British Biotechnology Journal
16(2): 1-8, 2016, Article no.BBJ.28905
ISSN: 2231–2927, NLM ID: 101616695

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Phytochemical Screening and Antimicrobial Activity of *Bryophyllum pinnatum* Extracts

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Authors' contributions

This work was carried out in collaboration between all authors. Authors LUA and JYD conducted the experiments and performed the statistical analysis. Authors MEK and JVA designed the study, wrote the protocol and managed the literature searches. Authors LUA and JOI wrote the first draft of the manuscript. All the authors read and approved the final manuscript.

Article Information

DOI: 10.9734/BBJ/2016/28905
Editor(s):
(1) Anil Kumar, School of Biotechnology, Devi Ahilya University, Madhya Pradesh, India.
Reviewers:
(1) Charu Gupta, Amity University UP, India.
(2) A. Harinatha Reddy, Sri Krishnadevaraya University, Anantapur 515 003, AP, India.
Complete Peer review History: http://www.sciencedomain.org/review-history/16389

Original Research Article

Received 11th August 2016
Accepted 2nd September 2016
Published 29th September 2016

ABSTRACT

The hexane, ethyl acetate and methanol extracts of the wood and stem bark of *Bryophyllum pinnatum* were investigated for their phytochemical constituents and activity against selected microorganisms. Phytochemicals found present were reducing sugars, saponins, steroids, tannins, alkaloids, flavonoids and phenols. The test microorganisms were *Staphylococcus aureus, Escherichia coli, Klebsiella pneumoniae, Shigella dysenteriae, Salmonella typhi, Pseudomonas aeruginosa, Candida albicans, Aspergillus fumigatus, Aspergillus niger, Microsporum spp* and *Trichophyton rubrum*. The Ethyl acetate extracts were the most effective against *S. aureus, E. coli, P. aeruginosa* and *K. Pneumonia* (MIC 5.0 mg/mL) and *S. dysenteriae, C. albicans, Microsporum spp,* and *T. rubrum* (MIC 10 mg/mL).

Keywords: Phytochemical screening; bioactive principles; antimicrobial activity; Bryophyllum pinnatum.

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1. INTRODUCTION

The use of natural products in human medicine is on the increase [1]. In India *Moringa oleifera* Lam (*Moringaceae*) is used as a hypocholesterolemic agent [2] while *Aloe barbadensis*is is used for its anti-inflammatory, astringent, emollient, antifungal, antibacterial antiviral and anti-parasitic activities [3,4]. Natural products are popular in many countries because the medicinal plants and herbs are readily available and affordable [1]. Countries such as Mali, Vietnam, China, Sri Lanka and India have integrated traditional with orthodox medicine in their health care delivery systems for more effectiveness [5,6]. It is therefore imperative to investigate these plants to find their effective constituents. Several reports have confirmed the use of *B. pinnatum* for different types of ailments as documented by [7-9].

Kalanchoe pinnata (synonym: *Bryophyllum pinnatum*) commonly known as "Miracle leaf", "Mexican Love plant", "Katakataka", "Cathedral Bells", "Air plant", "Life plant", "Goethe plant", "Wonder of the World" "African–never–die", resurrection plant, life plant, the belongs to the Crassulaceae family [10]. This plant is a water-storing perennial that grows about 1 to 1.5 m tall. The leaves are thick green, fleshy and distinctively scalloped. The stems are tall and hollow, bearing pendulous bell-like flowers [11]. In traditional medicine, *B. pinnatum* has been used to treat rheumatism, inflammation, hypertension and kidney stones [12]. The pounded fresh material is applied as a poultice for sprains, boils, abscess, eczema, infections, burns, carbuncle and erysipelas [13]. The availability of ascorbic acid in the plant provides the biochemical basis for the use of the plant extract in the treatment and prevention of cold and other diseases like prostate cancer. *Kalanchoe pinnata* extracts also have immunosuppressive effects [14]. The flavonoids, polyphenols, triterpenoids and other chemical constituents of the plant are speculated to account for the antinociceptive, anti-inflammatory, antihypertensive and anti-diabetic properties observed in the aqueous leaf extracts [15]. The extract also had neuro-sedative and muscle relaxant activities and produced a depressant action on the central nervous system of mice. These effects were attributed to bufadienolide and other water soluble constituents in the extract [16]. The aqueous leaf extracts of *B. pinnatum* have a strong analgesic potency comparable in a time and dose–dependent manner to a non-steroidal anti-inflammatory drug [17]. Most of these investigations were centered on the leaf extract of *B. pinnatum.* Studies on the plant stems are not many and this report is on the phytochemical screening and antimicrobial activities of the twigs.

