



ISSN NO. 2320-5407

Journal Homepage: -[www.journalijar.com](http://www.journalijar.com)

## INTERNATIONAL JOURNAL OF ADVANCED RESEARCH (IJAR)

Article DOI:10.21474/IJAR01/13904  
DOI URL: <http://dx.doi.org/10.21474/IJAR01/13904>



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Journal DOI:10.21474/IJAR01

### RESEARCH ARTICLE

#### ANTIOXIDANT PROPERTIES OF METHANOLIC EXTRACT OF *BRYOPHYLLUM PINNATUM* ON ALBINO WISTAR RATS

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#### Manuscript Info

##### Manuscript History

Received: 10 October 2021

Final Accepted: 14 November 2021

Published: December 2021

##### Key words:-

Bryophyllumpinnatum, Antioxidant, Crassulceae, Sprague Dawley, Wistar Rats

#### Abstract

Bryophyllumpinnatum, which belong to the family Crassulceae, is a succulent plant native to Madagascar, which is a popular houseplant and has become naturalized in tropical and subtropical areas. It has been in traditional medicine for the treatment of hypertension and kidney stones. Despite these acclaimed efficacies of its use in traditional medical practices, the effects on body organs are not well understood. The aim of this study was to evaluate antioxidant properties of Bryophyllumpinnatum. Fifteen adult Sprague dowley rats were use in this study split into three groups (group 1, 2 and 3). Group 1 animals served as the control, while group 2 and 3 served as the experimental groups. Acute toxicity, and antioxidants. The result of this study found LD<sub>50</sub> to be above 5000mg/kg. In vitro antioxidant test found the presence of alkaloids, saponins, thiamin, riboflavin etc. Antioxidant activity test result found an increase in GSH, GPx, a decrease in MDA and a stable CAT. In conclusion, we recommend its uses in human, but at a regulated doses because of its efficacy as stated in this study.

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#### Introduction:-

Bryophyllumpinnatum, which belong to the family Crassulceae, also known as the air plant, cathedral bells, life plant, miracle leaf (Ghasiet;al, 2011), and Africa never die. Bryophyllumpinnatum which is a perennial plant flourishes throughout the Southern part of Nigeria (Gill, 1992). It is known as miracle leaf or resurrection plant, amongst other names. It is called "ewe abamoda or Odundun" by the Yoruba tribe of Southwestern Nigeria, "Odaopue" among the Igbo's of Southeastern Nigeria. It is referred to as "du busi" in Chinese (Iwu, 1993; Ghasi et al., 2011).

It is a succulent, perennial plant, about 1 m (39 in) tall, with a fleshy cylindrical stem and reddish color for the youngest and it flowers most of the year (Esmail and Al Snafi, 2013).

Bryophyllumpinnatum has become naturalized in tropical and subtropical areas, inhabiting warm and temperate climates from sea level to 2,600 m (8,500 ft), occupying sites on rock in tropical evergreen and deciduous forests, as well as montane forests. It is native to Madagascar and Southern Africa and grows mainly in the tropics (Nagaratna and Prakash, 2015). It is also found in parts of Asia, Australia, New Zealand, the West Indies, the Philippines,

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Macaronesia, the Mascarenes, the Galapagos Islands, Melanesia, Polynesia, and Hawaii (Germplasm Resources Information Network (GRIN), 2007).

Bryophyllum Pinnatum reproduces via seeds and also vegetatively from leaf bulbils (Okwu and Josiah 2006; Okwu, 2007). B. pinnatum can easily be propagated vegetatively (through stems or leaf cutting).

Bryophyllum pinnatum has been recorded in Trinidad and Tobago as being used as a traditional treatment for hypertension (Lans, 2006).

In traditional medicine, the juice of the leaves is also used for kidney stones, although there is ongoing research and some scientific evidence for this use but further research is required. In the French West Indies, Kalanchoepinnata, called zebmaltet, is used in local application against headaches. For the people of the Amazon, kalanchoe has multiple uses: the Creoles use it roasted against inflammations and cancer and as an infusion, and as a popular remedy for fevers. Against headaches, Palikur rubs their forehead with a mixture of kalanchoe leaf juice with coconut oil (Hermann, 1983).

Phytochemical studies of Kalanchoepinnata have identified the presence of triterpenes, steroid, phenanthrene, flavonoid, flavones, chalcones, taraxasterol, auronos, phenolic acid, caffeic acid, syringic acid, malic, oxalic, ferulic acid and organic acid.

Bryophyllum pinnatum in traditional medicine is used for the treatment of earache, burns, abscesses, ulcer, insect bites, whitlow, diarrhea, cithiasis etc (okwu, 2007). In Eastern part of Nigeria, this herb is used to facilitate the dropping of the remnant of the umbilical cord (okwu and Josiah, 2006). The leaves of B. pinnatum is used on the epidermis for the treatment of fungi, inflammation and fevers (Egereonu and Mokwe, 2005).

Ethnomedically, B. pinnatum is used to induce vomiting of blood, treat acute and chronic bronchitis, pneumonia and other forms of respiratory tract infections (Mudi and Ibrahim, 2008).

