*: LUH 975, *J. curcas*: LUH 9752, and *G. pubescens*: LUH9755. The leaves were washed in distilled water and air-dried for three weeks at room temperature. The air-dried samples were separately ground into powdered form using an electric blender. The extraction of leaves samples were carried out according to the method of (Boulekbache-makhlouf, *et al.*, 2012). 200 g of each powdered sample was weighed into 2000 ml of ethanol and soaked for 72 hours at room temperature. Each sample was then filtered using muslin cloth into a Buchner funnel and No 1 Whatman's filter paper. The filtrate was evaporated under vacuum in a rotatory evaporator (40 °C) to obtain the crude extracts.

### **Qualitative Phytochemical Analysis**

The qualitative analysis of the phytochemical constituent of the selected plant extracts was assessed by the methods of Shaikh and Patil, (2020).

#### **Test for Alkaloids**

Mayer's test: 1 ml of concentrated HCl was added to 1 ml of extract (50 mg/ml) followed by few drops of Mayer's reagent. Formation of green or white precipitate indicates that alkaloid is present.

#### **Test for Phenols**

Ferric Chloride test: 1 ml of 5 % ferric chloride solution was added to 1 ml of extract (50 mg/ml). Formation of dark-green colour indicates that a phenol is present.

#### **Test for Flavonoids**

Lead acetate test: 1 ml of 10 % lead acetate solution was added to 1 ml of extract (50 mg/ml). Formation of yellow precipitate indicates that flavonoid is present

#### **Test for Saponins**

Foam test: 1 ml of distilled water was added to 1 ml of extract (50 mg/ml) and was shaken vigorously. Formation of the foam indicates that saponin is present.

#### **Test for Tannins**

Braymer's test: 1 ml of distilled water followed by 1 ml of 5 % ferric chloride solution was added to 0.5 ml of extract (50 mg/ml). Formation of blue-green colour indicates tannin is present.

#### **Test for Terpenoids**

5 mL of the sample (50 mg/ml) was mixed with 2 mL of  $\text{CHCl}_3$  in a test tube. Thereafter, 3 mL of concentrated  $\text{H}_2\text{SO}_4$  was carefully added to the mixture to form a layer. An interface with a reddish brown coloration indicates the presence of terpenoids.

#### **Test for Quinones**

0.5 ml of concentrated hydrochloric acid was added to 1 ml of extract (50 mg/ml). Formation of green colour indicates that quinone is present.

#### **Test for Cardiac glycosides**

Killer-killani test: 2 ml of glacial acetic acid and 1 ml of 5 % ferric chloride solution along with 1 ml of concentrated sulphuric acid was added to 1 ml of sample (50 mg/ml). Formation of blue colour at the interface indicates that cardiac glycoside is present.

#### **Test for Anthraquinones**

Few drops of 10 % ammonia solution were added to 1 ml of extract (50 mg/ml). Appearance of pink colour precipitate indicates the presence of anthraquinones.

#### **Test for Phlobatanin**

Few drops of 2% hydrochloric acid were added to 1 ml of extract (50 mg/ml). A red colour precipitate formation indicates the presence of phlobatannins.

#### **Test for Steroids**

2 ml of chloroform with 1 ml of sulphuric acid was added to 1 ml of extract (50 mg/ml). Formation of reddish brown ring at the interface indicates that steroids are present.

#### **Test for Courmarins**

1 ml of 10 % sodium hydroxide was added to 1 ml of the extract (50 mg/ml). A yellow colour formation indicates that coumarins is present

### **Quantitative Phytochemical Analysis**

#### **Determination of Total Phenolic Content (TPC)**

The total phenol content of the extract was determined by the method of (Singleton et. al., 1999). 0.2 ml of 100 mg/ml of the extract was mixed with 2.5 ml of 10 % Folin-ciocalteu's reagent and 2 ml of 7.5 % Sodium carbonate. The reaction mixture was subsequently incubated at 45 °C for 40 mins, and the absorbance was measure at 700 nm in the

spectrophotometer, Calibration curve was generated from 0.5 -15 mg/ml of Gallic acid that was used as standard phenol. The TPC was estimated as gallic acid equivalent per g of the extracts.

### **Determination of Total Flavonoids Content (TFC)**

The total flavonoids content of the extract was determined using a colorimetric assay advanced by (Bao, 2005). 0.3 ml of 5 % NaNO<sub>3</sub> was added to 0.2 ml of 100 mg/ml of the extract at time zero. 0.6 ml of 10 % AlCl<sub>3</sub> and 2 ml of 1 M NaOH was added to the mixture after 6 minutes followed by the addition of 2.1 ml of distilled water, after which the absorbance was read at 510 nm against the reagent blank and total flavonoids content was expressed as mg of Rutin equivalent. The calibration curve was generated from 0.05 - 5 mg/ml of rutin that was used as standard.

