



Comparative Effects of Solvents on the Herbal Extraction of Antidiabetic Phytochemicals

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Authors' contributions

This work was carried out in collaboration among all authors. All the authors contributed to the success of the work and its publication. Author SCI designed and supervised the work and edited the first draft. Author CVO participated in carrying out the work and she wrote the first draft. Author CII contributed in carrying out the laboratory analyses. Author WCO participated in collecting the materials used and author ECC participated in the statistical analysis. All authors read and approved the final manuscript.

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ABSTRACT

Aims: The study determined and compared the herbal extraction yields using water, ethanol and hydromethanol solvent and the solvent extracting the highest antidiabetic constituents.

Place: The study took place in the Department of Chemistry (Organic Laboratory), Federal University of Technology Owerri, Nigeria.

Methodology: The antidiabetic contents of *Moringa oleifera* (Moringa) and *Vernonia amygdalina* (bitter leaf) were extracted by soaking using water, ethanol and hydromethanol (1:1) as solvents. The phytochemicals analysis was done both qualitatively and quantitatively (using Spectrophotometer (UV-V15)). Data collected were statistically analysed using SPSS version 10 tools.

Results: The crude ethanolic extraction was found to give the highest extract yield of 46.06% and

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38.91% from Moringa and bitter leaf, respectively. There were more phytochemicals obtained from Moringa (28+) than from Vernonia crude extracts (21+). The antidiabetic phytochemicals identified in both plants included Steroids, Phenols, Cardiac glycosides and Terpenoids. Ethanol extracted the Glycosides, Terpenoids and Phenols in relative abundance.. Hydromethanol solvent extraction yielded the highest concentrations of Steroids from Moringa (59.87mg/100g) and bitter leaves (75.43mg/100g) as well as highest extraction of Cardiac glycosides from both plants. Water extracted the highest concentrations of Phenols from both Moringa (0.32mg/g); bitter leaf (0.25mg/g) and Terpenoids from Moringa.

Conclusion: This study suggests that the choice of solvent (s) for phytochemical extraction (s) should consider factors such as the plant material (s) and the phytochemical (s) involved. So, Water > Hydromethanol > ethanol could be used for extracting phytochemicals for diabetes therapy.

Keywords: Extraction; solvents; antidiabetic; phytochemicals; moringa sp.; vernonia sp.

1. INTRODUCTION

Medicinal plants are used in the treatment and prevention of diseases in various parts of the world. The therapeutic use of natural products from indigenous plants for ethnomedicinal and nutritional purposes had grown tremendous interest among scientists who now search for bioactive components [1,2].

Diabetes is a chronic disease characterized by a group of metabolic diseases associated with high blood sugar (hyperglycaemia), which occurs either when the pancreas does not produce enough insulin or when the body cannot effectively use the insulin it produces [3,4]. Diabetes mellitus, a globally prevalent non-communicable disease is currently the fourth leading cause of death in most developed countries [5]. Without effective prevention and control programs, the incidence of diabetes mellitus will continue to increase worldwide. Recent epidemiological studies indicate that the total number of patients affected by diabetes in 2004 was close to 190 million, a fig. likely to have reached 325 million (an increase of more than 70%) by 2005 [6]. The figs. show that the disease is a global health concern that requires serious effort by all nations towards arresting its scourge. Type 2 diabetes is one of the fastest growing public health problems in both developed and developing countries. It is estimated that the number of people with diabetes in the world will double in coming years, from 171 million in 2000 to 366 million in 2030 [7].

The success so far in the use of phytomedicinal products like Metformin in Diabetes II treatment justified the continued search for phytochemicals with more effective and safer antidiabetic actions

[8,9]. However, it is often common to observe that researchers on phytomedicines use different solvents in the extraction of the phytochemicals [10,11,12,13,14]. It is therefore pertinent to assess and compare the extractive yields of the commonly used solvents and their antidiabetic phytochemicals.

2. MATERIALS AND METHODS

2.1 Plants

Fresh leaves of *Moringa oleifera* and *Vernonia amygdalina* were obtained from local markets in Owerri-West Local Government Area of Imo State, Nigeria.

2.2 Chemicals

Chemicals used in this study included Methanol (extra pure) manufactured by LobaChemie LTD.107, Wodehouse road, Mumbai 400005, India. Ethanol (absolute for analysis) by Merck KGA, 64271 Darmstadt Germany.10% ferric chloride, chloroform, conc. sulphuric acid, acetic acid.

