PHYTOCHEMICAL AND PHARMACOLOGICAL EVALUATION OF SELECTED PLANTS

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ABSTRACT

Araucaria cookii, Bauhinia blakeana and Brassaia actinophylla are ornamental plants. The presence of various phytochemicals and pharmacologically important compounds in these plants can be exploited for their medicinal use. But there are no reports on the phytochemical and pharmacological evaluation of these plants and this study aims at investigating these. The plant extracts were prepared in different solvents like methanol, ethanol, ethyl acetate, acetone, hexane, water and chloroform. Qualitative analysis of phytochemicals were assessed. The anti-microbial, anti-oxidant and enzyme inhibitory activity was determined for all three plant extracts. Anti-bacterial activity against three gram negative bacteria, E. coli, Pseudomonas and Klebsiella was done and Araucaria cooki showed highest antibacterial activity among the three plants. Maximum antioxidant activity was seen in methanol extract of Brassaia actinophylla with 81% inhibition. The order of the antioxidant activity of the three plants are in the order B. actinophylla > A cookie > B. blakeana. The results of phytochemical analysis suggest that phytosteroids are present in all the three plants. Maximum inhibition against the tested enzymes was exhibited by hexane and chloroform extracts of A. cookii. Hemolytic activity was done and the hexane extract showed maximum haemolysis where as aqueous extracts showed minimum activity. From the results it is clear that the three plant extracts has pharmacological applications. This is the first report of antimicrobial, antioxidant and enzyme inhibitory activities of these three plant extracts. Further studies are needed to exploit the actual mechanism and active compounds of these plants.

Keywords: Araucaria Cooki, Bauhinia Blakeana, Brassaia Actinophylla, Antioxidant, Phytochemical

1. INTRODUCTION

Medicinal plants are the primary source of medicine for the treatment of human diseases in many rural areas of the developing countries (Chitme et al., 2004). About 80% of the world population relies on the traditional medicine for their primary health care (Owolabi and Omogbai, 2007). The medicinal value of the plant is due to the presence of various bioactive chemical constituents such as alkaloids, tannins, flavanoids and phenolic compounds (Hill, 1952). Therefore, the plants with the medicinal values have to be investigated to understand their safety and efficacy (Nascimento et al., 2000).

The main cause for diseases and ageing is due to the oxidative damage to the cell. Oxidative stress occurs due to the imbalance in the oxidants and antioxidants, resulting in the oxidative damage to the molecules. Free radicals attack lipids and DNA by inducing oxidations that cause membrane damage leading to cancer (Pietta, 2000; Cerutti, 1994). A potent free radical scavenger may serve as intervention for free radical mediated diseases (Ames et al., 1995). Recent reports showed
that plant products including polyphenolic compounds (e.g., flavanoids and tannins) and various plant extracts exhibit antioxidant activities (Iqbal et al., 2009; Kiselova et al., 2006).

Multiple drug resistance has been developed due to the use of commercial antibiotics in the treatment of infectious diseases. Therefore scientists have started looking for new antibiotics. There is a need to develop antimicrobials for the treatment of diseases from medicinal plants (Agrawal et al., 1996). The general antimicrobial activities of medicinal plants and plant products, such as essential oils, have been reviewed previously (Cowan, 1999; Kalemba and Kunika, 2003). The plant extracts and their phytochemicals having antimicrobial (antibacterial) properties plays an important role in the therapeutic treatments. The most important constituents of plants are alkaloids, tannins, flavonoids and phenolic compounds (Hill, 1952). The medicinal values of the selected plants depend upon the physiological action and their effects on human body (Edeoga et al., 2005).

Plants have long been used for the treatment of diabetes and the research on medicinal plants for the management of diabetes has attracted the interest of scientists (Ali et al., 2006; McCue et al., 2005). Alpha amylase and alpha glucosidase inhibitors are reported to be present in plant extracts (Ingrid and Matthias, 2006). Inhibition of these two enzymes helps in the management of type II diabetes mellitus.