The plant is considered a sedative, wound-healer, diuretic, anti-inflammatory and cough suppressant (Egereonu and Mokwe, 2005; Okwu and Josiah, 2006). It is employed for the treatment of kidney stones, gastric ulcers and oedema of the leg (Nassis, 1992).

Researches has evaluated the phytonutrient quantity and quantities properties of bryophyllum pinnatum (Nwali et al, 2012), such as quantities of alkaloids, saponins, flavinoids, tannins etc. the study was silent on the invitro antioxidants activities; such DPPH, TRAP, and Nitric oxide activities scavenging activities.

### **Aim Of Study**

The aim of this study was to access the antioxidant properties of Bryophyllum pinnatum (both on exogenous and endogenous).

### **Scope Of The Study**

This study is restricted to acute toxicity, phytochemical and antioxidant effect following administration of Bryophyllum pinnatum on albino wistar rats.

### **Methods:-**

Fresh leaves of B. pinnatum were collected from the botanical garden of the Abia State University, Uturu. The leaves were air dried, pulverized (400 g. The extract was then tested for the presence of phytochemicals using standard methods.

### **Ethical Approval**

Fifteen albino wistar rats weighing between 150g and 300g purchased from Anatomy Department Abia State University, Uturu, was use for this test as approved by the faculty of Basic medical science ethics committee, Abia State University Uturu.

**Plant Identification.**

The plant *Bryophyllum pinnatum* was identified and certified by Mrs Chikodi, a plant scientist and technologist in the department of Plant Science Biology (PSB), Abia State University, Uturu.

**Acute Toxicity Test**

The acute toxicity was done using Lorke's Method. They were injected with ethanolic extract of *Bryophyllum pinnatum* and were observed for a period of seven days.

**Phytochemistry Analysis****Qualitative phytochemical analysis of *B. Pinnatum***

Phytochemical analysis of methanolic extract of *B. Pinnatum* leaves was carried out by the standard methods as described by Sofowora (1990), Trease and Evans (1989), Brain and Turner (1975) and Evans (1996) which provided for the presence and absence of metabolites such as Saponins, Alkaloids, Flavonoids, Phenol, Saponin, Glycosides, Steroids and Tannins was carried out.

**Test for the presence of saponins**

An estimated 5 ml of extract was shaken vigorously with 5 ml of distilled water in a test tube and warmed. The formation of frothing stable foam) was taken as an indication of the presence of saponins.

**Test for the presence of flavonoids**

To 2 ml of extract, few drops of lead acetate solution were added. Formation of a yellow precipitate was an indication of the presence of flavonoids.

**Test for the presence of tannins**

Approximately 2ml of extract was mixed with 2ml distilled water and heated on a water bath. Few drops of ferric chloride ( $\text{FeCl}_3$ ) solution were added. Formation of a green coloured precipitate was an indication that tannins were present.

**Test for the presence steroids**

a. **Salkowski's test** :- a red color produced in the lower chloroform layer when 2 ml of organic extract was dissolved in 2 ml of chloroform and 2 ml concentrated sulphuric acid was added in it, indicates the presence of steroids.

b. **Liebermann Burchard test**: - development of a greenish color when 2 ml of the organic extract was dissolved in 2 ml of chloroform and treated with concentrated sulphuric acid and acetic acid indicates the presence of steroids.

**Detection of Steroids**

Two ml of acetic anhydride was added to five mg of the extracts, each with two ml of  $\text{H}_2\text{SO}_4$ . The colour was changed from violet to blue or green in some samples indicating the presence of steroids.

**Test for the presence of terpenoids**

2 ml of the organic extract was dissolved in 2ml of  $\text{CHCl}_3$  and evaporated to dryness. 2ml of conc.  $\text{H}_2\text{SO}_4$  was then added and heated for about 2 minutes. Development of a grayish color indicates the presence of terpenoids

**Detection of Terpenoids****Salkowski's Test**

Five mg of the extract of the leaves, flowers and seeds was mixed with two ml of chloroform and concentrated  $\text{H}_2\text{SO}_4$  (3ml) was carefully added to form a layer. An appearance of reddish brown colour in the inner face was indicates that the presence of terpenoids.

**Test for the presence alkaloids**

3 ml of extract was stirred with 3 ml of 1% HCl on steam bath. 1 ml of mixture was taken separately in two test tubes. Few drops of Dragendorff's reagent were added in one tube and occurrence of orange red precipitate was taken as positive. Two the second tube Mayer's reagent was added and appearance of buff colored precipitate was taken as positive test for presence of alkaloids.

**Detection of Alkaloids**

Extracts were dissolved individually in dilute hydrochloric acid and filtered. The filtrates were used to test the presence of alkaloids.

**Mayer's test:**

Filtrates were treated with Mayer's reagent. Formation of a yellow cream precipitate indicates the presence of alkaloids

**Wagner's test:**

Filtrates were treated with Wagner's reagent. Formation of brown/ reddish brown precipitate indicates the presence of alkaloids.

**Test for the presence glycosides**

To about 2 ml of extract with dilute HCl and 2 ml Sodium nitropruside in pyridine and sodium hydroxide solution were added. Formation of pink to blood red color indicates the presence of Cardiac glycosides.

