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## Extraction solvent polarity affects the antidiabetic activity of *Dioscorea bulbifera* L. (Dioscoreaceae) Tuber

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**Abstract**

This study investigated the antidiabetic activities of different methanolic concentrations of *D. bulbifera* extract. The pulverized dried tubers of *D. bulbifera* were cold macerated with different concentrations of methanol in water 30, 50, 70 % v/v. Hyperglycemia was induced by the administration of a single intraperitoneal dose of alloxan (120 mg/kg). Hyperglycemic mice were treated with various extracts at 50 and 200 mg/kg for 10 days. The trend of activity for the extracts was 70>50>30% methanol extract. Comparison within treatments revealed that 70% methanol extract exhibited significant difference compared to other concentrations at 50 mg/kg on Day 10 and with the 30% extract from days 6-10. At 200 mg/kg, 70% methanol extract showed higher antihyperglycemic activity than the standard (metformine) from day 3-10. The methanol concentration (70%) extracted more saponins compared to other concentrations of methanol. Abundance of saponins may therefore explain higher antidiabetic activity recorded by this extract.

**Keywords:** *Dioscorea bulbifera*, Diabetes, Solvent polarity, hyperglycemia

**Introduction**

Diabetes, characterized by hyperglycemia is a group of metabolic diseases that results from defects in either insulin secretion, insulin action, or both [1]. Diabetes is one of the top 10 causes of death and reduced life expectancy globally [2]. According to Shaw *et al.* [3], diabetes is being recognized as a world-wide epidemic as its prevalence has been persistently rising for the last few decades. If left untreated or not controlled properly, this metabolic condition may cause kidney failure, blindness, lower limb amputation, and other long-term consequences that impact significantly on the quality of life [4].

Natural products have contributed immensely and continue to play an invaluable role in the treatment of various ailments and in drug discovery process [5]. It has provided and remained a source of novel compounds with diversified structural arrangements. The advantages of drugs from natural products numerous as they are usually considered to be safer, cheaper, readily available and occasionally more efficacious than purely synthetic ones [6]. Presently, available typical therapies for diabetes are challenged by their inherent limitations and medicinal plants are being researched as a source of alternative therapies [7].

Plants provide one of the most essential source of antidiabetic compounds [8]. Herbal remedies continue to be more accessible and affordable than conventional antidiabetic drugs in many regions of the world. There has also been a surge in the demand for herbal remedies in societies with actually well-developed and modern health care systems to complement prescribed, modern therapies for several diseases, including diabetes [9]. *Dioscorea bulbifera* is one of the distinctive medicinal plants among the 600 species in the family dioscoreaceae which has found its importance in traditional medicine throughout the world [10]. Its aqueous extract has been reported to show antihyperglycemic activity in C57BL/6J mice and streptozotocin (STZ) treated Wistar rats [11]. Diosgenin isolated from *D. bulbifera* has been developed as a novel drug against type II diabetes [12].

Extraction is a crucial preliminary step in the analysis of medicinal plant as it is necessary to extract the desired bioactive component from the plant material for further separation and characterization. The selection of solvent systems largely depends on the specific nature of the bioactive compound being targeted [13]. Different solvent systems are available to extract the bioactive molecules from natural compounds. Optimized extraction conditions in a binary solvent system entails varying the composition of the system to achieve the most efficient solvent composition.

This invariably results in qualitative and quantitative bias among different systems [14]. This is also supported by the principle of 'like dissolves like', where bioactive molecules responsible for the desired pharmacological effects will have the same polarity with the most efficient solvent mixture. To this end, this present work was set to investigate the antidiabetic activities of three different methanolic concentrations of *D. bulbifera* extract in alloxan-induced diabetic mice.

## Materials and Methods

### Plant sample

Fresh tubers were collected in March, 2019 from a local market in Umuoji, Idemili North Local Government Area of Anambra State, and identified by Mrs. Amaka Onwudili of Pharmacognosy Department, Faculty of Pharmaceutical sciences, Nnamdi Azikiwe University Awka, Anambra State, Nigeria. The plant sample was dried at room temperature and pulverized into coarse powder.

### Experimental animals

Adult albino mice of either sexes, aged 12 weeks with body weights 23-28 g were obtained from the Animal House of the Department of Pharmacology and Toxicology, Faculty of Pharmaceutical sciences, Nnamdi Azikiwe University. They were housed in cages; and allowed free access to drinking water ad libitum and were fed with standard laboratory diet (UAC feed, Nigeria). The experiment was commenced after acclimatizing the animals for one week.