### **Antioxidant activity**

#### **Determination of ferric reducing property**

The reducing property of the extract was determined by the method described by Pulido et al. (2000). The extract (0.25 ml) of different concentration (50, 75, and 100 mg/ml) was mixed with 0.25 ml of 200 mM of Sodium phosphate buffer pH 6.6 and 0.25 ml of 1% potassium ferricyanide (KFC). The mixture was incubated at 50 °C for 20 min, thereafter 0.25 ml of 10 % trichloroacetic acid (TCA) was also added and centrifuged at 2000 rpm for 10 min, 1 ml of the supernatant was mixed with 1 ml of distilled water and 0.1 % of FeCl<sub>3</sub> and the absorbance was measured at 700 nm. Ascorbic acid was used as standard, with the result expressed in µg ascorbic acid equivalent/g dried sample (µg AAE/g dried sample).

#### **Determination of DPPH radical Inhibition**

The free radical scavenging ability of the extract against DPPH (1, 1- diphenyl-2-picrylhydrazyl) was determined using the method reported by Gyamfi et al. (1999). One milliliter (1 ml) of varied concentration (50, 75, and 100 mg/ml) of the extracts and that of blank (methanol) was mixed with 1 ml of 0.4 mM methanol solution of DPPH, the mixture was left in the dark for 30 min before measuring the absorbance at 516 nm. For the standard, same concentration of trolox was mixed with the DPPH in place of the extract. Percentage inhibition was calculated from equation 2.

$$\text{DPPH scavenging activity (\%)} = \frac{\text{Absorbance of blank} - \text{Absorbance of sample}}{\text{Absorbance of Blank}} \times 100 \dots \text{Eqn 2}$$

### **Determination of Alpha-amylase Inhibitory Activity**

The method used involved estimating the amount of reducing sugar produced by the activity of each enzyme on buffered starch. The substrate for assay was 0.5 ml of 0.5 % soluble starch, buffered with 0.2 ml of 0.1 M sodium acetate (pH 5.6). 0.3 ml (27.5 mg of porcine pancreatic amylase  $\alpha$ -amylase (PPA) in 100 ml of 20 mM of phosphate buffer containing sodium chloride), was added to the mixture, mixed, and incubated at 40 °C for 30 min in a water-bath. “One ml of DNSA solution was added to the mixture and boiled for 5 min. Four ml of distilled water was introduced after cooling before absorbance was read at 540 nm in spectrophotometer. Blank that consisted of 0.3 ml distilled water, 0.5 ml of 0.5 % soluble starch, 0.2 ml of buffer only was subjected to similar treatments” (Demoraes et al., 1999).

$$\alpha\text{-amylase Inhibitory Activity (\%)} = \frac{\text{Absorbance of blank} - \text{Absorbance of sample}}{\text{Absorbance of Blank}} \times 100 \dots \text{Eqn 3}$$

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### **Determination of Alpha-glucosidase Inhibitory Activity**

The effect of the plant extracts on  $\alpha$ -glucosidase activity was determined according to the method described by Kim et al. (2005) using  $\alpha$ -glucosidase from *Saccharomyces cerevisiae*. The substrate solution p-nitrophenylglucopyranoside (pNPG) was prepared in 20 mM phosphate buffer, and pH 6.9. 100  $\mu$ L of  $\alpha$ -glucosidase (0.3 U/mL) was pre-incubated with 50  $\mu$ L of the sample (50, 75, and 100 mg/ml) for 10 min. Then 50  $\mu$ L of 3.0 mM (pNPG) as a substrate dissolved in 20 mM phosphate buffer (pH 6.9) was then added to start the reaction. The reaction mixture was incubated at 37°C for 20 min and stopped by adding 2 mL of 0.1 M Na<sub>2</sub>CO<sub>3</sub>. The  $\alpha$ -glucosidase activity was determined by measuring the yellow-colored paranitrophenol released from pNPG at 405 nm.

$$\alpha\text{-glucosidase inhibitory activity(\%)} = \frac{\text{Absorbance of blank} - \text{Absorbance of sample}}{\text{Absorbance of Blank}} \times 100 \dots \text{Eqn 4}$$

### Statistical Analysis

The experimental data obtained were expressed as Mean $\pm$ SEM. The difference between the extract and standard were compared using one way Analysis of Variance (ANOVA) followed by Duncan multiple range test using the SPSS Software. P<0.05 was statistically significant.

## Results

### Phytochemical Composition of the plant extracts

Table 1 and 2 respectively shows the qualitative and quantitative phytochemical contents in the leaves of the selected plant extracts. Alkaloids, phenols, flavonoids, tannins, saponins, terpenoids, and cardiac glycoside were present in the ethanol leave extracts of the four plants (Table 1). Steroids were not detected in *G. pubescens* but present in the other three plant extracts. Furthermore, phlobatanin was only present in *V. amygdalina*, whereas anthraquinones was only detected in *S. alata*.

