QUALITATIVE AND QUANTITATIVE PHYTOCHEMICAL ANALYSIS OF MORINGA OLEIFERA AND VERNONIA AMYGDALINA

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Manuscript received: 07/01/2017   Accepted:09/10/2017        Published: December 2017

ABSTRACT

Plants have been known to be used in the treatment and management of diseases, and this is due to the presence of phytochemicals. Thus, the screening of plants, both quantitatively and qualitatively, has got the interest of researchers. Moringa oleifera and Vernonia amygdalina are two plants that have very rich application in trado-medicine for various purposes. Thus this study seeks to understand the phytochemical profile when both plants are mixed. Ethanol, lime and aqueous extracts were used, of which lime extract gave the highest percentage yield of 27.3%, while ethanol and aqueous extracts gave 13.6% and 11.2% respectively. The qualitative phytochemical screening showed that only phenols and tannins were detected in all the three extracts. Flavonoids were only detected in the aqueous extract and alkaloids detected in the ethanolic extract only. The quantitative analysis showed the presence of all the five phytochemical classes in all extracts with the lime extract having the lowest values for all phytochemical classes tested for. The aqueous extract showed a generally good concentration in all five phytochemicals. Saponins, tannins and flavonoids were highest in the aqueous extract of 13.2%, 49.32 mg/mL and 2.10 mg/mL respectively. Phenols and alkaloids were highest in the ethanolic extract of 5.48 G.A. Eq. mg/mL and 3.2% respectively. This result showed that the mixture of Moringaoleifera and Vernoniaamygdalina is a good dietary combination.

Keywords: Phytochemicals, Moringa oleifera, Vernonia amygdalina, bitter leaf, extracts, herbs
INTRODUCTION
From time immemorial, man depended on plants as sources of drugs and 75% – 90% of the rural populations of the world have still not emerged from the use of plants as herbal remedy (Kong et al., 2003). The phytochemicals present in plants are responsible for their use in medical practice especially, trado-medicine (Aliyu et al., 2011, Sexena et al., 2013). These phytochemicals have various protective functions for the plants, but not nutritional function; while at the same time they have various health benefits for man (Sexena et al., 2013). Phytochemicals include vitamins, terpenoids, phenolic acids, lignins, stilbenes, tannins, flavonoids, quinines, coumarines, alkaloids, amines and betalains (Gracelin et al., 2013).

Moringa oleifera (M. oleifera) is a plant that naturally grows in the tropics, which include areas like India, Africa, Arabia, South Asia and South America. It belongs to the family called Moringaceae (Vinnoth et al., 2012). It is a multifunctional plant as it is used for food, as water cleaning material, and used in biofuel production (Torres-Castillo et al., 2013). The leaves are known to provide Vitamin C, especially when raw, and are also a good source of Vitamin B (Patel et al., 2014). All these and many more makes M. oleifera a well-documented plant (Manikandan et al., 2016). The leaves, apart from vitamins also contain essential amino acids, proteins and minerals in high content which makes it a good and ideal nutritional supplement (Elangovan et al., 2014). Due to its high content in phytochemicals, and also other nutrients, trado-medical practitioners have employed it in the treatment and management of various diseases which include diabetes, arterial tension, stomach ulcers, diarrhea, gastric discomft, skin infections, etc. (Nweze and Nwafor, 2014). The trado-medical practitioners makes use of every part of the plant (Abalaka et al., 2012, Kasolo et al., 2011, Manikandan et al., 2016), little wonder why some refer to it as “miracle tree” (Vinoth et al., 2012). The plant has been shown to have some pharmacological uses. Various reports have shown that different solvents extracts of the plant, especially the leaves, is a good antibacterial agent (Abalaka et al., 2012, Adline and Devi, 2014, Manikandan et al., 2016). The focus on the leaves may be due to its high usage as a vegetable in human diet, though other parts of the plant are also rich in nutrients and phytochemicals. The antimicrobial function of the plant has also been reported (Imohiosen et al., 2014, Napolean et al., 2019 Ojiako, 2014). Other pharmacological reports indicate that it has antifungal (Vinoth et al., 2012, Patel et al., 2014) and antioxidant (Elangovan et al., 2014, Torres-Castillo et al., 2013) properties. The various phytochemical analyses and assays carried out on the leaves on M. oleifera indicates that it contains tannins, steroids and terpenoids, flavonoids, saponins, anthraquinones, alkaloids and reducing sugars (Kasolo et al., 2010). There is also the presence of essential and semi-essential amino acids in different amount (Isitua et al., 2015).

