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Pharmacognostic and Antioxidant activity of *Bryophyllum pinnatum*
Leaves

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ABSTRACT

Bryophyllum pinnatum is used in ethno medicine for treatment of earache, burns, abscesses, ulcer, insect bites, and diarrhoea. In Nigeria, this herb is used to facilitate the dropping of the placenta of a newly born baby. To search for new sources of safe and inexpensive antioxidants, the leaf of *Bryophyllum pinnatum* was screened for its antioxidant activity. Sample of leaf from *B. pinnatum* was tested for organoleptic, phytochemical, thin layer chromatography and antioxidant activity using standard procedure. The leaf was found to be simple, petiolated 6.8cm in size. Microscopically, the leaf had irregularly shaped epidermal cells with numerous anisocytic stomata on the lower surface. Chemomicroscopical evaluation revealed the presence of cellulose, tannins, starch, lignin, calcium oxalate, suberin, aleurone grain and mucilage with the exception of calcium carbonate. Phytochemicals which include alkaloids, phenols, flavonoids, saponins, tannins, carbohydrates and triterpenes were detected in both aqueous and methanolic extracts while anthraquinones was absent. Methanol extract when developed in Chloroform: Methanol (9:1) and virtualized with *p*-Anisaldehyde/H₂SO₄, it gave six spots. The DPPH radical scavenging ability of the extract showed the following trend Ascorbic acid > extract. These results suggested that *B. pinnatum* leaves have moderate antioxidant potentials. Further study is necessary for isolation and characterization of active antioxidant agents which can be used to treat various oxidative stress related diseases.

Key words: Chemomicroscopical, evaluation, organoleptic, phytochemical, antioxidant, activity, *Bryophyllum pinnatum*

1.0 INTRODUCTION

1.1 Discovery of Drugs

The total combination of knowledge and practices, whether explicable or not, used in diagnosing, preventing or eliminating physical, mental or social diseases and which may rely exclusively on past experience and observation handed down from generation to generation, verbally or in writing (WHO, 2008). Recently, there has been a renewed interest in natural product research due to the failure of alternative drug discovery methods to deliver many lead compounds in key therapeutic areas such as immunosuppression, anti-infectives, and metabolic diseases. These approved substances are representative of very wide chemical diversity and continue to demonstrate the importance of compounds from natural sources in modern drug discovery efforts (Chin *et al.*, 2006). Natural products have played a key role in pharmacy researches, as many medicines are either natural products or derivatives thereof. Indeed, it is estimated that about 40% of all medicines is either natural products or their semi-synthetic derivatives (Jacob, 2009).

1.2 Statements of problem

Despite the fact that the leaves of *B. pinnatum* have several medicinal properties, no standardization parameter has been assessed. Free radicals are implicated in various diseases which include cancers, fever, diabetes, inflammation, neurodegenerative diseases, rheumatoid arthritis etc. *B. pinnatum* has numerous folkloric uses, antioxidant being one but compound(s) responsible for this activity has been isolated and elucidated. Due to lack of standard parameters, proper identification and ascertaining quality and purity of the leaves of *B. pinnatum* in the events of adulteration has been thwarted. Reactive Oxygen Species (ROS) can damage the integrity of various biomolecules including lipids (Yla-Herttuala, 1999) proteins (Stadtman and Levine, 2000) and DNA (Marnett, 2000) leading to increased oxidative stress in various human diseases such as diabetes mellitus, neurodegenerative diseases, rheumatoid arthritis, cataracts, cardiovascular diseases, respiratory diseases as well as in aging process.