**Test for the presence phenols**

An estimated 10mg of the extract was dissolved in 5ml of distilled water, then treated with few drops of ferric chloride solution. A dark green or bluish black colour indicated the presence of phenolic compounds.

**Antioxidant Test****In Vitro Antioxidant Activities Of The Extracts**

The methods used by Marcocci et al., 1994; Mensor et al., 2001; Iwalewa et al., 2008; Benzie et al., 1999; and Ijioma et al., 2019 were adopted. The in vitro antioxidant studies carried out included Nitric oxide inhibition activity, Photometric Assay of 2, 2-Diphenyl-1-Picrylhydrazyl (DPPH), and Ferric Reducing Antioxidant Power. In each case result of test was compared with that of a standard (Vitamin C).

**Nitric oxide inhibition activities of the extracts**

Nitric oxide, generated from sodium nitropruside in aqueous solution at physiological pH, interacts with oxygen to produce nitrite ions which were measured by Griess reaction. The reaction mixture (3 ml) containing sodium nitropruside (10 mM) in phosphate buffer saline (PBS) and the extract from (50 - 800) µg/ml was incubated at 25°C for 150 min. After incubation, 0.5 ml of the reaction mixture was removed and 0.5 ml of Griess reagent (1% (w/v) sulfanilamide, 2% (v/v) H<sub>3</sub>PO<sub>4</sub> and 0.1% (w/v) naphthylethylenediamine hydrochloride) was added. The absorbance of the chromophore formed was measured at 546 nm.

**Photometric Assay of 2, 2-Diphenyl-1-Picrylhydrazyl (DPPH)**

The free radical scavenging activity of the extract was analyzed by the DPPH Assay using spectrophotometer. The crude extract at concentrations (25, 50, 100, 200 and 400) µg/ml each was mixed with 1 ml of 0.5 mM DPPH (in methanol) in a cuvette. The absorbance at 517 nm was taken after 30 minutes of incubation in the dark at room temperature. The experiment was done in triplicate. The percentage antioxidant activities were calculated as follows. % antioxidant activity (AA) = 100-[(ABS sample - ABS blank) ×100]/ABS control]. One milliliter of methanol plus 2.0 ml of the test extract was used as the blank while 1.0 ml of the 0.5 mM DPPH solution plus 2.0 ml of methanol was used as the negative control. Ascorbic acid (vitamin C) was used as reference standard.

**Ferric Reducing Antioxidant Power**

The ferric reducing antioxidant power was carried out as used by Benzie and Strain. The protocol involved is as follows:

**Reagents:**

1. Acetate buffer (300 mM), pH 3.6 (3.1 g sodium acetate.3H<sub>2</sub>O and 16 ml glacial acetic acid in 1000 ml buffer solution).
2. 2, 4, 6-triphridyl-s-triazine (TPTZ) (10 mM) in 40 mM HCL.
3. FeCl<sub>3</sub> 6H<sub>2</sub>O (20 mM) in distilled water.

FRAP working solution was prepared by mixing solution 1, 2 and 3 in the ratio of 10:1:1, respectively.

The working solution was freshly prepared in each test. The aqueous solution of known amount of ascorbic acid was used for calibration.

**Assay:** Blank FRAP reagent.

Sample: FRAP reagent (3 ml) and 100 µl sample solution at concentrations of 50, 100, 200, 400 and 800 µg/ml was mixed and allowed to stand for 4 minutes. Colometric readings were recorded at 593 nm, at 37°C. The ascorbic acid standard solution was tested in a parallel process. Calculations were made by a calibration curve.

### Experimental Animals

Thirty adult male Wistar albino rats of 12 weeks of age, obtained from the animal house of Abia State University, Nigeria were used for the study. The animals were given standard rodent chow and clean drinking water ad libitum. The animals were kept in a well-ventilated room with a 12 h light/dark cycle at room temperature. All animal experiments were approved by the Animal Research Ethics Committee of the University, in accordance to the guide for care and use of laboratory animals.

### Experimental Design

The animals were randomly distributed into three groups of 5 rats per group. The first group served as the control, the second groups were administered 400mg/kg of methanolic extract of *B. pinnatum* leaf extract by oral gavage daily for 28 days, respectively. The animals in the third group were administered 800mg/kg of ethanolic extract of *B. pinnatum* daily for 28 days. At the end of extract administration, the rats were sacrificed by cervical dislocation under deep diethyl ether anaesthesia.

### Statistical Analysis

The results are presented as mean±SEM for each group. Differences among groups were analysed using one-way analysis of variance (ANOVA) followed by Dunette's multiple comparison test. Data were analysed using SPSS Version 20 and values were considered significant at P<0.05.

## Results:-

### Result Of Acute Toxicity Evaluation

No death was recorded within the 24 hours and a further 7 days of the acute toxicity study, even at the highest dose of 5000 mg/kg body weight. The animals instead had normal disposition and were emotionally stable and all survived the period of the study.