Vernonia amygdalina (V. amygdalina) commonly called “Bitter Leaf” in English Language (Oguwike et al., 2013) is a member of the Asteraceae family, a small ever green shrub of the tropical African region, especially in West Africa (Ikhaijange-Happy et al., 2014). It is known by different names such as Onugbu (Igbo), Shiwaka (Hausa), Ewuro (Yoruba) and Kinologbo (Ijaw) (Kigagha and Onyema, 2015). It is usually used for dietary purposes, especially after washing the leaves to remove the bitter taste (Oboh and Masodje, 2009). The bitter taste is due to factors such as alkaloids, saponins, tannins and glycosides (Adetunji et al., 2013). In ethno-medicinal practice, the roots and leaves are used to treat fever, kidney problems, hiccups and stomach discomft. Its blood clothing properties and its ability to reduce blood glucose has also been exploited in the trado-medical practice (Udochukwu et al., 2015). Also reported are its uses as treatment of gastro-intestinal problems, malaria, tooth ache and fertility problems (Eyo et al., 2013). Some of its pharmacological activities are antibacterial (Adetunji et al., 2013, Kigagha and Onyema, 2015, Udochukwu et al., 2015), antimicrobial and hypolipaemic and anti-diabetic effect (Ikhaijange-Happy et al., 2014, Oboh and Masodje, 2009). It is also a good haemostatic control agent and a good blood sugar reducing agent (Oguwike et al., 2013). Proximate analysis of the leaves of V. amygdalina shows that it is a good dietary item. The phytochemical analysis of V. amygdalina shows that it contains proanthocyanidins, flavonoids, alkaloids and phenols (Momoh et al., 2015). Saponins and hydroxyanide were also found to be present (Evong et al., 2011).

It is evident from the literature that several studies have examined the phytochemical profile of M. oleifera and V. amygdalina (Evong et al., 2011, Isitua et al., 2015, Kasolo et al., 2010, Momoh et al., 2015). However, these studies only examined the plants independently of each other. Moreover, as part of efforts in our lab to understand the effect of the combination of these two plants on diabetes and hypertension, we investigate in this study the qualitative and quantitative phytochemical properties of the combination of M. oleifera and V. amygdalina.
MATERIALS AND METHODS

Collection and Preparation of Plant Leaves and Extracts

*M. oleifera* and *V. amygdalina* leaves were harvested at night from farm site in Anyigba Community, Kogi State, North Central Nigeria. Specimens were air-dried for three (3) weeks and then pulverized to powder. 90g of ground *V. amygdalina* leaves sample and 150g of ground *M. oleifera* leaves sample mixed together were soaked in 1000ml of ethanol, lime and distilled water (aqueous) for three (3) days. The extracts of the various solutions were then concentrated in-vacuo to give the ethanol, lime and aqueous extracts respectively.

Phytochemical Analysis

Qualitative Analysis

The qualitative phytochemical analysis was done using standardized procedures according to Tiwari et al (2011). The extract was subjected to phytochemical screening for alkaloids (Mayer’s test and Wagner’s test), saponins (Froth test and Foam test), tannins (Gelatin test), flavonoids (Alkaline reagent test and lead acetate test) and phenols.

**Alkaloids**

0.5 g of the extracts (ethanol, aqueous and lime) were mixed in 8ml of 1% HCL, this was warmed and filtered. 2ml of each the filtrates was treated with Mayer’s reagent. The present of alkaloid shows turbidity or precipitate formation.

**Tannins**

0.5 g of the extracts (ethanol, aqueous and lime) was dissolved in 2 ml of distilled water, 2 drops of diluted ferric chloride solution was added. A dark green or blue green coloration indicates the presence of tannins.

**Flavonoids**

0.5 g each of the extracts was dissolved in ethanol (5 ml) was added to a concentrated sulphuric acid (1 ml) and 0.5g of Mg. A pink or red coloration that disappear on standing (3 mins) indicates the presence of flavonoids.

**Saponins**

0.5g of each extract was added to 5ml of distilled water in a test tube. The solution was shaken vigorously and observed for a stable persistent froth for 20 min.

**Phenols**

10mg extracts were treated with 2 drops of ferric chloride solution. Formation of bluish black colour indicated the presence of phenol.

Quantitative Analysis


**Determination of Alkaloids**

0.5 g of ethanolic, aqueous and lime extracts were dissolved in 50mls of their respective solvents. Concentrated ammonium hydroxide was added to the extract solutions drop wise until precipitation was completed. The whole solutions were allowed to settle and the precipitates were collected via filtration (using Wattman filter paper no 24). The residues were weighed and the percentages of total alkaloid contents were calculated as;

\[
\text{Percentage of total alkaloids (%)} = \frac{W_2 - W_1}{W_i} \times 100
\]

Where  
\( W_1 = \text{Weight of filter paper} \)  
\( W_2 = \text{Weight of filter paper + Precipitate} \)  
\( W_i = \text{Weight sample} \)