_Brassaia actinophylla_ belongs to Araliaceae family, widely distributed in tropical rainforests in Australia and New Guinea. It is grown as decorative trees in the gardens. _Bauhinia blakeana_ is an evergreen tree with large thick leaves and striking purplish red flowers, sometimes called as ‘Hong Kong Orchid’. It is sterile and hybrid between _Bauhinia variegate_ and _Bauhinia purpurea_. Propagation is by cutting and air layering. There is no data found before about its biological activities. _Araucaria cookii_ is a tall tree, used as a decorative plant, commonly called as Christmas tree. In our study we have evaluated the various photochemical and pharmacological applications of _Araucaria cookii_, _Bauhinia blakeana_ and _Brassaia actinophylla_.

### 2. MATERIALS AND METHODS

#### 2.1. Plant Material and Extraction

Leaves of _Araucaria cookii_, _Bauhinia blakeana_ and _Brassaia actinophylla_ were collected from horticulture nursery of VIT University, Vellore. The leaves were shade dried, crushed, powdered and extracted (100 g mL\(^{-1}\)). Methanol, ethanol, hexane, chloroform, distilled water, acetone and ethyl acetate were the various solvents used for extraction. It was then kept undisturbed in orbital shaker for 5 days at room temperature. The supernatant was collected and utilised for the assessment of the various activities.

#### 2.2. Qualitative Analysis of Phytochemicals

The Following screening tests were carried for the various extracts of the three plants to detect the presence of phytochemicals.

#### 2.3. Detection of Saponins (Kokate, 1999)

About 0.5 mL of extract was dissolved in 5 mL of distilled water in a test tube. Persistent frothing on warming indicates the presence of saponins. The ability of saponins to produce frothing in aqueous solution was used as a screening test for the sample.

#### 2.4. Detection of Tannins (Trease and Evans, 1996)

About 0.5 mL of extract was dissolved in 5 mL of distilled water. To it, a few of neutral ferric chloride solution was added. Formation of blue precipitate indicates the presence of tannins.

#### 2.5. Detection of Phenols (Mace, 1963)

About 0.5 mL of extract was dissolved in 5 mL of distilled water. To it, a few drops of neutral 5% ferric chloride was added. A dark green colour indicates the presence of phenols.

#### 2.6. Detection of Flavonoids (Evans, 1997)

About 0.5 mL of extract was dissolved in 5 mL of distilled water. To it, a few drops of neutral 10% ammonium hydroxide solution. A yellow fluorescence indicated the presence of flavonoids.

#### 2.7. Detection of Phytosteroids (Finer, 1988)

About 50 \(\mu\)L of extract was treated with 2 mL of acetic anhydride. To it, 1-2 drops of conc. sulphuric acid was added along sides of the test tube. An array of colour showed presence of phytosteroids.

#### 2.8. Evaluation of Antioxidant Activity Using DPPH Model

The radical scavenging activity was determined using DPPH which was described by Menser et al. (2001). 2 mL of 0.3 mM alcoholic solution of DPPH was added to 2 mL of the samples extracted with
solvents like methanol, ethanol, water, ethyl acetate, hexane, chloroform, acetone. The samples were kept in the dark for 30 min after which the optical density was measured at 518 nm. The radical scavenging activity was determined by the following formula:

\[ AA\% = \frac{(A_{\text{control}} - A_{\text{sample}})}{A_{\text{control}}} \times 100 \]

where, \( A_{\text{control}} \) is the absorbance of free radical alone and \( A_{\text{sample}} \) is the absorbance of the free radical in the presence of extract. The optical density of samples was measured against methanol which was taken as blank.

2.9. Evaluation of \( \alpha \)-Amylase and \( \alpha \)-Glucosidase Inhibitory Activity

2.9.1. Inhibition Assay for Porcine \( \alpha \)-Amylase Activity

About 500 \( \mu \)L of extract was taken and mixed with 500 \( \mu \)L of 0.02 M sodium phosphate buffer (pH 6.9 with 0.006 M sodium chloride) containing \( \alpha \)-amylase solution (0.5 mg mL\(^{-1}\)) and incubated for 10 min at 25\(^\circ\)C. After incubation, 500 \( \mu \)L of 1% starch solution in 0.02 M sodium phosphate buffer was added to each tube at 5 s intervals. The reaction mixture was incubated at 25\(^\circ\)C for 10 min and finally the reaction was stopped with 1.0 mL of dinitrosalicylic acid color reagent. The test tubes were then incubated in boiling water bath for 5 min and cooled to room temperature. The reaction mixture was then diluted by adding 10 mL distilled water and absorbance was measured at 540 nm. Percentage of inhibition was calculated by this formula:

\[ \% \text{inhibition} = \frac{[A_{540\text{control}} - A_{540\text{extract}}]}{A_{540\text{control}}} \times 100 \]

