

Full Length Research Paper

Evaluation of the chemical composition of two Nigerian medicinal plants

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Nigerian medicinal plants (*Aspilia africana* and *Bryophyllum pinnatum*) were analyzed for their chemical composition, vitamins and minerals. The results revealed the presence of bioactive constituents comprising alkaloids (1.24 to 1.48 mg/100 g), saponins (1.46 to 1.72 mg/100 g), flavonoids (1.46 to 1.86 mg/100 g), phenols (0.06 mg/100g) and tannins (0.04 to 0.5 mg/100g). The medicinal plants contained ascorbic acid (26.42 to 44.03 mg/100 g), riboflavin (0.20 to 0.42 mg/100 g), thiamine (0.11 to 0.18 mg/100 g), and niacin (0.02 to 0.09 mg/100 g). These herbs are good sources of minerals such as Ca, P, K, Mg, Na, Fe and Zn. The importance of these chemical constituents is discussed with respect to the role of these herbs in ethnomedicine in Nigeria.

Key words: *Aspilia africana*, *Bryophyllum pinnatum*, bioactive compounds, ethnomedicine.

INTRODUCTION

In Nigeria, many indigenous plants are used in herbal medicine to cure diseases and heal injuries. Such medicinal plants include *Bryophyllum pinnatum* (Fam: Crassulaceae) and *Aspilia africana* (Asteraceae). *B. pinnatum* is an erect, succulent, perennial shrub that grows about 1.5 m height and reproduced from seeds and also vegetatively from leaf bubbils (Agoha, 1974). It is an introduced ornamental plant that is now growing as weed around plantation crops (Dalziel, 1955). *B. pinnatum* is used in ethnomedicine for the treatment of earache, burns, abscesses, ulcers, insect bites, diarrhea and lithiasis (Chopra et al., 1956; Agoha, 1974; Ofokansi et al., 2005). In Southeastern Nigeria, this herb is used to facilitate the dropping of the placenta of newly born baby (Dalziel, 1955). 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 shear butter or palm oil and rubbed on abscesses or other swellings. This is also applied on ulcers, burns and on the bodies of young children when they are ill (Agoha, 1974). Bryophyllin, potassium malate, ascorbic, malic and citric acids have been isolated from the leaves of *B. pinnatum* (McKenzie et al., 1985; Siddiqui et al., 1983; Singh, 1976; Ichikawa et al., 1986; Oliver, 1989).

A. africana is a perennial herb varying in height from 60 cm to about 1.5 m depending on rainfall. It is a common weed of field crops in West Africa and sometimes found in fallow land, especially the forest zones (Akobundu, 1987). The crushed leaves and flowers are used to stop bleeding and for treating wounds and sores (Agoha, 1974). In infusion of the leaves is taken by children and can also be mixed with clay as a medicine for stomach troubles (Dalziel, 1955).

The present study was designed to evaluate the minerals, vitamins and secondary metabolite constituents of *B. pinnatum* and *A. africana* commonly used in herbal medicine in Nigeria.

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MATERIALS AND METHOD

Plant materials

The experimental leaves were collected from Ubakala Village in Umuahia South Local Government Area of Abia State, Nigeria on 20th March 2002. The plant materials (leaves, flowers and fruits) were identified and authenticated by Dr A. Nmeregini of Taxonomy Section, Forestry Department, Michael Okpara University of Agriculture Umudike. The voucher specimens were deposited in the Forestry Department, Herbarium of Michael Okpara University of Agriculture, Umudike, Nigeria.

The leaves were air-dried for 10 days and milled into powder with the aid of an electrical grinder and finally stored in airtight bottles before analysis.

