Academic Sciences International Journal of Pharmacy and Pharmaceutical Sciences

ISSN- 0975-1491 Vol 6, Issue 1, 2014

Reserch Article

IN VITRO ANTIBACTERIAL AND ANTIOXIDANT ACTIVITY OF *Bryophyllum pinnatum* (Lam.) Kurz.

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Received: 25 Oct 2013, Revised and Accepted: 16 Nov 2013

ABSTRACT

Objective: *Bryophyllum pinnatum* (Lam.) Kurz. is a medicinal herb commonly used to treat ulcers, cough, diabetes and cancer. In this study, antibacterial and antioxidant activity of aqueous and methanolic extracts of root, stem, leaf and whole plant of *Bryophyllum pinnatum* (Lam.) Kurz. have been evaluated.

Method: In the present study, methanolic and aqueous extracts of root, stem, leaf and whole plant of *Bryophyllum pinnatum* have been used in the present investigation to study the superoxide, hydroxyl radical scavenging activity, iron chelating power and total antioxidant activity. Antibacterial effect was tested against six species of bacteria; three Gram-positive (*Corynebacterium diphtheriae, Micrococcus luteus* and *Bacillus subtilis*) and three Gram-negative (*Alcaligenes faecalis, Bordetella bronchiseptica* and *Serratia marcescens*). The tests were carried out using the minimum inhibitory concentration (MIC) and agar well diffusion method.

Results: In our results aqueous and methanolic extracts of root, stem, leaf and whole plant of *Bryophyllum pinnatum* showed abilities to scavenge hydroxyl and superoxide free radicals, IC_{50} values for hydroxyl radical (35.48, 37.15, 31.62, 28.13 mg/ml) and (50.18, 70.79, 32.35, 25.11 mg/ml) for aqueous and methanolic extracts of root, stem, leaf and whole plant respectively. IC_{50} values for superoxide radical (16.21 and 16.59 mg/ml) and (19.95 and 17.78 mg/ml) for aqueous and methanolic extracts of leaf and whole plant respectively. IC_{50} values for iron chelating power (63.09, 25.70 and 34.54 mg/ml) for aqueous extract of root, leaf and whole plant and (40.73 and 31.62 mg/ml) for methanolic extracts of leaf and whole plant respectively. Antibacterial activity was shown by both extracts of aqueous and methanolic extract of root, stem, leaf and whole plant of *Bryophyllum pinnatum* (Lam.) Kurz.

Conclusion: These findings suggest the excellent medicinal bioactivity of *Bryophyllum pinnatum* (Lam.) Kurz. and explain the popularity of this plant in the folk medicine as a remedy for different illnesses.

Keywords: *Bryophyllum pinnatum* (Lam.) Kurz., Antioxidant and Antibacterial activity.

INTRODUCTION

Plants derived natural products are the source of most active components of medications, which in turn play a significant role in the treatment or prevention of human illnesses. Tropical plants have been investigated intensively during the last decades in order to evaluate the possibility of developing new, sustainable, natural and affordable cosmetics and drugs [1].

Bryophyllum pinnatum (Lam.) Kurz., (Crassulaceae) Synonym: *Kalanchoe pinnata* (Lam.) Oken, *Bryophyllum calycinum* Salisb. It is commonly known as Zakham-e-hyat, Life plant, air or maternity plant, love plant, Canterbury bells, Cathedral bells, parnabija. It is a perennial herb growing widely and used in folkloric medicine in tropical Africa, tropical America, India, China and Australia, classified as a weed. The plant flourishes throughout the Southern part of Nigeria. This is the only *Kalanchoe* species found in South America, however, 200 other species are found in Africa, Madagascar, China and Java. A number of species are cultivated as ornamentals and are popular tropical house plants [2].

B. pinnatum is rich in alkaloids, triterpenes, glycosides, flavonoids, cardienolides, steroids, bufadienolides and lipids. The leaves contain a group of chemicals called bufadienolides which are very active. Bufadienolides like bryotoxin A, B, C which are very similar in structure and activity as two other cardiac glycosides, digoxin and digitoxin and possesses antibacterial, antitumor, cancer preventative and insecticidal actions [2].

Though there were many scientific validations attempted and reported on *Bryophyllum pinnatum* (Lam.) Kurz., very less studies highlighting its antibacterial and antioxidant properties have been reported with root, stem, leaf and whole plant parts.