1.3 Pharmacological Significance of *Bryophyllum pinnatum* (Lam.)

Bryophyllum pinnatum (Lam.), syn. *Kalanchoe pinnata* (Lam.), and *B. calycinum* (Salisb.) is a widely distributed perennial medicinal herb. It is native to Madagascar, but has been naturalized in several other regions, including the temperate regions of Asia, Australia, and New Zealand. *B. pinnatum* is known by some common names including, life plant, air plant, maternity plant, love plant, miracle leaf, cathedral bells, mother of thousands, leaf of resurrection plant, and *Lao di Sheng gen* (Aprioku and Igbe, 2017). The plant is locally called "Never Die" in Nigeria, and is very popular in folklore medicine. It has been used for the treatment of a variety of conditions in tropical America, India, China, Australia and Africa, including, rheumatism, body pain, arthritis, heartburn, skin ulcers, peptic ulcer, diabetes mellitus, microbial infections, and hypertension (Aprioku and Igbe, 2017). In Nigeria, the plant is particularly known for its effective wound healing properties and detachment of the umbilicus of infants. Pharmacological studies on *B. pinnatum* reported several biological activities some of which could authenticate the plant's traditional uses including, immunomodulatory, CNS depressant, analgesic, antiinflammatory, antimicrobial, antitumor, antiulcer, insecticidal, antidiabetic, anticonvulsant, antioxidant, and antihypertensive properties (Aprioku and Igbe, 2017). Studies have also reported a wide range of active phytochemicals such as alkaloids, triterpenes, glycosides, flavonoids, steroids, bufadienolides, lipids and organic acids. These compounds have been considered to be responsible for the plant's diverse pharmacological activities.

Although, folklore claims of many herbal remedies are yet to be authenticated scientifically, *B. pinnatum* has been reasonably studied with justification of most of the claims. This has enhanced the promotion of the use of *B. pinnatum* and other plants either as alternatives or to complement orthodox medicines. The objective of the present investigation is to establish some important pharmacognostic profile and antioxidant activity of *Bryophyllum pinnatum* leaves with the

hope of assisting in its standardization for quality and purity.

1.4 Antioxidant Activity of *Bryophyllum pinnatum*

Plants with medicinal properties are used for the treatment of diseases (Sofowora *et al.*, 2013). They produce several secondary metabolites with many important biological activities (Guerriero *et al.*, 2018). Some of these plant have anticoagulant and antioxidant activities (Felix, 2014).

Oxidation is a critical metabolic process that aid living functions. Metabolism of oxidation produces reactive oxygen species (ROS) that causes oxidative stress which leads to cell structure damages and contribute to pathogenesis of most inflammatory diseases, neurodegenerative cardiovascular diseases, and cancers by attacking biological molecules (Do Hoang *et al.*, 2018). Free radicals are unstable chemical species, which tend to trap electrons from the molecules in the immediate surroundings (Phaniendra *et al.*, 2015). These radicals may damage lipids, proteins and DNA leading to disease condition if not scavenged (Lobo *et al.*, 2010). Furthermore, antioxidants scavenge reactive species and upregulate antioxidant defences (Shadab *et al.*, 2012). Researchers have been focused on protecting the human body from oxidative damage caused by free radicals with the use of natural antioxidants (Godwin *et al.*, 2017). Phytochemicals are bioactive chemicals of plant origin. They are regarded as secondary metabolites because the plants that manufacture them may have little need for them. They are naturally synthesized in all parts of the plant body; bark, leaves, stem, root, flower, fruits and seeds. Any part of the plant body may contain active components (Kalyani and Bandita, 2014)

1.5 Hypotheses and Research Design

Leaves of *B. pinnatum* contains bioactive constituents with potent antioxidant properties. The aim of this work is to carry out pharmacognostic standardization and antioxidant analysis on *Bryophyllum pinnatum* leaves.

2.0 METHODS

2.1 Materials for Collection and Identification of Plant

The leaves of *Bryophyllum pinnatum* was collected in May, 2019 at Botanic Garden, Department of Plant Biology, Bayero University Kano. The plant was identified and authenticated in the Herbarium of the Plant Biology Department of Bayero University, Kano by Malam Bahawuddeen and was compared with a voucher specimen number of BUKSAN14.