The ethanol and methanol were purchased at Kentin Company LTD Okigwe road, Owerri, Imo state.

2.3 Equipment

The equipment used were obtained from the Department of Biochemistry, Federal university of Technology, Owerri (FUTO).These included weighing scale, conical flasks, stirrer, measuring cylinder, hot plate, water bath, test tubes, electric grinder and Spectrophotometer (UV-V15) with the model no- N4S, manufacturer – Searchtech Product no – 477517060317060004.

2.4 Preparation of Extracts

Fresh leaves of *Moringa oleifera* and *Vernonia amygdalina* were collected. They were air dried at room temperature for two weeks. The dried leaves were grinded into fine powder form using electric grinding machine. The powdered forms were extracted separately using the solvents: boiled water, ethanol and hydromethanol (50:50) in the soaking method [15]. The samples were soaked with each of the solvents for 48 hours and filtered using a muslin cloth and the solvents were evaporated using water bath.

2.5 Determination of Percentage Yields of the Extracts

The percentage yields of the extracts were determined by weighing the fine powder of the leaves before extraction and also weighing after extraction, then the percentage yield was calculated using the formula:

$$\text{Percentage yield of extract} = \frac{\text{Final weight of extract} \times 100}{\text{Initial weight of ground powder}}$$

2.6 Phytochemical Tests on the Extracts

Some phytochemical tests were carried out on the crude extracts to determine the presence of steroids, glycosides, phenols, terpenoids, alkaloids, flavonoids, oxalates, tannins, saponins. Phytochemicals were determined

qualitatively using the methods described [16,17].

The phytochemicals were also determined quantitatively using standard methods [18,19,20].

2.7 Quantitative Estimation of Steroids

1ml of the extract was transferred into 10ml of volumetric flask. Sulphuric acid (4N,2ml) and Iron (III) chloride (0.5%,2ml) were added, followed by potassium hexacyanoferrate(III) solution (0.5%,0.5ml).The mixture was heated in a water bath maintained at 70°C for 30 minutes with occasional shaking and diluted with distilled water. The absorbance was measured.

2.8 Statistical Analysis

Data collected were statistically analysed using SPSS 11.5 software for Analysis of the Variance (ANOVA). The T-test was used to compare two sets of data obtained on two comparable variables. Significant means were compared using Fisher's Least Significant Difference (FLSD) at 5% probability.

3. RESULTS

Figs. 1A and 1B shows the percentage crude extract yields of the three extraction solvents from the powdered samples of air-dried *Moringa oleifera* and *Vernonia amygdalina* leaves.

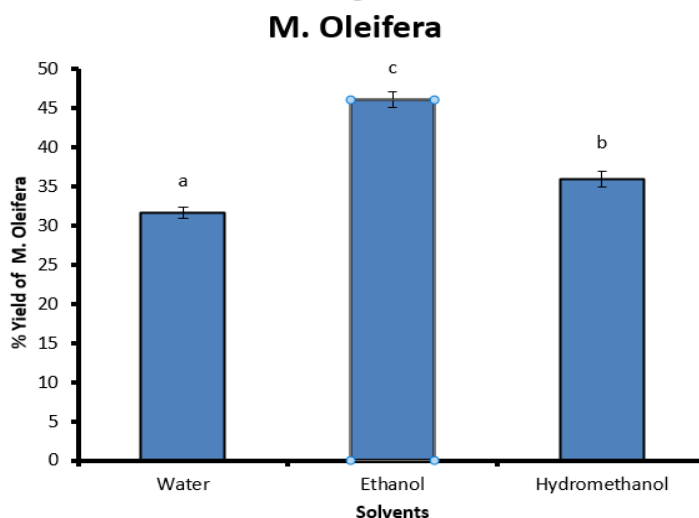


Fig. 1A. Comparing the percentage crude extract yield of *Moringa oleifera* using different solvents

Ethanol gave the highest crude extract yields of 46.06% and 38.91% from *Moringa sp.* and *Vernonia sp.*, respectively, followed by hydromethanol yields, as shown in Figs. 1A and 1B.

Table 1 showed that the three solvents could extract all the tested antidiabetic phytochemicals. All the phytochemicals tested positive in the two crude extracts. However, there were more phytochemicals obtained from *Moringa* (28+) than from *Vernonia* crude extracts (21+). The Glycosides and Phenols had their abundant yields with ethanol solvent in *Moringa sp.* whereas Steroids and Terpenoids had their abundant yields with water solvent in *Moringa sp.* mainly. Hydromethanolic yield of Glycosides in *Moringa* and Steroids in *Vernonia* were in comparative abundance.

