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Phytochemical Properties and Hypoglycemic Activity of the Aqueous Plant Extract *Vernonia amygdalina* (bitter leaf) and Rhizomes of *Zingiber officinale* (Ginger) on Blood Glucose Level in Normoglycemic Wistar Albino Rats

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ABSTRACT

The Phytochemical properties and hypoglycemic activity of the aqueous plant extract Vernonia amygdalina (bitter leaf) and rhizomes of Zingiber officinale (ginger) on blood glucose in Wistar albino rats were studied. The result of the phytochemical analysis of Vernonia amygdalina revealed that cardenoloids, flavonoids, Saponin glycoside carbohydrates, and terpinoids, Cardiac glycosides are present, while alkaloids, tannins and anthraquinones were absent. The phytochemical analysis *of Zingiber officinale revealed alkaloids, tannins, saponin glycoside carbohydrate, terpinoids, cardiac glycosides, and flavonoids present, while cardenolides and anthraquinones were absent. At 12 hours, post administration the rats treated with 800 mg/kg of the extracts had significant ($p > 0.05$) blood glucose decrease of 3.54 ± 0.61, 3.62 ± 0.29, and 3.88 ± 0.61 Mmol/L, respectively when compared with the zero values of 5.00 ± 0.34, 5.34 ± 0.44 and 5.48 ± 0.66 Mmol/L.*

Keywords: *Vernonia amygdalina, Zingiber officinale, phytochemical screening, hypoglycemic*

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INTRODUCTION

Vernonia amygdalina, commonly known as bitter leaf, is a popular leafy vegetable in Nigeria and is a common ingredient in many Nigerian and African soups and stews. The plant is known by different names in Nigerian languages or tribes such as Shuwaka (Hausa), Fulani (Gyada or Cirrii), Shuwaka (Kanuri), Shakshakai (Shuwa), and Mupukr (Babur). Vernonia is a genus of about 1000 species of forbs and shrubs, of which *Vernonia amygdalina* is the most prominent species and one of the pan-tropical species of the family *Asteraceae* (Johri *et al.,* 1997).

Zingiber officinale, commonly known as ginger, is a widely used spice in Nigerian cuisine. It is known by various names in different Nigerian languages and tribes. Some of these names include: Citta (Hausa)**,** Sinde (Fulfulde), Kajuwur (Kanuri), Zanjabil (Shuwa), and Takatafur (Babur/Bura). The English botanist William Roscoe named the plant *Zingiber officinale* in 1807. The genus name is from the Greek word 'zingiberis', which is derived from the Sanskrit word 'shringavera', aptly meaning 'shaped like a deer's antlers', while 'officinale' pertains to the medicinal properties of the rhizomes (Elzebroek and Wind 2008).

Blood sugar level is the amount of glucose in the blood, also known as serum glucose level, expressed in Mmol/L or Mg/dl. Hypoglycemia is experienced when the glucose level in the body is used up or when glucose is released into the bloodstream more slowly than it's needed, or when an excessive amount of insulin from the pancreas is released into the bloodstream (Ian and Soon, 2006). Relative hypoglycemia has been reported in neonates (Kar *et al*., 2003). Hypoglycemia may also occur due to insulin-secreting tumours of the pancreas and liver or as a response to ingestion of alcohol with symptoms such as weakness, headache, hunger, cold, sweat, muscle pain, paleness, memory loss, and coma (Acampora *et al*., 2002; Laakso, 2006).

Plant extracts have been used in the control of hyperglycemia as well as hypoglycemia, these include *Balanites aegyptiaca* (Kamel *et al*., 1991), *Pterocarpus marsupium* (Chakravarti *et al*., 1980) and *Cuminum nigrum* (Ahmad *et al*., 2000*), Opuntia streptocantha Lem, Trigonella foenum graecium, Momordica charantia, Ficus bengalensis, Polygala senega, Gymnema sylvestre, Allium sativum, Citrulus colocynthis,* Myrrh, black seed, heelteet, Fenugreek, Aloe and Artemisia. Synthetic agents such as glimepride, metformin, and many other forms of these agents have also been used in the management of hyperglycaemia and other related glycemic cases (Duncan, 2012). This study aims to determine the phytochemical properties and hypoglycemic activity of the aqueous plant extract *Vernonia amygdalina* (bitter leaf) and rhizomes of *Zingiber officinale* (ginger).

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MATERIALS AND METHOD

Plant Collection and Identification: Fresh leaves of *Vernonia amygdalina* were collected within the University of Maiduguri Campus, and fresh rhizome of *Zingiber officinale* was purchased from Maiduguri Monday Market, Borno State, Nigeria. The plants used were taken for identification since the taxonomy of the plant was known a long time ago.