2.2. Extraction

The leaves collected were air-dried for two weeks and size reduced with mortar and pestle. This was extracted by cold maceration using absolute methanol. 100g of the powdered leaves was added into Erlenmeyer flask with 500ml of the methanol and kept for 48 hours and shaken intermittently, then filtered. The extract was evaporated to dryness in an evaporator under reduced pressure and controlled temperature (40-60°C). After evaporation, the extract was preserved in a levelled air tight container for future (Evans, 2009).

2.3 Chemo-microscopic Studies on the powdered leaves of *Bryophyllum pinnatum*

Powdered sample (2g) was used for this study to detect the presence of cell wall materials and cell inclusions. Finely ground sample of plant was cleared in a test tube containing 70% chloral hydrate solution. It was then boiled on a water bath for about thirty minutes to remove obscuring materials. The cleared sample was mounted with dilute glycerol onto a microscope slide. Using various detecting reagents the presence of cell wall materials and cell inclusions was detected in accordance to WHO (2011) guidelines.

2.3.1 Cell wall Materials

2.3.1.1 Test for Cellulose

A drop or two of iodinated zinc chloride was added to the powdered sample and allowed to stand for a few minutes and observed under a microscope. It stained cellulose cell wall blue to blue- violet (WHO, 2011).

2.3.1.2 Test for Lignin

The powdered plant material was moistened on a slide with a small volume of phloroglucinol and allowed to stand for about two minutes or until almost dry. A drop

of hydrochloric acid was added and viewed under a microscope. Pink stained or cherry red observed indicates the presence of lignin (WHO, 2011).

2.3.1.3 Test for Suberized or Cuticular cell walls

Two drops of Sudan red was added to the cleared powdered sample and allowed to stand for few minutes and observed under a microscope. Orange red or red colour observed indicates the presence of suberin or cutin on the cell (WHO, 2011).

2.3.1.4 Test for Gum and Mucilage

To a small portion of the cleared powdered sample of the plant, a drop of ruthenium red was added. Appearance of pink coloration was considered positive for gums and mucilage (WHO, 2011).

2.3.2 Cell Inclusions

2.3.2.1 Test for Starch grains

To a small portion of the cleared powder sample of the plant, N/50 iodine was added. Appearance of blue-black or reddish-blue coloration on some grains would be considered positive for starch (WHO, 2011).

2.3.2.2 Test for Calcium oxalates and Calcium Carbonates

To a small portion of the cleared powdered sample of the plant, HCl was added, dissolution of crystals in the powdered drug without effervescence was considered positive for calcium oxalate while slow dissolution with effervescence was considered positive for calcium carbonate (WHO, 2011).

2.3.2.3 Inulin

A drop of 1-naphthol and that of sulphuric acid was added to the powdered sample and viewed under the microscope. Spherical aggregations of crystals of Inulin turned brownish red and dissolve (WHO, 2011).

2.3.2.4 Test for Tannins

To a small portion of the cleared powdered sample of the plant, 5% ferric chloride solution was added. Appearance of greenish black colour was considered as positive for tannins (WHO, 2011).

2.3.2.5 Phytochemical Screening

Phytochemical screening was carried out on the dried extract using simple chemical tests to detect the presence of alkaloids, saponins, tannins, flavonoids,

terpenoids, anthraquinones, and cardiac glycosides. The following methods were used:

2.3.2.6 Tests for carbohydrates

Molish's (General) Test for Carbohydrates:

To 1 ml of the filtrate, 1 ml of Molish's reagent was added in a test tube, followed by 1 ml of concentrated sulphuric acid down the test tube to form a lower layer. A reddish colour at the interfacial ring indicates the presence of carbohydrate (Evans, 2009).

2.3.2.7 Tests for Saponins

Frothing test

About 10ml of distilled water was added to a portion of the extract and was shaken vigorously for 30seconds. The tube was allowed to stand in a vertical position and was observed for 30mins. A honeycomb froth that persists for 10-15mins indicates presence of saponins (Evans, 2009).