Chemical analysis

The major elements, comprising calcium, phosphorus, sodium, potassium, magnesium and trace elements (iron and zinc) were determined according to the method of Shahidi et al. (1999). The ground plant samples were sieved with a 2 mm rubber sieve and 2 g of each of the plant samples were weighed and subjected to dry ashing in a well-cleaned porcelain crucible at 550°C in a muffle furnace. The resultant ash was dissolved in 5 ml of HNO₃/HCl/H₂O (1:2:3) and heated gently on a hot plate until brown fumes disappeared. To the remaining material in each crucible, 5 ml of deionized water was added and heated until a colourless solution was obtained. The mineral solution in each crucible was transferred into a 100 ml volumetric flask by filtration through a Whatman No 42 filter paper and the volume was made to the mark with deionized water. This solution was used for elemental analysis by atomic absorption spectrophotometer. A 10 cm-long cell was used and concentration of each element in the sample was calculated on percentage of dry matter. Phosphorus content of the digest was determined colorimetrically according to the method described by Nahapetian and Bassiri (1975). To 0.5 ml of the diluted digest, 4 ml of demineralised water, 3 ml of 0.75M H₂SO₄, 0.4 ml of 10% (NH₄)₆MO₇O₂₄.4H₂O and 0.4 ml of 2% (w/v) ascorbic acid were added and mixed. The solution was allowed to stand for 20 min and absorbance readings were recorded at 660 nm. The content of phosphorus in the extract was determined.

Preparation of fat free sample

2 g of the sample were defatted with 100 ml of diethyl ether using a soxhlet apparatus for 2 h.

Alkaloid determination

5 g of the sample were weighed into a 250 ml beaker and 200 ml of 20% acetic acid in ethanol was added and covered to stand for 4 h. This was filtered and the extract was concentrated using a water-bath to one-quarter of the original volume. Concentrated ammonium hydroxide was added drop wise to the extract until the precipitation was complete. The whole solution was allowed to settle and the precipitate was collected by filtration and weighed (Harborne, 1973; Obadoni and Ochuko, 2001).

Tannin determination

500 mg of the sample was weighed into 100 ml plastic bottle. 50 ml of distilled water was added and shaken for 1 h in a mechanical shaker. This was filtered into a 50 ml volumetric flask and made up

to the mark. Then 5 ml of the filtrate was pipetted out into a tube and mixed with 3 ml of 0.1 M FeCl₃ in 0.1 N HCl and 0.008 M potassium ferrocyanide. The absorbance was measured in a spectrophotometer at 120 nm wavelength, within 10 min. A blank sample was prepared and the colour also developed and read at the same wavelength. A standard was prepared using tannin acid to get 100 ppm and measured (Van-Burden and Robinson, 1981).

Determination of total phenols

For the extraction of the phenolic component, the fat free sample was boiled with 50 ml of ether for 15 min. 5 ml of the extract was pipetted into a 50 ml flask, then 10 ml of distilled water was added. 2 ml of ammonium hydroxide solution and 5 ml of concentrated amyl alcohol were also added. The samples were made up to mark and left to react for 30 min for colour development. The absorbance of the solution was read using a spectrophotometer at 505 nm wavelengths (Harborne, 1973; Obadoni and Ochuko, 2001).

Saponin determination

The samples were ground. 20 g of each plant samples were dispersed in 200 ml of 20% ethanol. The suspension was heated over a hot water bath for 4 h with continuous stirring at about 55°C. The mixture was filtered and the residue re-extracted with another 200 ml of 20% ethanol. The combined extracts were reduced to 40 ml over water bath at about 90°C. The concentrate was transferred into a 250 ml separator funnel and 20 ml of diethyl ether was added and shaken vigorously. 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 the oven to a constant weight. The saponin content was calculated in percentage (Obadoni and Ochuko, 2001).

Flavonoid determination

10 g of the plant samples were extracted repeatedly with 100 ml of 80% aqueous methanol at room temperature. The whole solution was filtered through Whatman filter paper no. 42 (125 mm). The filtrate was later transferred into a crucible and evaporated to dryness over a water bath and weighed (Boham and Kocipai, 1994).

Determination of riboflavin

5 g of the sample was extracted with 100 ml of 50% ethanol solution and shaken for 1 h. This was filtered into a 100 ml flask; 10 ml of the extract was pipetted into 50 ml volumetric flask. 10 ml of 5% potassium permanganate and 10 ml of 30% H₂O₂ were added and allowed to stand over a hot water bath for about 30 min. 2 ml of 40% sodium sulphate was added. This was made up to 50 ml mark and the absorbance measured at 510 nm in a spectrophotometer.

Determination of thiamin

5 g of the sample were homogenized with ethanolic sodium hydroxide (50 ml). It was filtered into a 100 ml flask. 10 ml of the filtrate was pipetted and the colour developed by addition of 10 ml of potassium dichromate and read at 360 nm. A blank sample was

prepared and the colour also developed and read at the same wavelength.

