PRELIMINARY PHYTOCHEMICAL SCREENING, IN-VITRO ANTIOXIDANT AND ANTIBACTERIAL ACTIVITY OF BEGONIA PECTA

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ABSTRACT

Although many plants are used as medicine traditionally they are going to be extinct day by day due to lack of knowledge and documentation. Among various plants Begonia pecta is one of them. The main aim of this study was to carry out phytochemical screening and evaluate in-vitro antioxidant and antibacterial activity of whole plant extract of B. pecta. The whole plant of B. pecta was extracted with methanol using double maceration process. The antioxidant activity of extract of different concentrations was determined using DPPH and NO scavenging method taking ascorbic acid as standard. The antibacterial activity was determined against 3 bacteria (Staphylococcus aureus, Klebsiella pneumonia and Escherichia coli) using well diffusion method. Phytochemical screening of the extract revealed the presence of flavonoid, phenol, tannins, and carbohydrates. The extract exhibited a dose dependent DPPH and NO radical scavenging activity comparable to that of standard. As compared to NO radical the extract showed better DPPH radical scavenging activity. At 100 µg/ml concentration, extract showed 78.77 ± 0.51% and 74.95 ± 0.44% inhibition of DPPH radical and NO radical respectively. Extract also showed moderate antibacterial activity at 3mg/ml concentration with abetter zone of inhibition (14 mm) for E. coli. This study showed that the plant possesses good antioxidant and antibacterial property which could be contributed to the presence of tannins, flavonoids and phenols. This implies future implications of the plant in...
drug development for treatment of various ailments including oxidative disorders and infections.

**KEYWORDS**: DPPH scavenging assay, NO scavenging assay, Antioxidant activity, Antibacterial activity, *Begonia pecta*.

**INTRODUCTION**

Free radicals or reactive oxygen species are part of healthy metabolism in all aerobic organisms including human but in limited concentration.\(^1\) Healthy cell balances the formation and elimination of free radicals creating a homeostatic state.\(^{1,2}\) Increase in free radical concentration above a critical level creates oxidative stress to cells and tissue leading to numerous pathological conditions like diabetes, cardiovascular and neurodegenerative diseases, amyloidosis, atherosclerosis, rheumatoid arthritis, ulcerative colitis and other degenerative diseases.\(^{1,2}\) Antioxidants are those compounds that inhibit the oxidation of other molecules, remove free radicals and thereby protect tissues from damage by stabilizing harmful free radicals.\(^{3,4}\) Most of the antioxidants used are manufactured synthetically at present but possess side effects when taken in vivo.\(^{5,6}\) Alternative to these synthetic antioxidants are the phytochemicals, derived from plant sources, which possess antioxidant property.\(^2\) Numerous plant species possess substantial amount of antioxidants like Vitamin C and E, carotenoids, flavonoids, tannins and can be utilized to scavenge the excess free radicals from body.\(^7\)

Infectious diseases are major causes of morbidity and mortality in the developing world and accounts for about 50% of all deaths.\(^8\) Antibiotics are powerful medicines that fight against bacterial infections and have greatly benefited the health related quality of human life.\(^9\) Till date number of antibiotics has been synthesized worldwide but resistance to these agents is emerging and has become a global concern posing threat to patients suffering from infectious diseases.\(^{10,11}\) This has created an urgent need for development of new antimicrobial agents and researchers are now turned towards medicinal plant sources for this purpose since natural products are considered safer than synthetic ones.\(^{12,13}\) According to WHO, medicinal plants would be the best source to obtain a variety of drugs including antibacterial agents and hence such plants should be investigated for their activity.\(^{13}\) The ultimate goal of this is to offer appropriate and effective antimicrobial drugs to the patient.\(^{10}\)
**Begonia pecta**, belonging to family, is a tuberous herb growing up to 10-18 cm tall with broadly ovate leaves and light pink flowers and flowers from July to September.\(^{[14,15]}\) It is locally called as “Magarkaanche” in Nepal and distributed throughout Nepal, Pakistan, India and Bhutan between 600-2800 m.\(^{[14,15]}\) Traditionally this herb has been utilized to treat various ailments in various forms like whole plant (pained nipple, appetizer), whole plant juice (headache, peptic ulcer, leech guard), paste (bleeding, ringworm, scabies, respiratory tract infections), root juice (conjunctivitis), tuber (renal calculi), leaf juice (fever, toe wounds), root infusion (constipation), leaf (dysentery), as well as decoction (colic, dyspepsia).\(^{[15-21]}\) Phytochemicals like vitexin, isovitexin, orientin and isoorientin (flavonoids) have been isolated from this plant.\(^{[14]}\) Previous studies have shown anti-inflammatory, hypoglycemic and cytotoxic properties of *B. pecta*.\(^{[22]}\)