2.3.2.8 Test for Flavonoids

Shinoda Test

A portion of the extract was dissolved in 1-2ml of 50% methanol in the heat metallic magnesium chips and few drops of concentrated hydrochloric acid were added. Appearance of red colour indicates presence of flavonoids (Evan, 2009).

2.3.2.9 Test for Alkaloid

Wagner's Test

Few drops of Wagner's reagents were added to a portion of the extract, whitish precipitate indicates the presence of alkaloid (Evans, 2009).

2.3.2.10 Test for Steroid and Triterpenes

Liebermann-Burchard's test

To a portion of the extract, equal volume of acetic acid anhydride was added and mixed gently. 1ml of concentrated sulphuric acid was added down the side of the test tube to form a lower layer. A colour change immediately and later indicates the presence of steroid and triterpenes. Red, pink or purple colour indicates the presence of Triterpenes while blue or blue green indicates steroids (Evans, 2009).

2.3.2.11 Test for Cardiac Glycoside

Kella-killiani's test

A portion of the extract was dissolved in 1ml of glacial acetic acid containing traces of ferric chloride solution. This was then transferred into a dry test tube

and 1ml of concentrated sulphuric acid was added down the side of the test tube to form a lower layer at the bottom. Observed carefully at the interphase for purple-brown ring, this indicates the presence of deoxy sugars and pale green colour in the upper acetic acid layer indicates the presence of cardiac glycosides (Evans, 2009).

2.3.2.12 Test for Tannins

Ferric chloride test

To a portion of the extract, 3-5 drops of ferric chloride was added. A greenish black precipitate indicates presence of condensed tannins while hydrolysable tannins give a blue or brownish blue precipitate (Evans, 2009).

2.3.2.13 Test for Anthraquinones

Bortrager’s test

To a portion of the extract in a dry test tube, 5ml of chloroform was added and shaken for at least 5mins. This was filtered and the filtrate shaken with equal volume of 10% ammonium solution, bright pink colour in the aqueous upper layer indicates the presence of free anthraquinones (Evans, 2009).

2.3.3 Thin Layer Chromatography Profile

TLC aluminum sheet of 20 x 20cm silica gel pre-coated plate using the one way ascending techniques was employed for this analysis. The plates were cut into sizes of 5 x 10 cm. The extract was dissolved in the initial extraction solvents and spots were applied manually on the cut plate using capillary tubes, after which the plates were dried and developed in solvents ratio Hexane: Ethyl acetate (8:2) in the chromatographic tank. Developed plates were sprayed using general detecting reagents (*p*-Anisaldehyde/H₂SO₄, 10% H₂SO₄ in methanol). It was viewed under UV 365nm) and heated at 110°C for 2

minutes where applicable. Number of spots, colour and retardation factors (Rf values) for each of the spots were determined and recorded while the chromatograms was scanned accordingly (Gennaro, 2000; Stahl, 2005).

2.3.4 Antioxidant Activity Procedure

The antioxidant activity of the plant extracts was measured in terms of radical scavenging ability, using a stable radical 2,2-diphenyl-1-picrylhydrazyl (DPPH) according to the modified method adopted from (Sani and Dailami, 2015). 200µl of 100µM methanol solution of DPPH were added to 100µL of various concentrations of the sample fractions in methanol (1000, 500, 250, 125, 62.5, 31.25, and 15.63µg/ml) and made to react in dark for 30mins time at room temperature. Absorbance of the blank, test and control were recorded at 517 nm. The experiment was performed in triplicate and scavenging activity was calculated by using the following formula and expressed as percentage of inhibition.

$$\% \text{ Inhibition} = \frac{\text{Absorbance of control} - \text{Absorbance of test}}{\text{Absorbance of control}} \times 100\%$$

The concentration corresponding to the 50%inhibition (IC₅₀) was determined using probit analysis by means of SPSS 16.0 software. The IC₅₀ values obtained are compared with that of ascorbic acid as a standard antioxidant (Sani and Dailami, 2015).

