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Evaluation of the Phytochemicals of the leaf of *Bryophyllum pinnatum* in Port Harcourt

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ABSTRACT

This study was carried out to determine the qualitative phytochemical screening of *Bryophylum pinnatum(BP) using aqueous leaf extract.* Fresh leaves of *Bryophyllum pinnatum* were collected from the Botanical garden of the University of Port-Harcourt and were identified and authenticated in the Department of Plant Science and Biotechnology, University of Port Harcourt. Phytochemical screening of the extracts was carried out at the Pharmacognosy Department, Faculty of Pharmacy, University of Port-Harcourt. The result of the phytochemical screening showed the presence of Saponins, Carbohydrates, alkaloids, tannins, phenols, flavonoids, triterpenoid, steroids and anthraquinone. Cardinolide and Cyanogenic glycosides were absent in the aqueous leaf extract of *Bryophyllum pinnatum* in the present study. When comparing the result of the present study and that of other studies carried out at different geographical locations, it was observed that, there were slight differences in the phytochemical Constituents that were present. This may be as a result of differences in the geographical location and physicochemical nature of the soil where the plants were collected from. Knowledge gained from this work will be useful to the anatomist and ethnomedicinal plant researchers.

Key Words: *Bryophyllum pinnatum,* Ethnomedicinal plant, Phytochemical screening,

INTRODUCTION

According to the World Health Organization, a medicinal plant is any plant in which one or more of its organs contain substances that can be used for the synthesis of useful drugs[1]. Plants should be investigated to better understand their properties, safety and efficacy[2]. Medicinal plants contains biologically active chemical substances such as saponins, tannins, essential oil flavonoids, alkaloids and other chemical[3] which have curative properties. These complex chemical substances of different composition are found as secondary plant metabolite in one or more of these plants[4]. There are several published reports describing the antimicrobial activity of various crude plant extracts either in single or in combinations[5]. It is estimated that there are about 2.5 million species of higher plants and the majority of these have not yet been examined for their pharmacological activities. Herbal extracts are fast becoming popular as natural antimicrobial preservatives or additives[6].

Despite the availability of orthodox medicine, many people living in Nigeria rely heavily on herbal medicine for the management of various diseases and heal injuries[7]. *Bryophyllum pinnatum* belongs to the family Crassulaceae and it is commonly used in the ethnomedical practices. It is found in the tropical Africa, America ,India and Australia[8]. In West Africa, *Bryophyllum Pinnatum* is found in tropical and subtropical areas. The Yoruba Ethnic group calls it Abamoda/eru-odundun; the Igbo's refer to it as OdaaOpue; the Edo people calls it Alupu; while the hausa's refered to it as Sutura[9], ShukaHalinka" or Karan Masallachi[10]. The Okrika people calls it Mbukiba Diri. According to Mahendra *et al.[11], this plant is* called *Pattharcaṭṭa* as a medicinal plant in India. The leaf of *Bryophyllum pinnatum* can be used as an anti-inflammatory, antihypertensive and wound healing agent[12,13,14]. In Brazil, it is used as a natural anti-inflammatory agent[15]. The Anti-inflammatory activity of *Bryophyllum Pinnatum* has also been reported using leaf extracts[16].

Nwali *et al.*[17] stated in their phytochemical study that *Bryophyllum Pinnatum* can be used in the management of stroke. Akinnibosun and Edionwe[18] evaluated the Phytochemicals of the Leaf Extracts of *Bryophyllum pinnatum*. They reported that the plant contains saponins, tannins, flavonoids, alkaloids, polyphenols and other chemicals. flavonoids and polyphenols possess neuroprotective and spasmolytic activity. They strengthen the walls of capillaries. For the purpose of prevention and treatment of ischemia or reperfusion injury, flavonoids and polyphenols have the potential to be used[19,20]. flavonoids and polyphenols have the potential to be used in the management of other neurodegenerative disease[19,20].

Morphology of *Bryophyllum pinnatum(BP):* The plant *Bryophyllum pinnatum* is a succulent herb that is found in Nigeria and India[8]. The leaves of the plant are either compound or simple. Around the stem of *Bryophyllum pinnatum* are the leaves. The calyx and the Corolla surrounds the fruit of *Bryophyllum pinnatum*. The plant has greenish fillaments at the inferior aspects of the plant. The flowers of *Bryophyllum pinnatum* are pendulous. The branches are stout[8]. The leaflets of *Bryophyllum pinnatum* are eliptic or Ovate. *Bryophyllum pinnatum* is found in Rivers State, Nigeria.

