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EVALUATION OF HYPOGLYCEMIC EFFECT AND PHYTOCHEMICAL CONSTITUENTS OF *VERNONIA AMYGDALIN* DEL. FAM. ASTERACEAE

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ABSTRACT: Purpose: Leaf of *Vernonia amygdalin* Del Fam. Asteraceae has been shown to have hypoglycemic activity. This work was designed to identify the secondary metabolites present in the plant leaf, test their lethality, and then determine the best solvent system for their extraction. **Methodology:** One kilogram of crushed leaves of the plant *Vernonia amygdalin* was extracted with 5.0 L of methanol using Sohlex extractor. The solvent systems used in thin layer chromatography were petroleum ether, N-hexane, diethyl ether, dichloromethane and methanol. The dichloromethane/Methanol fraction which showed the highest TLC separation was fractionated in a column packed with silica gel G254 and eluted gradiently at various ratio. Fractions that showed similar TLC characteristics on analysis were subjected to antidiabetic studies using Wister albino rats. LD50 was determined using Lorke,s method. Alloxan was used for hyperglycemic induction. Glibenclamide was used as the standard drug 1. Five groups of five rats per group were used. Blood glucose levels were determined using Accu check glucometer. Data was analyzed using analysis of variance with a randomized design at $p<0.05$. **Result:** Preliminary phytochemical analysis showed the plant contains alkaloids, saponins, flavonoids, terpenes, steroids. The LD50 of the extract is above 5000 mg/kg. The administration of Alloxan increased significantly $p<0.05$ the blood glucose level of rats. The crude methanol extract at the doses of 100 mg/kg and 200mg/kg gave percentage reductions of 60.53% and 53.08% of hyperglycemia within 24 h. Gglibenclamide gave 51.8% reduction. Methylene: methanol extract (7:3) gave the best separation. **Conclusion:** The findings showed that the extracts of *Vernonia* amygdalin have hypoglycemic activity.

INTRODUCTION: Medicinal plants are of utmost importance to the health of individuals and communities. The plant kingdom presents a wide field for prospecting effective and oral hypoglycemic agents.

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More than three hundred plant species have been reported to possess hypoglycemic activities [1]. The medicinal values of plants are derived from the fact that some of them contain some chemical substances that exert definite physiological action on the human system.

Herbal medicines, otherwise called phytomedicines are medicinal products of plant parts such as roots, leaves, flowers, barks, seeds, and fruits which are used to treat diseases and improve health [2]. The use of herbs as medicines pre-dates the use of synthetic drugs but the zeal to use herbal drugs seemed to

have declined due to the availability of numerous synthetic drug alternatives. However, overtime, several reports of adverse reactions due to synthetic drugs forced a return to natural medicines especially from herbs [3]. As more people return to herbal remedies, adulteration and substitution of these herbals are on the increase [4]. There is a need to ascertain, isolate, characterize, quantify and validate these compounds which are frequently desirable by pharmaceutical establishments for the manufacture of novel therapeutic remedies for the management of several health disorders [5]. The most important of these bioactive constituents are alkaloids, flavonoids, saponins, tannins, and other phenolic compounds. Secondary plant metabolites play important role in alleviating several ailments in traditional medicine and folk uses.

The vast and versatile pharmacological effects of medicinal plants are basically dependent on their phytochemical components [6].

The earliest known documentation of plant derived treatments for diabetes is present in the Ebbers Papyrus of about 1550 BC [7]. A plant becomes a medicinal plant only when its biological activity has been ethno pharmacologically reported or scientifically established [8]. Natural compounds of botanical origin are increasingly being investigated for the development of novel biocides [9]. Diabetes mellitus is a multisystem disease caused by increase in the sugar level of the blood.