2. MATERIALS AND METHODS

Stems of *Bryophyllum pinnatum* were collected from Aguluezechuwu in Aguata L.G.A of Anambra State Nigeria in August 2015. Although the plant is commonly known, it was confirmed by Dr B. A. Ayinde of the Department of Pharmacognosy, Faculty of Pharmacy, University of Benin, Nigeria. A herbarium specimen with voucher number FHI 107762 was deposited with the Forest Research Institute of Nigeria by Miss Joy Odimegwu. Impurities were carefully picked and the twigs were thereafter rinsed with tap water. The stem was separated into stem bark and woody portions and air dried at ambient temperatures to a constant weight over a period of four weeks. It was then powdered in a wooden mortar.

2.1 Microwave Assisted Extraction of Samples

A microwave assisted extraction was carried out using a domestic microwave oven according to methods described by [18]. The pulverized plant (150 g) was extracted by adding hexane, ethyl acetate and methanol (800 mL each) successively. The mixture of the plant material and solvent was heated for three minutes in (70 Watts/Defrost Function) using a modified domestic kitchen microwave (Mio-star, Model 7173.295, Germany) and repeated ten times with 14 minutes cooling intervals so the temperature does not rise above 70℃. The extracts were thereafter allowed to cool to ambient temperature (32 - 35℃).Pressure build up was vented after every successive heating. The extracts were filtered, air dried and then stored until required.

2.2 Phytochemical Screening

Crude extracts were subjected to phytochemical tests for the presence of anthraquinones, saponins, tannins, steroids, terpenes, reducing sugars, flavonoids and alkaloids using standard procedures as described by [19].

2.3 Test for Antimicrobial Activity

The antimicrobial activities of the hexane, ethyl-acetate and methanol extracts of the wood stem

and stem bark were determined using clinical isolates of some pathogenic microbes obtained from Department of Medical Microbiology Ahmadu Bello University Teaching Hospital Zaria. A Diffusion method was used for screening the extracts. Mueller Hinton Agar was used as growth medium for microbes, sterilized at 121℃ for 15 mins, poured into sterile petri dishes and allowed to cool and solidify.

The extracts (0.4 g) were dissolved in 10 mL of DMSO to obtain a concentration of 40 mg/mL. The sterilized medium was then seeded with the standard Inoculum (0.1 mL) of test microbes spread evenly over the surface of the medium with sterile swabs. Using a 6 mm standard cork borer a well was cut at the center of each inoculated medium. A concentration of 5 mg/mL of the extract was then introduced into each well on the inoculated medium. The inoculated medium was incubated at 37℃ for 24 hr, after which the medium was observed for the zones of inhibition of growth. The zones were measured with a transparent ruler [20].