Natural products including plants have been the basis of treatment of human diseases for thousands of years. Almost 60% of all new chemical entities introduced worldwide as a drug in last two decades may be traced to or inspired by natural products.\(^{[23]}\) *B. pecta* is a plant with huge scope in therapeutic purposes as suggested by its wide range of traditional uses.\(^{[15-21]}\) However, the therapeutic effects of *B. pecta* have been studied limitedly. This study has been designed to evaluate potential antioxidant as well as antibacterial activity of methanol extracts of whole plant of *B. pecta* using *in-vitro* methods.

**MATERIAL AND METHODOLOGY**

**Plant Materials**

Whole plant of *B. pecta* was collected from Bhakunde-4, Baglung, Nepal at the elevation range from 1,500-2,100 meters. The unnecessary parts of plants like dead parts and other materials were removed. The rest parts of plants were washed with water and shade dried for 15 days. The air dried plants were cut into small pieces for its chemical investigation. A specimen of the plant was used to prepare herbarium and then authenticated by botanist Dr. Manoj K.L. Das, Associate Professor and Head of Botany Department, Birendra Multiple Campus, Bharatpur, Chitwan, Nepal.

**Chemicals**

Ascorbic acid (LobaChemie), DiMethyl Sulphoxide (Alpha Chemika), DPPH (Wako Pure Chemical), Methanol (Fischer Scientific), Muller Hilton Agar (Himedia Laboratories), NEDD (LobaChemie), Sodium Nitroprusside (Fischer Scientific) and all the other required
chemicals were obtained from various chemical suppliers as well from the laboratory of Shree Medical and Technical College (SMTC), Bharatpur, Chitwan.

**Extraction**

Shade dried samples were first grinned into fine powders and then extracted using double maceration process where methanol was used as solvent. After solvent evaporation extractive yield for each extract was determined.

**Phytochemical Screening**

Phytochemical screening of methanol extract of whole plants of *B. pecta* was carried out to reveal the presence of various secondary metabolites according to the method described previously with some modification.[24]

**Alkaloids**

Plant extract (5 mg) was taken and dissolved in 10% v/v hydrochloric acid and filtered. The filtrate was subjected to tests for presence of alkaloids.

**Mayer's test**

Filtrate was treated with a few drops of the Mayer’s reagent (potassium mercuric iodide). The formation of white or pale yellow precipitate indicates the presence of alkaloids.

**Hager's test**

A few drops of Hager’s reagent (saturated picric acid solution) were added to filtrate. The formation of yellow ppt. confirms the presence of alkaloids.

**Wagner's test**

Filtrate was treated with a few drops of Wagner’s reagent (iodine in potassium iodide). A yellow or brown ppt. indicates the presence of alkaloids.

**Carbohydrates**

Plant extract (5 mg) was dissolved in 20 ml of distilled water and filtered. The filtrate was subjected to tests for presence of carbohydrates.
Molisch’s test
Two drops of alcoholic α-napthol solution were treated with filtrate in a test tube and 2 ml of conc. Sulphuric acid was added carefully along the sides of the test tube. The formation of violet ring at the junction indicates the presence of carbohydrates.

Benedict’s test
Filtrate was treated with Benedict’s reagent and heated on water bath. The formation of orange red precipitate indicates the presence of reducing sugars.

Fehling’s test
Equal parts of Fehling’s solution A and B i.e. 1-1 ml was added to the filtrate and boiled for few minutes. The formation of red or brick red ppt. indicates the presence of reducing sugars.

Glycosides
Plant extract (5 mg) was hydrolyzed with 10 ml of dil. Hydrochloric acid (10 % v/v) and then subjected to tests for presence of glycosides.

