Bayero Journal of Pure and Applied Sciences, 1(1):43 – 48
Received: October, 2008
Accepted: November, 2008

ACTIVITY OF *BRYOPHYLLUM PINNATUM* S. KURZ EXTRACTS ON RESPIRATORY TRACT PATHOGENIC BACTERIA

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ABSTRACT
Ethanol extract of Bryophyllum pinnatum (commonly known as 'Shuka halinka' or 'Karan masallachi' in Hausa) (BP1) was partitioned into n-hexane, chloroform, ethyl acetate and aqueous methanol soluble fractions and labeled BP1–01, BP1–02, BP1–03 and BP1–04 respectively. These fractions were subjected to antibacterial testing against respiratory tract pathogenic bacteria. The n-hexane soluble fraction showed activity against the selected microorganism with highest on Staphylococcus aureus (12mm), Klebsiella pneumonia (11mm) and Salmonella typhi (08mm); ethyl acetate soluble fraction showed mild activity against Escherichia coli (06mm), Staphylococcus aureus (07mm) and Salmonella typhi (07mm), at 10mg/ml. The n-hexane soluble fraction was subjected to activity guided column chromatography. This leads to isolation of three bioactive compounds: BP1-01-01, BP1-01-33 and BP1-01-65. The activity of this plant's extracts against the test bacteria coupled with the various phytochemical compounds present in the fractions is a pointer to the plant's potential as a source of drugs that can be used against respiratory tract pathogens.

Keywords: Bryophyllum pinnatum, respiratory tract pathogens, phytochemical screening, antibacterial activity

INTRODUCTION

Respiratory tract infections are clinical syndrome produced by the inflammation of the trachea, bronchi and bronchioles. These infections are caused by some bacterial pathogens (eg; *Staphylococcus aureus, Streptococcus pneumonia, Klebsiella pneumoniae, Herpes influenzae, Mycobacterium tuberculosis*), by virus (examples: adenovirus, Herpes simplex virus) and fungi (Macleod, 1974, Patrick, 2006)

Despite the progress made in the development of drugs and antimicrobial agents, occurrence of drug resistant microbes and the emergence of unknown disease causing microbes pose an enormous public health concern (Iwu, 1999). This fact has forced scientists to search for new antibiotics/antimicrobial compounds from various sources (Mann *et al*., 2007) such as the medicinal plants to replace those that have become in active.

Traditional medicine uses numerous plants parts for the treatment of respiratory diseases among which is this plant, *Bryophyllum pinnatum* S, Kurz (Wagner*,* 1999 and Dalziel *et al.,* 1958) selected for this research based upon its wide enthnomedicinal uses against respiratory tract diseases.

Enthnomedically, *B. pinnatum* is used among other applications to induce vomiting of blood, cut umbilical cord in new borns, expel Tay Tay worms, poultries for head cold, acute and chronic bronchitis pneumonia and other forms of respiratory tract infections (Medicineatourfeet.com, 2008, Dalziel, 1936). Various biological activities of *B.pinnatum* include works by Plangger *et al.,* (2005), Ojewole (2005), Gwehenberger *et al.,* (2004), Pal *et al.,* (1991), Reppas (1995), Yemitan *et al.,* (2005) and Mckenzie *et al.,* (1986, 1987). The antibacterial activity of the leaf juice of *B. pinnatum* was reported by Obaseiki-Ebor (1985). Flavonoids, polyphenols, and triterpenoids have been identified from the leaves of *B. pinnatum* (Ojewole, 2005). Quecetin-3-α-L-rhu-β-D-xyl; a flavonoid (Cao *et al.,* 2005), Bryophyllin B [1], a novel potent cytotoxic bufadienolide (Yamagishi *et al.,* 1989) and Malic acid (Sutton *et al.,* 1972) were isolated from the leaves of *B. pinnatum.*

Yet, more work is needed to ascertain the reliability of the use of this plant as antibacterial and hence the present. It is therefore, the aim of this work to determine the bio effects of the fractions from the plant under study (*B.pinnatum*) using antimicrobial susceptibility to respiratory tract pathogenic bacteria (i.e. *Klebsiella pneumoniae, Streptococcus pneumoniae, Staphylococcus aureus, Pseudomonas aureuginosa, Escherichia coli* and *Salmonella typhi*.). The overall effect would be try to uncover at least a single compound whose a chemotherapeutic index equal or exceed the drugs used to cure pneumonia and other respiratory infections.