In Table 2, the quantitative flavonoids, phenols, and tannin contents in the leaves of the plant extracts were revealed. Highest concentration of flavonoids, phenols, and tannin was observed in the extract of *Senna alata* followed by *V. amygdalina*. The least concentration of all the phytochemicals was observed in the ethanol extract of *Jatropha curcas*. Flavonoids, phenol and tannin concentration in the ethanol extract of *Senna alata* was  $0.27 \pm 0.002$  mg: mg of rutin per g of extract,  $10.63 \pm 0.017$  mg: mg of gallic acid per g of extract and  $6.72 \pm 0.06$  mg/g respectively, while the concentration in the extract of *V. amygdalina* was  $0.20 \pm 0.002$  mg: mg of rutin per g of extract,  $8.27 \pm 0.017$  mg: mg of gallic acid per g of extract, and  $7.98 \pm 0.03$  mg/g respectively. For the ethanol extract of *Jatropha curcas*, tannin was the most abundant of the phytochemicals, unlike the other plant extracts where phenol had the highest quantity among all the phytochemicals. *Jatropha curcas* content of the three phytochemicals: flavonoids, phenol and tannin was  $0.13 \pm 0.002$  mg: mg of rutin per g of extract,  $3.62 \pm 0.017$  mg: mg of gallic acid per g of extract and  $3.86 \pm 0.12$  mg/g respectively.

**Table 1: Qualitative Phytochemical Analysis of Ethanol Extract of the selected plants**

Phytochemical Test	<i>V. amygdalina</i>	<i>S. alata</i>	<i>G. pubescens</i>	<i>J. curcas</i>
Alkaloids	+	+	+	+
Phenols	+	+	+	+
Flavonoids	+	+	+	+
Tannins	+	+	+	+
Saponin	+	+	+	+
Cardiac glycoside	+	+	+	+
Terpenoid	+	+	+	+
Quinones	-	+	-	-
Coumarins	-	+	-	-
Steroids	+	+	-	+
Phlobatanin	+	-	-	-

Anthraquinones	-	+	-	-
	Present:+	Absent:-		

**Table 2: Quantitative Phytochemical Analysis of Ethanol Extract of the selected plant extracts**

Phytochemical	<i>Vernonia amygdalina</i>	<i>Senna alata</i>	<i>Grewia pubescens</i>	<i>Jatropha curcas</i>
Flavonoid (mg: mg of rutin per g of extract)	0.20±0.002	0.27±0.002	0.15±00.002	0.13±00.002
Phenol (mg: mg of gallic acid per g of extract)	8.27±0.017	10.63±0.017	7.88±00.017	3.62±0.0.017
Tannin (mg/g)	7.98±0.03	6.72±0.06	5.25±0.01	3.86±0.12

Values were expressed as mean±SEM of triplicate data.

#### ***In-vitro* antioxidant activities of the ethanol leave extracts of the selected plants**

The ferric reducing antioxidant power (FRAP) and the DPPH radical scavenging activity of the selected plant extracts is shown in Table 3a and b respectively. Concentration dependent difference, which is statistically significant, was observed in both the FRAP and DPPH radical inhibition studies. *Senna alata* showed the strongest reducing power followed by the *V. amygdalina* however at a level that is significantly ( $p < 0.05$ ) different from each other. Also, least antioxidant power against ferric was observed with *J. curcas* with the strongest reducing power as  $4.24 \pm 0.01$  mg AAE/g lower than the least antioxidant power of *Senna alata* and *V. amygdalina* extracts. Both *Senna alata* and *V. amygdalina* showed DPPH radical inhibition that is not significantly ( $p > 0.05$ ) different from that of trolox. the DPPH radical inhibition at 100 mg/ml of *Grewia pubescens* was also insignificantly ( $p > 0.05$ ) different from

that of the standard. Overall, the lowest DPPH radical inhibition was exhibited by the extracts of *J. curcas*.

**Table 3a: Ferric reducing antioxidant power of the selected plant extracts**

FRAP radical scavenging activity <b>mg AAE/g dried sample</b>				
Concentration	<i>V. amygdalina</i>	<i>Senna alata</i>	<i>Grewia pubescens</i>	<i>J. curcas</i>
50 mg/ml	4.77±0.01 <sup>a</sup>	5.15±0.01 <sup>a</sup>	3.65±0.01 <sup>a</sup>	1.48±0.01 <sup>a</sup>
75 mg/ml	7.13±0.01 <sup>b</sup>	7.75±0.01 <sup>b</sup>	5.98±0.01 <sup>b</sup>	3.05±0.01 <sup>b</sup>
100 mg/ml	9.06±9.06 <sup>c</sup>	10.36±0.01 <sup>c</sup>	7.41±0.01 <sup>c</sup>	4.24±0.01 <sup>c</sup>

Values were expressed as mean±SEM of triplicate data. Statistical significant difference (P<0.05) between concentrations was represented with different alphabets and data with different alphabets are significantly different.