Modified borntrager’s test
The extract was treated with few drops of ferric chloride solution and immersed in boiling water for about 5 minutes. The mixture was cooled and shaken with an equal volume of benzene. The benzene layer was separated and treated with ammonia solution. The formation of rose-pink color in the ammonia layer indicates the presence of anthranol glycosides.

Legal’s test
The extract was treated with few drops of sodium nitroprusside in pyridine and methanolic alkali. The formation of pink to blood red color indicates the presence of cardiac glycosides.

Saponins
Foam test
Plant extract (5 mg) was dissolved in methanol and small amount of extract was shaken with little quantity of water. If foam produced persists for 10 minutes it indicates the presence of saponins.
Resins

**Acetone-water test**

Plant extract (5 mg) was dissolved in methanol and treated with acetone. Small amount of water was added and shaken. Appearance of turbidity indicates the presence of resins.

Phenols

**Ferric chloride test**

Plant extract (5 mg) was dissolved in methanol and treated with few drops of ferric chloride solution. The formation of bluish black color indicates the presence of phenols.

Flavonoids

**Alkaline reagent test**

Plant extract (5 mg) was dissolved in methanol and treated with few drops of sodium hydroxide solution. The formation of intense yellow color, which becomes colorless on addition of dilute acid, indicates the presence of flavonoids.

Proteins and amino acids

**Xanthoproteic test**

Plant extract (5 mg) was dissolved in methanol and treated with few drops of concentrated nitric acid solution. The formation of yellow color indicates the presence of proteins.

Tannins

Plant extract (5 mg) was dissolved in methanol. Then extract were treated with 5 ml of 1% gelatin solution containing 10% NaCl. The formation of white precipitates indicates the presence of tannin.

Terpenoids

**Salwoskii test**

Plant extract (5 mg) was mixed with chloroform. 3 ml of concentrated sulphuric acid was added from sides of test tube to form a layer. Then reddish brown precipitates formed at interference indicate presence of terpenoids.

Fixed oil and fats

**Stain**

Small quantity of extract were taken and pressed between filters paper. Stain in filter paper indicates presence of fixed oils.
Antioxidant activity
The antioxidant activity of the B. pecta extract was determined using DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging assay and NO (Nitric Oxide) radical scavenging assay methods. Ascorbic acid was used as standard antioxidant in these methods.

DPPH radical scavenging assay
The DPPH radical free assay was measured according to a previously described method with some medication. In this, 2 ml of whole plant extract solution of different concentrations (100 µg/ml, 10 µg/ml and 1 µg/ml) were separately mixed with 2 ml of DPPH solution and left for 30 minutes for reaction. The absorbance of each was then measured by using UV-visible spectrophotometer at 517 nm against a blank solution. Control test solution was one where no sample was used. Similar process was repeated with ascorbic acid solution (1 µg/ml, 10 µg/ml and 100 µg/ml).

Radical scavenging activity of whole plant was calculated by using following formula;

\[
\text{Radical scavenging activity (\%) = \left( \frac{A_0 - A_S}{A_0} \right) \times 100}\%
\]

Where, \(A_0\) = Absorbance of control, \(A_S\) = Absorbance of sample.

NO radical scavenging assay
Preparation of griess reagent
0.25 g of NEDD (N-(1-Naphthyl)ethylenediamine) was mixed in sufficient deionized water to produce 250ml of 0.10% of NEDD solution. 1% sulfanilamide solution was prepared by dissolving 2.50 g of sulfanilamide in 5% phosphoric acid to produce 250ml. Finally 500ml of griess reagent was prepared by mixing NEDD solution and sulfanilamide solution which was then stored in refrigerator and used before 8 h.

