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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* actinophyla 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 Cookii*, *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 primaryhealth 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

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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 blakaena* 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 pupurea*. 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 treated with 5 mL of 10% ammonium hydroxide solution. A yellow fluorescence indicated the presence of flavonoids.

2.7. Detection of Phytosteroids (Finer, 1988)

About 50 µL 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:

$$AA\% = (A_{control} - A_{sample})/A_{control} *100$$

where, $A_{control}$ is the absorbance of free radical alone and A_{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 α-Amylase and α-Glucosidase Inhibitory Activity

2.9.1. Inhibition Assay for Porcine α-Amylase Activity

About 500 µL of extract was taken and mixed with 500 µL of 0.02 M sodium phosphate buffer (pH 6.9 with 0.006 M sodium chloride) containing α-amylase solution (0.5 mg mL^{-1}) and incubated for 10 min at 25°C. After incubation, 500 µL 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°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:

$$\%inhibition = [A_{540\ control}-A_{540\ extract}] \text{ X } 100/A_{540\ control}$$

2.10. Inhibition Assay for Yeast α- Glucosidase Activity

About 50 µL of extract was mixed with 100 µL of 0.1 M phosphate buffer containing α-glucosidase solution and incubated in 96 wells plate at 25°C for 10 min. After incubation, 50 µL of 5 mM p-nitro phenyl a-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°C for 5 min. After incubation, absorbance was recorded at 540 nm by micro-array reader and compared with control which had 50 µL of buffer solution in place of extract. The inhibitory activity was calculated by this formula:

$$\%inhibition = [(A_{control540} -A_{extract540})] *100/A_{control\ 540}$$

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°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 *invitro* condition in 96 well plate. 100 µL of 0.85% Nacl solution containing 10 mM Cacl2 was added to each well. The first well served as negative control and contained PBS buffer and the second well onwards contained 100 µL of sample extracts. The last well served as positive control containing 100 µL of 0.1% triton X-100 in 0.85% saline. Then 100 µL 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 acetateand 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

Extracts	Saponins	Tannins	Phenols	Flavonoids	Phytosteroids
Methanol	+	-	+	-	+
Ethanol	-	-	+	-	+
Ethyl acetate	-	+	-	-	+
Chloroform	-	-	+	-	+
Hexane	-	-	+	-	+
Water	+	+	-	-	+
Acetone	-	-	+	-	+

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

	Saponins	Tannins	Phenols	Flavonoids	Phytosteroids
Methanol	+	+	-	+	+
Ethanol	-	-	+	-	+
Ethyl acetate	-	+	-	-	+
Chloroform	-	+	-	-	+
Hexane	-	-	+	-	+
Water	-	+	-	+	+
Acetone	-	-	+	+	+

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

Extracts	Saponins	Tannins	Phenols	Flavonoids	Phytosteroids
Methanol	+	+	-	+	+
Ethanol	-	+	-	-	+
Ethyl acetate	+	-	+	-	+
Chloroform	-	-	+	-	+
Hexane	-	-	+	-	+
Water	-	-	+	+	+
Acetone	-	+	-	+	+