PLANT TAXONOMY

Kingdom:	Plantae
Phylum	Magnoliophyta
Class	Magnoliopsida
Order	Saxifragales
Family	Crassulaceae
Subfamily	Allioideae
Genus	*Bryophyllum*
Specific	*Bryophyllum Pinnatum Lam*

SYNONYMS

Bryophyllum Calycinum Salisb
Bryophyllum germinans Blanco
Cotyledon Calycina Roth
Cotyledon Calyculata Solex Sims
Cotyledon Calyculata Solander
Cotyledon pinnata Lam.
Cotyledon rhysophylla Roxb.
Crassulla pinnata
Crassuvia florependia comm. Ex /lam

Figure 2: *Bryophyllum pinnatum*

It has been observed that the same plant may have slight variation in the phytochemical constituents due to differences in locations or country where the same plant is found. There is the need to evaluate the phytochemicals of *Bryophyllum pinnatum gotten from Port Harcourt.* This is the driving force behind this study. This study was carried out to determine the qualitative phytochemical screening of *Bryophylum Pinnatum(BP) using aqueous extract.*

MATERIALS AND METHODS

Fresh leaves of *Bryophyllum pinnatum* were collected from the Botanical garden of the University of Port-Harcourt and were identified and authenticated in the Department of Plant Science and Biotechnology , University of Port Harcourt.

Phytochemical screening of the extracts was carried out according to methods described by[21,22].

A). Test for Alkaloids: Five grams of evaporated extract was boiled with 5 ml of 2 % HCL on a steam bath for 5 mins, the mixture was filtered after cooling, and the filtrate was shared into 3 test tubes A B and C. 1 ml portion of filtrate was treated with 2 drops of Mayer's reagent, a creamy white precipitate was observed. To confirm this result, 1 ml portion of the filtrate was treated with Dragendoff's reagent which gave a red precipitate to indicate the presence of alkaloids.

B). Test for Flavonoids: Five grams of extracts was introduced into a test-tube containing 10 ml ethyl acetate solution and heated in boiling water for 1 min, the mixture was filtered and 4 ml of filtrate was shaken with 1ml of 1 % aluminum chloride solution and left to stand for 10 mins. The formation of a yellow colouration in the presence of 1 ml of dilute ammonia solution, indicated the presence of flavonoids.

C). Test for Saponins: One gram of extract was boiled with 5ml of distilled water for 5 mins and the mixture was filtered while hot. To 1 ml of filtrate, two (2) drops of olive oil was added, the mixture was shaken and observed for the formation of emulsion, then 1 ml of the filtrate was diluted with 4ml of distilled water. The mixture was shaken and observed for the formation of stable frothing on standing, which indicated positive for saponins.

D). Test for Tannins: To 2 g of the sample, 5 ml of 45 % ethanol was added and boiled for 5 mins. The mixture was cooled and filtered. To 1 ml of the filtrate, three (3) drops of lead acetate solution was added. The formation of gelatinous precipitate indicates the presence of tannins. Also as a confirmation test, 1 ml of filtrate was treated with 0.5 ml of bromine water and the formation of a pale brown precipitate indicates the presence of tannins.

E). Test for glycosides: Two grams of samples were mixed with 30 ml of distilled water and boiled for 5 mins in a water bath. The mixture was cooled and filtered. To 5 ml of the filtrate, 0.2 ml of Fehling's solution A and B were added and boiled further in a water bath for 2 mins. A brick red colouration which indicates the presence of glycosides was noticed.

F). Test for Terpenes
3ml of chloroform was added to 0.5g of extract, shaken and filtered; then 10 drops of acetic anhydrate followed by 2 drops of conc. tetra oxosulphate six acid. A reddish brown coloration at the interface was looked out for as evidense for the presence of terpenes.

G). Test for Phlobotannins: An aqueous extract of the sample was heated with 1% aqueous hydrochloric acid. Deposition of a red precipitate shows evidence for presence of phlobotannins.