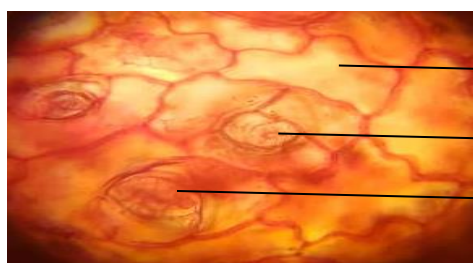
3.0 RESULTS

The leaves was found to have dark green and light green colour on the upper and lower surfaces, slightly rough texture with granules, characteristic smell, intense bitter taste, reticulate venation and 6-8cm in size.

Table 1: Organoleptic/ Macroscopic Features of B. pinnatum Leaves

Features	Description
Leaves structure	Simple , opposite
Size	6-8cm
Leaf apex	Obtuse
Venation	Reticulate
Petiole	Long petiole
Leaf margin	Crenate
Leaf surface	Globrous
Upper epidermis	Dark green

Lower epidermis	Light green
Odour	Characteristics smell
Taste	Bitter taste
Cells	Circular
Texture	Rough with granules



Subsidiary cells
Stomata pore
Guard cell

Plate 1: Anisocytic type of stomata viewed at X 40.

Table 2. Microscopic Examination of Bryophyllum pinnatum Leaves

Features	Description
Upper and lower epidermis	Cuticle is present
Cells	Parenchymatous and homogenous
Stomata	Stomata are abundant 18-20 stomata per mm ² , Anisocytic type

Chemo-microscopical examination of powdered leaves of *Bryophyllum pinnatum* revealed the presence of cellulose, tannins, starch, lignin,

calcium oxalate, suberin, aleurone grain and mucilage but calcium carbonate was absent.

Table 3. Chemomicroscopical Studies of Bryophyllum pinnatum Powdered Leaves

Constituents	Inference
Starch	+
Gum and Mucilage	+
Cellulose cell walls	+
Lignin	+
Aleurone grain	+
Calcium oxalate crystals	+
Calcium carbonate	-
Suberized/Cuticular cell wall	+
Inulin	+

Phytochemical screening of aqueous and methanol extracts revealed the presence of alkaloid, flavonoids, saponins, glycoside, triterpenes, steroids, tannins, carbohydrates and phenols while anthraquinones was absent.

Table 4. Qualitative Phytochemical Screening of Aqueous and Methanolic Extract of *Bryophyllum pinnatum* Leaves

Metabolites	Inference	
	Aqueous	Methanolic extract
Alkaloid	+	+
Flavonoid	+	+
Saponins	+	+
Cardiac glycoside	+	+
Tannins	+	+
Steroid	+	+
Triterpenes	+	+
Phenol	+	+
Anthraquinones	-	-
Carbohydrate	+	+

TLC Profile of *Bryophyllum pinnatum* methanol extract using solvent system Chloroform: Methanol (9:1). Nine spots were detected with *p-Anisaldehyde* spray and the Rf values were shown alongside the spots

Table 5. TLC Results of Methanolic Extract of *Bryophyllum pinnatum* Leaves

Extract	Solvent System	Number of Spots	Distance of Spots	RF-Value
Methanol extract	BT:EA:H ₂ O (6:2:2)	2	5.6	0.34, 0.79
	HE: EA (8:2)	1	5.7	0.42
	CLRF:ME (9:1)	6	5.9	0.23, 0.47, 0.62, 0.78, 0.89, 0.94

Key: BT(Butanol), EA (Ethyl acetate) , HE (Hexane), CLRF(Chloroform), H₂O (Water)

The trend of the inhibition of DPPH radical by the extract was concentration dependent with respect to the IC₅₀ values (concentration of the extract to cause 50% inhibition), the DPPH radical scavenging ability of the extract showed the following trend Ascorbic acid > extract.