It is a chronic non-communicable metabolic disorder which results from insulin deficiency or reduced effectiveness of insulin activity [10]. Waist circumference, body mass index, smoking habits, hypertension and total cholesterol level were significantly associated with Diabetes mellitus and these factors are potentially modifiable. Therefore, targeting the prevention strategy to such modifiable risk factors might reduce the prevalence of Diabetes mellitus in that area [11]. Increasing physical activity and promoting weight loss can reduce the risk for type 2 diabetes and improve pain management among adults with pre-diabetes and arthritis. Healthcare and public health PR actioners can address arthritis-specific barriers to physical activity among adults by promoting evidence based arthritic interventions (Sad oval) [12]. Chronic hyperglycemic patients live with a high risk of long term macro- and micro-vascular complications, such as cardiovascular diseases, nephropathy, retinopathy and neuropathy [13]. According to WHO speculations and projections, the prevalence of diabetes is likely to increase by 33%. Diabetes mellitus is worldwide growing epidemic disorder with 424.9 million people affected in 2017 and an estimated 48% increase in the number of diabetic individuals by the year 2045 [14]. The International Diabetes Federation (IDF) Atlas Guideline reports that currently, there are 352 million adults with impaired glucose tolerance who are at the risk of developing diabetes in the future. In 2017, it was estimated that 425 million people [15] suffered from diabetes mellitus and the number is expected to rise to 629 million by 2045 [16]. About 16% of affected pregnancies are in women with established pre-gestational diabetes mellitus and 84% in women with gestational diabetes mellitus [17]. From 2002-2012 type 1 and type 2 diabetes incidence has increased among U.S youths aged less than 20 years. From 2011-2015, both type 1 and type 2 diabetes incidence continue to increase among youths at 5 U.S sites included in the search for diabetes in youth study, especially among racial and ethnic minority populations [18].

Vernonia amygdalin Del Fam. (Asteraceae) known as bitter leaf is a small shrub of about 2-5 metres high. It has silvery skin and leaves with Characteristic bitter taste. The leaves are seriated, simple and finely glandular displaying few Lateral lines. Inflorescence is a capitulum producing dirty white flowers which are fragrant and usually bee infested. The aqueous extract of *Vernonia amygdalin* has demonstrated highest FECR(100%) compared to standard anthelminthic drugs (albendazole 99%, levamisole 96%, ivermectin 96%) against nematodes. Haemonchus contortus, *Trichostrongylus spp., Ostealgia ostertagi, oesophagostomum spp., Chabertia spp., Strongyloides* and *Paramphistomum spp*. tested [19]. Continued research on the pharmacological activity of *Vernonia amygdalin* has demonstrated its immunomodulatory activity in improving the level CD4 counts in both man and animals. Ezeonu *et al*. [20] analyzed the leaf extract of Vernonia amygdalin for its immunological effect in rats and reported a remarkable effect on CD4 count, haematological parameters and lipid profile.

Also, Olubumi *et al*. [21] evaluated concoctions containing ten medicinal plants including bitter leaf with potential antidiabetic activity for the presence of toxic elements. The anti-malaria effect of the plant to ascertain its use as antimalaria in traditional medicine has been verified by Challand *et al* [22].

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Collection and Preparation of the Plant Material: The leaves of *Vernonia amygdalin* were collected from Ejuona Obukpa area in Nsukka District of Enugu state in March 2014. The identity of the leaf was verified and ascertained by Mr. A.O Ozioko, a taxonomist with International Centre for Ethnomedicine and Drug development. A voucher number PCG/14/414 was assigned to the plant specimen and deposited in the herbarium of Department of Pharmacognosy and Environmental Medicine, University of Nigeria Nsukka, Enugu state Nigeria. The collected leaves were dried under shade and then crushed for size reduction. A 1 kg of the crushed leaves was extracted with 5.0 L of 95% methanol continuously in Soxhlet apparatus. The extract was concentrated under reduced pressure using rotatory evaporator.

Phytochemical Studies: The phytochemical classes of compounds in the extract were determined following standard method [23]. The phytochemical classes tested for are tannins, saponins, glycosides, carbohydrates, proteins, alkaloids, resins, flavonoids, terpenes and terpenoids, steroids and fats and oils.

Acute Toxicity: The oral acute toxicity test of the methanol extract was determined in mice using Lorke,s method [24]. Permission was obtained from the Animal use Ethics committee of the faculty of Pharmaceutical sciences, University of Nigeria Nsukka Nigeria with IAEC NO: FPSRE/UNN/14/0038 in accordance with the world animals' Ethics Charter of U.S.A. 1994. Nine rats were randomly divided into three groups (n=3). Orally administered to them were 10, 100, and 1000 mg/kg of methanol extract respectively and observed for 24 h for death. In the absence of mortality, 1600, 2900, and 5000 mg/kg of ME were administered through the same route to a fresh batch of the animals and the number of deaths in 24 h noted. The LD_{50} is estimated to the geometric mean of the highest non-lethal dose and the lowest lethal dose [25, 26].