### Extraction of Plant material

The pulverized sample was cold macerated with different concentrations of methanol in water thus: 30% (methanol: water 30:70 v/v), 50% (methanol: water 50:50 v/v) and 70% (methanol: water 70:30 v/v). To each of the concentrations, 300 g of the pulverized plant sample was macerated in 1.5 liters of the solvent for 48 h. It was sieved using muslin cloth and filtered with no 1 whatman filter paper. The filtrate was concentrated using rotary evaporator at 50 °C. The concentrated extracts were kept in airtight containers in a refrigerator (2-4 °C) until used.

### Phytochemical Analysis

The standard methods as described by Odoh *et al.*, was employed in the qualitative phytochemical analysis of the extracts [15].

### Acute toxicity and Lethality test

The acute toxicity and lethality of the crude extracts was determined in mice using Lorke's method [16]. A total of 13 mice were used for each extract obtained with various methanol concentrations; and the study was done in two phases. Phase 1 comprised of three groups of three mice per group. The groups received 10 mg/kg, 100 mg/kg and 1000 mg/kg respectively. The animals were constantly monitored for the next one hour, intermittently for the next 3 hours and finally at 24 hours for behavioural changes and mortality. From the result of the first phase, the second phase was carried out. In this phase, 4 groups of one mouse each were used. The groups received 3000, 4000 and 5000 mg/kg of the extract respectively. The animals were monitored as in phase one for behavioural changes and mortality.

### Hypoglycemic study

The hypoglycemic effect of hydro-methanol extract of

*Dioscorea bulbifera* was studied using alloxan induced hyperglycemia. Forty adult albino mice were used. They were grouped into eight groups of five mice per group. The animals were starved for 18 h, having access to drinking water prior to the experimental day. Their fasting blood glucose (FBG) was measured using One Touch® glucometer. Hyperglycemia was induced by the administration of a single intraperitoneal dose of alloxan (120 mg/kg), 24hrs after the alloxan administration, the animals received 2000 mg/kg glucose monohydrate orally. At 72 h post-alloxan induction, the FBG of the animals were checked to confirm hyperglycemia (FBG>160 mg/dl). The animals received treatment as follows:

Groups 1 and 2 received 50 and 200 mg/kg of 30% methanol extract while groups 3 and 4 were given 50 and 200 mg/kg of 50% methanol extract. Similarly, groups 5 and 6 received 50 and 200 mg/kg 70% methanol extract respectively. Groups 7 and 8 serving as control received 5 ml/kg 5% Tween 80 (vehicle control) and 500 mg/kg metformin (reference drug control) respectively. Treatment by daily oral route administration was adopted. Fasting blood glucose levels were measured subsequently on day 3, day 6, and day 10 of the treatment period.

### Statistical analysis

Statistical analysis was carried out using numerical data gotten from the study expressed as the mean values  $\pm$  standard error of mean. Differences among means of control and tested groups were determined using one-way analysis of variance (ANOVA). A probability level of less than 5 % ( $p < 0.05$ ) was considered significant.

## Results

### Phytochemical constituents

The Result of the distribution of phytochemicals in the extracts obtained with various methanol concentrations is as presented in Table 1. Alkaloids, flavonoids, tannins, saponins, steroids, terpenoids and cardiac glycosides were present in the extracts while tannins and steroids were absent. Alkaloids increases with decrease in methanol concentration while saponins increases with increase in methanol concentration making both phytochemicals to be abundant in 70% and 30% methanol extracts respectively. Other phytochemicals were evenly distributed in the extracts obtained with various methanol concentrations.

**Table 1:** The distribution of phytochemicals in extracts obtained with various concentrations of methanol

Phytochemicals	Hydro-methanol 70:30 v/v (30%)	Hydro-methanol 50:50 v/v (50%)	Hydro-methanol 30:70 v/v (70%)
Alkaloids	+++	++	++
Saponins	+	++	+++
Tannins	-	-	-
Flavonoids	++	++	++
Steroids	-	-	-
Terpenoids	++	++	++
Cardiac glycosides	+	+	+
Proteins	-	-	-
Carbohydrates	++	++	++
Reducing sugars	++	++	++

Where + = present; ++ relatively abundant; +++ abundant; - absence

### Median lethal Dose

No clear signs of toxicity or mortality were observed in all the extract-treated groups at all the tested doses. The median lethal dose (LD<sub>50</sub>) of the extracts in mice was estimated to be above 5000 mg/kg.