MATERIALS AND METHODS

Plant materials

Bryophyllum pinnatum S, KURZ leaves were collected from Rijiyar Zaki, Kano, Nigeria. They were identified and authenticated by Dr. B. S. Aliyu of Biological sciences (BUK), air dried and grounded into fine powder. A voucher specimen was deposited at the herbarium.

Extraction and partitioning

200g of powdered plant material was extracted by percolation for 10 days with 800ml absolute ethanol (Aldrich). The percolate was filtered and the Mack repercolated three times with 400ml each of absolute ethanol to ensure that no metabolites were left in the residue. All extracts from the plant were combined and concentrated at 40^0C under reduced pressure using rotavapor and weighed. 8g of this crude ethanol extract was separately dissolved in 200ml 60% aqueous methanol in Quick fit separatory funnel and partitioned with 100ml X 3 of n-hexane, chloroform, and ethyl acetate (all fron Aldrichin the given order). The afforded fractions were concentrated with Gallenkamp rotavapor (Vishnoi, 1979), weighed and labeled BP1-01, BP1-02, BP1-03, and BP1-04. Each of these fractions were tested for antibacterial activity, and phytochemically screened.

Phytochemical analysis of the fractions

The plant fractions i.e. BP1-01, BP1-02, BP1-03, and BP1-04 were phytochemically screened using standard techniques for the qualitative detection of reducing sugars, saponins, tannins, steroids, cardiac glycosides, alkaloids, flavonoids, flavonosides, resins, anthraquinones and phlobatannins.

Test for alkaloids (El-olemy *et al.,* 1994)

0.5g of each fraction was strirred with 5ml 1% HCl on steam bath. The solution was cooled and filtered. 1ml of the filterate was treated separately with drops of Mayer's, Dragendoff's and wagner's reagents; and formation of dirty/dark brown, yellow-brown or reddish brown precipitate respectively indicates the presence of alkaloids.

Test for saponins (Sofowora, 1984)

2ml of each fraction was vigorously shaken with distilled water and allowed to stand for awhile. A persistent frothing indicates the presence of saponins.

Test for phlobatannins (sofowora, 1984)

2ml of each plant's fraction was added to 5ml HCl. Formation of turbidity/precipitate indicates the presence of phlobatannins.

Test for steroids (Harbone, 1973)

I. Liebermann's test:- 2ml of ethanoic acid anhydride was added to 2ml of each fraction. The content was then cooled on ice for 5minutes. 1ml of concentrated H_2SO_4 was added along the walls of the test tube. The change in colour from violet to blue and then to green indicates the presence of steroidal nucleus (i.e. aglycone component of cardiac glycosides).
II. Salkowski's test:- 2ml of concentrated H_2SO_4 was added to 2ml of each fraction. Appearance of effervescence after which a clear reddish brown colour appeared at the interface confirms the presence of steroidal ring.

Test for cardiac glycosides (Sofowora, 1984)

2ml of each fraction was added to in succession, 3ml 3.5% Iron (III) Chloride $FeCl_3$, then 3ml ethanoic acid. This gave a green precipitate and a dark colored solution respectively. Finally, concentrated H_2SO_4 was carefully poured down the side of the test tube which resulted to formation of brownish-red layer, at the interface. This confirms the presence of cardiac glycosides.

Test for flavonoids (Harbone, 1973)

I. Sodium Hydroxide test:- 2ml of plants' fraction was acidified with 1% HCl and dissolved in 20% NaOH. A canary yellow colour indicates the presence of flavonoids.
II. Lead (II) acetate test:- 2ml of each fraction was treated with 10% $Pb(oAc)_2$ solution. A light yellow – milky precipitate indicates the presence of flavonoids.

Test for antraquinone (Ciulei, 1975)

2ml of each fraction was treated with 5ml of benzene. This gave two layers. The clear colourless upper layer was pipette and the organic layer treated with 3ml of 10% NH_3(aq). Change of colour from rose pink to red indicates anthraquinone.

Test for tannins (Sofowora, 1984)

2ml of each fraction was treated with 3drops of 5% $FeCl_3$. A dark black coloured precipitate in a very dark solution, which gives a green-black to blue-black colouration on dilution indicates the presence of tannins.