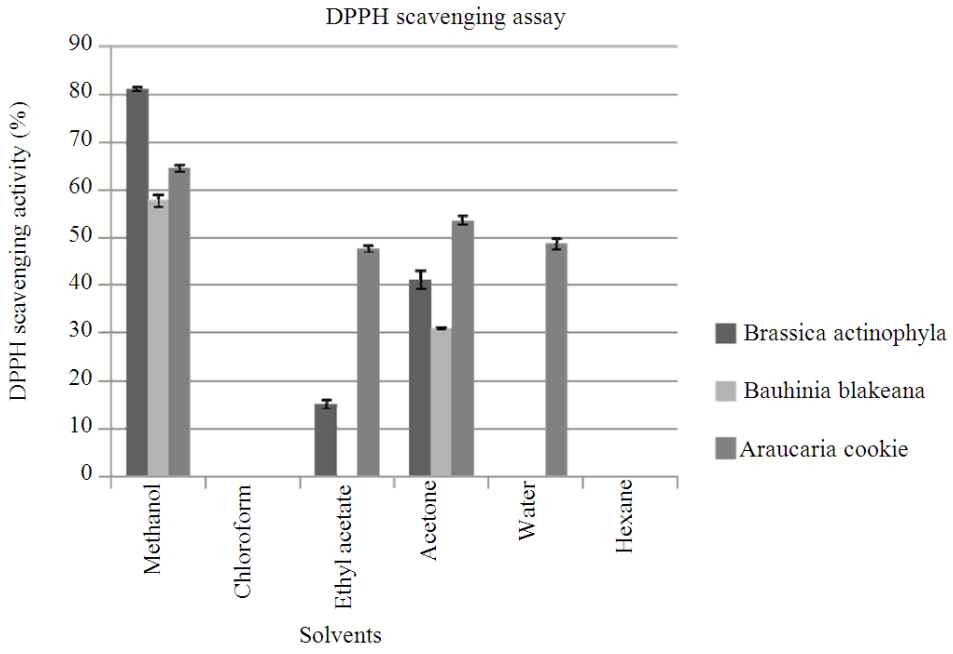

Fig. 1. DPPH Scavenging assay of the three plant extracts. Methanolic extract of Brassica actinophyla with maximum activity followed by *Araucaria cookii* and *Bauhinia blakeana*