Determination NO radical scavenging assay
NO radical scavenging activity was measured by previously described method with slight modification. One ml of test sample of different concentration were taken in separate test tubes and mixed with 1ml of sodium nitroprusside solution and incubated for 2.5 h at 29ºC. After 2.5 h, 2 ml of griess reagent was added to each and absorbance was noted in UV-visible spectrophotometer at 548 nm. Control test solution was one where no sample was used. Similar process was done with ascorbic acid solution (standard) of same concentrations that of extract (1 µg/ml, 10 µg/ml, 100 µg/ml).
The radical scavenging effects of test sample were calculated by:

\[
\text{Radical scavenging activity (\%) } = \left( \frac{A_0 - A_s}{A_0} \right) \times 100\%
\]  

(2)

Where, \( A_0 \) = Absorbance of control, \( A_s \) = Absorbance of sample

**Antibacterial activity**

**Bacterial strain and its growth condition**

The pure cultures of bacteria with their antibiotic resistance profiles were obtained from the Chitwan Medical College Bharatpur-10, Chitwan, Nepal. One gram-positive bacterium: *Staphylococcus aureus*, two gram-negative bacteria: *Escherichia coli* and *Klebsiella pneumonia* were used as test pathogens. The strains were maintained in Muller Hinton Agar (MHA) at 24°C and sub-cultured for routine use. Stock cultures were maintained at 24°C on slopes of MHA.[27]

**Well diffusion method**

The antibacterial activity of the extract was determined using well diffusion method as described previously[27] with slight modification.

The method involved used of MHA plates. 1 loop (5 mm) inoculums of cultured microorganism was swabbed uniformly and was allowed to dry for 30 min. Wells (7mm diameter) were made in each of these plates using sterile cork borer. About 100 μl of *B. pecta* extract solution was added by micropipette into the wells and allowed to diffuse at room temperature for 2 h. Control experiments comprising inoculums without plant extract were also set up. Ofloxacin, Ciprofloxacin and Cefpodoxime solutions were also added into separate wells. 10% DMSO was used as negative control. Media plates were incubated at 37 °C for 18 to 24 hin an incubator. After 24 h, zone of inhibition (in mm) were measured for each treatment by using plastic scale and it was recorded.

**RESULTS**

**Phytochemical screening**

The result on phytochemical screening of extract of *B. pecta* is shown in Table 1.
Table 1: Phytochemical screening of *B. pecta* extract.

<table>
<thead>
<tr>
<th>S.N.</th>
<th>Phytochemical test</th>
<th>Test performed</th>
<th>Inference</th>
<th><em>B. pecta</em> Extract</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Alkaloids</td>
<td>Mayer’s test</td>
<td>Yellow cream ppt.</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Wagner’s test</td>
<td>Brown/ reddish brown ppt.</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Hager’s test</td>
<td>Yellow color ppt.</td>
<td>-</td>
</tr>
<tr>
<td>2</td>
<td>Glycosides</td>
<td>Modified Borntrager’s test</td>
<td>Yellow color ppt.</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Legal test</td>
<td>Pink to blood red color</td>
<td>-</td>
</tr>
<tr>
<td>3</td>
<td>Saponins</td>
<td>Foam test</td>
<td>Foam that lasts for more than 10 minute</td>
<td>-</td>
</tr>
<tr>
<td>4</td>
<td>Flavonoids</td>
<td>Alkaline reagent test</td>
<td>Intense yellow color</td>
<td>+</td>
</tr>
<tr>
<td>5</td>
<td>Carbohydrates</td>
<td>Molisch’s test</td>
<td>Violet ring at the junction.</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Benedict’s test</td>
<td>Orange red ppt.</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Fehling’s test</td>
<td>Red ppt.</td>
<td>+</td>
</tr>
<tr>
<td>6</td>
<td>Phenols</td>
<td>Ferric chloride test</td>
<td>Bluish black ppt.</td>
<td>+</td>
</tr>
<tr>
<td>7</td>
<td>Fixed oil</td>
<td>Filter paper press test</td>
<td>Oily stain on filter paper</td>
<td>+</td>
</tr>
<tr>
<td>8</td>
<td>Resin</td>
<td>Acetone water test</td>
<td>Turbidity</td>
<td>+</td>
</tr>
<tr>
<td>9</td>
<td>Tannins</td>
<td>Gelatin Test</td>
<td>White ppt.</td>
<td>+</td>
</tr>
<tr>
<td>10</td>
<td>Protein</td>
<td>Xanthoproteic Test</td>
<td>Yellow color</td>
<td>+</td>
</tr>
<tr>
<td>11</td>
<td>Terpenoids</td>
<td>Salkwoskii test</td>
<td>Reddish brown ppt.</td>
<td>+</td>
</tr>
</tbody>
</table>