2.10. Inhibition Assay for Yeast \( \alpha \)-Glucosidase Activity

About 50 \( \mu \)L of extract was mixed with 100 \( \mu \)L of 0.1 M phosphate buffer containing \( \alpha \)-glucosidase solution and incubated in 96 wells plate at 25\(^\circ\)C for 10 min. After incubation, 50 \( \mu \)L of 5 mM p-nitro phenyl \( \alpha \)-D-glucopyranoside solution in 0.1 M phosphate buffer was added to each well at 5 s intervals. The reaction mixture was incubated at 25\(^\circ\)C for 5 min. After incubation, absorbance was recorded at 540 nm by micro-array reader and compared with control which had 50 \( \mu \)L of buffer solution in place of extract. The inhibitory activity was calculated by this formula:

\[ \% \text{inhibition} = \frac{[A_{\text{control}540} - A_{\text{extract}540}]}{A_{\text{control}540}} \times 100 \]

2.11. Determination of Antibacterial Activity

*E.coli*, *Pseudomonas* and *Klebsiella* are the test bacterial cultures used. The bacterial cultures were swabbed on to Muller Hinton agar media. A total of 6 mm diameter wells were punched into agar and filled with plant extracts (distilled water, hexane, methanol, ethanol, ethyl acetate, chloroform and acetone). The bacterial plates were then incubated at 37\(^\circ\)C for 48 h. The antibacterial activity was evaluated by measuring the zone of inhibition.

2.12. Evaluation of Hemolytic Activity

Human blood was obtained from of a healthy volunteer. Collected blood was washed 3 times in nine volumes of sterile 0.9% NaCl saline solution. After each washing, cells were centrifuged at 1000 rpm for 5 min and the supernatant was discarded. The final pellet was diluted 1/9 (V/V) in sterile 0.9% NaCl saline solution then 1/24(V/V) in sterile Dulbecco’s Phosphate Buffer (D-PBS). The hemolytic activity of crude extract was tested by Malogoli’s method under \textit{in vitro} condition in 96 well plate. 100 \( \mu \)L of 0.85% NaCl solution containing 10 mM CaCl\(_2\) was added to each well. The first well served as negative control and contained PBS buffer and the second well onwards contained 100 \( \mu \)L of sample extracts. The last well served as positive control containing 100 \( \mu \)L of 0.1% Triton X-100 in 0.85% saline. Then 100 \( \mu \)L of 2% suspension of human erythrocytes in 0.85% saline containing 10 mM CaCl\(_2\) was added to each well and incubated for 30 min at room temperature. It was then centrifuged and supernatant was used to measure the absorbance of the liberatedhaemoglobin at 540 nm. The average value was calculated from triplicate assay.

3. RESULTS

3.1. Phytochemical Analysis

The presence of different phytochemicals in the plant extracts are summarised in the Table 1-3. Saponin was detected in *Brassaia actinophyla* (methanol and aqueous extracts), *Bauhinia blakaena* (Methanol extract) and *Araucaria cookii* (methanol and ethyl acetate). Tanins were found in many extracts including ethyl acetate and aqueous extracts of *Brassaia actinophyla*, water, methanol, ethyl acetate and chloroform extracts of *Bauhinia blakaena* and methanol, acetone and ethanol extracts of *Araucaria cookii*. 
Table 1. Qualitative analysis of *B. actinophylla* for the presence of phytochemicals

<table>
<thead>
<tr>
<th>Extracts</th>
<th>Saponins</th>
<th>Tannins</th>
<th>Phenols</th>
<th>Flavonoids</th>
<th>Phytosteroids</th>
</tr>
</thead>
<tbody>
<tr>
<td>Methanol</td>
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<td>-</td>
<td>+</td>
<td>-</td>
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<tr>
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<td>-</td>
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<td>+</td>
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<tr>
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<td>+</td>
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<tr>
<td>Acetone</td>
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<td>-</td>
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</tbody>
</table>

Table 2. Qualitative analysis of *B. blakeana* for the presence of phytochemicals