### Result Of Qualitative And Quantitative

**Table 3:-** Result of qualitative and quantitative phytochemical analysis.

PARAMETER	QUALITATIVE	QUANTITATIVE (MG/100G)
Flavonoids	++	4.02 4.49 4.30
Alkaloids	+++	11.38 11.70 10.72
Tannins	++	2.72 2.97 2.67
Terpenoids	+	2.69 2.32 2.38
Cardiac glycoside	+	0.89 0.92 0.91
Saponins	+	1.95 1.70 1.90

Phenols	++	5.13 4.79 4.98
Steroids	+	2.13 2.10 1.98

Table3: Phytochemical analysis of the extract of *B. pinnatum* showing the result of qualitative and quantitative contents of the extract. The table showed the presence of Alkaloids, flavinoids, tannins and phenols in high quantities, while cardiac glycosides, steroids are in minimal quantity.

**Table 4:-** Result of quantitative vitamin analysis.

PARAMETER	QUANTITATIVE ( $\mu\text{G/G}$ )
Vitamin A	4.98 4.70 4.72
Vitamin B <sub>1</sub>	0.33 0.36 0.32
Vitamin B <sub>2</sub>	0.19 0.22 0.20
Vitamin B <sub>3</sub>	2.51 2.49 2.12
Vitamin C	15.34 15.72 15.40
Vitamin E	6.52 6.98 6.41

Table 4; shows the vitamin constituents in the extract of *Brophyllum pinnatum*. It shows the presence of vit A, Vit B<sub>1</sub>, Vit B<sub>2</sub>, Vit B<sub>3</sub>, Vit C, and Vitamin E.

#### Results Of In Vitro Antioxidant Evaluation Of The Extract

**Table 5:-** Nitric Oxide scavenging activity.

CONC. ( $\mu\text{G/ML}$ )	ACTIVITY OF THE EXTRACT (%)	ACTIVITY OF ASCORBIC ACID (%)
25	1.88 1.90 1.85	5.91 6.13 6.22
50	13.52 13.68 13.61	24.70 25.08 25.11
100	30.50 31.08 31.57	42.18 43.30 44.11
200	48.19 49.70 47.55	78.05 77.59 77.59
400	69.72 70.39 69.81	81.63 81.50 81.10

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Table 5; shows the scavenging activities of *B. pinnatum* extract against Nitric oxide in correlation to that of ascorbic acid. It showed that *B. pinnatum* has the ability to fight against free radicals, such as NO<sub>3</sub> when compared with ascorbic acid.

**Table 6:-** DPPH scavenging activity of the extract.

CONC.(μG/ML)	ACTIVITY OF THE EXTRACT (%)	ACTIVITY OF ASCORBIC ACID (%)
25	2.70	30.75
	2.85	31.88
	3.75	31.20
50	19.42	57.04
	19.65	57.31
	19.87	56.10
100	42.11	70.05
	41.50	70.87
	42.49	70.74
200	50.21	79.49
	50.95	78.14
	50.28	79.30
400	62.94	84.22
	66.08	83.59
	65.12	84.27

Table 6; showing antioxidant scavenging activities of *B. pinnatum* against DPPH in correlation with ascorbic acid. The table shows that *B. pinnatum* fights free radicals generated by DPPH.

**Table 7:-** FRAP activity of the extract.

CONC.(μG/ML)	ACTIVITY OF THE EXTRACT (μMOL)	ACTIVITY OF ASCORBIC ACID (μMOL)
25	0.25	1.30
	0.27	1.04
	0.20	1.02
50	0.79	2.52
	0.80	1.91
	0.72	2.05
100	1.97	3.41
	1.90	3.20
	2.05	3.05
200	2.95	5.41
	2.98	5.57
	3.05	5.02
400	5.71	7.81
	5.60	8.05
	5.44	7.94

Table 7; shows the antioxidant activities of *Bryophyllum pinnatum* against radicals generated by FRAP. It shows that *B. pinnatum* fights against free radicals generated by FRAP.