Figs. 2A – 5B compared the antidiabetic phytochemical concentrations present in *Vernonia amygdalina* (VA) and *Moringa oleifera* crude leaf extracts using different solvents.

In Fig. 2A, the extractions of Steroids by Hydromethanol and water are significantly higher than Ethanol extraction.

In Fig. 2B, the extractions of Steroids by Hydromethanol and water solvent are significantly higher than Ethanol extraction solvent.

In Fig. 3A, the extractions of Cardiac glycosides by Hydromethanol and Ethanol are significantly higher than Water extraction.

In Fig. 3B, the extractions of Cardiac glycosides by Hydromethanol and Ethanol are significantly higher than Water extraction.

In Fig. 4A, the extractions of Terpenoids by Ethanol and Water are significantly higher than Hydromethanolic extraction.

In Fig. 4B, the extractions of Terpenoids by Ethanol and Water are significantly higher than Hydromethanolic extraction.

In Fig. 5A, the extractions of Phenols by Water and Ethanol are significantly higher than Hydromethanolic extraction.

In Fig. 5B, the extractions of Phenols by Water and Ethanol are significantly higher than Hydromethanolic extraction.

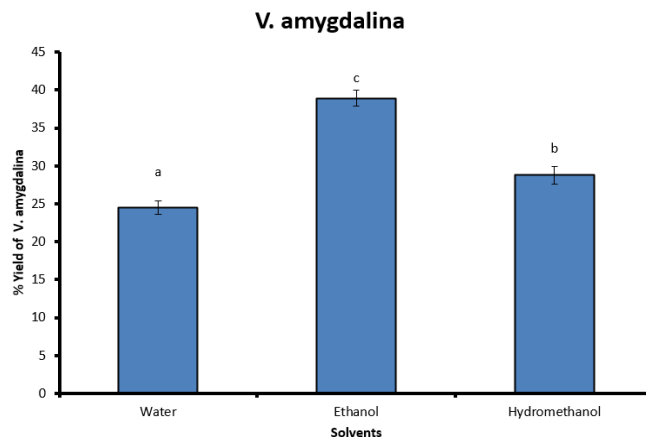


Fig. 1B. Comparing the percentage crude extract yield of *Vernonia amygdalina* using different solvents

Table 1. Phytochemicals present in *Moringa oleifera* (MO) and *Vernonia amygdalina* (VA) leaf extracts using different solvents

Phytochemicals tested	MO Leaf Extracts			VA Leaf Extracts		
	Water	Ethanol	Hydromethanol	Water	Ethanol	Hydromethanol
Steroids	+++	++	++	+++	++	+++
Glycosides	++	+++	+++	+	+	++
Phenols	++	+++	+	+	++	+
Terpenoids	+++	++	++	++	++	+

(+++ = abundant, ++ = less abundant, + = minute)

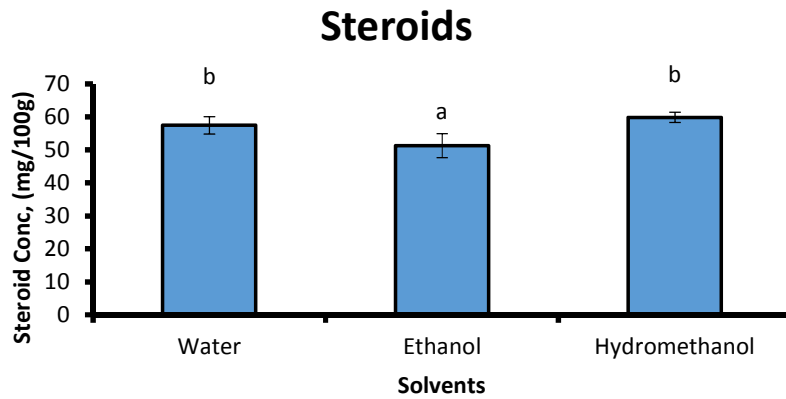


Fig. 2A. Comparing the concentrations of Steroids extracted from *Vernonia amygdalina* using different solvents