**Table 3b: DPPH radical scavenging activity of the selected plant extracts**

DPPH Radical Inhibition (%)					
Concentration	<i>V. amygdalina</i>	<i>Senna alata</i>	<i>Grewia pubescens</i>	<i>J. curcas</i>	Trolox
50 mg/ml	79.49±0.04 <sup>a*</sup>	86.30±0.04 <sup>a</sup>	71.95±0.04 <sup>a*</sup>	56.68±0.04 <sup>a*</sup>	92.27±0.04 <sup>a</sup>
75 mg/ml	88.07±0.04 <sup>b</sup>	89.06±0.04 <sup>b</sup>	75.95±0.04 <sup>b*</sup>	63.89±0.04 <sup>b*</sup>	93.64±0.04 <sup>b</sup>
100 mg/ml	89.38±0.04 <sup>c</sup>	91.61±0.04 <sup>c</sup>	80.14±0.04 <sup>c</sup>	66.12±0.04 <sup>c*</sup>	97.38±0.04 <sup>c</sup>

Values were expressed as mean±SEM of triplicate data. Statistical significant difference (P<0.05) between concentrations was represented with different alphabets and data with different alphabets are significantly different. \* shows significant difference compared to the standard

***In-vitro* Anti-diabetic potentials of the selected plant extracts**

In table 4, the  $\alpha$ -amylase and  $\alpha$ -glucosidase inhibition by the ethanol leave extract of the selected plants was demonstrated in a concentration dependent manner. The difference in the percentage inhibition of  $\alpha$ -amylase and  $\alpha$ -glucosidase was significantly difference ( $p<0.05$ ) among the tested extracts concentration. In addition, statistically significant ( $p<0.05$ ) difference was only observed in the  $\alpha$ -amylase inhibition between acarbose and *V. amygdalina* and *S. alata* when tested at 100 mg/ml. Whereas, no significant difference ( $p>0.05$ ) at all the tested concentration of the two extracts on comparing their  $\alpha$ -glucosidase inhibition with that of acarbose. In both the  $\alpha$ -amylase and  $\alpha$ -glucosidase inhibition, *V. amygdalina* and *S. alata* exhibited the most significant inhibitory properties among the plant extracts whereas *G. pubescens* and *J. curcas* showed somewhat similar potentials in inhibiting  $\alpha$ -amylase and  $\alpha$ -glucosidase inhibition.

**Table 4: *In-vitro*  $\alpha$ -amylase and  $\alpha$ -glucosidase inhibition by the selected plant extracts**

Alpha-amylase inhibition (%)					
Concentration	<i>Vernonia amygdalina</i>	<i>Senna alata</i>	<i>Grewia pubescens</i>	<i>Jatropha curcas</i>	Acarbose
50 mg/ml	32.24±0.12 <sup>a</sup>	38.81±0.12 <sup>a</sup>	27.10±0.12 <sup>a*</sup>	27.31±0.12 <sup>a*</sup>	37.37±0.12 <sup>a</sup>
75 mg/ml	40.66±0.12 <sup>b</sup>	51.33±0.12 <sup>b</sup>	32.85±0.12 <sup>b*</sup>	29.57±0.12 <sup>b*</sup>	47.64±0.12 <sup>b</sup>
100 mg/ml	43.74±0.12 <sup>c*</sup>	58.52±0.12 <sup>c*</sup>	37.78±0.12 <sup>c*</sup>	34.70±0.12 <sup>c*</sup>	74.74±0.12 <sup>c</sup>
Alpha-glucosidase inhibition (%)					
Concentration	<i>Vernonia amygdalina</i>	<i>Senna alata</i>	<i>Grewia pubescens</i>	<i>Jatropha curcas</i>	Acarbose
50 mg/ml	81.96±0.11 <sup>a</sup>	77.06±0.11 <sup>a</sup>	64.51±0.11 <sup>a*</sup>	43.73±0.11 <sup>a*</sup>	80.00±0.11 <sup>a</sup>
75 mg/ml	84.31±0.11 <sup>b</sup>	82.16±0.11 <sup>b</sup>	66.67±0.11 <sup>b*</sup>	62.16±0.11 <sup>b*</sup>	87.84±0.11 <sup>b</sup>
100 mg/ml	89.61±0.11 <sup>c</sup>	89.02±0.11 <sup>c</sup>	79.61±0.11 <sup>c*</sup>	74.31±0.11 <sup>c*</sup>	92.55±0.11 <sup>c</sup>

Values were expressed as mean±SEM of triplicate data. Statistical significant difference ( $P<0.05$ ) between concentrations was represented with different alphabets and data with different alphabets are significantly different. \* shows significant difference compared to the standard

## Discussion

In the recent time, more attention has focused on the consumption of herbal medicine instead of synthetic drugs, mainly because of some adverse effects associated with synthetic medicines more so, many of the synthetic drugs originated from plant (Alara and Abdurahman, 2019). While these plants are reported with strong medicinal efficacy against many human diseases, their therapeutic efficacy is usually attributed to their antioxidant constituents (Abdulmumeen *et al.*, 2016; Oriakhi *et al.*, 2014). “Different morphological organs of plants used in various treatment regimens include the leaves, barks, tubers, and root, which secrete phytochemicals such as alkaloids, terpenes and phenolic compounds” (Oriakhi *et al.*, 2014).