### Hypoglycemic effects of the extracts in alloxan induced diabetes

The animals showed pre-diabetic fasting blood glucose (FBG) of less than 100 mg/dl. However, 72 h post administration of alloxan produced elevated fasting blood glucose in the animals that were above 200 mg/dl (Figure 1). The extracts showed significant ( $p < 0.05$ ) reduction in blood glucose compared to the vehicle control group at both 50 and 200 mg/kg from days 3 – 10 (Figure 2). The trend of activity for the extracts based on the concentration of methanol used for the extraction was 70% > 50% > 30% v/v. Comparism within

treatments revealed that extract obtained with 70% methanol exhibited significant ( $p < 0.05$ ) difference with other methanol combinations at 50 mg/kg on Day 10 and with the 30% methanol obtained extract from days 6 - 10. At 200 mg/kg, 70% methanol extract showed higher antihyperglycemic activity than the standard (metformine) from day 3-10. Significant ( $p < 0.05$ ) differences between the 70% methanol extract and extracts obtained with other methanol concentrations (50 and 30%) were also detected from days 3-10 at 200 mg/kg.

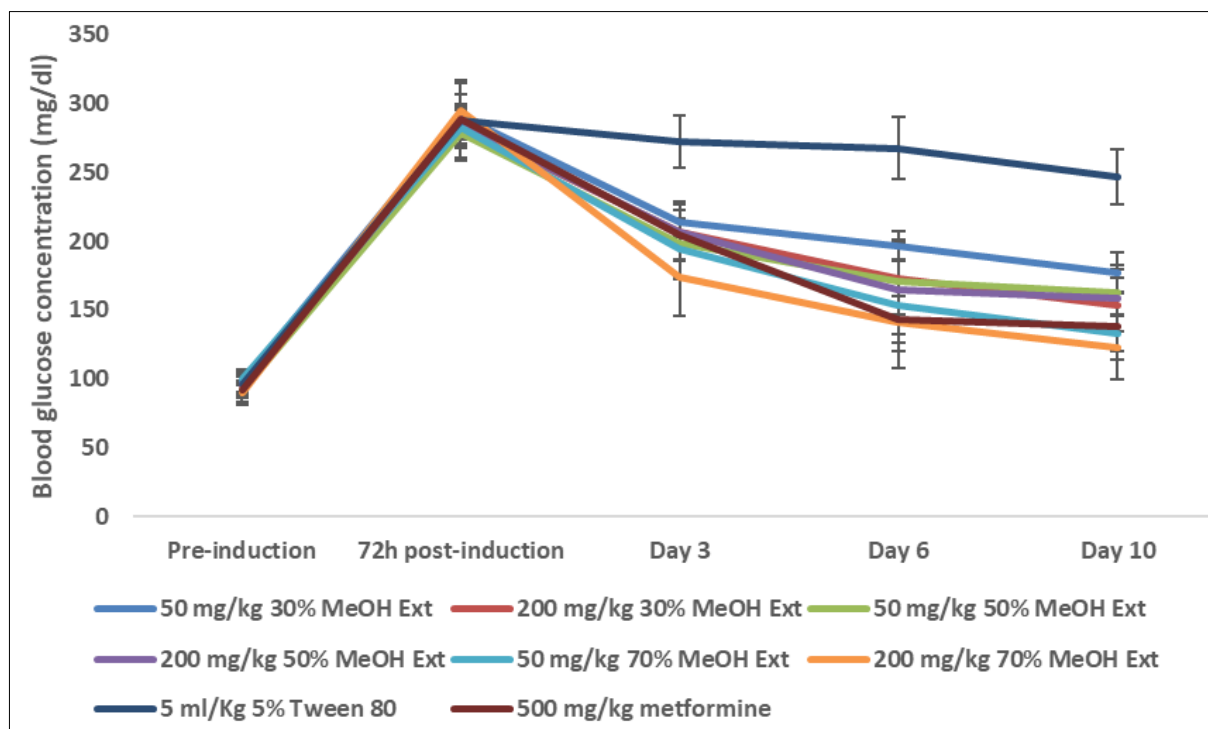
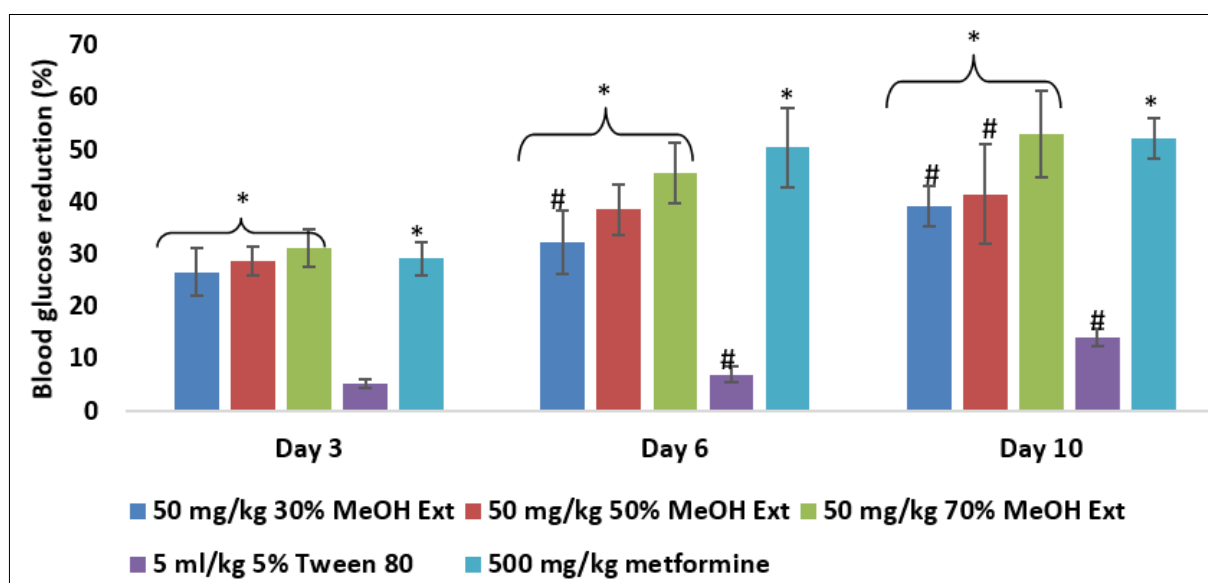
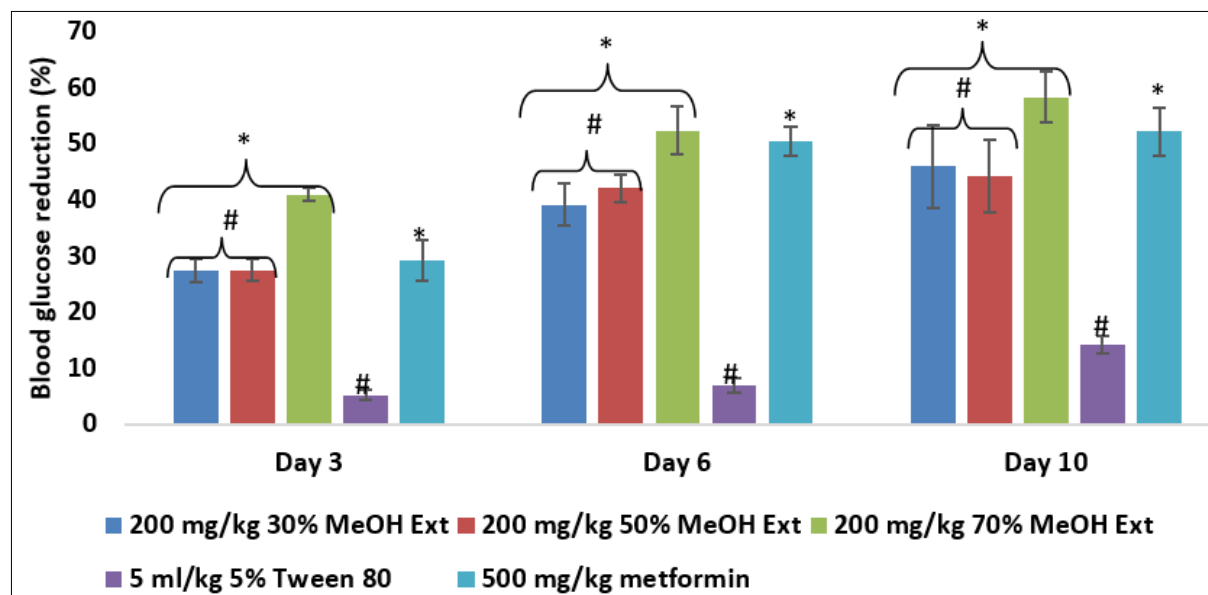