Experimental animals: Twenty-five adult Wistar albino rats of both sexes were used for the research. Healthy rats were purchased from the Faculty of Pharmacy, University of Maiduguri, Borno State. The rats were allowed to acclimatise in the laboratory and were maintained on chick mash. Distilled water *ad libitum* was given for one week. The study was conducted with strict adherence to the ethical procedure on the use of animals for experiments (National Institute of Health, 1985).

Preparation of the Aqueous plant extracts: The aqueous extract of *Vernonia amygdalina* (bitter leaf) was prepared from fresh leaves of the plant, rinsed thoroughly in clean water, and left overnight to dry. The dried leaves were then pulverised into powder using an electric blender. The powder from the leaves of *Vernonia amygdalina* was soaked in ionised water in various maceration jars for 24 hours by mechanical agitation at room temperature. The suspension was filtered using Whatman No. 1 filter paper, and the product gave a yield of 4.94g% w/w of light green colour product. Rhizomes of *Zingiber officinale* (Ginger) were crushed in a blender and air-dried. An air-dried powder was soaked and macerated in distilled water over 12 hours at room temperature before filtration to obtain 4.71g% w/w of coffee brown colour rhizome aqueous extract.

Qualitative Phytochemical Content Analysis of the Plant Extracts: The aqueous extract obtained was subjected to a phytochemical test using standard methods of Trease and Evans (1989) and Odebiyi and Sofowora (1978).

Tests for Carbohydrates (Molisch's test): A few drops of Molisch's solution were added to two (2) ml of aqueous solution of the extract; thereafter, a small volume of concentrated sulphuric acid was allowed to run down the side of the test tube to form a layer without shaking. The interface was observed for a purple colour, which is indicative of a positive for carbohydrates.

Tests for Carbohydrate (Barfoed's test): One (1) ml of aqueous solution of the extracts and 1mL of Barfoed's reagent were added into a test tube, and heated in a water bath for about 2 min. Red precipitate shows the presence of monosaccharides.

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A standard test for combined reducing sugars: One (1) ml of the crude solution of the extracts was hydrolysed by boiling with 5 ml of dilute hydrochloric acid. This was neutralised with a sodium hydroxide solution. The Fehling's test was repeated as indicated above, and the tube was observed for brick-red precipitate, which indicates the presence of combined reducing sugars.

Standard test for free reducing sugar (Fehling's test): Two (2) ml of the crude aqueous solution of the extracts in a test tube was added to a five (5) ml mixture of equal volumes of Fehling's solutions I and II and boiled in a water bath for about 2min. The brick-red precipitate indicates the presence of reducing sugar.

Test for ketones: Two (2) ml of the crude aqueous solution of the extracts were added to a few crystals of resorcinol and an equal volume of concentrated hydrochloric acid, and then heated over a spirit lamp flame and observed for a rose that shows the presence of ketones.

Test for Tannins (Ferric chloride test): Two (2) ml of the crude solution of the extracts was added to a few drops of 10% Ferric chloride solution (light yellow). The occurrence of blackish-blue colour indicates the presence of gallic tannins, and a green-blackish colour indicates the presence of catechol tannins.

Test for Phlobatannins (Hydrochloric Acid Test): Two (2) ml of the crude aqueous solution of the extracts was added to dilute hydrochloric acid and observed for red precipitate that indicates the presence of Phlobatannins.

Test for Saponins (Frothing Test): Three (3) ml of the crude solution of the extracts was mixed with 10 ml of distilled water in a test tube. The test tube was stopped and shaken vigorously for about 5 min, then allowed to stand for 30 min and observed for honeycomb froth, which is indicative of the presence of saponins.

Test for Cardiac glycosides: Two (2) ml of the crude aqueous solution of the extracts was added to 3 drops of a strong solution of lead acetate. This was mixed thoroughly and filtered. The filtrate was shaken with 5ml of chloroform in a separating funnel. The chloroform layer was evaporated to dryness in a small evaporating dish. The residue was dissolved in a glacial acetic acid containing a trace of ferric chloride; this was transferred to the surface of 2ml concentrated sulphuric acid in a test tube. The upper layer and interface of the two layers were observed for bluish-green and reddish-brown colouration, respectively, which indicates the presence of cardiac glycosides.

Test for Flavonoids (Shibita's reaction test): One gram of the crude aqueous extract was dissolved in methanol (50%, 1-2ml) by heating, then magnesium metal and 5-6 drops of

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concentrated hydrochloric acid were added. The solution when red is indicative of flavonols and orange for flavones.