“Plant leaves are major antioxidants source, which provides defense against several diseases, including cardiovascular diseases, cancer, cataracts, atherosclerosis, arthritis and neuro-degenerative diseases to mention a few” (Sarker and Oba, 2019). These antioxidant constituents generally belong to one of the classes including the phenolics, flavonoids, terpenoids, and tannins (Olasunkanmi *et al.*, 2022). Generally speaking, the phytochemicals in plants have unique biological functions. For example, glycoside possess sedative, anticancer and digestives properties (Galvano *et al.*, 2004; Guclu-Ustundag and Mazza, 2007; Zhou *et al.*, 2013), while plants rich in tannins act against diarrhea, stomach and duodenal tumours, and as anti-inflammatory (Khanbabaee and vanRee, 2001). Alkaloids are known for their sedative, antimalarial, and anticancer properties (Swain and Padhy, 2015). Flavonoids are good antioxidant and a principal example in this class is the quercetin: a natural antioxidant with wide usage (Olasunkanmi *et al.*, 2022). In this study, many of the phytochemicals were identified in the ethanol leave extracts of the selected plants. Abundance of the flavonoids, phenols, and tannin was found in *V. amygdalina*, *S. alata* and *G. pubescens*. Liu *et al.* (2019) and Asante *et al.* (2016) had reported the presence of steroids, polyphenolics, alkaloids, anthraquinones, lignans, flavonoids, and coumarins in *V.*

*amygdalina*. The methanol leaf extracts of *G. pubescens* was reported by Abdulmumeen *et al.* (2016) to contain similar phytochemicals. These important phytochemicals might have contributed to the antioxidant properties exhibited by the selected plant extracts. For instance, phyto-constituents in *S. alata* were associated with the reducing power and ABTS inhibitory properties of its methanol extracts (Madankumar and Pari, 2020). The water, ethanol, chloroform and petroleum ether extracts of *V. amygdalina* also exhibited significant FRAP, ABTS and DPPH radical inhibition with the water and methanol extracts as the best (Atangwho *et al.*, 2014). Just as observed in this study, the antioxidant activity demonstrated by *G. pubescens* was a moderate inhibition of DPPH (Abdulmumeen *et al.*, 2016). In this study, *V. amygdalina* and *S. alata* showed the best antioxidant activity and the effects could have been contributed by their rich tannin, phenolic, and flavonoid constituents.

Diabetes being one of the major cause of morbidity and mortality worldwide with geared interest towards finding a remedy, we, in this study assessed the potentials of the selected plant extracts as possible source of anti-diabetic drug. Postprandial control of hyperglycaemia is a major approach in diabetes treatment (Kwon *et al.*, 2008) and the approach involves carbohydrate digestive enzymes' regulation with consequent prevention of glucose absorption after meal (Vardhini *et al.*, 2013; Olalokun *et al.*, 2013). In other words, the two enzymes;  $\alpha$ -amylase and  $\alpha$ -glucosidase, when regulated may delay carbohydrate ingestion and glucose absorption thus attenuating post-prandial hyperglycaemia (Srivastava *et al.*, 2011). The role of alpha-amylase is the hydrolysis of starch releasing simple sugars like maltotriose, dextrin, and maltose (Alexander, 1992). Therefore, when inhibited, starch absorption after a carbohydrate meal may be limited (Jini and Sharmila, 2020) and in result prevent a rise in blood glucose concentration (Moorthy *et al.*, 2012; Tamil *et al.*, 2010) which is hallmark of diabetes mellitus. The other digestive enzyme i.e. alpha-glucosidase cleaves oligosaccharides and disaccharides to produce monosaccharaides (Chen and Guo, 2017) and

when inhibited, will cause reduction in circulating blood glucose level (Khan *et al.*, 2014; Zhou *et al.*, 2015). We hypothesize that the inhibition of  $\alpha$ -amylase and  $\alpha$ -glucosidase may be supportive to anti-diabetic therapy considering the two as the major enzymes involved in carbohydrate digestion, and that diabetes as a disease is characterized by hyperglycaemia. In this study, the efficacy of the selected plants as potent inhibitor of  $\alpha$ -glucosidase and  $\alpha$ -amylase was evidenced from the percentage of inhibition relative to acarbose. Four stigmastane-type steroid saponin viz; Vernoniacum B, vernonloside B1, vernonloside B2 and vernoamyoside E from *V. amygdalina* were responsible for its  $\alpha$ -amylase and  $\alpha$ -glucosidase inhibition (Kim, 2019). From the observation, vernoamyoside E exhibited more inhibitory potentials than the acarbose used as standard. Alara and Abdurahman. (2019) reported a similar inhibitory effect of the ethanol leaf extracts. Other in-vivo studies have validated the anti-diabetic potentials of the ethanol leaf extracts of *V. amygdalina* (Asante *et al.*, 2016; Yazid *et al.*, 2020). In the same vein, among the ethyl acetate, hexane and acetone extracts of *S. alata*, the acetone extract exhibited the strongest inhibitory potential against  $\alpha$ -amylase while the hexane extract exhibited the strongest inhibition against  $\alpha$ -glucosidase. As reported by Kazeem *et al.* (2015) the extracts exhibited similar inhibitory properties against  $\alpha$ -amylase and  $\alpha$ -glucosidase just as observed in the present study. The authors also demonstrated the anti-hyperglycaemia activity of the extracts from *S. alata*. The inhibitory potential of *S. alata* methanol extract was similarly reported by Madankumar and. Pari, (2020).

