PHYTOCHEMICAL COMPOSITION OF BRYOPHYLLUM PINNATUM LEAVES

B. U. Nwali, A.N.C Okaka, U.A. Ibiam, and P.M. Aja
Department of Biochemistry, Ebonyi State University, Abakaliki, P.M.B 053 Abakaliki, Nigeria

ABSTRACT
The qualitative and quantitative phytochemical analysis of Bryophyllum Pinnatum (African Never Die) which are commonly used as medicinal plant in Nigeria were carried out on both dry and fresh sample. The results revealed the presence of bioactive constituents comprising alkaloids (0.89 ± 0.02 µg/g and 0.37 ± 0.01 µg/g), Saponins (0.35 ± 0.01% and 0.17 ± 0.01%), Flavonoids (0.08 ± 0.01 µg/g and 0.03 ± 0.02 µg/g), and Tannins (1.24 ± 0.16% and 0.81 ± 0.02%) for both dry and fresh samples respectively. This shows that Bryophyllum Pinnatum contain appreciable amount of bioactive compounds. Medicinally, the presence of phytochemicals explains the role of this plant leaves in ethnomedicine in Nigeria.

KEY WORDS: Bryophyllum Pinnatum, bioactive compounds. Ethnomedicine, qualitative, quantitative, phytochemicals

INTRODUCTION
In Nigeria, many indigenous plants are used in herbal medicine to cure diseases and heal injuries (Okwu, 2006). Such medicinal plant includes Bryophyllum Pinnatum. The active ingredients of most of the commonly used conventional drugs were originally derived from plant part before their pharmaceutical mass production from synthetic chemical (Sofowara, 1993) Bryophyllum Pinnatum is an erect, succulent, perennial shrub that grows about 1.5m height and reproduces from seeds and also vegetatively from leaf bubble (Agotha, 1974). It is an introduced ornamental plant that is now growing as seed around plantation crops (Dalziel, 1995). Bryophyllum Pinnatum is used in ethno medicine for treatment of earache, burns, abscesses, ulcer, insect bites, diarrhea and Lithiasis (Chopra et al., 1956). In South Eastern Nigeria, this herb is used to facilitate the dropping of the placenta of a newly born baby (Daziels, 1995). The plant leaf is mildly exposed to heat and the juice extracted and applied to the baby’s placenta on daily basis. The crushed leaves as well as the extracted juice are mixed with palm oil and rubbed on abscesses or other smelling. This is also applied on ulcers, burns and on the body of young children when they are ill (Agotha, 1974). Bryophyllum Pinnatum is widely distributed especially in philippines and it is known as katakataka which is an adjective for astonishing or remarkable (Duster, 1985). The plant is easily propagated, found in thickets and open places. Bryophyllum Pinnatum, is usually applied externally. The bufadienolides which are active components of Bryophyllum Pinnatum possess antibacterial, anti tumors, cancer preventive and insecticidal actions (Fujita,2000). From upper respiratory infections and cough to stomach ulcer and infections of the skin, eyes and ears; it is widely known and used as “miracle leaf”. The present study was designed to evaluate the chemical constituents of Bryophyllum Pinnatum leaves commonly used in herbal medicine in Nigeria.

MATERIALS AND METHODS
Collection and preparation of samples
The leaves of Bryophyllum Pinnatum were collected from Abakaliki near Grace Court Hotels Ebonyi State of Nigeria. The leaves of Bryophyllum Pinnatum were identified by a taxonomist in the Department of Applied Biology of Ebonyi State University, Abakaliki, Nigeria. The leaves were destalked, washed and sun dried by constantly exposing the leaves to sunlight for 2-3 weeks and turning of the leaves to prevent fungal growth. The leaves, both fresh and dried were used by grinding them with electric blender; they were labeled and store in a container for analyses.

Qualitative phytochemical screening of Bryophyllum pinnatum
Phytochemical screening procedures carried out were adopted from the previous work on plant analyses (Ayodele, 2005). These analyses determine the biologically active compounds that are present in the leaf of Bryophyllum Pinnatum. Examples Alkaloids, Saponins, Tannins Flavonoids, Glycoside, etc.

