



## AMERICAN JOURNAL OF PHARMTECH RESEARCH

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### Effects of Different Solvents on Crude Anti nutritional Extracts Of Moringa Oleifera And Vernonia Amygdalina (Bitter Leaf)

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#### ABSTRACT

Antinutrients are important phytochemicals that also determine the safety of medicinal and nutritional plant parts. The antinutrients of *Moringa oleifera* and *Vernonia amygdalina* (bitter leaf) leaves were extracted by soaking, using water, ethanol and hydromethanol (1:1) as solvents. The phytochemical analyses were done both qualitatively and quantitatively (using Spectrophotometer: UV-V15). This study showed that Moringa and bitter leaf contain some antinutritional constituents, including Tannins, Oxalates, Saponins and Alkaloids. Hydromethanol extracted the highest concentration of Tannins (65.91%) from bitter leaf extract. Ethanol extracted the highest concentrations of Oxalates ( $5.2 \times 10^3$  Mg/100g) and Saponins (7,616.84  $\mu$ g/g) from both medicinal leaves and the highest concentration of Alkaloids from Bitter leaves only. Water extracted the highest concentrations of Alkaloids and Tannins from Moringa leaves only. Generally, ethanol solvent yielded highest crude extraction of antinutrients in the herbs. Therefore, it is also necessary to consider the antinutritional yield of a solvent in the choice of solvents for herbal production.

**Keywords:** Solvents, Antinutritional Extractions, Herbs.

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Received 10 April 2020, Accepted 05 May 2020

Please cite this article as: Iwuji Sc *et al.*, Effects of Different Solvents on Crude Anti nutritional Extracts Of Moringa Oleifera And Vernonia Amygdalina (Bitter Leaf). American Journal of PharmTech Research 2020.

## INTRODUCTION

Medicinal plants are sources of raw materials for pharmaceutical drug formulation [1]. The phytochemicals present in plants are responsible for their use in medical practice, especially in tradomedicine [2, 3 4, 5]. Phytochemicals have various protective functions for the plants where they do not have significant nutritional function, while at the same time they have various health benefits for man [2, 5, 6]. Phytochemicals include vitamins, steroids, phenolic acids, lignins, tannins, flavonoids, quinines, alkaloids, amines, etc. [3, 4, 5].

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 [3, 5].

The plant parts are dried immediately either in an artificial environment at low temperature or dried preferably under a shade (air-dried) so as to reduce the initial large moisture content to enable its prolonged storage life. The plant parts are grinded by mechanical/electrical grinders. Commonly, various solvents have been used to extract different phytochemicals. Phytochemicals can be extracted using a Soxhlet apparatus or by soaking in solvents. The resulting extract is filtered, concentrated in vacuo or by evaporation. This can then be used to determine the presence of phytochemicals [7]. These active ingredients could be useful in the treatment of diseases or could be toxic antinutritional.

Antinutritional factors are known as those biological compounds present in human or animal foods that reduce nutrient utilization or food intake, thereby contributing to impaired gastrointestinal and metabolic performance [8].

Among the antinutritional factors, the compounds most likely to cause adverse effects such as impaired intake, uptake, or utilization of other foods include protease inhibitors, lectins, antigenic proteins, particular types of oligosaccharides and polysaccharides, saponins, glucosinolates, condensed tannins, nonprotein amino acids, gossypol and biogenic amines [9].

It is therefore pertinent to assess the extractive potentials of the locally available solvents, determine their crude extract yields of antinutrients.

## MATERIALS AND METHOD

### Plants

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

### Chemicals

Chemicals used in this study included Methanol (extra pure) manufactured by Loba Chemie 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.

### **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 – Search tech Product no – 477517060317060004.

### **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 form were extracted separately using the solvents: boiled water, ethanol and hydromethanol (50:50) in the soaking method [10]. 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.

### **Phytochemical tests on the extracts for the presence of antinutrients**

Some phytochemical tests were carried out on the crude extracts to determine the presence of alkaloids, oxalates, tannins, saponins. Phytochemicals were determined qualitatively using the methods described [11, 12].

The phytochemicals were also determined quantitatively using standard methods [13, 14, 15].

### **Quantitative estimation of Saponins:**

Methanolic and water extract was dissolved in 80% methanol, 2ml of Vanilin in ethanol was added, mixed well and the 2ml of 72% sulphuric acid solution was added, mixed well and heated on a water bath at 600c for 10min, absorbance was measured at 544nm against reagent blank. Diosgenin is used as a standard material and compared the assay with Diosgenin equivalents.

### **Quantitative estimation of Tannins:**

1 gram of sample was dissolved in 10ml distilled water and agitated, left to stand for 30mins at room temperature. Each sample was centrifuged and the extract recovered. 2.5ml of the supernatant were dispersed into 50ml volumetric flask. Similarly, 2.5ml of standard Tannin acid solution was dispersed into a separate 50ml flask. A 10ml Folin Denis reagent was measured in each flask followed by 2.5ml of saturated Na<sub>2</sub>CO<sub>3</sub> solution. The solution was diluted to 50ml in the flask and

incubated for 90mins at room temperature. The absorbance of the samples was measured at 250nm with the reagent black at zero. The percentage Tannin was calculated.