The overall result in this study suggested that *V. amygdalina*, *S. alata* and *G. pubescens* are potential source of drug for the management of diabetes. The anti-oxidative property exhibited by the extracts may also be important in limiting diabetic complications and contribute significantly to the ability of the plants to provide remedy against oxidative stress related diseases.

## Conclusion

The present study substantiates the antioxidant and anti-diabetic potentials of the plants under study. Both *V. amygdalina*, and *S. alata* were the most abundant in phenol, tannins, and flavonoids and this content of the phytochemicals might have contributed to their strong antioxidant and anti-diabetic properties. The result of this study substantiates the report of other studies and reveals the difference in the efficacy of each of the selected plants.

## NOTE:

The study highlights the efficacy of "herbal" which is an ancient tradition, used in some parts of India. This ancient concept should be carefully evaluated in the light of modern medical science and can be utilized partially if found suitable.

## References

Abdulmumeen A. H., Oguntoye, S. O., Alli, S. O., Akomolafe, G. A., Aderinto, A., Otitigbe, A., ... & Aminu, R. O. (2016). Chemical composition, antimicrobial, and free radical scavenging activities of *Grewia pubescens*. *Chem Int*, 2(4), 254-261.

Abo, K. A., Fred-Jaiyesimi, A. A., & Jaiyesimi, A. E. A. (2008). Ethnobotanical studies of medicinal plants used in the management of diabetes mellitus in South Western Nigeria. *Journal of ethnopharmacology*, 115(1), 67-71.

Adedayo, O., Anderson, W. A., Moo-Young, M., Snieckus, V., Patil, P. A., & Kolawole, D. O. (2001). Phytochemistry and antibacterial activity of *Senna alata* flower. *Pharmaceutical biology*, 39(6), 408-412.

Alara, O. R., & Abdurahman, N. H. (2019). Antidiabetic activity and mineral elements evaluation of Vernonia amygdalina leaves obtained from Malaysia. *J. Res. Pharm*, 23(3), 514-521.

Alexander, R (1992). Maltodextrins: production, Properties, and applications. In: Schenk F, Hebeda R (ed.) Starch hydrolysis products; worldwide technology: production and applications, New York, 1992. p. 62–122.

Asante, D. B., Effah-Yeboah, E., Barnes, P., Abban, H. A., Ameyaw, E. O., Boampong, J. N., & Dadzie, J. B. (2016). Antidiabetic effect of young and old ethanolic leaf extracts of Vernonia amygdalina: A comparative study. *Journal of diabetes research*, 2016.

Atangwho, I. J., Egbung, G. E., Ahmad, M., Yam, M. F., & Asmawi, M. Z. (2013). Antioxidant versus anti-diabetic properties of leaves from Vernonia amygdalina Del. growing in Malaysia. *Food chemistry*, 141(4), 3428-3434.

Bao J. Y., Cai M., Sun G., Wang and H. Corke, (2005). Anthocyanins, Flavonoid and Free Radical Scavenging Activity of thines Bayberry (*Myrial rubia*) extracts and their colour properties and stability. *Journal of Agric Food Chem*. 53: 2327-2332.

Boulekbache-Makhlouf, L.B., Sakina, S., & Khodir, M. (2012). Antioxidant effects and phytochemical analysis of crude and chromatographic fractions obtained from Eucalyptus globulus bark. *African Journal of Biotechnology*, 11(42), 10048-10055.

Chen, G., Guo, M. (2017). Rapid screening for  $\alpha$ - glucosidase inhibitors from *Gymnema sylvestre* by Affinity Ultrafiltration-HPLC-MS. *Front. Pharmacol*. 8: 228–235

Chopra, R. N. (1956). Glossary of Indian medicinal plants. CSIR, New Delhi, p. 128.

de Freitas Junior, L. M., & de Almeida Jr, E. B. (2017). Medicinal plants for the treatment of obesity: ethnopharmacological approach and chemical and biological studies. *American journal of translational research*, 9(5), 2050.

DeMoraes, L.M.P, Filho, S.A., Ulhoa, C.J. (1999). Purification and some properties of an -amylase glucoamylase fusion protein from *Saccharomyces cerevisiae*". *World J. Microbiol. Biotechnol.* 15: 561-564

Farombi, E. O., & Owoeye, O. (2011). Antioxidative and chemopreventive properties of *Vernonia amygdalina* and *Garcinia biflavonoid*. *International journal of environmental research and public health*, 8(6), 2533-2555.

Fitzgerald, M., Heinrich, M., & Booker, A. (2020). Medicinal plant analysis: A historical and regional discussion of emergent complex techniques. *Frontiers in pharmacology*, 10, 1480.

