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

Comment [A1]: Replace by medicinal plant

Abstracts

For many years, medicinal plants have been a resource for healing in several local communities around the world and the phytochemicals in them are attributed to their many medicinal values. *Vernoniaamydalina*, *Sennaalata*, *Jatrophacurcas*, and *Grewiapubescens*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.

Comment [A2]: In vitro was write in italic through the manuscript

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 α-amylase and α-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 werehighest in*Sennaalata* (0.27±0.0.002, 10.63±0.0.017, and 6.72±0.06 mg/g respectively)followed by *V.* amygdalina (0.20±00.002, 8.27±0.0.017, and 7.98±0.03 mg/g respectively). While the least content of all was found in the extracts of*Jatrophacurcas*. Concentration dependent and statistically significant difference was observed in both the FRAP and DPPH radical inhibition of all the extracts. *Sennaalata*showed the strongest reducing power followed by the *V. amygdalina* however at a level that is significantly ($p<0.05$) lower than the reducing power of trolox. Both *Sennaalata*and *V. amygdalina* showed DPPH radical inhibition that is not significantly ($p>0.05$) different from that of vitamin C. α-amylase and α-glucosidase inhibition was also demonstrated in a concentration dependent manner. In both the α-amylase and α-glucosidase inhibition, *V. amygdalina*and *S. alatsa*exhibited the most significant inhibitory properties among the plant extracts.

Comment [A3]: Use a single form (S. alatsa or Sennaalata) for all plants

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.

Keywords: medicinal plants, phytochemicals, α-amylase, α-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 ethnomedical treatment of diverse aliments. Medicinal plants are generally sources of various phytochemicals, which as usually responsible for their biological activities (Ugboko*et al.*, 2020). Most knowledge of medicinal plant uses originated from ethnobotanical and ethnopharmacologicalrepositories (de Freitas and de Almeida, 2017) and this knowledge have ensured the preservation of the enthnomedicinal 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).

Sennaalata(*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 'Asunwonoyinbo' 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 Wister 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).*Vernoniaamygdalina*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 andOwoeye, 2011). The plant has also attracted attention due to it usage in providing remedy for diabetes mellitus and hypertension (Atangwho*et al.*,2014**).**

Grewiapubescens (family Maliaceae) 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). 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 *Vernoniaamydalina*, *Sennaalata*, *Jatrophacurcas*, and *Grewiapubescens*were collected around IyaIjado, 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 ^{o}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: 1ml of concentrated HClwas added to 1ml of extract followed by few drops of Mayer's reagent. Formation of green or white precipitate indicates that alkaloid is present.

Comment [A4]: Add the concentration used for each extract in all Tests

Test for Phenols

Ferric Chloride test: 1ml of 5% ferric chloride solution was added to 1ml of extract. Formation of dark-greencolour indicates that a phenol is present.

Test for Flavonoids

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

Test for Saponins

Foam test: 1ml of distilled water was added to 1ml of extract and was shaked vigorously. Formation of the foam indicates that saponin is present.

Test for Tannins

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

Test for Terpenoids

5 mL of the sample was mixed with 2 mL of $CHCl_3$ in a testtube. Thereafter, 3 mL of concentrated H_2SO_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

0.5ml of concentrated hydrochloric acid was added to 1ml of extract. Formation of green colourindicates that quinone is present.

Test for Cardiac glycosides

Killer-killani test: 2ml of glacial acetic acid and 1ml of 5% ferric chloride solution along with 1ml of concentrated sulphuric acid was added to 1ml of sample. 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 1ml of extract. Appearance of pink colour precipitate indicates the presence of anthraquinones.

Test for Phlobatanin

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

Test for Steroids

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

Test for Courmarins

1ml of 10% sodium hydroxide was added to 1ml of the extract. 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). 0.2ml of the extract was mixed with 2.5ml of 10% Folinciocalteau's reagent and 2ml of 7.5% Sodium carbonate. The reaction mixture was subsequently incubated at 45^{o}C for 40mins, and the absorbance was measure at 700nm in the spectrophotometer, garlic acid was used as standard phenol.

Comment [A5]: Specify the concentration used for each extract

Comment [A6]: What is garlic acid! I think you used gallic acid. Correct, please.
What is the concentration range used for the standard phenol

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% $NaNO_3$was added to 0.2 mlof the extract at time zero. 0.6 ml of 10% $AlCl_3$ and 2 ml of 1 M NaOHwas 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 Rutin equivalent.