### Statistical analysis

Data collected were statistically analysed using Analysis of 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.

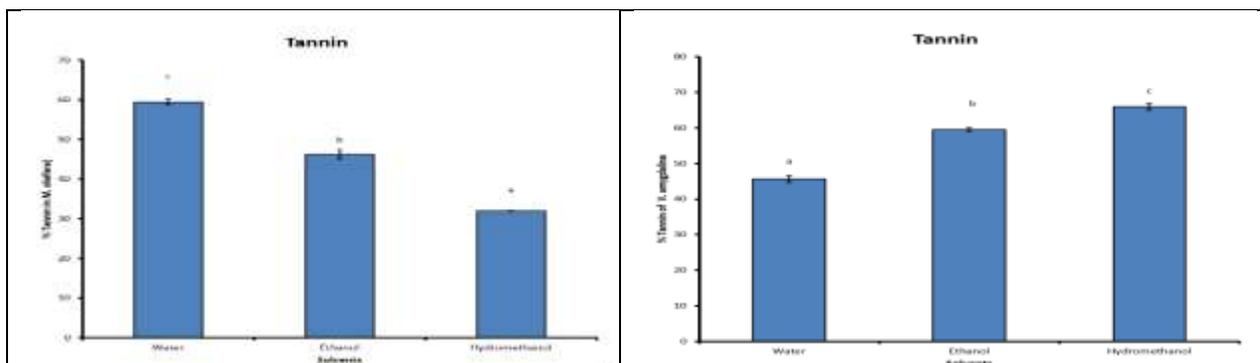
## RESULTS AND DISCUSSION

### Results:

The phytochemical analysis of the crude extracts showed the presence of some antinutrients in the two medicinal and nutritional plant leaves.

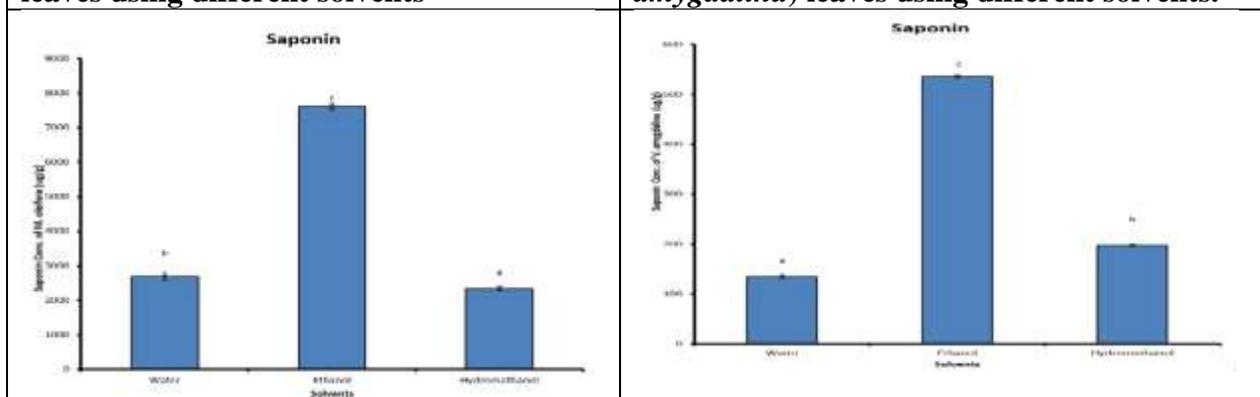
TABLE 1 below

**Table 1:** shows that the three solvents could extract the tested antinutrients. All the phytochemicals tested positive in the crude extracts.



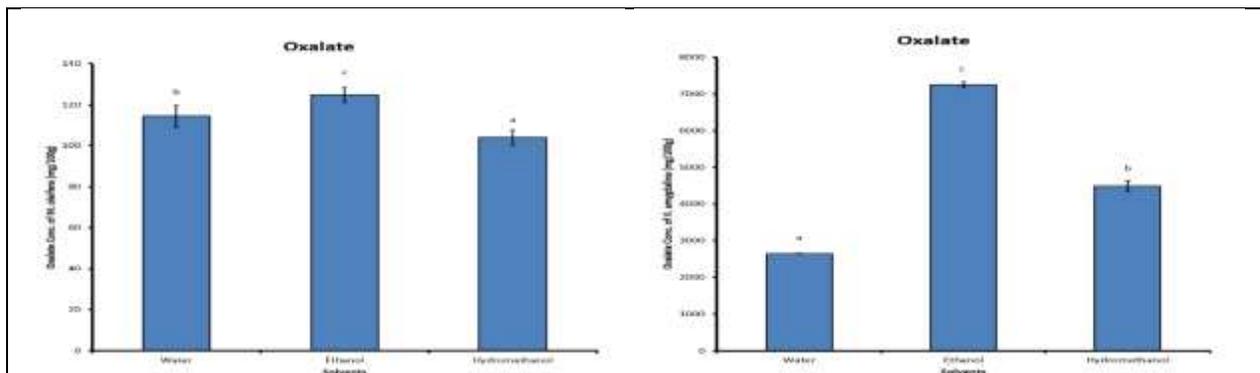
**Figure 1A:** Comparing the concentrations of Tannins extracted from *Moringa oleifera* leaves using different solvents

**Figure 1B:** Comparing the concentrations of Tannins extracted from bitter leaf (*Vernonia amygdalina*) leaves using different solvents.



