GSC Biological and Pharmaceutical Sciences

eISSN: 2581-3250 CODEN (USA): GBPSC2

Cross Ref DOI: 10.30574/gscbps

Journal homepage: https://gsconlinepress.com/journals/gscbps/

(RESEARCH ARTICLE)

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An assessment of phytochemical constituents of *Brophyllum pinnatum* (Odaa Opue)

I. I. Ujah [1,*] and C.K Onyishi [2]

[1] *Department of Applied Biochemistry Enugu State University of Science and Technology, Enugu State Nigeria*

[2] *Department of Pharmaceutical Microbiology and Biotechnology, Faculty of Pharmaceutical Sciences, Enugu State University of Science and Technology, Nigeria.*

GSC Biological and Pharmaceutical Sciences, 2023, 23(01), 154–159

Publication history: Received on 26 December 2022; revised on 09 April 2023; accepted on 12 April 2023

Article DOI: https://doi.org/10.30574/gscbps.2023.23.1.0494

Abstract

This study investigated the phytochemical composition of the leaf extract of the plant *Bryophyllum pinnatum* (Odaa Opuee). The qualitative analysis of *Bryophyllum pinnatum* results revealed the presence of terpenoids, flavonoids, and phenols, while the quantitative analysis were flavonoids (1.387 ± 0.020) terphenoids (1.560 ± 0.081), and phenol (1.193 ±0.009). The results of this research indicates that *Bryophyllum pinnatum* contain some bioactive compounds which could be of medicinal uses.

Keywords: Phytochmical; *Brophyllum pinnatum*; Terpenoids; Flavonoids; Phenols; Bioactive

1. Introduction

Bryophyllum pinnatum is one the indigenous shops in Nigeria which is used for herbal medicinal purposes (Okwu, 2006). *Bryophyllum pinnatum* plant is linked with different names similar as rejuvenation plant, air plant, love plant, phenomenon plant and life plant. It's an imperishable condiment used in folkloric drug in tropical Africa, India, Chain, Australia and Tropical America (Okwu, 2003). *Bryophyllum pinnatum* is classified as weed and the plant grows well throughout the southern part of Nigeria. It's a Crassulaceae condiment of about1.5 m altitudinous and reproduced through seeds and also vegetative form. It's has a altitudinous concave stems, lately dark green leaves that are distinctively scalloped and trimmed in red and dark bell like pendulous flowers (Yadav and Dixit, 2003). *Bryophyllum pinnatum* is distributed worldwide but growing primarily in the rain timber. It's sour in taste, sweet in the post digestive effect, has hot energy and the factory can fluently be propagated through stems or splint slice (David, 2000).

Bryophyllum pinnatum is extensively distributed especially in Philippines and it's known as katakataka which is an adjective for astonishing or remarkable (Duster, 1985). The factory is fluently propagated, plant in chaparrals and open places, the active constituents of utmost of the generally used conventional medicines were firstly deduced from factory part before their medicinal mass product from synthetic chemical (Sofowara 1993). But it's generally used in traditionally drug in different regions of India substantially to treat urinary monuments (Gill, 1990). It's an introduced cosmetic factory that's now growing as seed around colony crops (Dalziel, 1995).

Bryophyllum pinnatum is used in ethno drug for treatment of numerous conditions similar as observance pang, becks, abscesses, ulcer, nonentity mouthfuls, diarrhea and Lithiasis (Chopra et al., 1956). In South Eastern Nigeria, this condiment is used to grease the dropping of the placenta of a recently born baby (Daziel, 1995). The factory splint is mildly exposed to heat and the juice uprooted and applied to the baby's placenta on diurnal base. The crushed leaves as well as the uprooted juice are mixed with win canvas and rubbed on abscesses or other smelling. *Bryophyllum pinnatum* is generally applied externally. There may be secondary metabolites which are attained from different corridor of factory similar as alkaloid, flavanoid, tannin, glycoside, phenolic composites, which have remedial value. The factory is