Determination of niacin

5 g of the sample was treated with 50 ml of 1 N sulphuric acid and shaken for 30 min. 3 drops of ammonia solution were added to the sample and filtered. 10 ml of the filtrate was pipette into a 50 ml volumetric flask and 5 ml potassium cyanide was added. This was acidified with 5 ml of 0.02 N H₂SO₄ and absorbance measured in the spectrophotometer at 470 nm wavelengths.

Determination of ascorbic acid (vitamin C)

5 g of the sample was weighed into an extraction tube and 100 ml of EDTA/TCA (2:1) extracting solution were mixed and the mixture shaken for 30 min. This was transferred into a centrifuge tube and centrifuged at 3000 rpm for about 20 min. It was transferred into a 100 ml volumetric flask and made up to 100 ml mark with the extracting solution. 20 ml of the extract was pipette into a volumetric flask and 1% starch indicator was added. These were added and titrated with 20% CuSO₄ solution to get a dark end point (Barakat et al., 1973).

RESULTS AND DISCUSSION

Table 1 summarizes the quantitative determination of phytochemical constituents of *B. pinnatum* and *A. africana*. High quantity of flavonoids, saponins and alkaloids were found on *B. pinnatum* and *A. africana*. The flavonoid content was more on *B. pinnatum* (1.86 mg/100 g) than *A. africana*, which contains 1.46 mg/100 g flavonoid. The values of phenolic compounds and tannins were very trace on both plants.

Table 1. Phytochemical composition of the leaves of *bryophyllum pinnatum* and *Aspilia africana* expressed as mg/100 g dry weight.

Phytochemicals	<i>Aspilia africana</i>	<i>Bryophyllum pinnatum</i>
Alkaloids	1.24 ± 0.11	1.48 ± 0.02
Flavonoids	1.48 ± 0.20	1.72 ± 0.11
Phenols	1.46 ± 0.02	1.86 ± 0.11
Tannins	0.04 ± 0.10	0.51 ± 0.20

Results are mean of triplicate determinations on a dry weight basis ± standard deviation.

The mineral contents of both plants are shown in Table 2. Calcium was the most abundant macro element present ranging from 1.04 mg/100 g in *A. africana* to 0.32 mg/100 g in *B. pinnatum*. This is followed closely by phosphorus, which was present from 0.32 mg/100 g in *A. africana* to 0.18 mg/100 g in *B. pinnatum*. Zinc was present at 5.68 mg/100 g in *A. Africana*, while *B pinnatum* contains 5.38 mg/100 g of zinc. Iron content was 3.78

Table 2. Mineral composition of the leaves of *Bryophyllum pinnatum* and *Aspilia africana* on mg/100 g dry weight.

Minerals	<i>Aspilia africana</i>	<i>Bryophyllum pinnatum</i>
Macroelements		
Magnesium	0.12 ± 0.11	0.10 ± 0.20
Calcium	1.04 ± 0.30	0.32 ± 0.10
Potassium	0.03 ± 0.10	0.04 V 0.11
Phosphorus	0.32 ± 0.20	0.18 ± 0.22
Sodium	0.02 ± 0.11	0.02 ± 0.10
Microelements		
Iron	3.78 ± 0.30	1.85 ± 0.20
Zinc	5.68 ± 0.10	5.38 ± 0.11

Results are mean of triplicate determinations on a dry weight basis ± standard deviation.

Table 3. Vitamin composition of *Bryophyllum pinnatum* and *Aspilia africana* on mg/100 g dry weight.

Vitamin	<i>Aspilia africana</i>	<i>Bryophyllum pinnatum</i>
Ascorbic acid	26.42 ± 0.10	44.03 ± 0.20
Riboflavin	0.20 ± 0.01	0.42 ± 0.10
Thiamine	0.11 ± 0.20	0.18 ± 0.02
Niacin	0.09 ± 0.11	0.02 ± 0.10

Results are mean of triplicate determinations on a dry weight basis ± standard deviation.

mg/100 g in *A. africana* and 1.85 mg/100 g in *B pinnatum*.