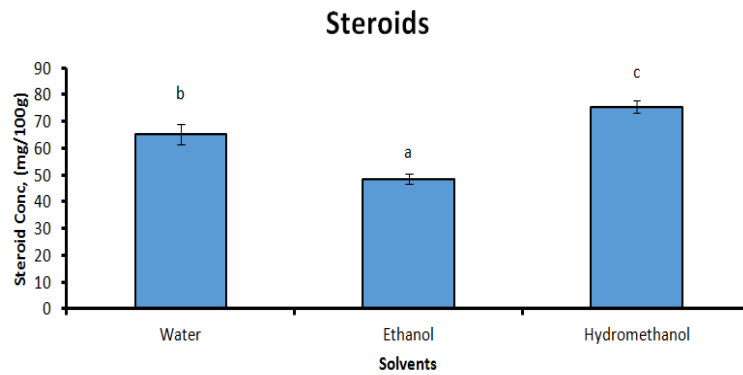


Fig. 2B. Comparing the concentrations of Steroids extracted from *Moringa oleifera* leaves using different solvents

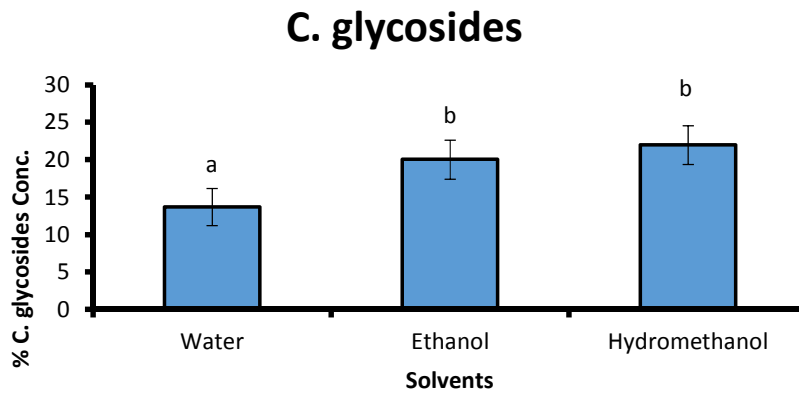


Fig. 3A. Comparing the concentrations of Cardiac glycosides extracted from *Vernonia amygdalina* using different solvents

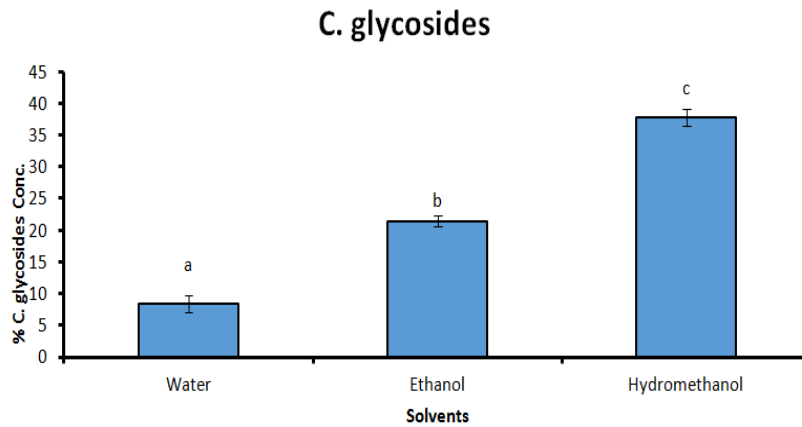


Fig. 3B. Comparing the concentrations of Cardiac glycosides extracted from *Moringa oleifera* leaves using different solvents

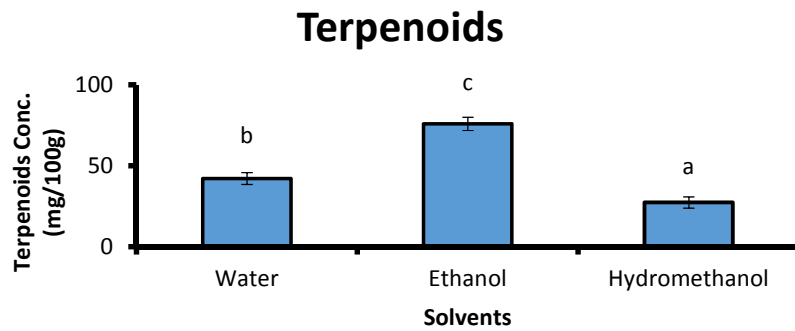


Fig. 4A. Comparing the concentrations of Terpenoids extracted from *Vernonia amygdalina* using different solvents