<table>
<thead>
<tr>
<th>Extracts</th>
<th>Saponins</th>
<th>Tannins</th>
<th>Phenols</th>
<th>Flavonoids</th>
<th>Phytosteroids</th>
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<tbody>
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<tr>
<td>Ethanol</td>
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<tr>
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<td>+</td>
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<td>+</td>
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<tr>
<td>Acetone</td>
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</tbody>
</table>

Table 3. Qualitative analysis of *A. cookii* for the presence of phytochemicals

<table>
<thead>
<tr>
<th>Extracts</th>
<th>Saponins</th>
<th>Tannins</th>
<th>Phenols</th>
<th>Flavonoids</th>
<th>Phytosteroids</th>
</tr>
</thead>
<tbody>
<tr>
<td>Methanol</td>
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<td>Ethanol</td>
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<td>Ethyl acetate</td>
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<tr>
<td>Acetone</td>
<td>-</td>
<td>+</td>
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</tr>
</tbody>
</table>

Fig. 1. DPPH Scavenging assay of the three plant extracts. Methanolic extract of Brassica actinophyla with maximum activity followed by *Araucaria cookii* and *Bauhinia blakeana*
Fig. 2. Alpha amylase and alpha glucosidase inhibition by *Brassaia actinophyla*

Flavonoids were not found in *Brassaia actinophyla* and was detected in water, methanol and acetone extracts of *Bauhinia blakaena* and in methanol and acetone extracts of *Araucaria cookii*. Phytosteroids was detected in all the extracts of the three plants taken under study.

3.2. DPPH Radical Scavenging Activity

The antioxidant activity of the three plant extracts is given in the Fig. 1. The antioxidant activity was found to be maximum in methanol extract of *B. actinophylla* having the highest value of 81% than the other two.
Fig. 4. Alpha amylase and alpha glucosidase inhibition by Bauhinia blakeana

Similarly, hexane extract of all the three plants possessed the least antioxidant activity. Some However, the order of antioxidant activity of the three plants are in the order \( B.\text{actinophylla} > A.\text{cookii} > B.\text{blakeana} \).

3.3. \( \alpha \)-Amylase and \( \alpha \)-Glucosidase Inhibition

From the results obtained, it was observed that the various extracts of the three plants exhibited greater \( \alpha \)-amylase inhibitory activity compared to \( \alpha \)-glucosidase activity. The results of \( \alpha \)-amylase and \( \alpha \)-glucosidase inhibition is given in the Fig. 2-4. Hexane and chloroform extracts exhibited highest \( \alpha \)-amylase inhibitory activity while methanol and ethanol extract exhibited the least inhibitory activity in all the three plants. Similarly, aqueous extract exhibited highest \( \alpha \)-glucosidase inhibitory activity in all the three plants. Extracts of \( A.\text{cookii} \) showed highest variations in \( \alpha \)-amylase and \( \alpha \)-glucosidase inhibitory activity while the other two \( B.\text{actinophylla} \) and \( B.\text{blakeana} \) showed moderate variations in their activities.

3.4. Antibacterial Activity

The antibacterial activity of the three plant extracts is given in the Table 4-7. Acetone extract of \( A.\text{raucaria} \) was found to be the most potent antibacterial agent in comparison to other extracts. Chloroform extract of \( A.\text{raucaria} \) showed similar antibacterial activity but less significant in comparison to acetone extract.
Tannins also have antibacterial (Akiyama et al., 2006) and antidiabetic (Andrade-Cetto et al., 2008) activities. In our study we are first time reporting the presence of tannins, flavonoids and saponins in these three plants. Tannins act as astringents (Halliwell and Gutteridge, 1989). From our results it is clear that tannins are present in the three plant extracts of all the three plants showed the maximum hemolytic activity while the aqueous extract exhibited the minimum hemolytic activity.

### 3.5. Hemolytic Activity Assay

The different extracts of the plants induced pronounced hemolysis on human blood which is summarised in Hexane extract of all the three plants. Tannins act as antioxidants (Halliwell and Gutteridge, 1989). Our results show that the acetone and chloroform extract of Araucaria cookii inhibited Pseudomonas.