Test for reducing sugars (Brain and Turner, 1975)

1.0g of each fraction was taken and diluted with 2ml distilled water. Fehling's solutions (A and B) were added and the mixture warmed. A brick-red precipitate at the bottom of the tube indicates reducing sugars.

Test for resins (Evans, 1995)

2.0g of each fraction was dissolved in 10ml of ethanoic acid anhydride. One drop of concentrated H_2SO_4 was added. The appearance of purple colour which rapidly changes to violet indicates the presence of resins.

Test for flavonosides (Ciulei, 1994)

2.0g of each fraction was dissolved in 50% CH_3OH(aq) with heating. Magnesium metal and 6 drops of concentrated HCl was then added. Appearance of red colour at the bottom of the tube indicates the presence of flavonosides.

Antibacterial activity testing of the fractions

The standard and local strains of microorganisms (as they are obtained within the state): *Staphylococcus aureus, Streptococcus pneumonia, Klebsiella pneumoniae, Escherichia coli, Pseudomonas aeruginosa* and *Salmonella typhi* were obtained from the pathology dept. of Murtala Muhammad Specialist Hospital, Kano, Nigeria. The cultures were checked for purity and maintained in slant nutrient agar. The nutrient culture was diluted in normal saline (0.85 w/v) such that the turbidity matched with 0.5 Macfarland standard thought to give a mean of $3.3X10^6$cfu/ml microbial population density (Mukhtar, 2006).

The paper disc technique described by Bauer-Kirby (1966) was in determination of the antibacterial activities of the fractions. 0.1g of each fraction was dissolved in 2ml dimethyl sulphoxide to afford a stock solution of 50mg/ml. Quantities; 0.2ml and 0.6ml of the stock solution were then dissolved in 0.8ml and 0.4ml of dimethyl sulphoxide to make 10 and 30mg/ml concentrations respectively (Abdulmann *et al.,* 2007). Filter paper disc (6mm diameter) were cut and sterilized (in Bijou bottles) in oven at 110^{o}C for 1hour. 28g of Biotech Nutrient agar was dissolved in 1 liter distilled water in a conical flask and autoclaved (to sterilized it) for 1 hour. 20ml of the sterilized medium was poured into the sterilized Petri dishes, covered and allowed to cool and solidify.

The sterilized filter paper disc in bijou bottles (100 pieces per bottle) were then impregnated with each of the plant fractions' concentration and even distribution was obtained with through shaking such that each of the 100 discs absorb 0.01ml equivalent to hold the mass of the antibiotic specified for the disc potency (British Pharmacopoeia, 1998).

The Petri dishes containing the nutrient agar were seeded with the test organisms by the spread plate technique (Mukhtar, 2006) and left for half an hour to dry. Then the antibacterial discs as well as the control i.e. discs containing only the solvent (DMSO-the negative control) were then aseptically pressed firmly using sterilized forceps onto the inoculated plates. Note that each concentration for every plant fraction was done in triplicate. After about five minutes pre-diffusion time, the set-up was then incubated at 35^{o}C for 18-24 hours, before they were examined for zone of inhibition. The diameters of the zones of inhibition growth were measured and expressed in millimeters (Mukhtar, 2006).

Column Chromatography of Fraction BP1-01

Silica gel (Merck 60-120 mesh, 60A, bet surface area 500m2/g, pore volume 0.75cm3) of 500g was packed in a column with petroleum ether. A portion of n-Hexane soluble fraction (BP1-01, 5.0 g) was loaded on the column and eluted in this order with n-hexane (2 liter), n-hexane:CH_2Cl_2 (4:1, 3.6 liter), n-hexane:CH_2Cl_2 (3:2; 2.6 liter), n-hexane:CH_2Cl_2 (1:1; 3.2 liter), n-hexane:CH_2Cl_2 (1:4, 1.6 liter), CH_2Cl_2 (1.0 liter), $CH_3CO_2C_2H_5$ (1.0 liter) and MeOH (1.0 liter), collecting fractions in portions of 200 ml. The fractions were evaporated, analyzed on TLC plates and fractions with same Rf were pooled together (Vishnoi, 1979).

RESULTS

Extraction and partitioning

Each of the crude extract/fraction was concentrated in vacuum and weighed. Table 01 give the masses obtained for the crude extracts and partitioned fractions.