*Corresponding author: I. I. Ujah

used for different pharmacological purposes similar as antidiabetic, antihypertensive, anti-leishmanial, antimicrobial, cancer preventative and insecticidal conduct (Fujita, 2000). The species is allowed to be toxic to beast, as it contains cardiac glycosides (Ayodele, 2001). In the traditional drug *Bryophyllum pinnatum* is also used in the treatment of respiratory infections, cough, stomach ulcer, and infections of the skin, eyes and cognizance (AOAC, 1990). This study therefore aimed at probing the phytochemical ingredients of the shops that have given the shops some of its medicinal parcels

2. Material and methods

2.1. Collection of sample

Fresh leave of *Bryophyllum* were obtained from Agbani in Nkanu West Local Government Area of Enugu State, Nigeria. It was then identified by Prof. Helen Nwamba of Enugu State University of Science and Technology.

2.2. Sample preparation

The samples were dried at room temperature for three weeks after which it was ground to fine powder using grinding machine

2.3. Extraction

For aqueous extraction, the weighing balance was zeroed and the filter papers were placed on the weighting balance, a quantity 0.5 g of the sample were weighed and poured into a reagent bottle. A volume, 10 ml of distilled water was added to facilitate extraction. After some minutes, proper filtration was carried out using filter paper and the filtrate analysed to determine the presence and quantity of phytochemicals such as tannins, alkaloids, saponins glycosides, terpenoids, flavonoids, steroids and phenols. .

2.4. Qualitative Phytochemical Analysis

The qualitative phytochemical analysis were done following Harborne (1998); Trease and Evans (1989) protocols. The preliminary analysis involved testing for the presence of flavonoids, terpenoids, steroids, saponins, alkaloids, tannins, glycosides and phenols.

2.5. Test for Tannins

Extract (0.1 g) was stirred with 10 ml of distilled water and then filtered. Few drops of 1 % ferric chloride solution were added to 2 ml of each filtrate. The presence of a blue-black or blue-green precipitate indicated the presence of tannins (Trease and Evans, 1989).

2.6. Test for the Alkaloids

A quantity of the extract (0.1 g) was dissolved individually in dilute Hydrochloric acid and filtered.Hager's Test: Filtrates were treated with Hager's reagent (saturated picric acid solution). Presence of alkaloids was confirmed by the formation of yellow coloured precipitate (Trease and Evans, 1989).

2.7. Test for Saponins

A quantity of each extract (0.1 g) was boiled with 5 ml of distilled water and filtered. To each filtrate, about 3 ml of distilled water was further added and shaken vigorously for about 5 minutes. Frothing which persisted on warming was taken as an evidence for the presence of saponins (Sofowara, 1993).

2.8. Test for Glycosides

Each extract (0.1 g) was mixed with 30 ml of distilled water and heated on a water bath for 5 minutes. To 5ml of each of the filtrates, 0.2 ml of Fehling's solution A and B were added until it turned alkaline. The solutions were heated on a water bath for 2 minutes. A brick-red precipitate indicated the presence of glycoside (Sofowara, 1993).

2.9. Test for Terpenoids

Each extract (0.1 g) was dissolved in ethanol. Acetic anhydride (1 ml) was added, followed by the addition of concentrated H_2SO_4. A change in colour from pink to violet showed the presence of terpenoids (Sofowara, 1993).

2.10. Lead Ethanoate Test for Flavonoids

A quantity (0.1 g) of each extract was dissolved in water and filtered. To 5 ml of each of the filtrates, 3 ml of lead ethanoate solution was added. Appearance of a buff – coloured (pale yellow-brown) precipitate indicated the presence of flavonoids (Trease and Evans, 1989).

2.11. Liebermann-Buchard Test for Steroids

To 0.1 g of each extract, 2 ml of acetic acid was added. The solution was cooled well in ice followed by the careful addition of concentrated tetraoxosulphate (VI) acid (H_2SO_4). Colour development from violet to blue or bluish-green indicated the presence of a steroidal ring (Sofowara, 1993).