2.4 Minimum Inhibition Concentration (MIC)

The minimum inhibitory concentration was determined using the Broth dilution method. Mueller Hinton broth was prepared 10 mL by dispensing into test-tubes, sterilized at 37℃ for 6 hours and allowed to cool. McFarland's turbidity standard scale number 0.5 was prepared to give a turbid solution. Dilution of the test microbes was in normal saline until turbidity matched that of the McFarland's scale and the concentration of the test microbes was taken to be 105×10^8 cfu/mL. The extracts in the sterile broths were diluted serially to obtain 40 mg/mL, 20 mg/mL, 10 mg/mL, 5 mg/mL and 2.5 mg/mL concentrations. The initial concentration of the extract was obtained by dissolving the extract (0.1 g) in the sterile broth (40 mL).The test microbes were then inoculated into the different concentrations [21] and incubated at 37℃ for 24 hr. There after the test tubes were observed for turbidity or growth. The lowest concentration of extracts in the sterile broth which showed no turbidity was recorded as the minimum inhibition concentration (MIC) [21,22].

2.5 Minimum Bactericidal and Minimum Fungicidal Concentration (MBC and MFC)

These were carried out to determine the concentration of extracts that could stop the growth of the test microbes. Mueller Hinton Agar was prepared, sterilized at 121℃ for 15 min, poured into sterile Petri dishes and allowed to cool. The contents of the test tube with the determined MIC was then sub-cultured onto prepared media, incubated at 37℃ for 24 hr. after which the plates were observed for any colony growth. MBC/MFC plates with lowest concentration of extract without a colony growth were considered as MBC/MFC [21].

3. RESULTS AND DISCUSSION

Phytochemical screening of the stem bark extracts of *Bryophyllum pinnatum* showed the presence of reducing sugars, saponins, tannins, flavonoids and phenolic compounds in the methanol, steroids and terpenoids in hexane and ethyl acetate while alkaloids were only detected in ethyl acetate and methanol (Table 1) extracts. The stem wood extracts showed the presence of saponins, tannins, flavonoids and phenols in the methanol while steroids and terpenoids were detected in the hexane, ethyl acetate and methanol, reducing sugars and alkaloids in the ethyl acetate and methanol. Antraquinones were not detected in any of the extracts (Table 1).

The extracts showed varying degrees of antibacterial and antifungal activities against tested pathogens (Table 2) and all organism bioassay were done in triplicates. The activities of the hexane, ethyl acetate and methanol extracts compared with that of standard antibiotics (Ciprofloxacin and Fluconazole) and appeared to have a broad spectrum of activity. The ethyl acetate extracts of stem bark and wood showed high inhibition zones against *Escherichia coli* and *Klebsiella pneumonia (*28 mm) respectively while the hexane extracts (stem bark and wood) showed the lowest inhibition zones (20 mm) against *S. aureus, Shigella dysenteriae, Microsporum sp, Escherichia coli* and *Trichophyton rubrum*. The extracts were ineffective against *Salmonella typhi, Aspergillus fumigatus* and *Aspergillus niger.* Isolates of *E.coli* were found to be resistant to Ciprofloxacin [23], while *B. pinnatum* bark and wood stem extracts were sensitive. Ciprofloxacin may not be used for minor infections caused by *Staphylococi* or *P. aeruginosa* [24] but *B. pinnatum* wood stem extracts with good sensitivity (Table 2) could be used. The results (Table 3) show that ethyl acetate extracts were the most effective against *S. aureus, E. coli, P. aeruginosa and K. pneumoniae* with MIC of 5 mg/mL followed by 10 mg/mL against

Table 1. Phytochemical screening of stem bark and stem wood extracts of *B. pinnatum*

Class of compound	Hexane		Ethyl acetate		Methanol	
	Stem bark	Stem wood	Stem bark	Stem wood	Stem bark	Stem wood
Reducing sugars	-	-	-	+	+	+
Antraquiunones	-	-	-	-	-	-
Saponins	-	-	-	-	+	+
Steroids	+	+	+	+	-	+
Tannins	-	-	-	-	+	+
Alkaloids	-	-	+	+	+	+
Flavonoids	-	-	-	-	+	+
Phenols	-	-	-	-	+	+
Terpenoids	-	+	+	+	+	+

Key = + present, – below detectable limits

Table 2. Sensitivity/Zone of Inhibition (mm) of extracts against test microorganisms