Phytochemical analysis

Phytochemical analysis of these fractions indicated the presence of reducing sugars, terpenoids, steroids, cardiac glycosides, tannins, phlobatannins, saponins, anthraquinone, flavonosides, flavonoids, and resins. Results are shown in table 02

Antibacterial test

Tables 03, 04 and 05 below gave the extent of sensitivity of test organisms (zone of inhibition) results at various concentrations of 10, 30 and 50 mg/ml of all the test fractions respectively. The n-hexane fraction (BP1-01) showed the highest activity against the selected microorganism at all the concentration, having strong activity against *Klebsiella pneumoniae, Staphylococcus aureus* and *Salmonella typhi*, but mild against *E .coli* and *Pseudomonas aeruginosa*. The chloroform fraction (BP1-02) and ethyl acetate fraction (BP1-03) showed activity against some P. aureginosa and Strept. pneumoniae at lower concentrations (10 and 30 mg/ml).

The n-hexane fraction was subjected to guided column chromatography packed with silica gel. This led to the isolation of three pooled fractions (BP1-01-01, BP1-01-33, and BP1-01-65) that were found to have melting point ranges of 66-68 ℃, 77-79 ℃ and 83-85 ℃ respectively (as shown in table 06. When these fractions were subjected to activity against *Strept. pneumoniae, Klebsiella pneumoniae, Staph. aureus, E.coli, P.aureginosa* and *Salmonella typhi*, fractions BP1-01-01 and BP1-01-65 show mild activity on *Klebsiella pneumonia, S. typhi* and *Strept. pneumoniae.* But fraction BP1-01-33 shows moderate activity against all the selected pathogenic bacteria as shown in table 07. Qualitative analysis of these tree fractions revealed that they contain only carbon, hydrogen and oxygen (Vishnoi, 1979).

Table1: physiochemical properties of crude extract and partitioned fractions

S/N	Crude/fraction	Weight(g)	Colour/texture
1	BP1	22.00	Dark green solid
2	BP1-01	5.62	Dark jelly-like solid
3	BP1-02	2.32	Dark green waxy solid
4	BP1-03	3.82	Orange waxy solid
5	BP1-04	8.20	Black waxy solid

Key: BP1; crude EtOH, BP1-01; n-hexane, BP1-02; $CHCl_3$, BP1-03; EtOAc, BP1-04; aqueous MeOH soluble fractions from the leaves extract of *Bryophyllum pinnatum*.

Table 2: results of phytochemical analysis

2° metabolite group	BP1-1	BP1-2	BP1-3	BP1-4
Reducing sugars	+	+	+	+
Tannins	-	-	+	+
Steroids	+	+	+	+
Flavonoids	+	+	+	+
Flavonosides	-	+	+	+
Alkaloids	-	+	-	-
Tannins	-	-	+	+
Phlabotannins	+	+	-	-
Cardiac glycosides	+	+	+	-
Resins	+	+	-	+
anthraquinone	+	-	-	-

Key: Present (+), Absent (-)

Table 03 zones of inhibition (in mm) of the plant's fractions (10 mg/ml)

Bacteria species	BP1	BP1-01	BP1-02	BP1-03	BP1-04
E. coli	08	06	01	06	00
P.aureginosa	10	08	10	00	00
K.pneumoniae	07	11	00	00	00
S. aureus	10	12	00	07	00
S. typhi	08	08	00	07	00
S.pneumoniae	08	10	00	00	00

Table 04 zones of inhibition (in mm) of the plant's fractions (30 mg/ml)

Bacteria species	BP1	BP1-01	BP1-02	BP1-03	BP1-04
E. coli	12	10	04	09	00
P.aureginosa	15	08	11	00	07
K.pneumoniae	10	20	05	00	00
S. aureus	11	22	00	12	00
S. typhi	10	09	03	10	02
S.pneumoniae	12	12	07	00	00

Table 05 zones of inhibition (in mm) of the plant's fractions (50 mg/ml)

Bacteria species	BP1	BP1-01	BP1-02	BP1-03	BP1-04
E. coli	04	15	00	15	00
P.aureginosa	13	09	13	09	13
K.pneumoniae	05	08	02	08	02
S. aureus	13	09	13	09	13
S. typhi	05	08	02	08	02
S.pneumoniae	00	20	07	20	07

Table 06: Some physiochemical properties of bioactive compound from *B. pinnatum*

Bioactive compound	Mass (mg)	Melting point(°C)	Colour	Texture
BP1-01-01	50	66-68	White	Non crystalline
BP1-01-33	70	77-79	White	Crystalline
BP1-01-65	80	83-85	White	crystalline

