

Original Article

PRELIMINARY PHYTOCHEMICAL ANALYSIS AND *IN VITRO* ANTIOXIDANT ACTIVITY OF
ARAUCARIA COLUMNARIS BARK PEEL AND *COSMOS SULPHUREUS* FLOWERS

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ABSTRACT

Objective: Four different extracts of *Araucaria columnaris* (bark peel) and *Cosmos sulphureus* (flowers) were screened for their phytochemical composition, and free radical scavenging activities.

Methods: DPPH method was used to test the antioxidant activity for extracts.

Results: Among the different extracts tested, the methanol extract of both the plant species showed significant radical scavenging activities. Phytochemical analysis of the extracts revealed that the radical scavenging activities might be due to the presence of flavonoids, tannins and phenolic compounds.

Conclusion: The results obtained suggest that *Araucaria columnaris* (bark peel) and *Cosmos sulphureus* (flowers) could be exploited in the treatment of various diseases like cancer, cardiovascular diseases and infection diseases.

Keywords: *Cosmos sulphureus*, *Araucaria columnaris*, Antioxidant activity, DPPH, Phytochemicals, Radical scavenging

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INTRODUCTION

Medicinal plants are resources of new drugs and many of the modern medicines are produced indirectly from plants [1]. In the last years, interest in medicinal plants as an alternative to synthetic drugs is more and more increasing because of safety concerns, particularly against oxidative stress [2, 3]. Oxidative stress is potential when there is an imbalance between ROS (Reactive Oxygen Species) production and cellular antioxidant activity [4]. Oxidative stress is implicated in over hundred human disease conditions, such as cancer, cardiovascular diseases, aging and neurological disorders. This free radical induced oxidative stress can be prevented by the intake of sufficient amount of antioxidants [5]. Antioxidants are molecules that are capable of neutralizing the harmful effects of the ROS through the endogenous enzymatic defence system such as the superoxide dismutase (SOD), glutathione peroxidase (GPX) and catalase (CAT) in human system [6, 4]. Several studies have shown that the mechanism underlying polar antioxidant involves reactions with the hydroxyl (OH) group present in phenolics. Indeed, phenolics are composed of one or more aromatic rings bearing one or more hydroxyl groups and are therefore potentially able to quench free radicals by forming stabilized phenoxy radicals and most of the current antioxidants isolated so far from flowering plants are simple phenolic compounds which owe their properties to the mere fact that their aromatic hydroxyl moieties react with free radicals [7].

Research has quite convincingly shown that foods and herbs rich in antioxidants reap health benefits. Foods may possibly enhance antioxidant levels because foods contain a lot of antioxidant substances [8]. Potential sources of antioxidant compounds have been found in several types of plant materials such as vegetables, fruits, leaves, oilseeds, cereal crops, barks and roots, spices and herbs, and crude plant drugs [9]. Natural products, mainly obtained from dietary sources provide a large number of antioxidants. Phytoconstituents are also an important source of antioxidant and capable to terminate the free radical chain reactions [10, 11].

Araucaria columnaris belongs to the family Araucariaceae and genus *Araucaria*. It is distributed throughout the New Caledonia and Peshawar. It is commonly used as an ornamental plant all around the world [12, 13].

Cosmos sulphureus Cav. is a member of the family Asteraceae [14]. It is cultivated as an ornamental plant or it has been found as growing wild weeds along the roadsides and other undisturbed areas. *Cosmos* flowers are available in three different colours, orange, yellow and red. Flower capitulum composed of the disc and ray florets (7-8 petals like), straight and long-stalked. The height of the plant is 90-120 cm and has compound leaves. Flowering season is October to December [15]. The present study was undertaken to investigate the phytochemical and antioxidants properties of bark peel extract of *Araucaria columnaris* and flower extracts of *Cosmos sulphureus* with a view to assess the potentials of both the plants as a source for phenolic antioxidants.

MATERIALS AND METHODS

Plant material

The bark peel of *Araucaria columnaris* was collected from Lalbagh Botanical Garden, Bangalore and *Cosmos sulphureus* flowers were collected from different Botanical gardens situated in and around Bangalore. Both the plant materials collected were air-dried and ground to fine powder.