2.12. Ferric Chloride Test for Phenols

About 0.1 g of each extract was boiled with distilled water and then filtered. To 2 ml of each filtrate, few drops of 10 % ferric chloride solution were then added. A green-blue or violet colouration indicated the presence of a phenolic hydroxyl group (Trease and Evans, 1989).

2.13. Quantitative Phytochemicals Analysis

2.13.1. Determination of Alkaloids (Harborne, 1973)

The sample was weighed (1.0 g) using electric weighing balance into a 250 ml beaker; 100 ml of 10 % acetic acid in ethanol was added to the sample and covered. The mixture was allowed to stand for four hours for proper extraction to take place. The sample was filtered with filter paper and the extract was concentrated on a water bath to one quarter of the original volume. A volume, 20 ml of ammonium hydroxide was added drop wisely to form precipitate of the alkaloid in the filtrate. The filtrate was weighed with NH_4OH and filtered. The filter paper was weighed before using it to filter. After filtering, the filter paper and the precipitate were dried in an oven at 40 ^{0}C and weighed. The alkaloid content was determined using the following formula.

$$\text{Concentration of alkaloid} = \frac{W2-W1}{W3}$$

Where W_1 = weight of empty filter paper
W_2 = weight of the alkaloid and filter paper
W_3 = weight of sample used

2.14. Determinations of Saponins

A quantity of (1.0 g) of the sample was weighed using an electric weighing balance into 250 ml conical flask and soaked with 100 ml of 20 % ethanol for three (3) minutes and heated for three (3) hours at 55 ^{0}C for proper extraction then filtered. The residue was re-extracted with another 100 ml of 20 % ethanol. The two extracts were combined and heated to 40 ml at 90 ^{0}C on a water bath. The concentrate was transferred into a 500 ml separating funnel and 20 ml of diethylether was added and shaken vigorously, the upper layer was discarded. The purification process was repeated and 60 ml of n-batanol was added, the lower layer was discarded while the upper layer was collected. The combined n-butanol extract was washed with 10 ml of 5 % aqueous NaCl and the lower layer was discarded while the upper layer was collected in a weighed beaker and heated to dryness. The beaker is allowed to cool in desiccators and re-weighed. The saponin content was determined using the following formula.

$$\text{Concentration of saponin} = \frac{W2-W1}{W3}$$

Where
W_1 = weight of empty beaker
W_2 = weight of beaker + sample after heating
W_3 = weight of sample used

2.15. Determination of Tannins (Robinson, 1981)

Extract of the sample was weighed (1.0 g) into a plastic bottle and 50 ml of distilled water was added and shaken for 3 hours in a vibrator. The sample was filtered into a 50 ml volumetric flask and made up to mark. A volume, 5 ml of the filtrate was dispensed into a test tube and mixed with 2 ml of 0.1m $FeCl_2$ in 0.1N HCl and 0.008 M potassium

ferrocyamide, the absorbance was measured at 120 nm for 10 mins. The tannin concentration was determined using the following relation.

$$\text{Concentration of tannin} = \frac{Abs\ x\ Path\ length}{100\ x\ weight\ of\ sample\ used}$$

Where
Abs = value of absorbance read
D.F = dilution factor

2.16. Determination of Flavonoids (Bohn and Kocipal-Abyassan, 1994).

Extract (1.0 g) was repeatedly extracted with 100 ml of 80 % aqueous methanol at room temperature; the solution was shaken for 30 mins and filtrate was transferred into a weighed beaker and evaporated to dryness over a water bath and weighed again. The time for the first extraction was 1 hour, 45 mins for the second extraction and 30 mins for the third extraction. Flavonoid was determined using the following formula.

$$\text{Concentration of Flavonoid} = \frac{W2-W1}{W3}$$

Where
W_1 = weight of empty beaker
W_2 = weight of beaker + sample after drying
W_3 = weight of sample used