Test for the presence of alkaloids
About 2g of the ground sample were used. A quantity of about 1g each of powdered sample was boiled in 2ml of 1% Hydrochloric acid in a water bath for 5minutes. The mixture was allowed to cool and filtered and the filtrate was shared in equal proportion into 5 test tubes and labeled A, B, C, D, and E. 1ml portion of the filtrate was treated with drops of the following reagents. With Dragendorff’s reagent a red precipitate was shown. With mayer’s reagent a creamy white coloured precipitate indicated the presence of alkaloids (Harborne, 1973).

Test for the presence of saponins
About 0.5g of the sample was boiled with 5ml of distilled water for 5 minutes. Mixture was filtered while still hot and the filtrate was then used for the following tests (Treas and Evans, 1996). To 1ml of the filtrate, 2 drops of olive oil was added, the mixture was shaken and...
observed for the formation of emulsion. 1ml of the filtrate was diluted with 5ml of distilled water. The mixture was vigorously shaken and observed for frothing (Trease and Evans, 1996).

**Test for the presence of flavonoids**
About 0.2g of the sample was introduced into 10mls of ethylacetate and heated in boiling water for 3 minutes. The mixture was then filtered and the filtrate used for the following tests. About 4ml of the filtrate was shaken with 1ml of dilute ammonia solution (1%). The layers were allowed to separate. A yellow colouration observed at ammonia layer, which indicates the presence of flavonoids (Harborne, 1973).

**Test for the presence of tannins**
About 2g of the sample was boiled with 5ml of 45% ethanol for 5 minutes. The mixture was cooled and littered. 1ml of the filtrate was added to 3 drop of lead sub- acetate solution. A gelatinous precipitates were observed which indicates the presence of Tannins. Another 1ml of the filtrate was diluted with distilled water and 2 drops of ferric chloride added. A transient greenish to black colour indicates the presence of Tannins (Trease and Evans, 1996).

**Test for the presence of glycosides**
About 2g of the sample was added to 10ml of distilled water and it was heated for 5mins in a water bath, filtered using whatman’s No1 filter paper. Then 2mls of the filtrate was added to 0.2ml of fehling solution A and fehling solution B until it turns alkaline and heated in a water bath for 5mins. A light blue colouration observed which indicates the absence of glycosides (Oloyede, 2005).

**Quantitative phytochemical analyses of Bryophyllum pinnatum**

**Determination of alkaloids**
0.5g of the sample was dissolved in 96% ethanol – 20% H₂SO₄ (1:1). 1ml of the filtrate was added to 5ml of 60% tetraoxosulphate (vi) and allowed to stand for 5mins. Then 5ml of 0.5% formaldehyde was added and allowed to stand for 3 hours. The reading was taken at absorbance of 565nm (Harborne, 1980).

**Determination of flavonoids**
Flavonoids in the test sample was determined by the acid hydrolysis of spectrophotometric method. O.5g of processed plant sample was mixed with 5ml of dilute HCl and boiled for 30mins. The boiled extract was allowed to cool and filtered. 1ml of the filtrate was added to 5ml of ethylacetate and 5ml of 1% NH₃. Then the absorbance was read at 420nm (Harborne, 1976).

**Determination of saponins**
0.5g of the samples was added to 20mls of INHCL and was boiled for 4 hours. After cooling it was filtered and 50mls of petroleum ether was added to the filtrate and evaporated to dryness. 5mls of ethanol was added to the residue. 0.4mls of each was taken into 3 different test tubes. 6mls of ferrous sulphate reagent was added into each followed by 2mls of conc. H₂SO₄. They were thoroughly mixed after 10mins and the absorbance taken at 490nm (Oloyode, 2005).

**Determination of tannins**
The follin-Denis spectrophotometric method (1980) was used. 1g of the sample was dispersed into 10ml distilled water and agitated, left to stand for 30 mins at room temperature and shaken every 5 minutes. At the end of 30minutes it was centriged and the extract gotten 2.5mls of supernatant excess and 2.5mls standard tannic acid solution was dispersed in separate 50mls volumetric flask. 1.0ml of follin-Denis reagent was measured into each followed by 2.5mls of standard Na₂CO₃ solution. The mixture was diluted to mark in the flask (50ms) and incubated for 90 minutes at room temperature. The absorbance was measured at 250nm spectrophotometrically.