Extraction

Successive solvent extraction [16, 17] procedure was adopted for the preparation of various extracts *Araucaria columnaris* and *Cosmos sulphureus*. The powdered plant materials were subjected to successive extraction using Soxhlet apparatus with solvents in their ascending order of polarity. The solvents used were petroleum ether (60-80 °C), chloroform, methanol and distilled water. The extracts were filtered using Whatman no. 1 and further dried by using rotary evaporator until semi-solid is obtained.

Preliminary phytochemical screening

The phytochemical screening was carried out using standard procedures [18]. The tests for phytochemical screening include:

A. Tests for Carbohydrates

i. Benedict's test: Equal volumes of Benedict's reagent and test solution were mixed in a test tube and heated in boiling water bath

for 5 min. Appearance of green, yellow or red indicated the presence of carbohydrates depending on the amount of reducing sugar present in test solution.

B. Test for Proteins

i. **Million's test:** 3 ml of test solution was mixed with 5 ml of Million's reagent. White ppt. forms; when made warm turns brick red or ppt. dissolves giving red coloured solution indicating the presence of proteins.

C. Tests for Amino acids

i. **Tyrosine test:** 3 ml of test solution was heated with 3 drops of Million's reagent. Dark red colour formed shows the presence of Amino acids.

D. Tests for Fats and Oils

i. **Filter paper test:** Filter paper was treated with the test solution and dried. Permanent oil stain on the filter paper indicated the presence of Fats and Oils.

E. Tests for Steroid

i. **Liebermann's reaction:** 3 ml of test solution was mixed with 3 ml acetic anhydride. Heated and cooled. On addition of few drops of H₂SO₄, the appearance of blue colour indicated the presence of steroids.

F. Tests for Volatile oils

i. **Hydro distillation method:** The test solution was hydrodistilled. Volatile oil was separated from the distillate. The filter paper was treated with volatile oil and dried. The filter paper is not permanently stained with volatile oil.

G. Tests for Glycosides

i. **Liebermann's test:** 3 ml of test solution was mixed with 3 ml acetic anhydride. Heated and cooled. On addition of few drops of H₂SO₄, appearance of blue colour indicated the presence of Glycosides

H. Tests for Saponins

i. **Foam test:** Persistent foam was observed when test solution was shaken vigorously with water indicating the presence of saponins.

I. Test for Coumarins

i. **Odour test:** Aromatic odour indicated presence of coumarin glycosides

ii. **Alkalinity test:** Alcoholic extract when made alkaline showed blue or fluorescence indicating the presence of coumarins.

J. Tests for Flavonoids

i. **Sulphuric acid test:** On addition of sulphuric acid (66% or 80%) flavones and flavonols dissolve into it giving a deep yellow solution. Chalcones and aurones gave red or red-bluish solutions. And orange to red colours indicated the presence of Flavanes.

ii. **Lead acetate solution test:** to a small quantity of residue, lead acetate solution was added. Yellow coloured ppt. formed indicated the presence of flavonoids.

K. Tests for Alkaloids

i. **Tannic acid test:** Test solution treated with tannic acid solution gave buff coloured ppt. indicating the presence of alkaloids.

L. Tests for Tannins and Phenolic compounds

i. **5% FeCl₃ test:** To 2-3 ml of aqueous or alcoholic extract, few drops FeCl₃ (5%) was added. Deep blue-black colour observed indicated the presence of Tannins and Phenolic compounds.

Determination of antioxidant activity (DPPH assay)