Test of pathogen	ESB	HSB	MSB	EWS	HWS	MWS	Ciprofloxacin	Fluconazole
Staphylococcus aureus	S (25)	S (20)	S (22)	S (27)	S (22)	S (23)	S (35)	-
Escherichia coli	S (26)	S (21)	S (23)	S (28)	S (20)	S (25)	S (40)	-
Klebsiella pneumonia	S (28)	S (22)	S (24)	R	R	R	S (37)	-
Shigellady senteriae	S (24)	S (20)	S (21)	S (26)	S (21)	S (22)	S (39)	-
Salmonella typhi	R	R	R	R	R	R	S (41)	-
Pseudomonas aeruginosa	R	R	R	S (27)	S (24)	S (26)	-	-
Candida albicans	S (25)	S (21)	S (23)	S (25)	S (21)	S (23)	-	-
Aspergillus fumigatus	R	R	R	R	R	R	-	S (32)
Aspergillus niger	R	R	R	R	R	R	-	S (31)
Microsporum sp	S (24)	S (20)	S (21)	S (25)	S (21)	S (23)	-	S (30)
Trichophyton rubrum	R	R	R	S (26)	S (20)	S (22)	-	S (33)

Key = S = Sensitive; R: Resistant, Numeric value in brackets = Diameter of zone of inhibition in millimeters; ESB: ethyl acetate stem bark, HSB: hexane stem bark; MSB: methanol stem bark; EWS: ethyl acetate wood stem; HWS: hexane wood stem; MWS: methanol wood stem; Bacteria: Ciprofloxacin; Fungi: Fluconazole

Table 3. Minimum inhibitory concentration of extracts against test microbes

Test of pathogen	ESB					HSB					MSB					EWS					HWS					MWS				
	40 mg/mL	20 mg/mL	10 mg/mL	5 mg/mL	2.5 mg/mL	40 mg/mL	20 mg/mL	10 mg/mL	5 mg/mL	2.5 mg/mL	40 mg/mL	20 mg/mL	10 mg/mL	5 mg/mL	2.5 mg/mL	40 mg/mL	20 mg/mL	10 mg/mL	5 mg/mL	2.5 mg/MI	40 mg/mL	20 mg/mL	10 mg/mL	5 mg/mL	2.5 mg/mL	40 mg/mL	20 mg/mL	10 mg/mL	5 mg/mL	2.5 mg/mL
Staphylococcus aureus	-	-	O#	+	++	-	-	O#	+	++	-	-	O#	+	++	-	-	-	O#	+	-	-	O#	+	++	-	-	O#	+	++
Escherichia coli	-	-	O#	+	++	-	-	O#	+	++	-	-	O#	+	++	-	-	-	O#	+	-	-	O#	+	++	-	-	O#	+	++
Klebsiella pneumonia	-	-	-	O#	+	-	-	O#	+	++	-	-	O#	+	++															
Shigellady senteriae	-	-	O#	+	++	-	-	O#	+	++	-	-	O#	+	++	-	-	O#	+	++	-	-	O#	+	++	-	-	O#	+	++
Salmonella typhi																														
Pseudomonas aeruginosa																-	-	-	O#	+	-	-	O#	+	++	-	-	O#	+	++
Candida albicans	-	-	O#	+	++	-	-	O#	+	++	-	-	O#	+	++	-	-	O#	+	++	-	-	O#	+	++	-	-	O#	+	++
Aspergillus fumigates																														
Aspergillus niger																					-	-	O#	+	++	-	-	O#	+	++
Microsporum sp	-	-	O#	+	++	-	-	O#	+	++	-	-	O#	+	++	-	-	O#	+	++	-	-	O#	+	++	-	-	O#	+	++
Trichophyton rubrum																-	-	O#	+	++										

KEY => No colony growth, o#; MBC/MFC +; low colony growth ++; moderate colony growth; ESB: ethyl acetate stem bark, HSB: hexane stem bark; MSB: methanol stem bark; EWS: ethyl acetate wood stem; HWS: hexane wood stem; MWS: methanol wood stem