In the present study, *in vitro* antibacterial and antioxidant activity of aqueous and methanolic extracts of root, stem, leaf and whole plant of *Bryophyllum pinnatum* (Lam.) Kurz. have been evaluated by using hydroxyl and superoxide radical scavenging activity, iron chelating power and total antioxidant activity (phosphomolybdate method), antibacterial activity by using MIC and agar well diffusion method.

MATERIAL AND METHODS

Collection of plant material

Root, stem, leaf and whole plant of *Bryophyllum pinnatum* (Lam.) Kurz. were collected Kalyan region. The voucher specimen of the plant was authenticated from Blatter Herbarium, Department of Botany, St. Xavier's College, Mumbai. All plant parts were washed properly under running tap water, shade dried, powdered and stored in an airtight bottle.

Preparation of extracts

Root, stem, leaf and whole plant powder (50 g) of *Bryophyllum pinnatum* (Lam.) Kurz. was macerated separately in 100 ml of methanol and distilled water for 24 hours in mechanical shaker at 120 rpm. The contents were filtered through Whatman filter paper No. 1 and residues were further macerated thrice using same procedure. The filtrates obtained at each step were combined and evaporated separately for root, stem, leaf and whole plant in water bath (60±2º C). These extracts were used for *in vitro* antioxidant and antibacterial activities.

Antioxidant activity

Total antioxidant activity of aqueous and methanolic extracts of root, stem, leaf and whole plant of *Bryophyllum pinnatum* (Lam.) Kurz. was determined according to the method of Prieto *et al*. [3]. Hydroxyl radical scavenging activity was evaluated as per the method of Elizabeth and Rao [4] and superoxide radical scavenging activity was measured by the reduction of NBT according to reported method [5]. The ferrous ion chelating activity was evaluated by a standard method [6].

Antibacterial activity

Total six organisms including three Gram negative bacteria *viz*., *Alcaligenes faecalis* (NCIM 2262), *Bordetella bronchiseptica* (NCIM

5390) and *Serratia marcescens* (NCIM 5061) and three Gram positive bacteria *viz*., *Corynebacterium diphtheriae* (NCIM 2253), *Micrococcus luteus* (NCIM 2704) and *Bacillus subtilis* (NCIM 2010) were used for the study. The bacterial cultures were obtained from National Collection of Industrial Microorganisms (NCIM), Pune, India. The bacterial cultures were maintained on Nutrient Agar (NA) slants and stored at 4°C.

Determination of Minimum Inhibitory Concentration

The minimum inhibitory concentration (MIC) of aqueous and methanolic extracts of root, stem, leaf and whole plant of *Bryophyllum pinnatum* (Lam.) Kurz. were determined against all the six selected bacteria separately. Concentration ranging from 1.00 – 300 mg/ml of aqueous and methanolic extracts of root, stem, leaf and whole plant was prepared and 500 µl of each dilution was incubated with 2.5 ml of Mueller Hinton Broth containing 0.1 ml of bacterial suspension at 37°C for 24 hours. After incubation the tubes were examined for bacterial growth by observing turbidity. The MIC was determined as minimum concentration that showed no visible growth. The experiments were carried out in triplicates.

Determination of antibacterial activity

Antibacterial activity was carried out using aqueous and methanolic extracts of plant parts viz., root, stem, leaf and whole plant of *Bryophyllum pinnatum* (Lam.) Kurz. by agar well diffusion method.

Antibacterial activity was determined by measuring the diameter (mm) of zone of inhibition. For the determination of zone of inhibition, the concentration of root, stem, leaf and whole plant extracts were calculated on the considering their respective MIC values. Concentrations with 2 - 4 times of MIC values are used to determine the zone of inhibition. 1.0 ml of culture suspension was added in 20 ml of sterile molten nutrient agar, mixed thoroughly and poured in pre - sterilized petri dishes under aseptic conditions. The agar was allowed to solidify at room temperature. Using a sterile cork - borer, wells of 7 mm diameter was prepared in seeded plates. Three equi-distant wells were prepared. 200 µl of plant extract (prepared in a particular solvent) was added in each test well by using sterile micropipettes. These plates were allowed to stand for 30 min at 4º C for pre- diffusion. The plates were incubated at 37º C for 24 hours. After incubation the diameter of the zone of inhibition was measured in mm. Ciprofloxacin (5µg/ml) was used as positive control and respective solvents were used as negative control.