Radical scavenging activity of the extracts was determined by measuring the decrease in absorbance of 2, 2-Diphenyl-1-picrylhydrazyl radical (DPPH•) at 517 nm [19]. The DPPH assay is a widely used method to evaluate the ability of antioxidants [20] to scavenge free radicals which are known to be a major factor in biological damages caused by oxidative stress. This assay is known to give reliable information concerning the antioxidant ability of the tested compounds [21-23]. This method is based on the ability of DPPH radical to react with hydrogen donor species such as phenolics and flavonoids present in the extracted material. Upon receiving a proton from the donor species it loses its color and becomes yellow. As the concentration of phenolic compounds increases, their DPPH radical scavenging activity also increases [24]. The crude extracts were weighed and dissolved in DMSO (10 mg/ml). This was considered as pure sample extract for testing the antioxidant property. 0.3 mmol solution of DPPH was prepared in 100% methanol. To 1 ml of this solution, three different concentrations 100 µl, 300 µl and 500 µl of sample extract and standard solution (Ascorbic acid) were added separately. The final volume was made up to 4 ml by adding 100% methanol to each sample mixture and also for a standard solution (Ascorbic acid). The same reaction mixture without the extracted sample but with an equivalent amount of standard phosphate buffer was taken as control. All the sample mixtures and control were shaken thoroughly and kept in dark at room temperature for 30 min. The absorbance of the reaction mixtures was measured at 517 nm [25]. The radical scavenging activities were expressed as a percentage of inhibition and calculated according to the following equation.

$$\text{DPPH radical scavenging activity (\%)} = \left[\frac{(\text{Abs}_{\text{control}} - \text{Abs}_{\text{sample}})}{\text{Abs}_{\text{control}}} \right] \times 100$$

Where *Abs_{control}* is absorbance control and *Abs_{sample}* is absorbance test sample.

RESULTS AND DISCUSSION

Preliminary phytochemical screening

The phytochemical analysis conducted on *Araucaria columnaris* and *Cosmos sulphureus* extracts revealed the presence of proteins, coumarins, saponins, amino acids, flavonoids, tannins, and phenolic compounds (table 1 and table 2). These phytochemical compounds are known to support bioactive activities in medicinal plants and thus responsible for the antioxidant activities of this plant extract used in this study.

Table 1: Results of the qualitative test for preliminary phytochemical analysis of *Araucaria columnaris* (bark peel) extracts

Phytochemical constituents	Tests	Blank	Control	Petroleum ether	Chloroform	Methanol	Aqueous
Carbohydrates	Benedict's test	-	+	-	-	-	-
Proteins	Million's test	-	+	-	-	+	-
Amino acids	Tyrosine test	-	+	-	-	-	-
Fats and oils	Filter paper test	-	+	-	-	+	+
Steroids	Liebermann-Burchard Test	-	+	-	-	-	-
Glycosides	Liebermann's test	-	+	-	-	-	-
Saponins	Foam test	-	+	-	-	-	+
Coumarins	Odour test	-	+	-	-	-	+
	Alkalinity test	-	+	-	-	-	-
Flavonoids	H ₂ SO ₄ test	-	+	-	-	++	+
	Lead acetate solution test	-	+	-	-	++	+
Alkaloids	Tannic acid test	-	+	-	-	-	-
Tannins and phenolic compounds	5% FeCl ₃ test	-	+	-	-	++	+

+++ (High), ++ (Moderate) + (low) and - (Nil)

Table 2: Results of the qualitative test for preliminary phytochemical analysis of *Cosmos sulphureus* (flower) extracts

Phytochemical constituents	Tests	Blank	Control	Petroleum ether	Chloroform	Methanol	Aqueous
Carbohydrates	Benedict's test	-	+	-	-	-	-
Proteins	Million's test	-	+	-	-	-	-
Amino acids	Tyrosine test	-	+	-	-	-	+
Fats and oils	Filter paper test	-	+	-	-	+	+
Steroids	Liebermann-Burchard Test	-	+	-	-	-	-
Glycosides	Liebermann's test	-	+	-	-	-	-
Saponins	Foam test	-	+	-	-	-	+
Coumarins	Odour test	-	+	-	-	-	-
Flavonoids	Alkalinity test	-	+	-	-	-	-
	H ₂ SO ₄ test	-	+	-	-	+++	++
Alkaloids	Lead acetate solution test	-	+	-	-	+++	-
	Tannic acid test	-	+	-	-	-	-
Tannins and phenolic compounds	5% FeCl ₃ test	-	+	-	-	++	+

+++ (High), ++ (Moderate), + (low) and - (Nil)

Determination of antioxidant activity (DPPH assay)

The *in vitro* antioxidant assay of both the plant extracts (fig. 1 and fig. 2) reveals significant antioxidant potential compared with standard Ascorbic acid. The methanol extracts of both the plants *Araucaria columnaris* and *Cosmos sulphureus* showed highest

antioxidant activity. The percentage inhibition of *Araucaria columnaris* and *Cosmos sulphureus* at the concentration of 500 µl were 90.37 % and 89.87 % respectively, compared to ascorbic acid (94.05%). The extracts showed increased antioxidant activity with the increase in concentration (µl) of the extracts.