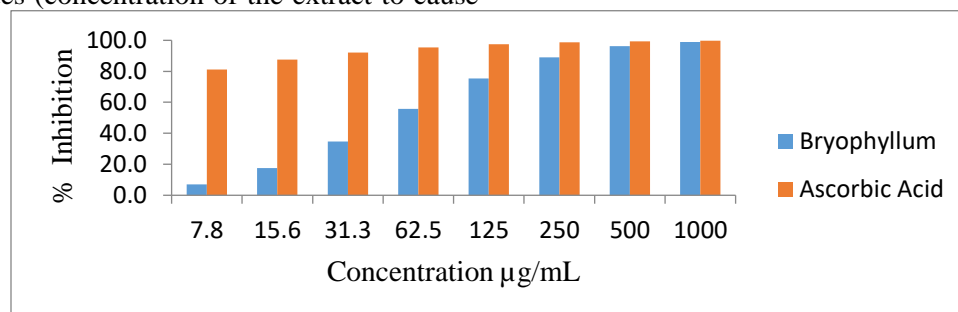


Figure 1. DPPH Antioxidant Activities of *Bryophyllum pinnatum* Leaves

Table 6: Antioxidant Activities of the Methanol Extract of *Bryophyllum pinnatum* Leaves

Sample	IC ₅₀ (µg/mL)
<i>B. pinnatum</i> methanol extract	51.78
Ascorbic Acid	0.79

Free radical scavenging ability of methanol extract of *Bryophyllum pinnatum* leaves was evaluated using DPPH radical. Ascorbic acid was used as positive control. It was determined that methanol extract of *Bryophyllum pinnatum* leaves possessed higher

4.0 DISCUSSION

Any medicinal plant requires detailed study prior to its use because; the therapeutic efficacy is absolutely dependent on the quality of the plant material used. The original and basic approach towards pharmacognosy includes study of morphological system, study of the cell structures and organization and study of tissue system, which still holds a key in the identification of the correct species of the plant and also to help us to differentiate between closely related species of the same genus. It is also first step to standardize a drug, which is the need of the day (Dhru *et al.*, 2010).

The microscopy of *Bryophyllum pinnatum* leaves along the upper and lower section revealed features such as cuticle, Parenchymatous cells, Anisocytic stomata (plate I). These anatomical features of the internal structures of plant drugs provide salient diagnostic characteristics for the identification of both entire and powdered crude drugs and detection of adulterants in plant materials (Ghani, 1990). Macro and microscopical evaluation of crude drugs are targeted at identification of precise variety and search for contaminants in plant materials (WHO, 1996). The macroscopic and microscopic evaluation is a reliable, cost effective and time saving method for establishing the right identification of herbal materials (Jeremiah *et al.*, 2019).

radical scavenging ability of 95.23% ,95.70% and 95.39% at the highest concentration of 1000 µg/mL, 500 µg/mL and 250 µg/mL respectively and was compared with standard where it showed 99.7% activity. (Figure 1).

Chemomicroscopical examination of the powdered leaves of *Bryophyllum pinnatum* revealed the presence of cellulose cell wall, lignified cell wall, tannins, starch, calcium oxalate and cutin while calcium carbonate was found to be absent (Table 3). The microscopic structures are most valuable in the identification of powdered drug as their identification is largely based on the form, the presence or absence of certain cell types and cell inclusions (Jeremiah *et al.*, 2019; Namadina *et al.*, 2019).