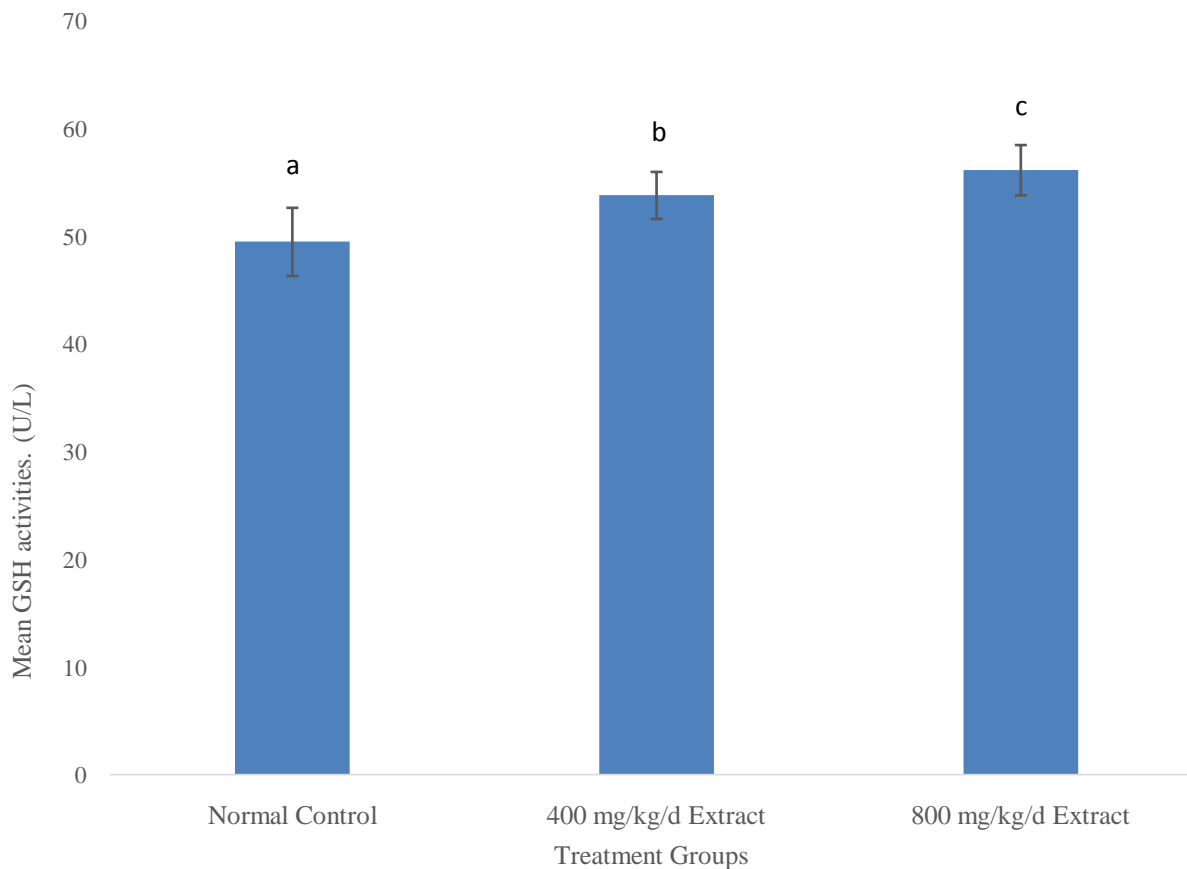
### Result Of Antioxidant Activities:-

**Table 8:-** Antioxidants.

Treatment groups	GSH (U/L)	GPx (U/L)	CAT (U/L)	MDA (mmol/L)
Normal Control	49.49±1.17 <sup>a</sup>	44.41±2.10 <sup>a</sup>	11.05±0.42 <sup>a</sup>	0.70±0.11 <sup>b</sup>
400 mg/kg/d Extract	53.81±2.18 <sup>b</sup>	46.78±1.17 <sup>b</sup>	10.67±0.94 <sup>a</sup>	0.58±0.94 <sup>a</sup>

800 mg/kg/d Extract	56.15±2.34 <sup>c</sup>	47.24±1.64 <sup>b</sup>	10.96±1.64 <sup>a</sup>	0.55±1.08 <sup>a</sup>
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Values are presented as mean ± deviation (n = 10) and values with different superscripts are significantly different from any paired mean at P < 0.05



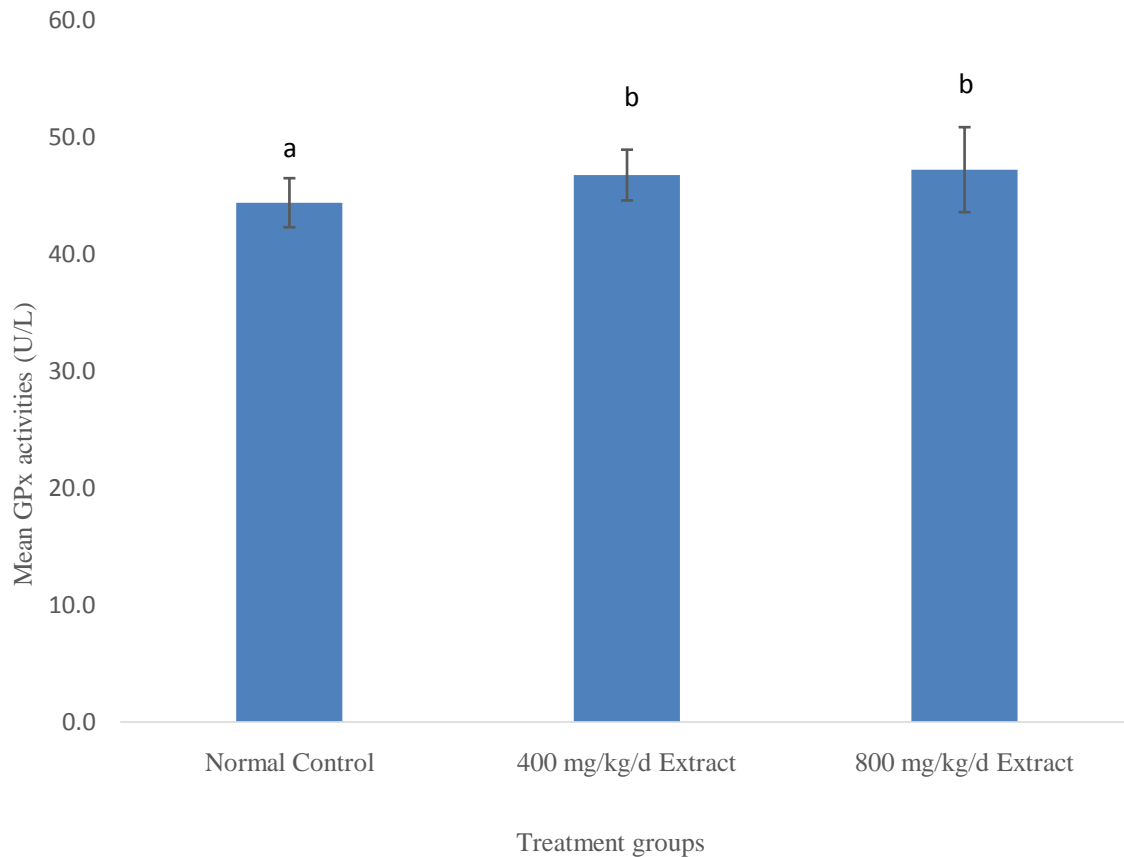
Each bar represent mean ± standard deviation (n = 10)