Table 07 zones of inhibition (in mm) of the isolates obtained from column chromatography of BP1-01 (20 mg/ml)

Bacteria species	BP1	BP1-01-01	BP1-01-33	BP1-01-65
E. coli	10	00	07	10
P.aureginosa	13	10	07	00
K.pneumoniae	09	08	10	10
S. aureus	13	10	07	00
S. typhi	09	08	10	10
S.pneumoniae	10	00	08	07

DISCUSSION

Results of phytochemical analysis (table 2) indicates the presence of reducing sugars, tannins, phlobatannins, steroids, cardiac glycosides, flavonoids, flavonosides, resins, alkaloids and saponins in all or some fractions of the two plants. Anthraquinone is observed only in the n-hexane soluble fraction of *B.pinnatum.* On the hand, flavonoids, polyphenol and terpenoids have been identified from previous studies on the leaves of *B.pinnatum* (Ojewole, 2005) and various bioactive compounds have been isolated from extracts of the plant (Cao, *et al.,* 2005, Yamagishi, *et al.,* 1989 and Sutton *et al.,* 1993).

Antibiotic resistance of pathogenic bacteria to current drugs has necessitated the investigation into new, safe, efficient and cost effective antibacterial as alternative agents for controlling the infectious diseases (Mann *et al.,* 2007). The extent of sensitivity of the test organisms on the plant fractions (*B. pinnatum*) was measured by measuring the zone of inhibition after 18-24 hours of inoculation (table 03-05 and 07). The readings complied with acceptable standards of activity, potency and sensitivity as the case might be when the diameter of zone of inhibition is equal or greater than that of the control (6mm). Otherwise, the fraction fails the test.

The guided column chromatography yielded fractions BP1-01-01 obtained as a white non-crystalline solid; 50 mg with m.p. 66-68°C, BP1-01-3 obtained as a white crystalline solid, 70 mg; with m.p.77-79°C and BP1-01-65 obtained as a white non-crystalline solid, 80 mg; with m.p. 83-85°C. All of which showed a remarkable activity against the tested pathogenic bacteria. The findings lend credence to the traditional use of the plant as medicine for respiratory and other related infections particularly those caused by the test organism susceptible to the plant's fractions.

CONCLUSION

The activity of the isolates from *B. pinnatum* leaves extracts on the tested respiratory infection causing pathogenic bacteria confirms the traditional use of the plant for curing respiratory tract infections, and thus meets with the objective of this research in its effort to uncover at least a single compound whose a chemotherapeutic index equal or exceed the drugs used to cure pneumonia and other respiratory infections. Based on this, further work is in progress to purify and characterize these bioactive obtained.

REFERENCES

Bauer, A.W and Kirby, W.M. (1966): Antimicrobial Susceptibility Testing by Standard Single Disc Method: *American Journal of Clinical Pathology*. **45**:493-496.

Brain, K.K and Turner, T.D. (1975): *The Practical Evaluation of Phytopharmaceuticals.* Wright sciencechica. Bristol; p 57-58

BP (1993): Standard Pharmacopoeia Details and Assay Methods for Antimicrobials. *British Pharmacopoeia*. Her Majesty stationary office, London. **2**:1-1012

Cao, H., Xia, J., Lu, B., and Chen, G. (2005): The Separation and Identification of Flavonoids from the Leaves of B. pinnatum. *PubMed*. Link: www.scirus.com

Ciulei, I. (1994): *Methodology for Analysis of Vegetable Drugs*. Chemical Industries branch Division of industrial operations, UNIDO; Romania; 24, 26 and 67

Dalziel, J.M. (1936): *Useful Plants of West Africa.* Crown Agent for Overseas Government and Administration.

Dalziel, J.M., and Hutchinson, J.I. (1958): Flora of Tropical West Africa. **1**: (2). Crown Agent for overseas Government and Administration.

El Olemy, M.M., Al-Muhtadi, F.J. and Afifi, A.A. (1994): *Experimental Phytochemistry. A Laboratory Manual.* King Saud University press. 8-9

Evans, W.C. (1995): *Textbook on Pharmacognocy.* 13th Edition. Bailiere-Tindall, London.