**RESULT AND DISCUSSION**
Table 1 summarizes the qualitative phytochemical analyses of Bryophyllum pinnatum. The result shows the presence of alkaloid, saponins, flavonoids, tannins and absence of glycosides. The result of quantitative determination of phytochemical constituents of Bryophyllum pinnatum is shown in table 2. High quantity of Tannins alkaloids and Saponins were found on Bryophyllum pinnatum. The values of flavonoids were very trace on the plant.

**TABLE 1. Qualitative phytochemical data of Bryophyllum Pinnatum leaves**

<table>
<thead>
<tr>
<th>Phytochemicals</th>
<th>Leaves</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alkaloids</td>
<td>+ve</td>
</tr>
<tr>
<td>Saponins</td>
<td>+ve</td>
</tr>
<tr>
<td>Tannins</td>
<td>+ve</td>
</tr>
<tr>
<td>Flavonoids</td>
<td>+ve</td>
</tr>
<tr>
<td>Glycosides</td>
<td>-ve</td>
</tr>
</tbody>
</table>

**TABLE 2. Phytochemical composition (Quantitative) of the leaves of Bryophyllum Pinnatum expressed as mg/100g dry and fresh weight.**

<table>
<thead>
<tr>
<th>Phytochemical</th>
<th>Dry</th>
<th>Fresh</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 Alkaloids</td>
<td>0.89 ± 0.10</td>
<td>0.37 ± 0.06</td>
</tr>
<tr>
<td>2 Saponins</td>
<td>0.35 ± 0.01</td>
<td>0.17 ± 0.01</td>
</tr>
<tr>
<td>3 Flavonoids</td>
<td>0.08 ± 0.01</td>
<td>0.03 ± 0.02</td>
</tr>
<tr>
<td>4 Tannins</td>
<td>0.24 ± 0.16</td>
<td>0.81 ± 0.02</td>
</tr>
</tbody>
</table>

Results are mean + standard deviation of triplicate determination on both dry and fresh samples.
DISCUSSION
A phytochemical analysis is very useful in the evaluation of some active biological compound of some medicinal plants. The qualitative and quantitative analyses of Bryophyllum Pinnatum were carried out in both dry and fresh samples. Alkaloids, Saponins, Flavonoids and Tannins were revealed to be present in Bryophyllum Pinnatum (table 1 and 2). This shows its possible medicinal values (Okwu, 2003). The high Saponins content of Bryophyllum Pinnatum justifies the use of the extract from this plant to stop bleeding and in treating of wounds (Okwu, 2004). Saponin has the property of precipitating and coagulating red blood cells. Some of the characteristics of Saponins include formation of foams in aqueous solutions, hemolytic activity, cholesterol binding properties and bitterness (Sodipo et al; Okwu, 2004). These properties prove a high medicinal value of the extract from Bryophyllum Pinnatum apart from Saponins, other secondary metabolite constituent of Bryophyllum Pinnatum found include alkaloids, tannins and flavonoids. Flavonoids are potent water-soluble Antioxidants and free radical scavengers, which prevent oxidative cell damage, have strong anti-cancer activity (Okwu, 2006). As antioxidants, flavonoids from these plants provide anti-inflammatory activity (Okwu, 2004). This may be the reason Bryophyllum Pinnatum is used in treatment of wounds, burns and ulcer in herbal medicine. Tannins have astringent properties, hasten the healing of wounds and inflamed mucous membranes. These, also explains the reason why traditional medicines healers in South Eastern Nigeria often use Bryophyllum Pinnatum in treating wounds and burns (Aghoha, 1974). The alkaloids level of Bryophyllum Pinnatum shows that the sample can be used as a CMS stimulant and as a powerful pain relievers (Stray, 1998). This study therefore has provided some biochemical basis for the ethanomedical use of extracts from Bryophyllum Pinnatum in treatment and prevention of infections. As a rich source of phytochemical Bryophyllum Pinnatum can be a potential source of useful drugs.

REFERENCES