*Note: ‘+’ means positive result, ‘-’ means negative result.*

Antioxidant activity

**DPPH radical scavenging assay**

The results of DPPH radical scavenging assay performed on *B. pecta* extract are shown in Figure 1 and 2. The percentage DPPH radical scavenging activities for different concentration of *B. pecta* extract i.e.1 µg/ml, 10 µg/ml and 100 µg/ml was found to be 11.22±0.84%, 35.88 ± 1.84% and 78.77 ± 0.51% respectively and that of standard was 17.77 ± 0.51%, 44.66± 1.00% and 81.55 ± 1.17% respectively. The IC$_{50}$ value of whole plant extract was found to be 8.41µg/ml and that of standard was 9.04µg/ml.

![DPPH free radicals scavenging activity of *B. pecta* plant extract and standard.](image)

*Fig 1: DPPH free radicals scavenging activity of *B. pecta* plant extract and standard.* Each value are expressed as mean ± SD (n=3).
Fig 2: IC50 of *B. pecta* extract and standard using DPPH radical scavenging assay.

**NO radical scavenging assay**

From NO radical scavenging assay, percentage NO radical scavenging activities for different concentration of whole plant extract i.e. 1 µg/ml, 10 µg/ml and 100 µg/ml was found to be 10.48 ± 0.72%, 35.71 ± 0.57%, 74.95 ± 0.44% and that of standard was 15.71 ± 0.86%, 44.38 ± 0.72% and 84.10 ± 0.88% respectively. The IC50 value of whole plant extract was found to be 8.80 µg/ml and that of standard was 8.44 µg/ml. The results are shown in Figure 3 and 4.

![Graph showing IC50 values](image1)

Fig 3: NO free radicals scavenging activity of *B. pecta* plant extract and standard.

Each value are expressed as mean ± SD (n=3).
Antibacterial activity

The whole plant extract of *B. pecta* at concentration of 3 mg/ml was used to evaluate the antibacterial property against *E. coli*, *S. aureus* and *K. pneumoniae*. The study revealed that among used bacteria, the plant extract showed moderate zone of inhibition towards *E. coli* i.e. 14 mm. The results are shown in Table 2.

**Table 2: Zone of inhibition of plant extract as compared to standard.**

<table>
<thead>
<tr>
<th>Sample concentration (3mg/ml)</th>
<th>Zone of inhibition diameter (mm)</th>
<th>Gram positive bacteria</th>
<th>Gram negative bacteria</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td><em>S. aureus</em></td>
<td><em>E. coli</em></td>
</tr>
<tr>
<td><em>B. pecta</em> extract</td>
<td></td>
<td>13</td>
<td>14</td>
</tr>
<tr>
<td>Ofloxacin</td>
<td></td>
<td>37</td>
<td>31</td>
</tr>
<tr>
<td>Ciprofloxacin</td>
<td></td>
<td>34</td>
<td>29</td>
</tr>
<tr>
<td>Cefpodoxime</td>
<td></td>
<td>28</td>
<td>30</td>
</tr>
<tr>
<td>10% DMSO</td>
<td></td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

Note: “-” indicates the no zone of inhibition. Diameter of well was 7mm

**DISCUSSION**

The therapeutic effects of medicinal plants can justifiably be attributed to phytochemicals in them especially the flavonoids, alkaloids, sterols, terpenoids, phenolic acids, stilbenes, lignans, tannins and saponins. These phytochemicals possess biological properties like antioxidant, antimicrobial, immune modulation, antineoplastic, anti-diabetic and so on. The phytochemical screening tests performed in the methanol extract of whole plant of *B. pecta* showed the presence of flavonoids, carbohydrates, phenols, resins, tannins, fixed oil,
terpenoids and protein (Table 1). These phytochemicals present in the plant extract could be responsible behind the use of B. pecta as a traditional medicine.