**Determination of Total Phenolic Contents in the Plant Extracts**

The concentration of phenols in plant extracts was determined using spectrophotometric method as described by Singleton et al.,(1999). Methanolic solution of the extract in the concentration of 1 mg/ml was used in the analysis. The reaction mixture was prepared by mixing 0.5 ml of methanolic solution of extract, 2.5 ml of 10% Folin-Ciocalteu’s reagent dissolved in water and 2.5 ml 7.5% NaHCO3. Blank was concomitantly prepared, containing 0.5 ml methanol, 2.5 ml 10% Folin-Ciocalteu’s reagent dissolved in water and 2.5 ml of 7.5% of NaHCO3. The samples were thereafter incubated in a thermostat at 45°C for 45 mins. The absorbance was determined using spectrophotometer at \( \lambda_{max} = 765 \text{ nm} \). The samples were prepared in triplicate for each analysis and the mean value of absorbance was obtained. The same procedure was repeated for the standard solution of garlic acid and the calibration line was construed. Based on the measured absorbance, the concentration of phenols was read (mg/ml) from the calibration line; then the content of phenolics in extracts was expressed in terms of garlic acid equivalent (G.A. Eq. mg/mL).
Determination of the Total Flavonoid (Aluminum Chloride Colorimetric Method)

The total flavonoid content was determined using aluminum chloride colorimetric method according to Nabavi et al., (2008). Quercetin was used to make the standard calibration curve. The standard calibration curve was prepared using quercetin solutions of various concentrations as follow; 0.1, 0.5, 1.0, 2.5 and 5.0 mg/ml dissolved in 80% ethanol. The diluted standard quercetin solutions (0.5 ml) were separately mixed with 1.5 ml of 95% ethanol, 0.1 ml of 10% aluminum chloride, 0.1 ml of 1 M potassium acetate and 2.8 ml of distilled water. After mixing, the solution was incubated for 30 min at room temperature. The absorbance of the reaction mixtures were measured at 415 nm wavelength with Visible spectrophotometer of model 721 (England). The amount of 10% aluminum chloride was substituted by the same amount of distilled water in blank. Similarly, 0.5 ml of ethanolic, aqueous, and lime extracts solutions were reacted with aluminum chloride for determination of flavonoid content as described above.


500 mg of the sample in each case was taken in a plastic bottle, and 50 ml of distilled water was added. Then it was shaken in a mechanical shaker for 1 h, and filtered in a 50 ml volumetric flask made up to the mark. 5 ml of the filtrate was pipetted out into the test tube and mixed with 2 ml of 0.1 M FeCl3 in 0.1 N HCl and 0.008 M K4Fe(CN)6 (potassium ferrocyanide). The absorbance was measured at 120 nm within 10 min.

Saponin Determination (Obadoni and Ochuko, 2001)

20 g of each ground sample was put into a conical flask and 100 cm3 of 20% aqueous ethanol was added. Then the flask was heated on a hot water bath for 4 h, with constant stirring at about 55°C. The mixture was then filtered and the residue was again extracted with another 200 ml 20% ethanol. The combined extract was reduced to 40 ml on a hot water bath at about 90°C. The concentrate was transferred into a 250 ml separating funnel, added 20 ml diethyl ether in it followed by vigorous shaking. The aqueous layer was recovered while the ether layer was discarded. The purification process was repeated. 60 ml of n-butanol was added. The combined n-butanol extracts were washed twice with 10 ml of 5% aqueous sodium chloride. The remaining solution was heated in a water bath. After evaporation the samples were dried in oven, weighed and saponin content was calculated as percentage.

RESULTS AND DISCUSSION

![Figure 1: Bar Chart showing extracts yield from each solvent extraction](image)

Table 1: Table showing percentage yield from each solvent extraction

<table>
<thead>
<tr>
<th>Extract</th>
<th>Ethanol</th>
<th>Aqueous</th>
<th>Lime</th>
</tr>
</thead>
<tbody>
<tr>
<td>Weight of Soaked Sample (g)</td>
<td>240</td>
<td>240</td>
<td>240</td>
</tr>
<tr>
<td>Yielded Extract (g)</td>
<td>32.7</td>
<td>27.0</td>
<td>65.6</td>
</tr>
<tr>
<td>Percentage Yield (%)</td>
<td>13.6</td>
<td>11.2</td>
<td>27.3</td>
</tr>
</tbody>
</table>

Table 2: Qualitative Phytochemical Screening Analysis of the Mixture of M. oleifera and V. amygdalina