### Table 7. EC50 values of different plant extracts

<table>
<thead>
<tr>
<th>Extracts</th>
<th>Methanol</th>
<th>Ethanol</th>
<th>Water</th>
<th>Chloroform</th>
<th>Hexane</th>
<th>Ethyl acetate</th>
<th>Acetone</th>
</tr>
</thead>
<tbody>
<tr>
<td>B. actinophylla</td>
<td>111.0</td>
<td>107.0</td>
<td>26.94</td>
<td>42.93</td>
<td>122.56</td>
<td>108.42</td>
<td>83.00</td>
</tr>
<tr>
<td>B. blakeana</td>
<td>145.0</td>
<td>66.160</td>
<td>16.33</td>
<td>67.51</td>
<td>109.60</td>
<td>138.38</td>
<td>123.91</td>
</tr>
<tr>
<td>A. cookii</td>
<td>30.80</td>
<td>126.94</td>
<td>38.22</td>
<td>87.21</td>
<td>118.52</td>
<td>92.590</td>
<td>54.710</td>
</tr>
</tbody>
</table>

**EC50 value- amount of extract (µg mL⁻¹) required to lyse 50% of erythrocytes**

The acetone and chloroform extract of Araucaria cookii inhibited Pseudomonas.

### 4. DISCUSSION

Araucaria cookii, Bauhinia blakeana, Brassaiia actinophylla are non medicinal plants and are used for ornamental purposes. In our study we are first time reporting the presence of tannins, flavonoids and saponins in these three plants. Tannins act as antioxidants (Halliwell and Gutteridge, 1989). From our results it is clear that tannins are present in the three plant extracts and they can be used as astringents. Tannins also have antibacterial (Akiyama et al., 2001) activity. Saponins are diverse family of secondary metabolites with many medicinal values, which has antimicrobial activity (Osburn, 2003) and extremely toxic to cold-blooded animals, but toxicity to mammals is low (Dini et al., 2001).

On the other hand phenolic compounds and flavonoids act as free radical scavengers and antioxidants. Plant antioxidants are safer than synthetic antioxidants (Gurpreet et al., 2006). The antioxidant activity of the plant could be related to hydroxyl group due to their polar nature (Prasad et al., 2005). Various plant species have been tested for antioxidant activity using DPPH assay (Wong et al., 2006; Annan and Houghton, 2008; Dall’Acqua et al., 2008; Borneo et al., 2009; Rohman and Man, 2010) and maximum activity was exhibited by Rupus ulmifolius with an IC₅₀ value of 5.1 µg mL⁻¹ (Dall’Acqua et al., 2008) and the lowest activity were exhibited by Thelesperma megapotamicum with the IC₅₀ value of 2000 µg mL⁻¹ (Borneo et al., 2009).

Antioxidant activity of the leaves of Calophyllum rubiginosum exhibited antioxidant activity with an IC₅₀ value of 0.11 µg mL⁻¹, 0.23 and 4.5 µg mL⁻¹ for DCM, Methanol and hexane extracts (Taher et al., 2010).

Plants and the plant constituents have received much attention in the treatment of diabetes, as researchers have identified hypoglycaemic agents from medicinal plants (Youn et al., 2004). Plant extracts containing α-amylase and α-glucosidase inhibitors have been reported (Shirwaikar et al., 2005) but not been identified in Araucaria cookii, Bauhinia blakeana and Brassaiia actinophylla. Flavonoids and polyphenols are natural antidiabetic agents (Andrade-Cetto et al., 2008). Plant phytochemicals has inhibitory activities against enzymes responsible for carbohydrate hydrolysis and subsequently lowers postprandial hyperglycemia and helps in the management of diabetes which was observed in vivo (Mai et al., 2007). Hemolytic activity of these extracts was checked with human erythrocytes. The IC₅₀ values for all the extracts for hemolytic activity are less than 200 µg mL⁻¹. From the results it is clear that none of the plant extracts possessed hemolytic activity against human erythrocytes.

### 5. CONCLUSION

The findings of our study show that the three plants extract has antioxidant, antimicrobial and enzyme inhibitory potential, due to the presence of various phytochemicals in the plant extracts. The phytochemical and pharmacological evaluation of the plant extracts states that these three ornamental plants possess medicinally important metabolites. Further Studies on the bioactive compounds of these plants in vivo should be carried out for the development of new compounds for the treatment of diabetes. Hemolysis at a concentration of 200 µg mL⁻¹ is considered to be active.

### 6. ACKNOWLEDGEMENT

We thank the management of VIT University for providing necessary facilities to carry out our research work.
7. REFERENCES