The anti-oxidant activity of B. pecta was evaluated using two methods; DPPH radical scavenging assay and NO radical scavenging assay methods. DPPH test is widely used method which is based on reduction of methanolic DPPH (deep violet color) solution in presence of antioxidant resulting in the formation of non-radical DPPH-H (pale yellow color). The discoloration of DPPH shows scavenging potential of extract. The reduction of DPPH is determined by the decrease in its absorbance at 517 nm. In this study DPPH radical scavenging based antioxidant potential of plant extract was also reported with the help of IC_{50}, defined as the amount of antioxidant required to inhibit 50% of DPPH free radical under experimental condition. A high DPPH radical scavenging activity is associated with a lower IC_{50}. The extract studied showed a significant DPPH radical scavenging activity comparable to that of standard at each concentration (Figure 1). This activity was concentration dependent as well (R^2 = 0.92), i.e. the inhibition of free radicals increased with increase in concentration, showing maximum DPPH radical scavenging activity (78.77 ± 0.51 %) at 100 µg/ml concentration. This activity was comparatively higher than that observed in methanol extract of whole plants other species of Begonia (B. malabarica and B. floccifera) in previous studies. Similar results were obtained from NO radical scavenging assay as well. NO radical scavenging assay methods are based on principle that sodium nitroprusside in aqueous solution at physiological pH spontaneously generates nitric oxide which interacts with oxygen to produce nitrite ions that can be estimated using Griess reagent and scavengers of nitric oxide compete with oxygen, leading to reduced production of nitrite ions. The extract showed a significant NO radical scavenging activity as compared to the standard at each concentration. This activity was also found to be concentration dependent (R^2 =0.90), showing maximum NO radical scavenging activity (74.95 ± 0.44%) at 100 µg/ml concentration.

The observed free radical scavenging activity or the anti-oxidant activity could be due to presence of flavonoids and phenols in the extract. Almost all flavonoids have the capacity to act as antioxidants. Various mechanisms have been suggested for this activity shown by flavonoids, most common ones being radical scavenging, decrease in number of leukocyte immobilization and interaction with enzyme systems. Specifically, the observed DPPH radical scavenging activity could be due to presence of flavonoids like orientin and isorientin.
which have been previously isolated from *B. pecta* and had shown significant DPPH radical scavenging activity as well.[36] On the other hand phenolic compounds possess anti-oxidant activity due to presence of resonance stabilized phenoxy groups that neutralized the free radicals.[28] Comparatively the extract showed a higher DPPH radical scavenging activity as compared to NO radical scavenging activity. Possible reason for this could be that the extract may contain less number of flavonoid free from hydroxyl group (OH) which is required to scavenge NO free radical.[37]

This study also evaluated the antimicrobial activity of the extract using well diffusion method. A moderate activity was shown by the extract against the test pathogens (*E. coli, S. aureus* and *K. pneumonia*). The extract showed similar inhibitory effects against gram positive and gram negative bacteria (Table 2). This indicated the extract possess a broad spectrum of action. The presence of tannins as observed in phytochemical screening might be the agent behind the observed moderate antimicrobial activity.[38] Although flavonoid and phenol containing plant shows good antibacterial activity but tannins are major chemical required to inhibit the growth of bacteria.[39] However, this antimicrobial activity was almost negligible as compared to the standard (table). This may be probably due to presence of less concentration of tannins in extract.

DPPH free radical scavenging activity shown by the extract in this study suggests its potential use in treatment of various disorders pathologically linked to free radical like diabetes, cardiovascular and neurodegenerative diseases, amyloidosis, atherosclerosis, rheumatoid arthritis, ulcerative colitis and other degenerative diseases.[1,2] Nitric oxide has also been linked with various diseases. Although nitric oxide is an important chemical mediator generated by endothelial cells, macrophages, neurons and involved in the regulation of various physiological processes; excess concentration of nitric oxide is implicated in the cytotoxic effects observed in various disorders like AIDS, cancer, alzheimer’s and arthritis.[34] Observation of NO free radical scavenging activity in this study suggests potential use of the *B. pecta* extract in treatment of these conditions.

**CONCLUSION**

This study showed the potential antioxidant activity of methanol extract of whole plant of *B. pecta* using DPPH and NO radical scavenging assay method. Also, the extract showed a moderate antimicrobial activity against microorganism selected for the study. So in future, this plant could be used for commercial production of antimicrobial drug. Further
investigations to determine anti-oxidant activity through in-vivo methods and antimicrobial activity against other test pathogens could be considered.

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