Bars with different superscripts are significantly different from any paired mean at P < 0.05

**Fig 1.0:-** Glutathione activity levels (U/L).

The bar chart shows glutathione activity level is significantly increased in the treatment groups that received 400mg/kg and 800mg/kg following Bryophyllumpinnatum administration when compared to the control group. This result showed that Bryophyllumpinnatum extract increases glutathione levels in cells and organ, and it does so in a dose dependent manner as shown in fig1.0.



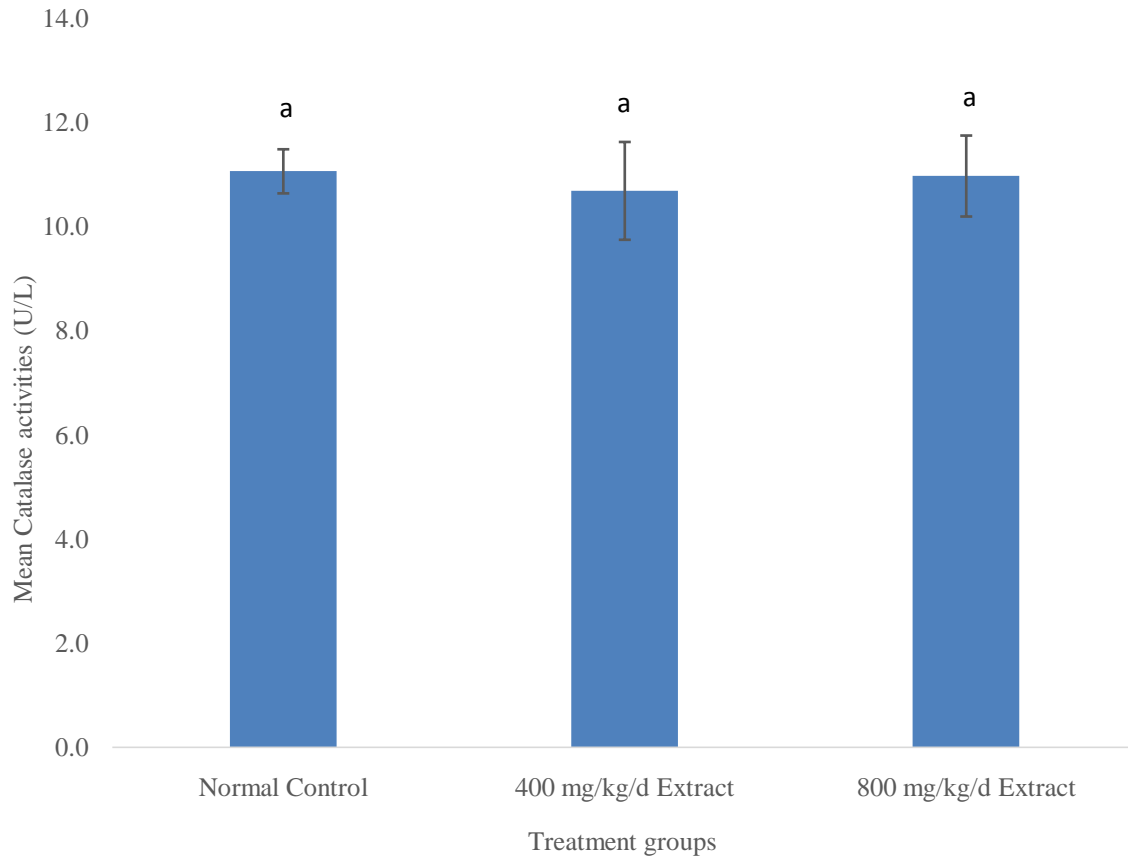


Each bar represent mean  $\pm$  standard deviation (n = 10)

Bars with different superscripts are significantly different from any paired mean at  $P < 0.05$

**Fig 1.1:-** Glutathione peroxidase activities (u/L).

The figure shows glutathione peroxidase level is significantly increased in the treatment groups that received 400mg/kg and 800mg/kg following Bryophyllumpinnatum administration when compared to the control group. The catalyzation of GPx to reduce free radicals in cells increases as the dose of B.pinnatum is increased. This result presented in table 11 showed the ability of B. pinnatum in reducing free radicals by catalyzation of GPx. This catalyzation is also dose dependent.