Galvano, F., La Fauci, L., Lazzarino, G., Fogliano, V., Ritieni, A., Ciappellano, S., Battistini, N.C., Tavazzi, B., Galvano, G. (2004). Cyanidins: metabolism and biological properties. *J. Nutritional Biochem.* 15 (1): 2–11.

Gu'c,lu"-U"stu"ndag",O., Mazza, G. (2007). Saponins: properties, applications, and processing. *Crit. Rev. Food Sci. Nutr.* 47 (3): 231–258.

Gupta, D. D., Haque, M. E., Islam, M. N., Mondal, M. S. I., & Shibib, B. A. (2010). Antimicrobial and Cytotoxic activities of *Jatropha curcas* (Euphorbiaceae). *Dhaka University Journal of Pharmaceutical Sciences*, 9(2), 139-142.

Gyamfi, M. A., Yonamine, M., & Aniya, Y. (1999). Free-radical scavenging action of medicinal herbs from Ghana: *Thonningia sanguinea* on experimentally-induced liver injuries. *General Pharmacology: The Vascular System*, 32(6), 661-667.

Hennebelle, T., Weniger, B., Joseph, H., Sahpaz, S., & Bailleul, F. (2009). *Senna alata*. *Fitoterapia*, 80(7), 385-393.

Jini, D., Sharmila S. (2020). Green synthesis of silver nanoparticles from *Allium cepa* and its in vitro anti-diabetic activity. *Materials Today: Proceedings* 22: 432–438

Kazeem, M. I., Azeez, G. A., & Ashafa, A. O. (2015). Effect of senna alata (L) roxb (fabaceae) leaf extracts on alpha-amylase, alpha-glucosidase and postprandial hyperglycemia in rats. *Tropical Journal of Pharmaceutical Research*, 14(10), 1843-1848.

Khan, K.M., Rahim, F., Wadood, A., Kosar, N., Taha, M., Lalani, S., Khan, A., Fakhri, M.I., Junaid, M., Rehman, W., Khan, M., Perveen, S., Sajid, M., Choudhary, M.I. (2014). Synthesis and molecular docking studies of potent  $\alpha$ -glucosidase inhibitors based on biscoumarin skeleton. *Eur. J. Med. Chem.* 81: 245–252.

Khanbabaee, K., van Ree, T. (2001). Tannins: classification and definition. *Royal Soc. Chem.* 18 (6): 641–649.

Kim, Y. H. (2019). In vitro study on  $\alpha$ -amylase inhibitory and  $\alpha$ -glucosidase of a new stigmastane-type steroid saponin from the leaves of *Vernonia amygdalina*. *Natural Product Research*, DOI:10.1080/14786419.2019.1607853

Kim, Y. M., Jeong, Y. K., Wang, M. H., Lee, W. Y., & Rhee, H. I. (2005). Inhibitory effect of pine extract on  $\alpha$ -glucosidase activity and postprandial hyperglycemia. *Nutrition*, 21(6), 756-761.

Kwon, Y.I., Apostolidis, E., Shetty, K. (2008). Inhibitory potential of wine and tea against  $\alpha$ -amylase and  $\alpha$ -glucosidase for management of hyperglycemia linked to type 2 diabetes. *J. Food Biochem.* 32(1): 15–31

Liu, X., Tian, W., Wang, G., Xu, Q., Zhou, M., Gao, S., & Chen, H. (2019). Stigmastane-type steroids with unique conjugated  $\Delta^7, 9(11)$  diene and highly oxygenated side chains from the twigs of *Vernonia amygdalina*. *Phytochemistry*, 158, 67-76.

Madankumar, D., & Pari, S. (2020). Evaluation of Invitro Antidiabetic and Antioxidant activity Using Methanolic Extract of Medicinal Plants (*Senna alata* and *Senna hirsuta*). *Solid State Technology*, 4962-4973.

Moorthy, N. S., Ramos, M. J., Fernandes, P. A. (2012). Studies on alpha-glucosidase inhibitors development: magic molecules for the treatment of carbohydrate mediated diseases. *Mini Rev. Med. Chem.* 12(8): 713-720.

Nayak, B. S., & Patel, K. N. (2010). Pharmacognostic studies of the *Jatropha curcas* leaves. *International journal of pharmtech research*, 2(1), 140-143.

Njan, A. A. (2012). Herbal medicine in the treatment of malaria: *Vernonia amygdalina*: an overview of evidence and pharmacology. *Toxicity and Drug Testing*, 167-186.

Olaokun, O. O., McGaw, L. J., Eloff, J. N., Naidoo, V. (2013). Evaluation of the inhibition of carbohydrate hydrolysing enzymes, antioxidant activity, and polyphenolic content of extracts of ten African *Ficus* species (Moraceae) used traditionally to treat diabetes. *BMC Complement Altern. Med.* 13 (1): 94.