Table 4. Minimum bactericidal/fungicidal concentration of the extract against the test microbe

Test of pathogen	ESB					HSB					MSB					EWS					HWS					MWS				
	40 mg/mL	20 mg/mL	10 mg/mL	5 mg/mL	2.5 mg/mL	40 mg/mL	20 mg/mL	10 mg/mL	5 mg/mL	2.5 mg/Ml	40 mg/mL	20 mg/mL	10 mg/mL	5 mg/mL	2.5 mg/Ml	40 mg/mL	20 mg/mL	10 mg/mL	5 mg/mL	2.5 mg/mL	40 mg/mL	20 mg/mL	10 mg/mL	5 mg/mL	2.5 mg/mL L	40 mg/mL	20 mg/mL	10 mg/mL	5 mg/mL	2.5 mg/mL
Staphylococcus aureus	-	O#	+	++	+++	O#	+	++	+++	+++	O#	+	++	+++	+++	-	-	O#	+	++	O#	+	++	+++	+++	-	O#	+	++	+++
Escherichia coli	-	O#	+	++	+++	O#	+	++	+++	+++	-	O#	+	++	+++	-	-	O#	+	++	O#	+	++	+++	+++	-	O#	+	++	+++
Klebsiella pneumoniae	-	-	O#	+	++	O#	+	++	+++	+++	-	O#	+	++	+++															
Shigella dysenteriae	-	O#	+	++	+++	O#	+	++	+++	+++	O#	+	++	+++	+++	-	O#	+	++	+++	O#	+	++	+++	+++	O#	+	++	+++	+++
Salmonella typhi																														
Pseudomonas aeruginosa																-	-	O#	+	++	-	O#	+	++	+++	-	O#	+	++	+++
Candida albicans	-	O#	+	++	+++	O#	+	++	+++	+++	-	O#	+	++	+++	-	O#	+	++	+++	O#	+	++	+++	+++	-	O#	+	++	+++
Aspergillus fumigates																														
Aspergillus niger																														
Microsporum sp	-	O#	+	++	+++	O#	+	++	+++	+++	O#	+	++	+++	+++	-	O#	+	++	+++	O#	+	++	+++	+++	-	O#	+	++	+++
Trichophyton rubrum																-	O#	+	++	+++	O#	+	++	+++	+++	O#	+	++	+++	+++

KEY => No colony growth, o#; MBC/MFC, +; low colony growth ++; Moderate colony growth +++; High colony growth; - = No zone of inhibition; ESB: ethyl acetate stem bark, HSB: hexane stem bark; MSB: methanol stem bark; EWS: ethyl acetate wood stem; HWS: hexane wood stem; MWS: methanol wood stem

(S. *dysenteriae, C. albicans, Microsporum* sp, and *T. rubrum*) respectively. *Bryophyllum pinnatum* ethyl acetate extracts had the most effective (MBC/MFC) (10 mg/mL) against *S. aureus, E. coli, K. pneumonia* and *P. aeruginosa* while hexane extracts were the least effective against all the test microbes at MBC/MFC of 40 mg/mL (Table 4).

The inhibitory effect of extracts of *B. pinnatum against* pathogenic strains makes the plant a potential drug development candidate for treatment of ailments caused by these pathogens. The ethyl acetate extract of the wood stem had the higher activity against both bacterial and fungal isolates. The presence of the detected secondary metabolites in the plant (Table 1) supports the usefulness of the plant [25,26]. These classes of compounds exert various physiological activities and saponins in particular play a positive role in cholesterol metabolism [27].

4. CONCLUSION

This study shows that stem extracts of *B. pinnatum* have antibiotics properties. They have both anti-bacteria and ant-fungal effects. These results support that traditional medicine use of the stem of *B. pinnatum* in traditional medicine.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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Peer-review history:
The peer review history for this paper can be accessed here:
http://sciencedomain.org/review-history/16389