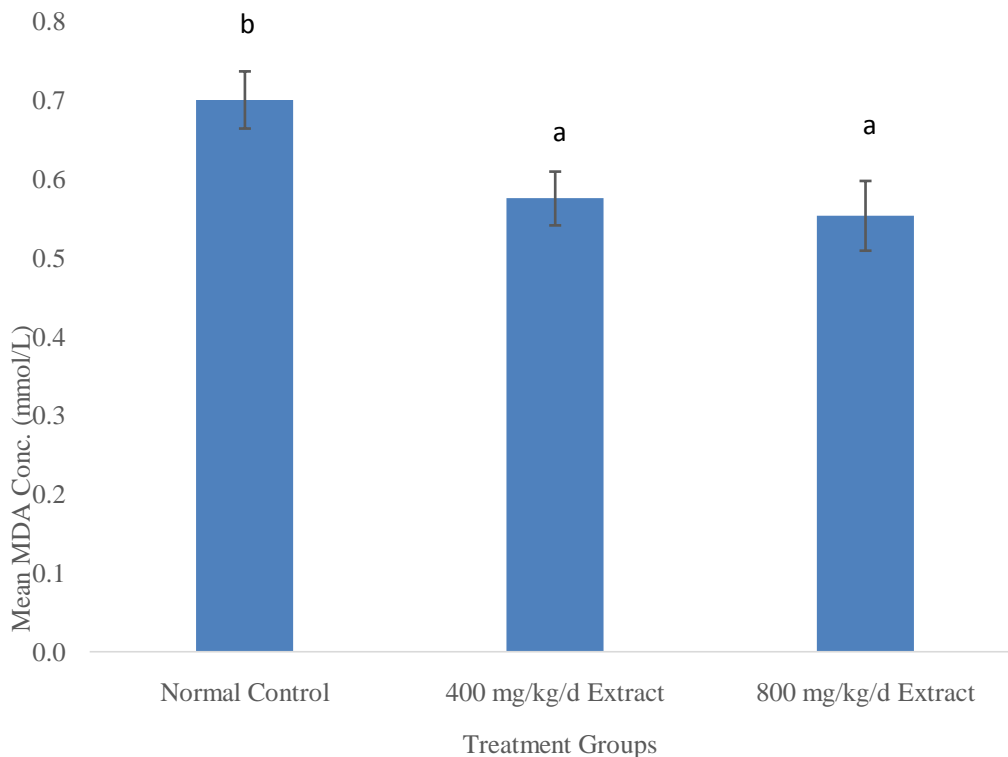


Each bar represent mean  $\pm$  standard deviation (n = 10)

Bars with different superscripts are significantly different from any paired mean at  $P < 0.05$

**Fig 1.2:-** catalase activity level (u/L).

The figure showed that there were no significant different in catalase activity level in the treatment groups that received 400mg/kg and 800mg/kg following *Bryophyllumpinnatum* administration when compared to the control group. The result showed that the activities of catalase remained unchanged in control group and the experimental groups following *B. pinnatum* administration.



Each bar represent mean  $\pm$  standard deviation (n = 10)

Bars with different superscripts are significantly different from any paired mean at  $P < 0.05$

**Fig 1.3:-** Malondialdehyde concentration (mmol/L).

The figure shows malondialdehyde activity level is significantly decreased in the treatment groups that received 400mg/kg and 800mg/kg following *Bryophyllumpinnatum* administration when compared to the control group. The decrease in malondialdehyde concentration in the result of the experimental groups shows that *B. pinnatum* extract decreases oxidative stress in a dose dependent manner as presented in fig 1.3.

## Discussion And Conclusion:-

### Acute Toxicity Testing

Acute toxicity describes any adverse effect that occurs within 24 hours of administering any agent or substance. To be described as acute toxicity the effects should be noticed within 14 days of giving any substance.

Animals in the phase one testing were observed to have normal physical activity as those in the control group, while animals in the phase two testing had normal activity, but were always clustering together and calm. Our study concluded that the  $LD_{50}$  of methanolic extract of *B. pinnatum* were above 5000mg/kg, reason being that no death occurred across the group within 24hrs of the toxicity testing and 14 days after the testing there was no fatality across the groups.

### Phytochemical properties of *b. Pinnatum*

The phytochemical analysis was done using standard method as described by Sofowora (1990) and Evans (1989). The analysis reveals the presence of Alkaloids (11.70 mg/100g), Flavonoids (4.49 mg/100g), and phenols (5.13 mg/100g) in high quantity as shown in table 6. These phytochemicals helps to reduce oxidative stress and fight against free radicals. This is in agreement with the study done by Nwali et al, (2012) on the phytochemical properties of *Bryophyllumpinnatum*.

The result of the quantitative analysis also show the presence of thiamine (Vit B1) riboflavin (Vit B2), niacin (Vit B3), Tocopherol (Vit E), Vitamin A, and Vitamin C. Each of these vitamins plays a vital role in an organism and a vitamin deficiency occurs when the body is deficient in any of it. *B. Pinnatum* is a source of this nutrients, it could supplement for any of this vitamin deficiency.