The results of phytochemical analysis revealed that the crude methanolic extract of *Bryophyllum pinnatum* leaves contains flavonoids, alkaloids, tannins, saponins, cardiac glycosides, phenol, steroid and terpenoids; while anthraquinones was absent. These primary and secondary metabolites in plants have numerous functions. Crude, pure and isolated alkaloids and their synthetic derivatives have been used as analgesic, antispasmodic and bactericidal agents (Sary, 1998; Okwu and Okwu, 2004). Flavonoids have been shown to provide antibacterial, anti-inflammatory, antiallergic, antimutagenic, antiviral, antineoplastic, anti-thrombotic and vasodilatory activity (Alan and Miller, 1996). Flavonoid also has immense antioxidant and anti-inflammatory activity because of its ability to scavenge hydroxyl radicals, super oxide anions and lipid peroxy radicals (Okwu, 2004; Okwu and Josiah, 2006). Tanins have been used in the treatment of wounds especially those emanating from varicose ulcers and haemorrhoids (Njoku and Akumufula, 2007) and is able to stop bleeding during circumcision (Edeoga *et al.*, 2005).

The phytochemical constituents especially the secondary metabolites could be useful as guide to chemotaxonomic markers (Jonathan and Tom, 2008) that will aid in chemo taxonomical classification system and further phylogenetic studies in Crassulaceae family.

Methanol extract when developed in Chloroform: Methanol (9:1) and virtualized with *p*-Anisaldehyde/H₂SO₄, it gave six spots with their R_f values and were purple and blue-black in colours. The Thin Layer Chromatography chemical screening is usually done to target isolation of new or very important constituent present in the plant extracts which has marked pharmacological activities and also serves as an important tool in recognizing how metabolite for isolation behaved and can be purified; hence, channeling scientific efforts towards the desired compound(s) and prevent waste of resources and time (Patra *et al.*, 2012). The success of separation of bio-molecules by chromatographic technique is markedly influence by the suitability of the separating solvent systems largely influences the successful and also rely upon an ideal range of partition coefficient (k) for each target compound(s) (Ito, 2005).

The effect of antioxidants on DPPH radical scavenging is thought to be due to hydrogen donating ability. DPPH is a stable free radical and it accepts an electron or hydrogen radical to become a stable diamagnetic molecule (Kannan *et al.*, 2012). When these molecules are formed, the absorbance decreases and the DPPH solution decolourises from violet colour to pale yellow. The degree of discolouration is an indication that the plant extract has the potential to scavenge free radicals as a result of its ability of hydrogen donation. More yellowish colour of DPPH

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is an indicator stronger antioxidant activity of the extracts (Sowunmi and Afolayan, 2015). The trend of the inhibition of DPPH radical by the extract was concentration dependent with respect to the IC₅₀ values (concentration of the extract to cause 50% inhibition), the DPPH radical scavenging ability of the extract showed the following trend Ascorbic acid > extract. It is interesting to note that the lower the IC₅₀ value, the higher the scavenging activity of the plant extract (Sowunmi and Afolayan, 2015). The DPPH test intends to measure the hydrogen atom or electron donor capacity of the extracts to the stable radical DPPH formed in solution (Orisakeye *et al.*, 2015). The activity was expressed as the concentration of sample necessary to give a 50% reduction in the sample absorbance (IC₅₀). Root extract exhibited considerable DPPH free radical scavenging activity as indicated by their IC₅₀ values of 2.35 mg/ml (Orisakeye *et al.*, 2015).

5.0 CONCLUSION

The established pharmacognostic standards for the powder of *Bryophyllum pinnatum* leaves could be used as a diagnostic tool for the standardization and identification of this medicinal plant for its purity and quality in the future and hence, inclusion into the pharmacopoeia for official use.

B. pinnatum extracts have some secondary metabolites namely alkaloids, tannins, flavonoids, cardiac glycosides and saponins. These were confirmed by thin layer chromatography profiles. These contribute in the effect of the plant. The extract have free radicals scavenging effects that are dose dependent. This supports the basis for the use of this plant traditionally for different ailments. The effects are probably due to phenolic compounds, flavonoids, tannins and some elements in the plant.

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