Comment [A7]: Specify the concentration used for each extract

Comment [A8]: What is the concentration range used for the 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 (0.25ml) of different concentrationwas mixed with 0.25ml of 200mM of Sodium phosphate buffer pH 6.6 and 0.25ml of 1% potassium ferricyanide (KFC). The mixture was incubated at 50°C for 20min, thereafter 0.25ml of 10% trichloroacetic acid (TCA) was also added and centrifuged at 2000rpm for 10min, 1ml of the supernatant was mixed with 1ml of distilled water and 0.1% of $FeCl_3$ and the absorbance was measured at 700 nm.

Determination of DPPH radical Inhibition

The free radical scavenging ability of the extract against DPPH (1, 1- diphenyl-2-picryhydrazyl) was determined using the method reported by Gyamfi et al. (1999). One milliliter (1 ml) of varied concentration of the extractswas mixed with 1 ml of 0.4mMmethanol solution of DPPH the mixture was left in the dark for 30min before measuring the absorbance at 516nm.

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). Crude enzyme extract(0.3ml) was added to the mixture, mixed, and incubated at 40°C for 30min 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).

Determination of Alpha-glucosidase Inhibitory Activity

Comment [A9]: Specify the rang concentration used for each extract

Comment [A10]: Specify the rang concentration used for each extract

Comment [A11]: What is the origin of the alpha-amylase used, it can be obtained from plants, animals and microorganisms

Comment [A12]: Normally the alpha-amylase is incubated at 37 ° C, so how you have sure that the enzyme has integrated into the reaction knowing that you have not used a negative control to validate this protocol

The effect of the plant extracts on α-glucosidase activity was determined according to the method described by Kim et al. (2005) using α-glucosidase from *Saccharomyces cerevisiae*. The substrate solution p-nitrophenylglucopyranoside (pNPG) was prepared in 20 mM phosphate buffer, and pH 6.9. 100 μL of α-glucosidase (0.3 U/mL) was pre-incubated with 50 μL of the sample for 10 min. Then 50 μL 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_2CO_3. The -glucosidase activity was determined by measuring the yellow-colored paranitrophenol released from pNPG at 405 nm.

Comment [A13]: Specify the rang concentration used for each extract

Statistical Analysis

The experimental data obtained were expressed as Mean±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 *Sennaalata*followed by *V. amygdalina.* The least concentration of all the phytochemicals was observed in the ethanol extract of *Jatrophacurcas.* Flavonoids, phenol and tannin concentration in the ethanol extract of *Sennaalata* was 0.27±0.0.002, 10.63±0.0.017 and 6.72±0.06 mg/grespectively, while the concentration in the extract of *V. amygdalina*was 0.20±00.002, 8.27±0.0.017, and 7.98±0.03 mg/g respectively. For the ethanol extract of *Jatrophacurcas*, tannin was the most abundant of the phytochemicals, unlike the other plant extracts where phenol had the highest quantity among all the phytochemicals. *Jatrophacurcas* content of the three phytochemicals: flavonoids, phenol and tannin was 0.13±00.002, 3.62±0.0.017 and 3.86±0.12 mg/g respectively.

Comment [A14]: Why does this value contain two points? It must be corrected.

Comment [A15]: The unit must be specified for each value
Example: TPC, Total Phenolic Content (mg /g: mg of Gallic Acid per g of extract); TFC, Total flavonoid content (mg/g:
mg of rutin per mg of extract)

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

Phytochemical Test	*V. amydalinaS.alata*	*G.pubescens*	*J.curcas*	
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	*Vernoniaamygdalina*	*Sennaalata*	*Grewiapubescens*	*Jatrophacurcas*
Flavonoid mg/g of rutin	0.20±00.002	0.27±0.0.002	0.15±00.002	0.13±00.002
Phenol mg/g of garlic	8.27±0.0.017	10.63±0.0.017	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 3. Concentration dependent difference, which is statistically significant, was observed in both the FRAP and DPPH radical inhibition studies. *Sennaalata*showed the strongest reducing power followed by the *V. amygdalina* however at a level that is significantly ($p<0.05$) lower than the reducing power of trolox. Also, least antioxidant power against ferric was observed with *J. curcas*with the strongest reducing power as 4.24±0.01 mg/glower than the least antioxidant power of *Sennaalata*and *V. amygdalina* extracts. Both *Sennaalata*and *V. amygdalina* showed DPPH radical inhibition that is not significantly ($p>0.05$) different from that of vitamin C. the DPPH radical inhibition at 100 mg/ml of *Grewiapubescens*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.*

Comment [A16]: How did you calculate the FRAP radical scavenging activity in mg/g?you must write the formula used and explain the method.