Test for Flavonoids (**Pew's test):** Five (5) ml of the crude solution of the water extracts was added to 0.1g of metallic zinc and 8 ml of concentrated sulphuric acid. The reaction mixture was observed for red colour formation, indicative of flavonols.

Effect of Aqueous plant extract on blood glucose level of normal albino rats: A total of twenty-five (25) Wistar albino rats were used for this study. Twenty-five (25) rats purchased were divided into five (5) groups (A -E) of five (5) rats each. Group A was used as a control (naive). Groups (B - D) were administered graded doses (800mg/kg) of aqueous extract of *Vernonia amygdalina* (Bitter Leaves) and *Zingiber officinale* (Ginger), and Group E (Positive control) was administered Metformin. Blood sugar level was determined using a glucose test strip and glucometer at 1, 6, 12, 18, and 24 hours post-oral extract administration (Asatoor and King, 1954).

Blood glucose Determination: Blood was obtained from the tail vein of each rat before the administration of the extract and thereafter, at 1, 6, 12, 18, and 24 hours post-extract administration was used for the determination of blood glucose level, respectively (Asatoor and King, 1954).

Statistical Analysis: The data generated were analysed statistically. The results are therefore presented as Mean ± Standard deviation, and differences between means were assessed using a one-way analysis of variance (ANOVA), and a posttest was done using the Dunnett comparison test (Mead and Curnow, 1982).

RESULTS AND DISCUSSION

Table 1: Qualitative Phytochemistry of the crude aqueous extracts of *Vernonia amygdalina* and *Zingiber officinale*

Phytochemistry	Tests	Inference	
		Vernonia amygdalina (Bitter Leaves)	*Zingiber officinale* (Ginger)
Alkaloids	1. Dragendorff's	-	+
	2. Mayer's Reagent	-	+
Tannins	1. Ferric chloride	-	-
	2. Lead acetate	-	+
Saponin glycosides	1. Frothing	+	+

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Carbohydrates	1. Molisch's	+	+
	2. Monosaccarides	-	-
	3. Combine reducing sugar	+	+
	4. Free reducing sugar	+	+
	5. Ketone's	-	+
Terpinoids	1. Hydrochloric acid	+	+
Cardiac glycosides	1. Salkowski's	+	+
	2. Liebermann–Burchard	+	+
Cardenolides	1. Keller-Killians	+	-
Flavonoids	1. Shinoda's	+	+
	2. Ferric chloride	+	-
	3. Lead acetate	+	+
	4. Sodium hydroxide	+	+
Anthraquinones	1. Free anthroquinones	-	-
	2. Combined anthroquinones	-	-

Key: + Present - Absent

The qualitative phytochemical analysis of the aqueous leaf extracts of *Vernonia amygdalina* and *Zingiber officinale* revealed that saponins, carbohydrates, terpinoids, cardiac glycosides, cardenolides, and flavonoids were the most abundant compounds present; alkaloids and tannins were only present in *Zingiber officinale,* and anthraquinones were absent in both products (Table 1).

Table 2: The effect of aqueous extracts of *Vernonia amygdalina* leaves, *Zingiber officinale rhizome* and their combination on Mean blood glucose level (Mmol/L) of normoglycemic albino rats

Group A = Control (Distilled H_2O)

Treatment/Oral Doses	**Time (hours) and X**						
	0	**1**	**6**	**12**	**18**	**24**	
A	5.94 + 0.27[a]	6.86 + 0.52[a] (1.55%)	6.44 + 0.83[a] (8.41%)	6.94 + 0.26[a] (16.8%)	6.86 + 0.52[a] (15.5%)	6.44 + 0.83[a] (8.41%)	
B	5.00 + 0.34[a]	5.22 + 0.31[a] (4.40%)	3.96 + 0.67[b] (-20.8%)	3.54 + 0.61[b] (-29.2%)	3.86 + 0.58[b] (-22.8%)	3.54 + 0.24[b] (-29.2%)	
C	5.34 + 0.44[a]	6.54 + 0.47[a] (22.5%)	4.68 + 0.66[b] (-12.4%)	3.62 + 0.29[b] (-32.2%)	3.50 +0.31[b] (-34.5%)	3.48 + 0.36[b] (-34.8%)	
D	5.48 + 0.66[a]	5.68 + 0.36[a] (0.04%)	3.96 + 0.38[b] (-27.7%)	3.88 + 0.6[b] (-29.2%)	4.08 + 0.75[b] (-25.5%)	3.78 + 0.73[b] (-31.0%)	
E	5.12 + 0.36[a]	4.92 + 0.55[a] (3.90%)	3.52 + 0.83[b] (-31.3%)	4.34 + 0.6[b] (-15.2%)	4.42 + 0.66[b] (-13.7%)	4.42 + 0.43[b] (-13.7%)	

Group B = *Vernonia amygdalina*(800mg/kg)
Group C = *Zingiber officinale*(800mg/kg)
Group D = *Zingiber officinale Vernonia amygdalina* (800mg/kg)
Group E = Metformin (500mg/kg)
X = Mean + SD
a – significantly ($P < 0.05$) same or higher than zero-hour value
b – Significantly ($P < 0.05$) lower than zero-hour value
Numbers in bracket indicates percentage increase (+) or decrease (-) in glucose level when compared with zero hour.