2.17. Determination of Steroids (Okeke and Eloku Method)

Extract (1.0 g) was dispersed in 100 ml of distilled water into a conical flask; the mixture was shaken for 3 hours and allowed to stand overnight. It was then filtered, the filtrate was eluted with 10 ml normal ammonium hydroxide solution, 2 ml of the elute was put into a test tube and mixed with 2ml of chloroform and also 3 ml of acetic hydride was added to the mixture, followed by 2ml of concentrated H_2SO_4 drop wisely. The absorbance was measured in a Spectrophotometer at 420 nm.The steroid concentration was determined using the following relationship

$$\text{Concentration of steroids} = \frac{Abs\ x\ Path\ length}{100\ x\ weight\ of\ sample\ used}$$

2.18. Quantification of Terpenoid Content (Harborne, 1973)

A quantity (0.1 g) of the extract was weighed out separately, macerated with 20 ml of ethanol and filtered through Whatman No. 1 filter paper. The filtrates (1 ml) were pipetted out and 1 ml of 5 % phosphomolybdic acid solution was added and shaken. Gradually 1 ml of concentrated H_2SO_4 was added to each. The mixtures were left to stand for 30 minutes. Ethanol (2 ml) was added and absorbance was measured at 700 nm.

$$\text{Concentration of terpenoid} = \frac{AbsxPathlength}{100\ xweightofsampleused}$$

2.19. Quantification of Glycoside Content (Harborne, 1973)

The extract (0.1 g) was weighed out separately, macerated with 20 ml of distilled water and 2.5 ml of 15 % lead acetate was added and filtered. Chloroform (2.5 ml) was added to the filtrates, shaked vigorously and the lower layer collected and evaporated to dryness. Glacial acetic acid (3 ml) was also added together with 0.1 ml of 5 % ferric chloride and 0.25 ml of concentrated H_2SO_4. The mixture was shaken and put in the dark for 2 hours. Absorbance was measured at 530 nm.

$$\text{Concentration of glycoside} = \frac{AbsxPathlength}{100\ xweightofsampleused}$$

3. Results

Table 1 Qualitative Phytochemicals

Phytochemicals	Abundance
Alkaloid	ND
Flavonoid	+++
Saponin	ND
Steroid	ND
Tannin	ND
Glycoside	ND
Terpenoid	+++
Phenol	+++

+ = minimally present; ++ = moderately present; +++ = highly present; ND= not detectable

Table 2 Quantitative Phytochemicals

Phytochemical parameters	Concentration (mg/g)
Flavonoids	1.387 ± 0.020
Terpenoids	1.560 ± 0.081
Phenol	1.193 ± 0.009

4. 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 dry samples which revealed the presence of flavonoid, terpenoid and phenol. In Nigeria and other west Africa countries, its fleshy leave of *Bryophyllumpinnatum* extract is used as herbal remedy for an array of human disorders including hypertension, diabetes, bruises , wounds , boils , insect bite , headache and body pains (Quazi Majaz, 2001). These properties prove a high medicinal value of the extract and other secondary metabolite constituent of *Bryophyllum pinnatum* which includes; flavonoid, terpenid and phenol. 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; this may be the reason *Bryophyllum pinnatum* is used in treatment of fevers, bruises and ulcer in herbal medicine (Okwu, 2004). Terpenoid have astringent properties, hasten the healing of wounds and inflamed mucous membranes. This also explains the reason why traditional medicines healers in South Eastern Nigeria often use *Bryophyllum pinnatum* in treating wounds and burns (Aghoha, 1974). The phenols level of *Bryophyllum pinnatum* shows that the sample can be used as a CMS stimulant and as powerful pain relievers (Stray, 1998). This study has provided some phytochemical constituents of the plant of *Bryophyllum pinnatum*.

5. Conclusion

The phytochemical analysis *Bryophyllumpinnatum* revealed the presence of flavanoid, terpenoids and phenols. These constituents could be of medicinal use.

Compliance with ethical standards

Acknowledgments

The Authors wish to acknowledge the Department of Applied Biochemistry Enugu State University of Science & Technology where this research was carried out.

Disclosure of conflict of interest

There is no conflict of interest.

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