Table 3: *In-vitro* antioxidant activity of the selected plant extracts

FRAP radical scavenging activity (mg/g)					
Concentration	***V. amygdalina***	***Sennaalata***	***Grewiapubescens***	***J. curcas***	**Trolox**
50 mg/ml	$4.77\pm0.01^{a*}$	$5.15\pm0.01^{a*}$	$3.65\pm0.01^{a*}$	$1.48\pm0.01^{a*}$	11.19 ± 0.01^{a}
75 mg/ml	$7.13\pm0.01^{b*}$	$7.75\pm0.01^{b*}$	$5.98\pm0.01^{b*}$	$3.05\pm0.01^{b*}$	14.46 ± 0.01^{b}
100 mg/ml	$9.06\pm9.06^{c*}$	$10.36\pm0.01^{c*}$	$7.41\pm0.01^{c*}$	$4.24\pm0.01^{c*}$	18.68 ± 0.01^{c}
DPPH Radical Inhibition (%)					
Concentration	***V. amygdalina***	***Sennaalata***	***Grewiapubescens***	***J. curcas***	**Vitamin C**
50 mg/ml	$79.49\pm0.04^{a*}$	86.30 ± 0.04^{a}	$71.95\pm0.04^{a*}$	$56.68\pm0.04^{a*}$	92.27 ± 0.04^{a}
75 mg/ml	88.07 ± 0.04^{b}	89.06 ± 0.04^{b}	$75.95\pm0.04^{b*}$	$63.89\pm0.04^{b*}$	93.64 ± 0.04^{b}
100 mg/ml	89.38 ± 0.04^{c}	91.61 ± 0.04^{c}	80.14 ± 0.04^{c}	$66.12\pm0.04^{c*}$	97.38 ± 0.04^{c}

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

Comment [A17]: you did not mention the positive controls (Trolox and Vitamine C) in the material and methods part, it is necessary to add

Comment [A18]: How did you calculate the FRAP radical scavenging activity in mg/g? You must write the formula used and explain the method.

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

In table 4, the α-amylase and α-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 α-amylase and α-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 α-amylase inhibition betweenacarbose 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 α-glucosidase inhibition with that of acarbose. In both the α-amylase and α-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 α-amylase and α-glucosidase inhibition.

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

Alpha-amylase inhibition (%)					
Concentration	***Vernoniaamygdalina***	***Sennaalata***	***Grewiapubescens***	***Jatrophacurcas***	**Acarbose**
50 mg/ml	32.24±0.12^{a}	38.81±0.12^{a}	27.10±0.12^{a*}	27.31±0.12^{a*}	37.37±0.12^{a}
75 mg/ml	40.66±0.12^{b}	51.33±0.12^{b}	32.85±0.12^{b*}	29.57±0.12^{b*}	47.64±0.12^{b}
100 mg/ml	43.74±0.12^{c*}	58.52±0.12^{c*}	37.78±0.12^{c*}	34.70±0.12^{c*}	74.74±0.12^{c}
Alpha-glucosidase inhibition (%)					
Concentration	***Vernoniaamygdalina***	***Sennaalata***	***Grewiapubescens***	***Jatrophacurcas***	**Acarbose**
50 mg/ml	81.96±0.11^{a}	77.06±0.11^{a}	64.51±0.11^{a*}	43.73±0.11^{a*}	80.00±0.11^{a}
75 mg/ml	84.31±0.11^{b}	82.16±0.11^{b}	66.67±0.11^{b*}	62.16±0.11^{b*}	87.84±0.11^{b}
100 mg/ml	89.61±0.11^{c}	89.02±0.11^{c}	79.61±0.11^{c*}	74.31±0.11^{c*}	92.55±0.11^{c}

Values were expressed as mean±SEM of triplicate data. Statistical significant difference (P<0.05) between concentrations was represented with different alphabets. *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). Whilethese 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, whichsecrete 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¨Ustundagand 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 leave 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; α-amylase and α-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-glucosidasecleaves oligosaccharides and disaccharides to produce monosaccharaides (Chen and Guo, 2017**)** and when inhibited, willcause reduction in circulating blood glucose level (Khan *et al.,* 2014; Zhou *et al.,* 2015). We hypothesis that the inhibition of α-amylase and α-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 α-glucosidase and α-

amylase was evidenced from the percentage of inhibition relative to acarbose. Four stigmastane-type steroid saponinviz; Vernoniacums B, vernonloside B1, vernonloside B2 and vernoamyoside E from *V. amygdalina*were responsible for it α-amylase and α-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 leave 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 α-amylase while the hexane extract exhibited the strongest inhibition against α-glucosidase. As reported by Kazeem***et al.*** (2015)the extracts exhibited similar inhibitory properties against α-amylase and α-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 tothe 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

Comment [A19]: References must be organized by an alphabetical order

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