Twenty-five Wilstar albino rats were used for this determination. The animals were randomly divided into five groups (n=5). Group1 received 2 mg/kg of distilled water with Tween 80 while group 11 received 5mg/kg of Glibenclamide. Groups 111 and 1V received 200, 400 mg/kg of methanol/ dichloromethane extract respectively. Same procedure was repeated using diabetic rats induced by intraperitoneal injection of 120 mg/kg of alloxan monohydrate at the same doses of methanol/ dichloromethane extract. Blood samples were collected from the tail veins of rats at 0, 1, 3, 6 and 12 h respectively after treatment. The blood glucose levels were determined using one touch Accucheck glucometer.

Statistical Analysis: Data obtained were analyzed using one-way analysis of variance (ANOVA) SPSS version 14 software and expressed as mean SEM. Differences between means were regarded significant at $p< 0.05$.

RESULTS:

TABLE 1: PRELIMINARY PHYTOCHEMICAL ANALYSIS

Alkaloids	+++
Carbohydrate/Reducing sugar	+++
Glycosides	+
Saponins	+++
Tannins	+++
Flavonoids	++
Steroids and Terpenoids	+++
Protein	+++
Fats and Oils	+
Resins	-

Key: +++= Highly abundant ++= Moderately abundant + = Low in abundance - Not present

TABLE 2: RESULT OF ACUTE TOXICITY TEST

	Phase II		Phase II		Phase III	
Treatment	Dose mg/kg	Mortality	Dose mg/kg	Mortality	Dose mg/kg	
Extract	10	0/3	2000	0/3	4250	0/3
	100	0/3	3000	0/3	4750	0/3
	1000	0/3	4000	0/3	5000	0/3

TABLE 3: THIN LAYER CHROMATOGRAPHY (TLC) ON DICHLOROMETHANE FRACTION (DCM)

Solvent Bands	No of	Visible color of Bands
Chloroform: Methanol	0	No color separation
Chloroform petroleum ether	2	Light green, green and yellow
Chloroform: n-hexane	3	Light green and yellow
Chloroform di ethyl ether	4	Green, light brown, yellow and yellowish brown
Methanol: Dichloromethane	5	Light brown, yellow, greenish yellow, thick brown and yellowish brown

TABLE 4: RF VALUES OF TLC BANDS OF DICHLOROMETHANE EXTRACTS USING METHANOL: (7:3)

Band	RF value	Color
1	0.140	Faint brown
2	0.288	Green
3	0.153	Yellow
4	0.510	Dark brown
5	0.598	Yellowish brown

TABLE 5: EVALUATION OF HYPOGLYCEMIC ACTIVITY OF THE DCM FRACTION, GLIBENCLAMIDE, TWEEN 20 AND NORMAL SALINE

Treatment	Mean Blood Sugar (MBS) Level (mg/dL)							Percentage reduction (%)
	0h	1h	3h	6h	9h	12h	24h	
100mg per kg	345.00±47.75	310.25±50.16	238.00±26.37	107.50±32.67	95.50±28.59	81.00±21.73	77.00±42.00	60.5374
200mg per kg	371.25±86.66	340.00±113.61	270.00±75.72	168.25±22.72	124.0±18.63	73.00±12.12	61.00±3.00	53.0829
300mg per kg	368.25±65.80	394.50±72.80	316.25±58.35	236.75±54.21	152.25±30.02	113.50±33.89	58.25±3.301	58.9927
Glibenclamide (10mg/kg)	165.5.81	158.4±1.92	137.54±3.14	79.30±4.51	85.65±0.54	90.5±1.60	78.32±1.40	51.78
Tween 20 (2mg/kg)	176.7±0.26	176.7±0.11	175.82±0.56	174.02±1.47	176.34±0.77	176.24±0.77	174.14±0.78	1.52

Values are expressed as plus ± minus SEM, n=5, percentage reduction of blood level shown at the last column.

DISCUSION: The result of phytochemical analysis shows that methanol extract of the plant contains tannins, alkaloids, and carbohydrates, reducing sugar, proteins, glycosides, saponins, resins, fats and oil. Acute toxicity test showed that the drug is safe at the maximum dose of 5000 mg/kg. This agrees with Lorke, s method of 1983. Also, TLC analysis showed RF values of 0.140, 0.288, 0.153, 0.150 and 0.598 at various ratios using gradient elusion technique. The percentage yield of the extract is 20%. This revealed that the result is in line with the standard recommended by the British Pharmacopoeia.