H). Test for Cardiac Glycosides (Cardenolides): About 0.5g of the ext. was dissolved in 2ml of glacial acetic acid comprising one drop of 1% of $FeCl_3$ solution. This was then underlayed with 1ml of conc. tetra oxosulphate six acid. A brown ring of the interface indicates deoxy-sugar characteristics of cardenolides. A violet ring would appear beneath the brown ring, whereas in the acetic acid layer, a greenish ring would form in the thin layer.

(1.) Keller-killian test: About 0.5g of the ext. was dissolved in 2ml of glacial acid comprising 1 drop of $FeCl_3$ solution. This was underlayed with 1ml of conc. Sulphuric acid. A brown ring obtained at the interface indicates the presence of a deoxy-sugar characteristic of cardenolides.

(2.) Kedde test: 1ml of the plant extract was mixed with 1ml of a 2% solution of 3, 5-dinitrobenzenoic acid, concentrated with 1ml of a 5% solution of NaOH. An immediate violet colour indicates the presence of cardenolide the colour fading gradually through reddish brown to brownish-yellow with precipitate of a whitish crystalline solid.

I). Test for Triterpenoid: (1.) Salkowski's test: 0.5g of the plant extract was dissolved in 2ml of chloroform. Conc. tetra oxosulphate six acid was cautiously added to produce a layer. A reddish-brown colour at the interface indicates the presence of a steroidal nucleus (aglycone of cardiac glycoside).

(2) Lieberman-Burchard's test: 0.5g of the plant extract was dissolved in 2ml of acetic anhydride and dipped well in ice water. Conc. tetra oxosulphate six acid was cautiously poured on the side of the test tube to produce a layer. Colour changes from violet to blue to green indicates the presence of steroidal nucleus (aglycone of cardiac glycoside).

J). Test for Anthraquinones (Bomtrager's test)
(a.) Free Hydroxyl Anthraquinone: 5mg of the extract was agitated with 10ml of chloroform filtered and 5ml of 10% ammonia solution would then be added to the filterate, and the mixture shaken.

(b.) Combined Anthraquinone: 5mg of the plant extract will be boiled with 10ml aqueous sulphuric acid and filtered while hot. The filterate will then be shaken with 5ml of benzene, the benzene will separate and half its own volume of 10% ammonia solution added.

K. Test for Simple Sugar: (a.) Molisch's test: To 2ml of the plant extract, 2 drops of 10% of alcohol solution of alpha naphthol was added and inclined at 45° angle. 2ml of conc. tetra oxosulphate six acid was added in order to produce a layer below the extract solution without mixing. A deep violet ring was produced to indicate the presence of carbohydrate.

(b.) Fehling test: 2ml of well-mixed Fehling's solution was separately boiled and added with 2ml of the plant extract. A change of colour from yellow to red (red ppt. of cuprous oxide) indicates a positive reduction test.
L. Test for terpenoids: 0.5ml of acetic anhydride mixed with 1ml of ext., and a few drops of conc. tetra oxosulphate six acid. A bluish green ppt. shows presence of terpenoids.

M. Test for Phenol: Prepare by dissolving 5g of $FeCl_3$ in 100ml of distilled water. This gives the working $FeCl_3$ test reagent. Transfer 2ml each of the 0.1% of plant extract into test tube. Add drops of 5% $FeCl_3$ reagent. A green/ dark green/ blue/ blue black coloured complexes when treated with a solution of $FeCl_3$ shows the presence of Phenols.

RESULTS

The result of the Qualitative Phytochemical screening of the leaf of *Bryophyllum pinnatum* is shown in Table 1. The Qualitative Phytochemical screening of the leaf of *Bryophyllum pinnatum* showed positive results for Saponins, flavonoids, tannins, alkaloids,phenols,anthraquinone, triterpenoids and carbohydrates. The Phytochemical screening showed negative for cardinolide and cyanogenic glycosides.