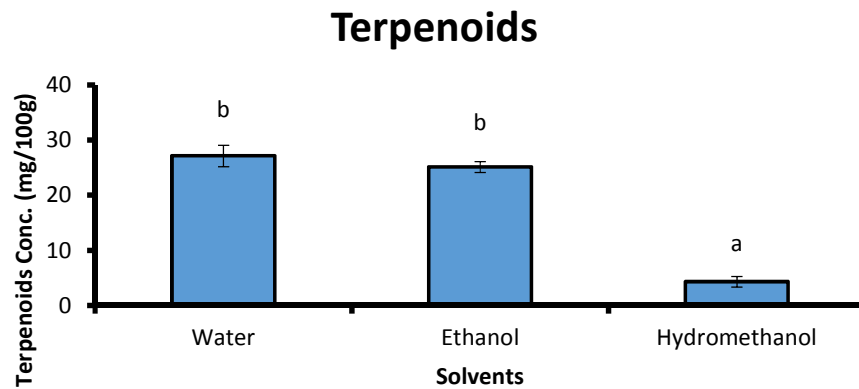


Fig. 4B. Comparing the concentrations of Terpenoids extracted from *Moringa oleifera* leaves using different solvents

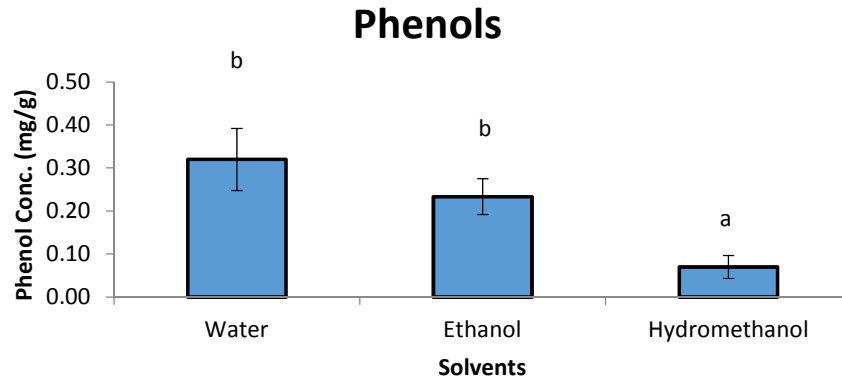


Fig. 5A. Comparing the concentrations of Phenols extracted from *Vernonia amygdalina* using different solvents

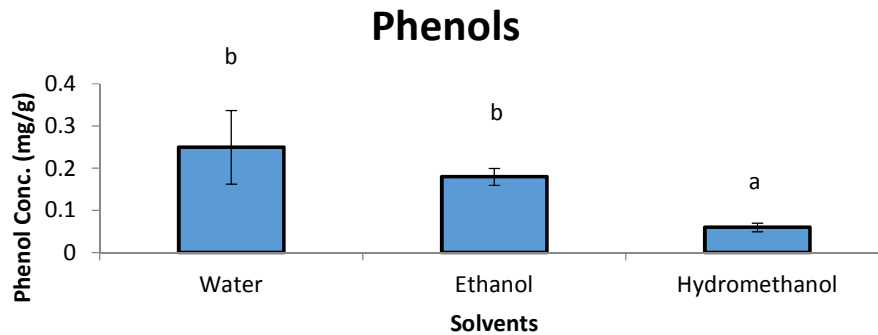


Fig. 5B. Comparing the concentrations of Phenols extracted from *Moringa oleifera* leaves using different solvent

4. DISCUSSION

4.1 Crude Extraction of Antidiabetic Phytochemicals from *Moringa Oleifera* Leaves Using Different Solvents

The soaking extraction method was adopted in this study because most herbs are commonly prepared in the developing world by soaking with different solvents. From previous studies, the antidiabetic phytochemicals present in *Moringa oleifera* are Steroids, Terpenoids and Phenols [21,22,23,24].

Phenols are classified into different groups as a function of phenol rings in the structure and their main classes include phenolic acids, flavonoids, stilbenes and lignans [25]. Flavonoids are the

most studied group of polyphenols. There are various plant-derived phenolic compounds which are currently used for treatment and inhibition of inflammatory pathways that are important in diabetic prevention strategies. These include: Apigenin [26] Diosmin [27] Quercetin [28]. Kaempferol [29] Narcinegin [30] Catechin [31,32] Curcumin [33,34].