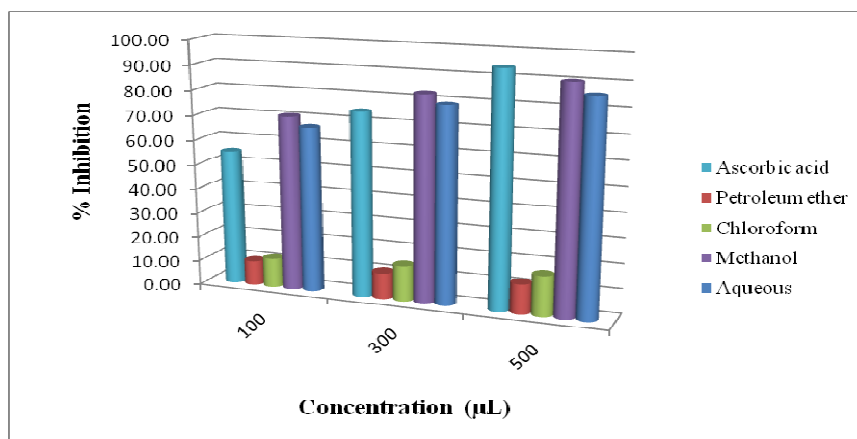


Fig. 1: Antioxidant properties of *Araucaria columnaris* plant extract in comparison with standard ascorbic acid as determined with DPPH method

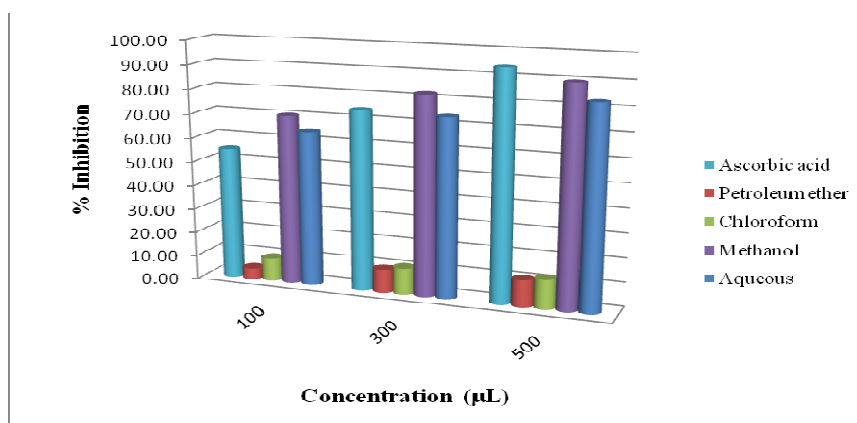


Fig. 2: Antioxidant properties of *Cosmos sulphureus* plant extract in comparison with standard ascorbic acid as determined with DPPH method

CONCLUSION

As antioxidants play a vital role in fighting against many diseases, especially those that are due to oxidative stress, it is important to identify which natural plants are effective in fighting against the free

radicals. Based on the results obtained, the radical scavenging activity is concentration-dependent, as it increases when the concentration changes from 100 µl to 300 µl to 500 µl. The investigation confirms the *in vitro* antioxidant potential of solvent extracts of *Araucaria columnaris* and *Cosmos sulphureus*, with results

comparable to those of the standard compound ascorbic acid and can, therefore, be proposed as new potential sources of natural additives for the food and/or pharmaceutical industries. The phytochemical screening revealed the presence of flavonoids, tannins and phenolic compounds in methanol and aqueous extracts of both the plant species. However, the components responsible for the antioxidant activity of the extracts were not identified and further work should be conducted to isolate and identify these bioactive compounds.

CONFLICTS OF INTERESTS

All authors have none to declare

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