Table 1: Qualitative Phytochemical screening of aqueous leaf extract of *Bryophyllum Pinnatum*

Phytochemical Constituents Assesed	Aqueous Extracts: Presence(+) Absence(-)
Alkaloid	+
Saponin	+
Tannin	+
Flavonoid	+
Phenol	+
Anthraquinone	+
Triterpenoid	+
Carbohydrates	+
Cardenolide	-
Cyanogenic Glycoside	-

Key: + =(Present) - = Absent

DISCUSSION

Bryophylum Pinnatum plant is used in Nigeria for wound healing and during umbilicus detachment of new born babies or infants[23].The plant is known as Zakham-ehayat[24]. Flavonoids and other antioxidant phytochemical constituents of *Bryophylum pinnatum* prevents oxidative damage of cells. They reduces oxidative stress[23]. Akinnibosun and Edionwe[18], reported that,saponins has antioxidant and anti-inflamatory activity. Akinnibosun and Edionwe[23] also observed in their phytochemical screening of *Bryophyllum pinnatum* that, flavonoids, alkaloids, saponins and reducing sugar were present in the aqueous extract. Steroids, tanins and cardiac glycosides were absent in their aqueous extract during the phytochemical screening. This is not in line with the result of the present study. In the present study, saponins, carbohydrates, alkaloids, tannins, phenols, flavonoids, triterpenoid, steroids and anthraquinone were present. Tanins and steroids which were absent in the study of Akinnibosun and Edionwe[23] , was found to be present in our study. The *Bryophyllum pinnatum* in the study of Akinnibosun and Edionwe[23] was collected from Benin City , Edo State. In the study conducted by Santosh *et al*.[25], it was observed that steroids and carbohydrates were absent in aqueous leaf extract of *Bryophyllum pinnatum*. This is not in line with the present study. Carbohydrates and steroids were present in our study. In the study of Santosh *et al*.[25], it was observed that Tannin, flavonoids, alkaloids and saponins were present. This is in line with our study. In the study of Santosh *et al.*[25], the leaves of *Bryophyllum pinnatum* plant were collected from rural area of Bhopal (M.P), India. Osunlana *et al*.[26], studied the qualitative phytochemical screening of *Bryophyllum pinnatum* . The *Bryophyllum pinnatum* in their study was collected from Lagos state, in the South West of Nigeria. They observed that, cardiac glycosides, flavonoids, Phlobotannins, steroids were absent. This is not in line with the result in the present study. Anthocyanosides were absent in their study which is in line with the result of the present study. Sani *et al.*[27] studied the leaves of *Bryophyllum pinnatum.* The plant was collected from the outskirts of Kaduna North, Kaduna state, Nigeria. They observed that steroids and tannins were absent in the aqueous extract. This is not in line with present study. Steroids and tannins were present in the present study. Our study is in line with the study carried out in Bayelsa State by[12]. The phytochemicals present in the aqueous leave extract of *Bryophyllum pinnatum* in their study is in line with ours. The *Bryophylum Pinnatum* in the present study was collected from the Botanical Garden of University of Port Harcourt. The differences in phytochemicals may be as a result of geographical location differences and physicochemical nature of the soils were plants were collected from. Sofowora[28] , stated that tannins, saponins and flavonoids are phytochemicals found in medicinal plants. These phytochemicals have a curative property. Kayode and Kayode[4], observed that, these phytochemicals are found in plants as secondary metabolites and they are therapeutically useful. Recently, about thirty percent of pharmacological drugs produced are from plant extract. This could be directly from plant or indirectly from the plant extract[29,30,31]. The antihypertensive activity of extracts *Bryophylum pinnatum* has been studied using different animal models such as cats, guinea pigs and rats[32,33,34]. The anti-oxidant and anti-inflammatory activity of *Bryophylum pinnatum* has also been studied[35,16]. Natural products (i.e. medicinal plants) with intrinsic antioxidant property constitute an ideal choice for maximum therapeutic effects with minimal risk of adverse effects[36]. The phytochemical screening of *Bryophylum Pinnatum* showed the presence of triterpenoids, tannins and flavonoids. Tannins and flavonoids are well known to have antioxidant properties, anti-inflammatory and antiproliferative activity[37]. Knowledge of the phytochemical screening of *Bryophylum pinnatum will be relevant to the Anatomist and ethnomedicinal plant researcher. It will enable one*

to appreciate the slight variations in the phytochemical constituents of Bryophylum pinnatum due to geographical locations and physicochemical differences of soil.

CONCLUSSION

This study has provided a reference data on the qualitative phytochemical analysis of *Bryophyllum pinnatum* collected from Port Harcourt City. It will be useful to the Anatomist, Ethnomedicinal researchers and medical scientist.

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