It was also observed that the methanol extracts ME gave 60.53% reduction in hypoglycemic activity within 24 h at the dose of 100 mg/kg while 200 mg/kg dose gave 53.08% within the same time range. The standard drug glibenclamide at this same dose yielded 51.80%. This is a clear evidence that the methanol extract compared favorably with the standard drug-glibenclamide [27]. It was equally observed that Metformin which belongs to the same class of sulphonylureas as glibenclamide exerted hypoglycemic effect by acting as insulin sensitizer. This is in agreement with the hypoglycemic effect of the methanol extract when compared with the effect of the standard drug glibenclamide. Moreover, it could also be inferred that another possible mechanism of action of the plant *Vernonia amygdalin* may be due its constituents which are rich in polyphenols known to possess antioxidant activity. The high percentage reduction of blood glucose level by the extracts is an indication that methylene chloride or dichloromethane is a good menstruum for extraction of hypoglycemic constituents of *Vernonia amygdalin.*

The hypoglycemic action of the extract could be due to a possible enhancement of the peripheral utilization of glucose or increase in the pancreatic secretion of insulin from the cells of Langerhans or its release from bound insulin [29]. This opinion led credence to the fact that despite pre-treatment of the rats with alloxan which was known to cause permanent destruction of pancreatic ß-cells [30], the hypoglycemic effect was still observed in alloxan induced diabetic rats. However, Ota and Uril [31]. suggested that another approach to treat hyperglycemic condition is to use drugs that lower the glucose absorption by competitively inhibiting intestinal carbohydrate hydrolyzing enzymes and to increase inflammatory conditions involved in the pathology of type 2 diabetes. Also, some studies suggest that increase in weight could be attributed

to the protective effect of the plant extract against degradation of structural proteins, lipids, and muscle wasting, possibly due to improvement of glycemic control *via* enhancement of insulin secretion or /and action [32]. From the thin layer chromatographic studies, the methanol-dichloromethane extract 7:3 yielded the best separation of the methanol extracts with five visible bands.[33] reported that *Vernonia amygdalin* showed the capacity to stimulate glucose uptake in brain tissues thereby enhancing antioxidative activities and modulation of functional chemistry and dysregulation of metabolic pathways. It has also been demonstrated by *ex-vivo* analysis of infusion of the plant extract, that *Vernonia amygdalin* had the ability to increase glutathione level, superoxide dismutase enzyme and catalase activities while concomitantly depleting malondialdehyde level and DNA fragmentation in Iron 11 induced hepatic injury [34].

Vernonia amygdalin showed neuroprotective action on Nitrobenzene induced neurodegenerative disease in Wistar albino rat [35]. Akah [36] reported the presence of saponins in the aqueous leaf extract of the plant. Saponins present in plants have been suggested to act as possible anticarcinogens. There is a need to ascertain, isolate, characterize, quantify and validate these compounds which are frequently desirable by pharmaceutical establishments for the manufacture of novel therapeutic remedies for the management of several health disorders [37].

The presence of phytochemicals in plants has been indicated to be responsible for their hypoglycemic activities. Plants secondary metabolites encompass several classes of structurally diverse natural products arising from the Shikimate-phenylpropanoids flavonoid pathways. Plants require these compounds for pigmentation, growth, reproduction, resistance to pathogens and for many other functions and they represent the adaptive characteristics that were subjected to natural selection during evolution [38]. Slaven *et al* [39] and [40] have investigated and reported that treatments with microsphere loaded with chemical and biological agents were used for enhancing secondary metabolite production in plant cell culture. Higher plant secondary metabolite share would also have an important impact on human health by improving the antioxidant and nutrient intake through the human diet [41]. Inoculation with *Arbusca mycorrhizal* fungi enhances phenolics content and increases the antioxidant activity of the lettuce leaves, but efficient formulation demands a carrier material for living micro-organism which must keep its functional properties after application. Yaduma *et al.* [42] reported that *Khaya senegalensis* and *Vernonia amygdalin* have high anthraquinone content in the stem-bark extract with *Annona senegalensis* stem bark in significant quantity. Gastroprotective effect of Methanol extract of *Vernonia amygdalin* has a protection in aspirin induced ulceration in the GIT. This was done by increased PH of the gastric juice and decreased secretion and reversed ulceration of the hematological parameters [43]. Phytochemicals with antioxidant activity were also proven to revert atherosclerotic cardiovascular complications such as coronary artery diseases by reducing the elevated serum lipid levels in diabetic patients [44].

CONCLUSION: Leaf of *Vernonia amygdalin* Del. Fam. Asteraceae possesses hypoglycemic potential and this justifies its use in Ethnomedicine for the treatment of diabetes mellitus and as an admixture in the preparation of other traditional diabetic remedies.

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CONFLICT OF INTEREST: The authors hereby declare that there was no conflict of interest whatsoever, during and after the compilation of this paper prior to its submission for publication.

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