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The effect of the aqueous extract of *Vernonia amygdalina* leaves, *Zingiber officinale* rhizome and their combination on the blood glucose level of normoglycemic rats is presented in Table 2 below. The groups treated with 800 mg/kg of the extracts 24 hours post-administration showed a significant ($P< 0.05$) decrease in blood glucose values of 3.54 $\pm$ 0.24, 3.48 $\pm$ 0.36 and 3.78 $\pm$ .73 Mmol/L compared with the zero-hour values, respectively. The rats treated with the standard drug (Metformin) had decreased blood glucose levels by 3.52 $\pm$ 0.83, 4.34 $\pm$ 0.61, 4.42 $\pm$ 0.66 and 4.42 $\pm$ 0.43 Mmol/L. The rats in the control groups had their blood glucose values maintained between 5.94 $\pm$ 0.27, 5.00 $\pm$ 0.34, 5.34 $\pm$ 0.44, 5.48 $\pm$ 0.66 and 5.12 $\pm$ 0.36 Mmol/L, respectively.

The aqueous extracts of *Vernonia amygdalina* leaves, *Zingiber officinale* rhizome and their combination significantly ($p<0.05$) reduced blood glucose in the treated normoglycemic albino rats at 6, 12, 18 and 24 hours post-administration. The standard drug (Metformin) significantly ($P < 0.05$) reduced blood sugar by 31.3% lower than that reduced by *Vernonia amygdalina*, *Zingiber officinale* and their combination 6 hours post oral administration, respectively.

The study indicated that the hypoglycemic potency of the products, *Zingiber officinale*, *Vernonia amygdalina* and their combination is as stated in the statement, while the standard drug (Metformin) had the least hypoglycaemic activity. *Zingiber officinale* aqueous product had a better hypoglycemic activity compared to the hypoglycemic effect of *Vernonia amygdalina*, and their combination is best. The better effect of the *Zingiber officinale* product exhibited may be due to the presence of more phytochemicals in the preparation than the rest of the compounds used during the experiment. The combined products of *Vernonia amygdalina* and *Zingiber officinale* also exhibited a better hypoglycemic effect than the standard drug (Metformin).

The hypoglycemic effect of these products used in these hypoglycemic studies may be due to the presence of plant metabolites such as alkaloids, tannins, saponins, terpinoids and flavonoids as reported by some researchers (Platel, 1997; Abdel–Zaher *et al*., 2005; Sharma *et al*., 2009; Patel *et al*., 2015; Huang *et al*., 2010; Kumar *et al*., 2010; Kunyanga *et al*., 2011; Barrera, 2012; Manach *et al*., 2013).

Naturally occurring carbazole alkaloids isolated from Murraya koenigii have also been fingered as the anti-diabetic principles of that plant (Sharma *et al*., 2009). The anti-diabetic activity of *Gymnema sylvester* has been reported to be linked to the presence of glycosides contained therein (Verma *et al*., 2008). Bioactive flavonoid glycosides isolated from *Jatropha curcus* have also been shown to possess hypoglycemic and antidiabetic activity in streptozotocin-induced diabetic rats. Hypoglycaemic and hypolipidaemic potentials of the diterpenes from *Croton cajucara* have also been demonstrated in alloxanized rats (Silva et al., 2005).

This study also revealed that the standard drug (Metformin) may be used to manage chronic hyperglycemia, while the plant products could be used to manage chronic hyperglycemic conditions.

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CONCLUSION

The aqueous extracts of *Vernonia amygdalina* leaves, *Zingiber officinale* rhizome, and their combination have been found to significantly ($P<0.05$) reduce blood glucose levels in treated normoglycemic albino rats at 6, 12, 18, and 24 hours post oral administration. The standard drug (Metformin) significantly ($P < 0.05$) reduced blood sugar by 31.3% lower than that reduced by *Vernonia amygdalina*, *Zingiber officinale* and their combination 6 hours post oral administration. The hypoglycemic effect of these products used in this hypoglycemic study may be due to the presence of plant metabolites such as alkaloids, tannins, saponins, terpinoids and flavonoids as reported by some researchers.

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