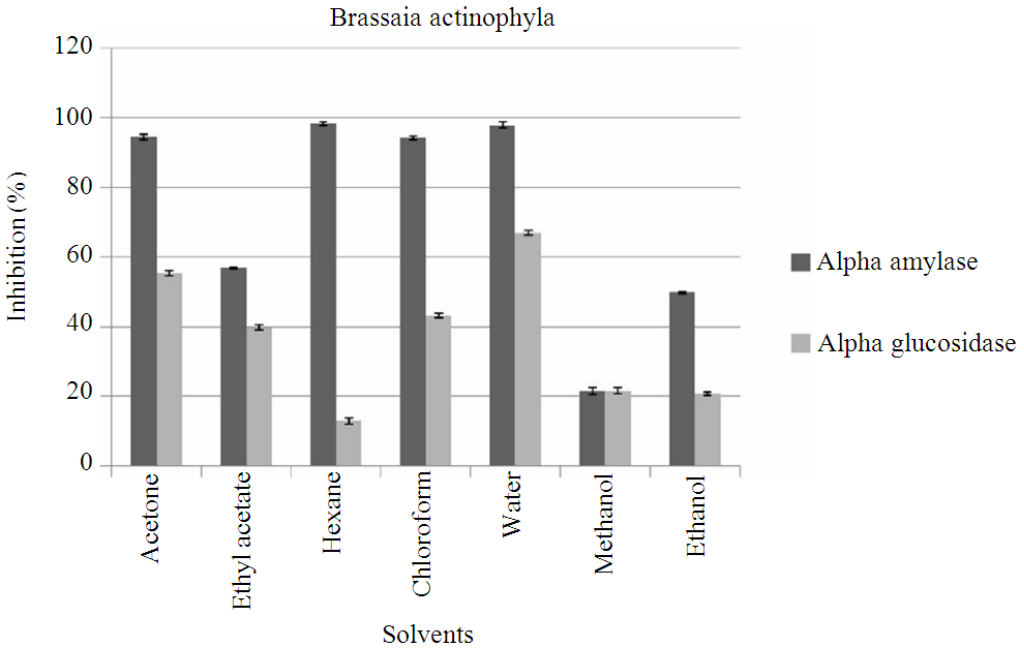

Fig. 2. Alpha amylase and alpha glucosidase inhibition by *Brassaia actinophyla*

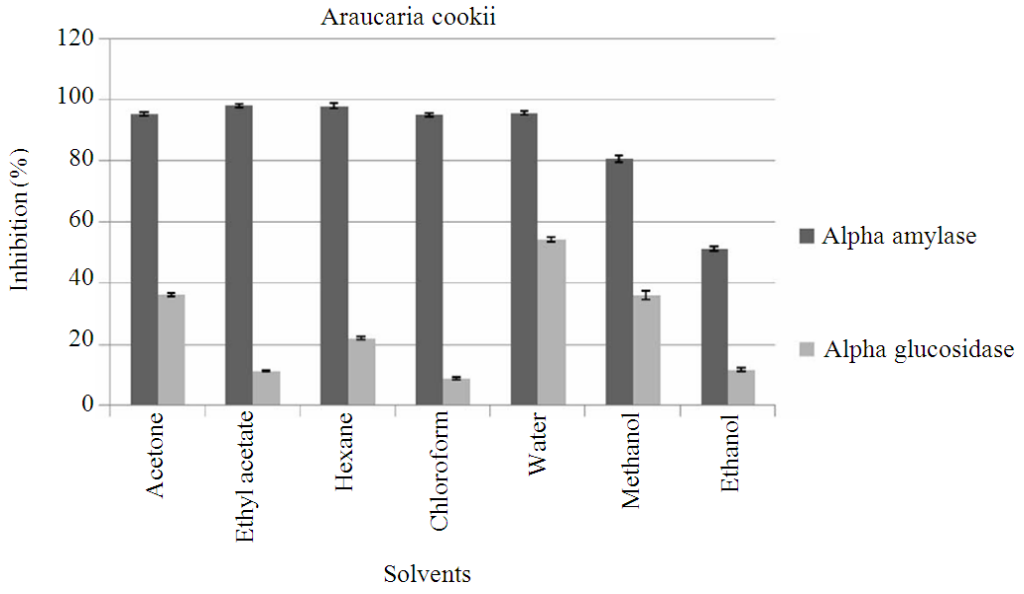

Fig. 3. Alpha amylase and alpha glucosidase inhibition by *Araucaria cookie*

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.