Fig 1: Effect of methanol extracts (MeOH Ext) on blood glucose concentration





\*  $p < 0.05$  compared to 5 ml/kg 5% Tween 80 (vehicle control group); #  $p < 0.05$  compared to 70% methanol extract (70% MeOH Ext)

Fig 2: Effect on methanol extracts on blood glucose reduction

## Discussion

Bioactive phytochemicals are obtained from plant materials mainly through extraction process. However, this technique is subject to different factors that influence the process efficiency such as the type and concentration of solvent used in the extraction process [17]. Bioactive compounds can commonly be extracted by means of different organic solvents or a mixture of these solvents with water [18].

Among the available organic solvents, methanol has been widely used for extraction of phytochemicals because of its high polarity and its ability to produce high extraction yields [18]. It also requires lower temperature for concentration when compared to other polar solvents. Owing to the variety of bioactive compounds contained in plant materials and their differing solubility properties, varying solvent polarity through alteration of its concentration with water affects the type and quality of phytochemicals that can be extracted from plants [19].

Saponins are plant secondary metabolites with wide pharmacological applications. Plant species containing saponins, as well as isolated saponins phytochemicals and saponin rich extracts have been widely patronized in the ethnomedicine especially in the treatment of diabetes [20]. This phytochemical has been reported to stimulate secretion of insulin as well as its actions, regeneration of beta cells islets and activation of enzymes which are responsible for glucose utilization [21]. The superior blood glucose lowering effect of the saponin rich extract of *D. bulbifera* may be explained by the concentration of this phytochemical in the hydro-methanol (30:70) extract.

The mechanisms underlying blood glucose lowering effect of *D. bulbifera* extract and isolated compound have been reported by other studies [22]. The active principle has also been identified as diosgenin (steroidal saponine), a major phytoconstituent of *D. bulbifera* [23]. Diosgenin from *D. bulbifera* has been reported to exhibit  $\alpha$ -amylase and  $\alpha$ -glucosidase inhibitory effect [22]. It was also reported to exhibit a significant glucose lowering effect after supplementation in Wistar rats [24]. Similarly, diosgenin from other dioscorea species (*D. esculanta*) was also reported to control hyperglycemia in the type 1 diabetes rat model

through an increased muscular GLUT4 translocation as well as activation of muscular GLUT4 signaling pathway by increased phosphorylation of key proteins [25]. Diosgenin have application in other types of diabetes like gestational diabetes. It has been reported to improve gestational diabetes in pregnant mice by improving glucose, insulin tolerance and increased hepatic glycogen content [26]. Given these documented scientific findings about saponins in general and diosgenin saponin isolated from *Dioscorea bulbifera* and other dioscorea species, it is not surprising that the saponin rich extract produced by extraction with 30:70 v/v hydro-methanol produced superior antidiabetic activity more than other extracts with lower abundance of this phytochemical. The extracts were also found to exact blood glucose lowering effect in a trend that follows the degree of abundance of its saponin content.

Alkaloids are more soluble in water than alcohol and a higher aqueous extractive value in water has been reported by other studies [27] which are consistent with the outcome of this study. Different alkaloids from medicinal plants have been reported to affect multiple targets to lower hyperglycemia associated with both type I and II diabetes mellitus [28]. Carbazole alkaloids like koenimbine, koenidine, mahanimbine have been reported to stimulate glucose uptake through increased translocation of GLUT4 on skeletal muscle cell surface [29]. Alkaloids like trigonelline have been reported to decrease blood glucose in alloxan induced diabetes through regeneration of islet cells [30]. Other alkaloids such as conophylline and ephedrine have also been reported to possess the ability to regenerate  $\beta$ -cells following their chemical destruction [31, 32]. Although alkaloids have been reported for their antidiabetic activities, the abundance of this phytochemical in the extracts obtained with other methanol concentrations failed to show better antidiabetic activity compared to saponin rich extract obtained with 70% methanol solvent. This observation suggested that the antidiabetic lead phytochemical of *D. bulbifera* may be saponin which may have worked synergistically with other phytochemicals.

## Conclusion

*D. bulbifera* showed hypoglycemic activity that was superior in 70% methanol extract. This methanol concentration



extracted more saponins compared to other concentrations of methanol. Abundance of saponins may therefore explain higher antidiabetic activity recorded by the extract obtained with 70% methanol which further supports saponins as the lead antidiabetic principle in *D. bulbifera*.

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