RESULTS

In the present work, intro antioxidant and antibacterial activity of different parts of *Bryophyllum pinnatum* (Lam.) Kurz. was studied.

Total antioxidant activity

Total antioxidant activity is a quantitative assay, since the antioxidant activity is expressed as the number of equivalents of Ascorbic acid. The assay is based on the reduction of Mo (VI) to Mo (V) by the sample analyte and the subsequent formation of a green phosphate/Mo (V) complex at acidic pH with the maximal absorption at 695nm. The linear equation of ascorbic acid for total antioxidant activity was found to be y=2.676x with r^2= 0.9979. The antioxidant activity of aqueous and methanolic extract of root, stem, leaf and whole plant was found to be 69.42±1.42, 24.66±2.18, 86.57±1.42 and 101.80±2.18 µg. Whereas, total antioxidant activity of methanolic extract of root, stem, leaf and whole plant was found to be 60.85±1.42, 18.47±2.18, 73.23±2.18 and 79.42±1.42 µg of ascorbic acid/ g dry weight.

Hydroxyl radical scavenging activity

This assay shows the abilities of the extract and standard ascorbic acid to inhibit hydroxyl radical-mediated deoxyribose degradation in an Fe^{3+}-EDTA-ascorbic acid and H_2O_2 reaction mixture. The results are shown in figure 2. The IC_{50} values (Table 1) of aqueous & methanolic extracts of root, stem, leaf and whole plant extract and standard in this assay were 35.48, 37.15, 32.35, 28.13 & 50.18, 70.79, 31.62 and 25.11 mg/ml respectively. The IC_{50} value of the extract was less than that of the standard.

Superoxide radical scavenging activity

The superoxide radicals generated from dissolved oxygen by PMS-NADH coupling can be measured by their ability to reduce NBT. The decrease in absorbance at 560 nm with the plant extract and the reference compound ascorbic acid indicates their abilities to quench superoxide radicals in the reaction mixture. As shown in figure 3, the IC_{50} values (Table 1) of aqueous & methanolic extracts of leaf and whole plant extract and standard in this assay were 16.21, 16.59, 19.95 and 17.78 mg/ml respectively.

Iron chelating power

Ferrozine produces a violet complex with Fe^{2+}. In the presence of a chelating agent, complex formation is interrupted and as a result the violet color of the complex is decreased. The results demonstrated that formation of the ferrozine-Fe^{2+} complex is inhibited in the presence of the test and reference compounds. The IC_{50} values shown by aqueous extract of root and leaf were 63.09 and 25.70 mg/ml respectively. IC_{50} value for methanolic extract of leaf and whole plant were 40.73 and 31.62 mg/ml respectively. Mannitol showed IC_{50} value of 1.95 mg/ml of the ferrous ion chelating ability **(Table 1).**

Antibacterial activity

The antibacterial activity of the extracts of the leaves and stem of the plant is presented in Table 2. The antibacterial activity of the methanol extracts of the stem was found to be higher than of the root, leaf and whole plant. The methanolic extract of stem showed activity against all the organisms at MIC range of 100 to 140 mg/ml against the entire test organism. Aqueous extract of leaf was only active against *Bacillus subtilis* and *Alcaligenes faecalis*, while methanolic extract of leaf was found to inactive against all the test organism **(Table 2 and 3)**.

Table 1: Antioxidant potential of aqueous and methanolic extracts of *Bryophyllum pinnatum* (Lam.) Kurz.

IC_{50} values (mg/ml)								
Plant parts	**Root**		**Stem**		**Leaf**		**Whole Plant**	
	Aq	**MeOH**	**Aq**	**MeOH**	**Aq**	**MeOH**	**Aq**	**MeOH**
Hydroxyl	35.48	50.18	37.15	70.79	31.62	32.35	28.13	25.11
Superoxide	NA	NA	NA	NA	16.21	19.95	16.59	17.78
Iron Chelating power	63.09	NA	NA	NA	25.70	40.73	24.54	31.62

Keys: Aq= Aqueous extract; MeOH= Methanolic extract.