<table>
<thead>
<tr>
<th>S/No</th>
<th>Phytochemical</th>
<th>Ethanol Extract</th>
<th>Lime Extract</th>
<th>Aqueous Extract</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Alkaloids</td>
<td>+++</td>
<td>Nil</td>
<td>Nil</td>
</tr>
<tr>
<td>2.</td>
<td>Tannins</td>
<td>++</td>
<td>+</td>
<td>+++</td>
</tr>
<tr>
<td>3.</td>
<td>Flavonoids</td>
<td>Nil</td>
<td>Nil</td>
<td>+</td>
</tr>
<tr>
<td>4.</td>
<td>Saponins</td>
<td>+</td>
<td>Nil</td>
<td>++</td>
</tr>
<tr>
<td>5.</td>
<td>Phenols</td>
<td>++</td>
<td>+</td>
<td>++</td>
</tr>
</tbody>
</table>

Table 3: Table showing result of Quantitative Determination of Phytochemicals in the M. oleifera and V. amygdalina Mixture

<table>
<thead>
<tr>
<th>Extract</th>
<th>Alkaloids (%)</th>
<th>Phenolics (G. A. E. mg/mL)</th>
<th>Flavonoids (mg/mL)</th>
<th>Tannins (mg/mL)</th>
<th>Saponins (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ethanol</td>
<td>3.2±0.2</td>
<td>5.48±1.2</td>
<td>1.97±1.1</td>
<td>45.59±0.5</td>
<td>8.0±1.3</td>
</tr>
<tr>
<td>Aqueous</td>
<td>2.0±0.4</td>
<td>0.89±0.9</td>
<td>2.10±0.8</td>
<td>49.32±0.4</td>
<td>13.2±1.3</td>
</tr>
<tr>
<td>Lime</td>
<td>1.0±1.0</td>
<td>0.25±1.3</td>
<td>1.54±0.8</td>
<td>44.27±0.8</td>
<td>2.6±1.0</td>
</tr>
</tbody>
</table>

The various solvents extractions showed varied yield with the lime solvent extract having the highest residue of 65.6 g (Figure 1), which corresponds...
to 27.3% yield (Table 1). The ethanol extract has a percentage yield of 13.2% (32.7g) while the aqueous extract had a percentage of 11.2% (27.0g). The high pH of lime may be responsible for its ability to extract the high amount observed.

The qualitative phytochemical screening (Table 2) showed that none of the five (5) phytochemical classes tested for were present in all the extract. The five phytochemical classes tested for include alkaloids, tannins, flavonoids, saponins and phenols. Alkaloids were detected highest in the ethanol extract while tannins showed highest detection in the aqueous extract. Flavonoids were not detected in the ethanol extract and also alkaloids were not detected in the aqueous extract. Three of the phytochemical classes, flavonoids, alkaloids and saponins, were not detected in the lime extract. This may be due to the high polarity of the lime which allows polar compounds to be extracted. The less polar ethanol and aqueous extract show better detection to more of the phytochemicals classes tested for.

The quantitative determination (Table 3) shows that tannins have the highest concentration in the three extracts there were expressed in mg/mL, while saponins have the higher percentage content between the saponin and alkaloid determinations that were expressed as percentages.

Alkaloids have been reported to be good in reducing headache and fever due to their analgesic and antibacterial properties (Wadood et al., 2013, Yadav and Agarwala, 2011). Thus, only the ethanolic extract of the sample would be good as an analgesic. In trado-medicine, the use of locally brewed alcohol would also serve in extracting the alkaloids for use. Tannins were found in all the three extracts, but has its highest value in the aqueous extract. Thus, the aqueous extract can be employed in helping in the management and treatment of cancers (Gnanaraja et al., 2014). The high concentration is also in consonance with its ability as a good anticancer agent. Flavonoids were only detected in the aqueous extract and which is also the highest concentration in the quantitative analysis. Thus, the aqueous extract would be good in modifying the body’s reaction to allergies, virus and carcinogens (Gnanaraja et al., 2014). Phenols have been known to be good antiaging, anticarcinogen, good in cardiovascular protection etc. (Yadav and Agarwala, 2011). Thus a mixture of bitter leaf and Moringa would be a good dietary item as phenols were present in all the extract. Saponins were detected highest in the aqueous extract and this also was supported by the value in the quantitative analysis of 13.2%. This would be a good antifeeding agent and the aqueous extract would have good detergent properties (Chaieb, 2010).

**CONCLUSION**

The results show that the mixture of these two plants is good source of phytochemicals. Though the qualitative analysis did not detect some of the phytochemicals in some of the extracts, the quantitative analysis shows that they are present. The result also shows that the phytochemicals are best extracted with water or ethanol for maximum percentage presence of the phytochemicals. The combination of these two plants would serve as a better dietary and medicinal benefit, owing to their synergistic effect.

**ACKNOWLEDGEMENT**

We acknowledge financial support from Nigeria Tertiary Education Trust Fund (TETFund) and thank Mr. Egwumah Christian for help with the phytochemical analysis as well as for valuable discussions on some part of this project. We also thank the laboratory staff in the Department of Anatomy, Kogi State University, Anyigba for their laboratory assistance.

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