A study showed that single and combined extracts of *Moringa* and bitter leaves have hepatoprotective effects in streptozotocin (STZ) induced diabetes using Wistar strain rats and may be safer in preventing diabetes induced damage to the liver [35]. The antidiabetic content of these plant leaves are extracted with the aid of solvents.

From this study, Hydromethanol extraction yielded highest concentrations of Steroids and

Cardiac glycosides from *Moringa* leaves whereas Water solvent extraction yielded the highest concentrations of Phenols and Terpenoids from *Moringa* followed by Ethanol solvent.

4.2 Crude Extraction of Antidiabetic Phytochemicals from *Vernonia amygdalina* Leaves Using Different Solvents

The antidiabetic contents determined in *Vernonia amygdalina* are Terpenoids, Phenols and Steroids [36]. Plants steroids or steroid glycosides are some of the most naturally occurring plant constituents that have found therapeutic applications [37]. Diosgenin and Cevadine are examples of plants steroids [38].

Terpenoids are among the most widespread and chemically diverse groups of natural products. They are classified as mono-, di-, tri-, and sesquiterpenoids depending on the number of carbon atoms. Monoterpenes include terpinen-4-ol, camphor, menthol, limonene, diterpenes are considered to be resins, taxol, forskolin. Triterpenes include α - amyrin, ursolic acid and oleanic acid, sesquiterpene, e.g. sesquiterpene lactones, artemisinin [39].

A study reported the antihyperglycemic effects of bitter leaf in diabetes and suggested that the consumption of bitter leaf extract is most probably prophylactic for the highly prevalent primary diabetes mellitus [40]. Bitter leaves are rich in steroidal Saponins, Sesquiterpene lactones and Flavonoids. These natural products may be responsible for the antidiabetic properties of the plant leaves [41, 42, 43, 44].

From this study, Hydromethanol extraction yielded highest concentrations of Glycosides and Steroids from bitter leaves. Ethanol extracted the highest concentrations of Terpenoids (75.90mg/100g) whereas Water extraction yielded the highest concentrations of Phenols from bitter leaves (0.25mg/g).

Terpenoids have antihyperglycemic activity; they help in uptake of the glucose in the muscle and the inhibition of the glucose absorption in the gastrointestinal tract, insulin release activity and insulin mimetic property [44,45,46].

The study showed that water extract of Phenols from *Moringa* produced the highest phenol

content of 0.32mg/g, followed by the water extract of VA (0.25mg/g) and that the ethanolic extract of *Moringa* (0.23mg/g of Phenols) is higher than ethanolic extraction from VA (0.18mg/g of Phenols).

Steroid is one of the plant constituents which possess antidiabetic properties [13]. Comparing the steroids extractions in this study, hydromethanolic extract of VA gave the highest (75.43mg/100g), followed by water extract of VA (65.30mg/100g) then hydromethanolic extract of MO (59.873mg/100g).

Ethanol gave the highest crude extract yield of 46.06% in MO and 38.91% in VA. This agreed with a previous work [47]. So ethanol is the commonest solvent for the crude extraction of most phytochemicals for diabetes therapy whereas hydromethanol and water have more selective capacities.

The findings also agreed with the report that the extracts caused significant decrease in serum glucose levels in the order of ethanolic extract >hydroalcoholic extract >aqueous extract >ethyl acetate>chloroform extract >n-hexane extract [48].

Previous studies showed that the dried MO leaf powder extracted using ethanol caused significant reduction in blood glucose level of streptozotocin-induced diabetic and normoglycaemic rats [49]. *Moringa oleifera* (MO) is very important in both plants and animals [50, 51].

5. CONCLUSION

Due to the variety of bioactive compounds contained in plant medicinal materials and their differing solubility in different solvents, the optimal solvent for each phytochemical depends on the particular type of plant, its materials, and the compounds that are to be isolated. Ethanol is the commonest solvent and it has the highest extract yield of of 46.06% and 38.91% from *Moringa* sp. and *Vernonia* sp., respectively, followed by hydromethanol yields. Water extract of antidiabetic Phenols from *Moringa* produced the highest phenol content of 0.32mg/g, followed by the water extract of VA (0.25mg/g). Consequently, water > Hydromethanol > Ethanol are preferentially recommended in the crude extraction of phytochemicals for diabetes therapy.

DISCLAIMER

The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

FUNDING

This research was designed, funded and carried out by the authors. The materials used were readily available in the locality of the research.

CONSENT

This is not applicable.

ETHICAL APPROVAL

This is not applicable.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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