Table 2: Antibacterial potential of aqueous extract of *Bryophyllum pinnatum* (Lam.) Kurz

Zone of Inhibition in mm (ZOI)					
Plant part	**Root**	**Stem**	**Leaf**	**Whole plant**	**Ciprofloxacin**
Organism					
Alcaligenes faecalis	21.77±0.83	16.55±0.19	23.22±0.76	19.44±0.83	29.33±1.76
Bordetella bronchiseptica	15.77±0.50	17.11±0.69	NZI	20.55±1.34	29.33±1.76
Serratia marcescens	13.22±1.26	NZI	NZI	19.44±0.83	29.33±1.76
Corynebacterium diphtheriae	13.00±0.33	17.11±0.69	NZI	16.88±1.07	29.33±1.76
Micrococcus luteus	NZI	NZI	NZI	19.55±0.83	29.33±1.76
Bacillus subtilis	20.33±1.20	20.66±1.45	20.44±0.38	18.88±0.50	29.33±1.76

Table 3: Antibacterial potential of methanolic extract of *Bryophyllum pinnatum* (Lam.) Kurz.

Zone of Inhibition in mm (ZOI)					
Plant part	**Root**	**Stem**	**Leaf**	**Whole plant**	**Ciprofloxacin**
Organism					
Alcaligenes faecalis	NZI	21.44±0.19	NZI	17.66±0.33	29.33±1.76
Bordetella bronchiseptica	16.66±1.20	20.66±1.15	NZI	NZI	29.33±1.76
Serratia marcescens	NZI	14.22±0.50	NZI	13.22±0.19	29.33±1.76
Corynebacterium diphtheriae	20.11±0.50	19.66±1.00	NZI	14.88±0.76	29.33±1.76
Micrococcus luteus	16.55±0.69	17.77±0.19	NZI	14.55±0.19	29.33±1.76
Bacillus subtilis	14.55±0.83	13.55±0.19	NZI	16.55±0.38	29.33±1.76

Values are Mean± S. D. of three determinants, Zone of inhibition includes diameter of well, NIZ= No Zone of Inhibition

DISCUSSION

In living systems, free radicals are constantly generated and they can cause extensive damage to tissues and biomolecules leading to various disease conditions, especially degenerative diseases, and extensive lysis [7]. Many synthetic drugs protect against oxidative damage but they have adverse side effects. An alternative solution to the problem is to consume natural antioxidants from food supplements and traditional medicines [8-9]. Recently, many natural antioxidants have been isolated from different plant materials [10-11].

Hydroxyl radicals are the major active oxygen species causing lipid peroxidation and enormous biological damage [12]. They were produced in this study by incubating ferric-EDTA with ascorbic acid and H_2O_2 at pH 7.4, and reacted with 2-deoxy-2-ribose to generate a malondialdehyde (MDA)-like product. This compound forms a pink chromogen upon heating with TBA at low pH [13]. When root, stem, leaf and whole plant extracts of *Bryophyllum pinnatum* (Lam.) Kurz. was added to the reaction mixture, it removed the hydroxyl radicals from the sugar and prevented the reaction. The IC_{50} value indicates that the plant extract is a better hydroxyl radical scavenger than the standard ascorbic acid.

Superoxide anion is also very harmful to cellular components [14]. Robak and Glyglewski [15] reported that flavonoids are effective antioxidants mainly because they scavenge superoxide anions. As shown in figure 3, the superoxide radical scavenging activities of the plant extract and the reference compound are increased markedly with increasing concentrations. The results suggest that the plant extract is a more potent scavenger of superoxide radical than the standard ascorbic acid.

Metal chelating capacity is significant since it reduces the concentration of the transition metal that catalyzes lipid peroxidation [16]. According to the results, the plant extract is not as good as the standard mannitol; but the decrease in concentration dependent color formation in the presence of the extract indicates that it has iron chelating activity.

The potential for developing antimicrobials from higher plants appears rewarding as it will lead to the development of a phytomedicine to act against microbes. Plant-based antimicrobials have enormous therapeutic potential as they can serve the purpose with lesser side effects that are often associated with synthetic antimicrobials [17]. Continued further exploration of plant- derived antibacterial is needed today.

CONCLUSION

The results from present study indicate that *Bryophyllum pinnatum* (Lam.) Kurz. possess antioxidant properties and could serve as free radical inhibitors or scavenger or, acting possibly as primary antioxidants. The antibacterial properties of *Bryophyllum pinnatum* (Lam.) Kurz. was effective. Lot of attention is being devoted to natural sources of antioxidant and antibacterial materials, the data obtained in this study might suggest a possible use of *Bryophyllum pinnatum* (Lam.) Kurz. as a source of natural antioxidant and antibacterial agents.

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