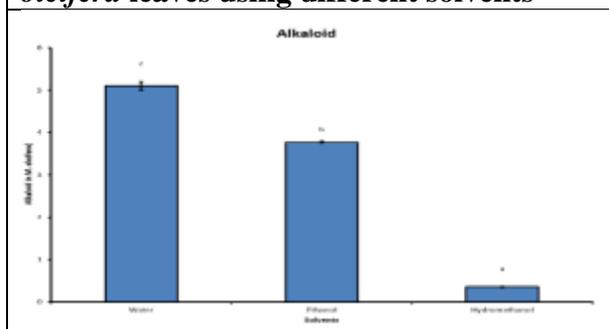
**Figure 2A:** Comparing the concentrations of Saponins extracted from *Moringa oleifera* leaves using different solvents

**Figure 2B:** Comparing the concentrations of Saponins extracted from *Vernonia amygdalina* leaves using different solvents

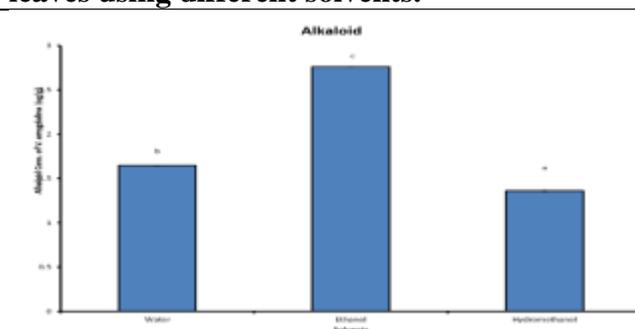


**Figure 3A: Comparing the concentrations of Oxalates extracted from *Moringa oleifera* leaves using different solvents**

**Figure 3B: Comparing the concentrations of Oxalates extracted from *Vernonia amygdalina* leaves using different solvents.**



**Figure 4A: Comparing the concentrations of Alkaloids extracted from *Moringa oleifera* leaves using different solvents**



**Figure 4B: Comparing the concentrations of Alkaloids extracted from bitter leaf leaves using different solvents**

Ethanol extracted the highest concentrations of Oxalates ( $5.2 \times 10^3$  Mg/100g) and Saponins (7,616.84 µg/g) from both crude extracts and highest concentration of Alkaloids from Bitter leaf extract. Water extracted the highest concentrations of Alkaloids and Tannins from *Moringa*. Hydromethanol extracted the highest concentration of Tannins (65.91%) from bitter leaf extract.

## DISCUSSION

Plant materials had been reported to have medicinal and nutritional properties [5, 6, 16] as well as other chemicals [3] like antinutrients [17]. Antinutrients are substances which interfere with the metabolism and utilization of body nutrients; examples are phytates, oxalates, tannins and saponins etc. In this study, antinutritional phytochemicals are crudely extracted from *Moringa oleifera* leaves using different solvents: water, ethanol and hydromethanol. *Moringa* leaves have been found to contain little quantities of the antinutrients [18, 19] which are also evident from the results obtained. *Moringa* leaves have also been found to contain small quantities of Saponins at levels that are relatively harmless to human health. Thus, *Moringa* leaves are consumed with no side effects. Ethanolic extraction of Saponin from *Moringa oleifera* gave the highest yield significantly more than the other two extraction solvents which makes it a better extraction solvent for Saponin from *Moringa oleifera*.

It can be observed that aqueous crude extraction of Tannin in *Moringa oleifera* gave the highest yield more than the ethanolic extraction and significantly more than the hydromethanolic extract.

The aqueous extract of alkaloid in *Moringa oleifera* gave a much higher yield than the other extraction solvents especially the hydromethanolic extract which gave a significantly very low yield as compared to the other two.

The importance of bitter leaf has been reviewed [20] and its medicinal properties had been studied [16, 21]. Antinutritional phytochemicals are also crudely extracted from *Vernonia amygdalina* leaves using different solvents. Tannins which have great beneficial effects in the human body are found in moderate quantities in bitter leaf. These moderate quantities of Tannin content in bitter leaf is known to be the dosage at which it exerts its beneficial effects [22, 23]. Hydromethanolic extractions of Tannin were observed to give the highest extract yields.

Ethanolic extraction of Saponin, Alkaloids and Oxalates from bitter leaf gave the highest extract concentrations compared with other solvents.

## CONCLUSION

There are a variety of bioactive compounds contained in plant materials and they have differing solubility properties in different solvents. The optimal solvent for extraction depends on the particular plant materials, and the phytochemicals that are to be isolated. In most cases in this study, ethanolic solvent yielded more concentrations of antinutrients from the investigated medicinal and nutritional leaves of *Moringa oleifera* and *Vernonia amygdalina*.

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