Results of analysis of *B. pinnatum* and *A. africana* showed that the plants are rich in vitamins (Table 3). Ascorbic acid (vitamin C) was found to be 44.03 mg/100 g in *B. pinnatum* and 26.42 mg/100 g in *A. africana*. Riboflavin, thiamine and niacin were also detected in both plants.

The presence of phenolic compounds in the plants indicates that these plants may be anti-microbial agent. This agreed with the findings of Ofokansi et al. (2005) who reported that *B. pinnatum* is effective in the treatment of typhoid fever and other bacterial infections, particularly those caused by *Staphylococcus aureus*, *Esterichia coli*, *Bacillus subtilis*, *Pseudomonas aeruginosa*, *klebsiella aerogenes*, *klebsiella pneumoniae* and *Salmonella typhi*. These findings supported the use of *B. pinnatum* in treating the placenta and navel of newborn baby, which not only heals fast but also prevent the formation of infections (Okwu, 2001, 2003).

The high saponin content of *A. africana* and *B. pinnatum* justifies the use of the extracts from these plants to stop bleeding and in treating wounds. 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., 2000; Okwu, 2004). These properties bestow high medicinal activities on the extracts from *B. pinnatum* and *A. africana*.

Apart from saponins, other secondary metabolite constituents of *B. pinnatum* and *A. africana* detected include the alkaloids and flavonoids. Pure isolated alkaloids and their synthetic derivatives are used as basic medicinal agents for their analgesic, antispasmodic and bactericidal effects (Stray, 1998; Okwu and Okwu, 2004). They exhibit marked physiological activity when administered to animals. Flavonoids, on the other hand are potent water-soluble antioxidants and free radical scavengers, which prevent oxidative cell damage, have strong anticancer activity (Salah et al., 1995; Del-Rio et al., 1997; Okwu, 2004). Flavonoids in intestinal tract lower the risk of heart disease. As antioxidants, flavonoids from these plants provide anti-inflammatory activity (Okwu, 2004). This may be the reason *B. pinnatum* and *A. africana* have been used for the treatment of wounds, burns and ulcers in herbal medicine. Tannins have stringent properties, hasten the healing of wounds and inflamed mucous membranes. These perhaps, explain why traditional medicine healers in Southeastern Nigeria often use *A. africana* and *B. pinnatum* in treating wounds and burns (Agoha, 1974).

Calcium was the most abundant macro element in the plants. Normal extra cellular calcium concentrations are necessary for blood coagulation and for the integrity, intracellular cement substances (Okaka and Okaka, 2001). Thus, the potentials of *A. africana* to stop bleeding and its use in treating wounds could be as a result of its high calcium content. The lower sodium content of *A. africana* and *B. pinnatum* might be an added advantage due to the direct relationship of sodium intake with hypertension on human (Dahl, 1972). The presence of zinc in the plants could mean that the plants can play valuable roles in the management of diabetes, which result from insulin malfunction (Okaka and Okaka, 2001).

These plants are good sources of ascorbic acids, riboflavin, thiamin and niacin (Table 3). Natural ascorbic acid is vital for the body performance (Okwu, 2004). Lack of ascorbic acid impairs the normal formation of intercellular substances throughout the body, including collagen, bone matrix and tooth dentine. A striking pathological change resulting from this defect is the weakening of the endothelial wall of the capillaries due to a reduction in the amount of intercellular substances (Hunt et al., 1980; Okwu, 2004). Therefore, the clinical manifestations of scurvy hemorrhage from mucous membrane of the mouth and gastrointestinal tract, anemia, pains in the joints can be related to the association of ascorbic acid and normal connective tissue metabolism (Hunt et al., 1980, Okwu, 2004). This function of ascorbic acid also accounts for its requirement for normal wound healing. As a result of the availability of ascorbic acid in *B. pinnatum* and *A. africana* these plants are used in herbal medicine for the treatment of common

cold and other diseases like prostate cancer (Okwu, 2003, 2004).

This study, therefore, has provided some biochemical basis for the ethnomedical use of extracts from *B. pinnatum* and *A. africana* in the treatment and prevention of infections. As rich source of phytochemicals, minerals and vitamins *B. pinnatum* and *A. africana* can be a potential source of useful drugs.

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