### **Antioxidant Activity**

Increased production of reactive oxygen/nitrogen species and decreased capacity of antioxidant defenses in the body leads to oxidative stress (Fidrianny et al., 2015; Ferry and Roussel, 2011). Generation of reactive oxygen/nitrogen species (ROS/RNS) is inevitable for aerobic organisms and in healthy cells, and it occurs at a controlled rate (Sohal and Orr, 2012). Under conditions of oxidative stress, ROS/RNS production is dramatically increased, resulting in subsequent alteration of membrane lipids, proteins, and nucleic acids (Mwihia, 2017). Oxidative damage of these biomolecules is associated with aging and a variety of pathological events, including atherosclerosis, carcinogenesis, ischemia reperfusion injury, and neurodegenerative disorders (Graves, 2012). From the result of this research, ROS/RNS generated in cells were significantly reduced, hence the increase in glutathione (GSH) levels and reduction in malondialdehyde (MDA) as shown in fig 4.1 and fig 4.4.

### **Exogenous Defense Mechanism**

To maintain homeostasis in the redox system and protect the body against ROS and RNS, humans have evolved complex antioxidant systems, which work to avert deleterious effects of oxidative stress (Rahman et al., 2012). The body has two defense systems which are exogenous and endogenous mechanisms. The exogenous sources includes ascorbic acid, beta-carotene, tocopherols which are derived from dietary supplements.

In this study ascorbic acid (vitamin C) was used as a standard to check for the scavenging activities of the extract on NO, FRAP, and DPPH according the method of Mensor et al., (2019). This method is based on the ability of *B. pinnatum* to reduce ferric ion ( $Fe^{3+}$ ) to ferrous ion ( $Fe^{2+}$ ). The  $Fe^{2+}$  formation is examined by asorbance capacity of 517nm. Increase in asorbance at this wavelength indicates an increase in reducing power of FRAP. The findings of this current study has shown a concentration dependent increase in asorbance values of methanolic extracts of *B. pinnatum*, depicting an appreciable ferric reducing antioxidant ability as shown in table 10 of the results.

On Nitric Oxide inhibition activities. Nitric oxide generated from sodium nitropusside interact with oxygen to produce nitrite ions which were measured which were measured by Griess reaction. The degree of asorbance was measured at 546nm according to Ijiomaetal., (2019). This present study has shown an increase in scavenging activity of nitric oxide in a concentration dependent manner (See table 8). This study is in agreement with work done by Mensor et al., (2001).

Photometric assay of 2,2-diphenyl-1-picrylhydrazyl (DPPH) scavenge activities was measured using spectrophotometer. The asorbance of methanol extract of *B. pinnatum* was measured at 517nm after 30 minutes of incubation (Ijioma et al., 2019). In the current study we found that DPPH scavenging activities of methanolic extract of *B. pinnatum* exhibited a concentration-dependent relationship as seen in table 9 of results.

### **Endogenous Defense of *Bryophyllum Pinnatum***

Endogenous sources of antioxidant defense includes superoxidase dismutase, catalase, glutathione and glutathione peroxidase, they catalyze free radicals quenching reactions (28-30).

Glutathione is an antioxidant produced in cells. GSH is capable of preventing damage to important cellular components caused by reactive oxygen species (ROS) such as free radicals and lipid peroxide (White et al., 2003). In our study, glutathione increases in a dose dependent manner ( $p > 0.05$ ) across the groups. This suggests that *B. pinnatum* increased GHS in the tissues tested for GHS, by fending off ROS and free radicals in the organs.

Glutathione peroxidase (GPx), is a cytosolic enzyme that catalyzes the reduction of hydrogen peroxide to water and lipid peroxide to alcohols. Research have shown that low GPx in serum maybe a contributing factor to vitiligo (Zedan et al., 2015), diabetes and multiple schlerosis (Socha et al., 2014). GPx also increases across the groups in a dose-dependent manner in this current study.

Catalase together with GPx decomposes hydrogen peroxide to water and oxygen. It also protect cells and tissues against oxidative stress. No, significant ( $p>0.05$ ) change were observed for the CAT between the control groups and the experimental groups.

Malondialdehyde is a natural occurring compound and it is a maker for oxidative stress. It results from lipid peroxidation of polyunsaturated fatty acids (Nair et al., 2008). High levels of MDA may depict tissue damage in an animal, such as abnormal spermatozoa, osteoarthritis (Collodel et al., 2015). In our study, there was a significant decrease in the levels of MDA inferring that *Bryophyllum pinnatum* can reduce damage caused by high MDA levels in cells and tissues. This study is in agreement with Chioma et al., (2017).

### Conclusion:-

The phytochemical properties of *B. pinnatum* shows it contains phenols, flavonoids, riboflavin and other important vitamins.

The scavenging activity of *Bryophyllum pinnatum* on Nitric oxide, FRAP and DPPH has shown it can fight free radicals. It has also shown to decrease MDA levels in the animals administered it and increases GHS in the groups administered *B. pinnatum*.

This research has shown promising and effective antioxidant properties in both exogenous and endogenous system and its safe human consumption.

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