Gwehenberger, B., Rist, L., Huch, R., and von Mandach, U. (2004): Effect of *B.pinnatum* versus Fenoterol on Uterine Contractility. *European Journal of Obstetric. Gynecology and Reproductive Biology.* 15; **113**(2):164-71. Link: www.scirus.com

Harborne, J.B. (1975): *Phytochemical Methods: A guide to Modern Techniques of Plant Analysis.* 1st edition. Chapman and Hall Ltd. London. 160

Iwu, M.W. (1999): New Antimicrobials of Plant Origin. In J. Janick (ed) *Perspectives on New Crops and their Uses.* ASHS press, Alexandria, VA. 457-462

Macleod, J. (1974): *Davidson's Principles and Practice of Medicine.* 11th edition. Churchill Livingstone. Edinghbough , London, New York. 312-425.

Mann, A. Amupitan, J.O., Oyewale, A.O., Okogun, J.I. and Ibrahim, K. (2007): *Antimicrobial Activity and Phytochemical Analysis of two Nigerian Medicinal plant used for Treatment of Respiratory Diseases*. A paper presented at the 5th ChemClass conference; A.B.U Zaria.

Mckenzie, R.A., Franke, F.P. and Dunster, P.J. (1987): The Toxicity to Cattle of Bufadienolide content of six *Bryophyllum* species. *Aust. Vet. J.* **64**(10):298-301. Link: www.scirus.com

Mckenzie, R.A., and Dunster, P.J. (1986): Hearts and Flowers: *Bryophyllum* Poisoning of Cattles. Aust. Vert. J. **63**(7):22-7. Link: www.scirus.com

Medicineatyourfeet (2008): www.medicineatyourfeet.com

Mukhtar, M.D. (2006): *Microbiological Method for Antimicrobial Drug Quality Assessment*. Biological Science Dept. B.U.Kano.

Obaseiki-ebor, E.E. (1985): Preliminary Report on the in-vivo Antibacterial Activity of *B. pinnatum* Leaf Juice. *Afri. J. Med. Med Sci.* **14**(3-4):199-202. Link: www.scirus.com

Ojewole, J.A. (2005): Antinociceptive, Anti-inflammatory and Antidiabetic Effects of *B. pinnatum* (Crassulaceae) Leaf aqueus Extract. *Journal of Enthnopharmacol.* 13; **99** (1):13-9. Link: www.scirus.com

Pal. S., and Nag Chaudhuri, A.K. (1991): Studies of the Anti-ulcer Activity of a *B.pinnatum* Leaf Extract in Experimental Animals. *J. Enthnopharmacol.* **33**(1-2):97-102.Link: www.ncbi.nih.gov

Patrick, L.C. (2006): *Bronchitis; Acute and Chronic.* Link: www.edicine.com/ped/topic

Plangger, N., Rist, L., Zimmermann, R and von Mandach, U. (2005): Intravenous Tocolysis with *B.pinnatum* is better tolerated than Beta-agonist Application. *European Journal of Obstert. Gynecology and Reprod. Biology.* 1; **124**(2):168-72. Link: www.scirus.com

Reppas, G.P. (1995): *B.pinnatum* Poisoning to Cattles. *Aust. Vert. Journal.* **72**(11):425-7. Link: www.scirus.com

Sofowora, S.A. (1984): *Medicinal plants and Traditional Medicine in Africa*. Spectrum books Ltd. 1st edition. 150-151 and 162-172.

Sutton, B.G. and Osmond, C.B. (1972): Dark Fixation of CO_2 by Crassulaceaen Plant: Evidence for single Carboxylation Step. *Plant physiol.* **50**(3):360-365.

Vishnoi, N.K. (1979): *Advanced Practical Organic Chemistry*. Vicas publishing house PVT Ltd, Ghaziabad, India

Wagner, W.L. and Daniel, P. (1999): *Manual of Flowering plants of Hawaii.* Revised edition. Bernice P. Bishop Museum Publication. University of Hawaii.

Yamagishi, T., Haruna, M., Yan X.Z., Chang, J.J., and Lee, K.H. (1989): Antitumor Agent, 110. Bryophyllin B, a novel Potent Cytotoxic Bufadienolide from *B.pinnatum. Journal of Natural Products.* **52**(5):1071-9. Link: www.scirus.com

Yemitan, O.K. and Salahdeen, H.M. (2005): Neurosedative and Muscle Relaxant Activities of Aqueous Extract of *B.pinnatum. Fitoterapia.* **76**(2):187-93. Link: www.scirus.com