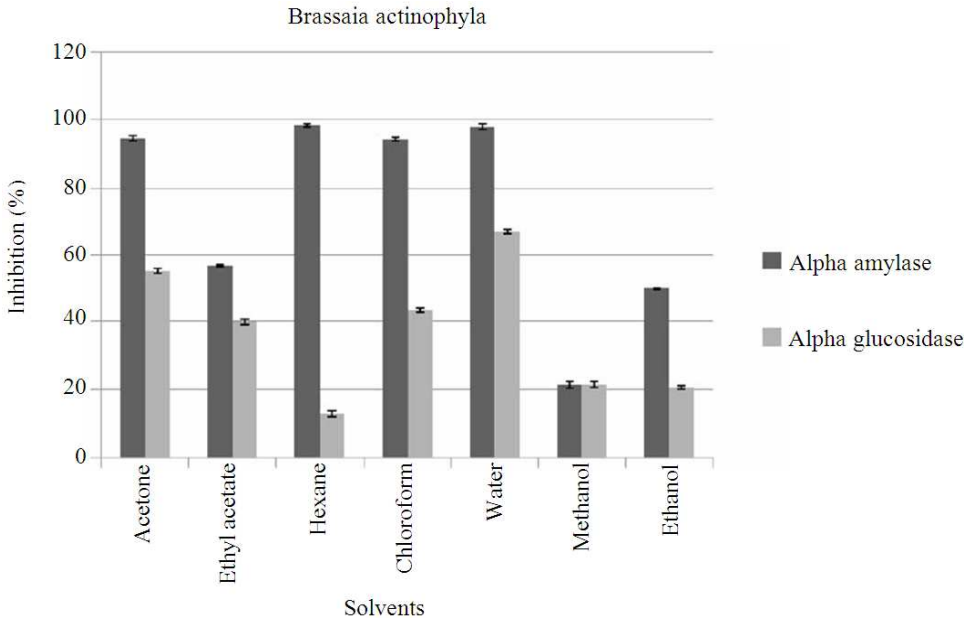

Fig. 4. Alpha amylase and alpha glucosidase inhibition by *Bauhinia blakeana*

Table 4. Antibacterial activity of three plant extracts against *Pseudomonas*

Extracts	*A.cookii*	*B.actinophylla*	*B.blakeana*
Ethanol	R	R	R
Methanol	R	R	R
Ethyl acetate	R	R	R
Hexane	R	R	R
Chloroform	2 mm	R	R
Water	R	R	R
Acetone	5 mm	R	R

Table 5. Anti bacterial activity against *E.coli*

Extracts	*A.cookii*	*B.actinophylla*	*B.blakeana*
Ethanol	R	R	R
Methanol	R	R	R
Ethyl acetate	R	R	R
Hexane	R	R	R
Chloroform	R	R	R
Water	R	R	R
Acetone	R	R	R

Table 6. Anti bacterial activity against *Klebsiella sp*

Extracts	*A.cookii*	*B.actinophylla*	*B.blakeana*
Ethanol	R	R	R
Methanol	R	R	R
Ethyl acetate	R	R	R
Hexane	R	R	R
Chloroform	R	R	R
Water	R	R	R
Acetone	R	R	R

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.actinophylla*>*A cookie*>*B.blakeana.*

3.3. α-Amylase and α-Glucosidase Inhibition

From the results obtained, it was observed that the various extracts of the three plants exhibited greater α-amylase inhibitory activity compared to α-glucosidase activity. The results of α-amylase and α-glucosidase inhibition is given in the **Fig. 2-4**. Hexane and chloroform extracts exhibited highest α-amylase inhibitory activity while methanol and ethanol extract exhibited the least inhibitory activity in all the three plants. Similarly, aqueous extract exhibited highest α-glucosidase inhibitory activity in all the three plants. Extracts of *A.cookii* showed highest variations in α-amylase and α-glucosidase inhibitory activity while the other two *B.actinophylla* and *B.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 *Araucaria cookii* was found to be the most potent antibacterial agent in comparison to other extracts. Chloroform extract of *Araucaria cookii* showed similar antibacterial activity but less significant in comparison to acetone extract.

Table 7. EC_{50} values of different plant extracts

Extracts	Methanol	Ethanol	Water	Chloroform	Hexane	Ethyl acetate	Acetone
B.actinophylla	111.0	107.07	26.94	42.93	122.56	108.42	83.00
B.blakeana	145.0	66.160	16.33	67.51	109.60	138.38	123.91
A.cookii	30.80	126.94	38.22	87.21	118.52	92.590	54.710

EC_{50} value- amount of extract (μg mL^{-1}) required to lyse 50% of erythrocytes

The acetone and chloroform extract of *Araucaria cooki* inhibited *Pseudomonas*.

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 showed the maximum hemolytic activity while the aqueous extract exhibited the minimum hemolytic activity.

4. DISCUSSION

Araucaria cookii, *Bauhinia blakeana*, *Brassaia 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 (Osbourn, 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 IC_{50} value of 5.1 μg mL^{-1} (Dall'Acqua *et al*., 2008) and the lowest activity were exhibited by *Thelesperma megapotamicam* with the IC_{50} value of 2000 μg mL^{-1} (Borneo *et al*., 2009). Antioxidant activity of the leaves of *Calophyllum rubiginosum* exhibited antioxidant activity with an IC_{50} value of 0.11 mg mL^{-1}, 0.23 and 4.5 mg 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 *Brassaia 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 helpsin the management of diabetes which was observed in vivo (Mai *et al*., 2007). Hemolytic activity of these extracts was checked with human erythrocytes. The IC_{50} 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 ext racts 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.

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