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

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. IC₅₀ 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. IC₅₀ 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. IC₅₀ 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., (Grassulaceae) Synonym: Kalanchoe pinnata (Lam.) Oken, Bryophyllum calycinum Salsib. is commonly known as Zakhm-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, cardiacnolides, 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 Microorganism (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 37ºC 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.

**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 + 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 Fe²⁺-EDTA-ascorbic acid and H₂O₂ reaction mixture. The results are shown in figure 2. The IC₅₀ 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 IC₅₀ 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 IC₅₀ 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 Fe²⁺. 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-Fe²⁺ complex is inhibited in the presence of the test and reference compounds. The IC₅₀ values shown by aqueous extract of root and leaf were 63.09 and 25.70 mg/ml respectively. IC₅₀ value for methanolic extract of leaf and whole plant were 40.73 and 31.62 mg/ml respectively. Mannitol showed IC₅₀ 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).

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

<table>
<thead>
<tr>
<th>IC₅₀ values (mg/ml)</th>
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<tbody>
<tr>
<td>Plant parts</td>
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<tr>
<td>Root</td>
</tr>
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<td>Stem</td>
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<td>Leaf</td>
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<td>Whole Plant</td>
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<td>Hydroxyl</td>
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<td>Superoxide</td>
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<td>Iron Chelating power</td>
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**Keys:** Aq= Aqueous extract; MeOH= Methanolic extract.

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

<table>
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<th>Zone of Inhibition in mm (ZOI)</th>
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<tbody>
<tr>
<td>Plant part</td>
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<tr>
<td>Root</td>
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<tr>
<td>Alcaligenes faecalis</td>
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<td>Bordetella bronchiseptica</td>
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<tr>
<td>Serratia marcescens</td>
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<td>Corynebacterium diphtheriae</td>
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<tr>
<td>Micrococcus luteus</td>
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<td>Bacillus subtilis</td>
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</table>
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.

REFERENCES

3. Prieto P, Pineda M, Aguilar MM. Spectrophotometric quantification of antioxidant capacity through the formation of a phoshomolybdenum complex specific application to the determination of vitamin E. Analytical Biochemistry 1999; 269: 337-341.