Olasunkanmi, A.A., Fadahunsi, O.S., Adegbola, P.I. (2022). Gas Chromatography-Mass Spectroscopic, highperformance liquid chromatographic and In-silico characterization of antimicrobial and antioxidant constituents of *Rhus longipes*(Engl). *Arabian Journal of Chemistry*. 15: 103601

Oriakhi, K., Oikeh, E. I., Ezeugwu, N., Anoliefo, O., Aguebor, O., & Omoregie, E. S. (2014). In vitro antioxidant activities of extracts of Vernonia amygdalina and Ocimum gratissimum leave. *J Pharm Bioresour*, 11, 58-65.

Palanichamy, S., Nagarajan, S., & Devasagayam, M. (1988). Effect of Cassia alata leaf extract on hyperglycemic rats. *Journal of Ethnopharmacology*, 22(1), 81-90.

Patil, D., Roy, S., Dahake, R., Rajopadhye, S., Kothari, S., Deshmukh, R., Chowdhary, A. (2013). Evaluation of Jatropha curcas Linn. leaf extracts for its cytotoxicity and potential to inhibit hemagglutinin protein of influenza virus. *Indian J Virol*; 24(2):220-6.

Pulido, R., Bravo, L., & Saura-Calixto, F. (2000). Antioxidant activity of dietary polyphenols as determined by a modified ferric reducing/antioxidant power assay. *Journal of agricultural and food chemistry*, 48(8), 3396-3402.

Re, R., Pellegrin N., Proteggente A., Pannala A., Yang M., Rice-Evans C. (1999). Antioxidant activity applying an improve ABTS Radication decolourization assay. *Free Rad. Biol. Med.* 26: 1231-1237

Sarker, U., Oba, S., 2019a. Protein, dietary fiber, minerals, antioxidant pigments and phytochemicals, and antioxidant activity in selected red morph Amaranthus leafy vegetable. *PLoS ONE* 14, (12) e0222517.

Shaikh, J. R., & Patil, M. (2020). Qualitative tests for preliminary phytochemical screening: An overview. *International Journal of Chemical Studies*, 8(2), 603-608.

Singleton, V. L., Orthofer, R., & Lamuela-Raventós, R. M. (1999). [14] Analysis of total phenols and other oxidation substrates and antioxidants by means of folin-ciocalteu reagent. In *Methods in enzymology* (Vol. 299, pp. 152-178). Academic press.

Srivastava, A.K., Srivastava, S., Srivastava, S.P., Raina, D., Ahmad, R., Srivastava, M.N. (2011). Ethanolic extract of a marine mangrove *Xylocarpus granatum*. *J Pharm Biomed. Sci.* 9: 1-12

Swain, S.S., Padhy, F.N. (2015). In vitro antibacterial efficacy of plants used by an Indian aboriginal tribe against pathogenic bacteria isolated from clinical samples. *J. Taibah Univ. Med. Sci.* 10 (4), 379–390.

Tamil, I.G., Dineshkumar, B., Nandhakumar, M., Senthilkumar, M., Mitra, A. (2010). *Indian J. Pharmacol.* 42: 280–282.

Ugboko, H. U., Nwinyi, O. C., Oranusi, S. U., Fatoki, T. H., & Omonhinmin, C. A. (2020). Antimicrobial importance of medicinal plants in Nigeria. *The Scientific World Journal*, 22;2020:7059323

Vardhini, R.S., Naik, B. R., Neelima, M., Ramesh, B.P., Sciences, P. (2013). Screening and production of  $\alpha$ -amylase from *Aspergillus Niger* using zero, value material for solid-state fermentation. *Int. J. Pharm. Pharm. Sci.* 5 (1): 55–60

Woradulayapinij, W., Soonthornchareonnon, N., & Wiwat, C. (2005). In vitro HIV type 1 reverse transcriptase inhibitory activities of Thai medicinal plants and *Canna indica* L. rhizomes. *Journal of ethnopharmacology*, 101(1-3), 84-89.

Yakubu, M. T., Adeshina, A. O., Oladiji, A. T., Akanji, M. A., Oloyede, O., Jimoh, G. A., & Afolayan, A. J. (2010). Abortifacient potential of aqueous extract of *Senna alata* leaves in rats. *Journal of reproduction and contraception*, 21(3), 163-177.

Yazid, F., Hasanah, N. B., Rosmalena, H. M., & Prasasty, V. D. (2020). Antidiabetic and antioxidant potential of *Vernonia amygdalina* leaf extract in alloxan-induced Sprague-Dawley rats. . *OnLine Journal of Biological Sciences.* 2020, 20 (4): 190.200

Zhou, Q., Liang, D., Deng, A., Zhang, J., Wu, C., Nie, Z., Jiang, J., Wang, Y.I. (2013). Antitussive, expectorant, and bronchodilating effects of ethanol extract of *Sorghum bicolor* (L.) Moench roots. *J. Ethnopharmacol.* 149 (1): 297–302.

Zhou, X.L., Liang, J.S., Zhang, Y., Zhao, H.D., Guo, Y., Shi, S.Y. (2015). Separation and purification of  $\alpha$ -glucosidase inhibitors from *Polygonatum odoratum* by stepwise high-speed counter-current chromatography combined with sephadex LH-20 chromatography target-guided by ultrafiltration-HPLC-screening. *J. Chromatogr.* 985: 149–154.

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