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Antioxidant and Antimicrobial Activity of *Araucaria cookii* and *Brassaia actinophyla*

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Abstract: *Araucaria cookie* is an ornamental plant, which are evergreen conifer found in India and in many other European countries. Similarly *Brassaia actinophylla* is also an ornamental plant with its native from Java, Australia and in U.S. Though these plants are used for various purposes, the medicinal properties of the plants were not investigated. In our study, the two ornamental plants were chosen for screening both antioxidant and antimicrobial activity. The Leaves of the plants were used for preparing crude extract and was prepared by Soxhlet extraction method. For the extraction of the leave extracts, different solvents viz., methanol, chloroform and petroleum ether were used based on our preliminary data. The obtained extracts were condensed and stored. For the antioxidant and antimicrobial activity, the extractions were prepared into various concentrations. For the antioxidant activity DPPH was used as scavenger of the free radicals which showed the inhibition of percentage for *Araucaria cookie* was 63% and the inhibition percentage for *Brassaia actinophylla* 41%. For the antimicrobial activity the extracts were checked against two bacterial and two fungal pathogens. The phytochemical analysis assists in the study of the antioxidant and antimicrobial activity as to the probable compounds responsible for the activity. The result thus obtained provides a report of *Brassaia actinophylla* as a possible source of antioxidants and also the use of both extracts as a probable antimicrobial agent.

Key words: *Araucaria cookii*, *Brassaia actinophylla*, antimicrobial activity, antioxidant activity, phytochemicals

INTRODUCTION

Plants are now becoming an important source of medicines. Around 80% of the entire world population is immensely dependant on medicinal plants. This is because of the inadequate supply of modern drugs. Moreover, these medicines are easily available and cheaper than the modern drugs present. According to the study of the World Health Organization (WHO), there is a huge scarcity of availability of basic medicines all over the world, whether it is a rural area or rapidly growing modernized cities (WHO). So, these medicinal plants offer a better alternative to that of the conventional modernized drugs with less side-effect and with more effectiveness. One of the major advantages of the medicinal plants is that these are easily available to the rural areas and also these can be used as a path of income for people living below the poverty level. The medicinal plants which were or still in use in many traditional therapeutic systems have the potential to cure many complicated diseases (WHO). In India one of the well-known traditional medicinal system is the Ayurveda which is a 5000 years old system is still in use. This system is mainly dependent on different herbs which not only provides a remedy to different complicated diseases but also provides source of different nutritional supplements (Dahanukar *et al*., 2000). Therefore, interest is rising to use these plants in modern chemotherapeutic system because the allopathic medicinal system may provide a better and faster cure but because of its high prices and side-effect, forcing the mass to use these herbal plants as an alternative (Kala, 2005). Therefore, a new search have started to find a herbal source in the field in pharmaceuticals, herbal remedies, as a flavoring agent, usage in cosmetic production and producing perfumes from the essential oils and other herbal products (Kumar *et al*., 2000). *Araucaria cookii* commonly known as Christmas tree; it is an ornamental plant, widely grown in tropical lands usually an evergreen conifer. It grows around 6-7 feet tall and is a perennial tree (Silba, 1986). This is found in New Zealand, Southern California, Mexico and Hawaii. *Brassaia actinophylla* (Beasley, 2006) (or Umbrella tree is a native to Java, New Guinea and Queensland, Australia and in U.S., it is a "tub plant" called as *Schefflera actinophylla* (Beasley, 2006). It grows up to 100 feet tall with compound leaves forming umbrella-like symmetrical heads with leaflets of 12 inches long.) There has always been a keen interest in searching of plants

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exhibiting antioxidant activities. Many of the complicated diseases are mainly due to free radicals which causes the oxidative damages. These antioxidants helps in preventing this oxidant damages but metal chelating, elimination of the free radicals, catalytic metals and on the oxygen rich species (Elzaawely and Tawata, 2012; Shahidi *et al*., 1992). There have reported works on polyphenols and antioxidants (Akond *et al*., 2011). There are oxygen rich species which are continuously been generated inside the body. These species are called as the Reactive Oxygen Species (ROS). Recent studies by Elzaawely and Tawata (2012) and by Gupta *et al*. (2007) reports that these ROS are responsible for many diseases viz., arthritis, atherosclerosis, diabetes mellitus and cancer. On daily consumption of tea, wine, fruits, vegetables and spices provides a better source of antioxidants (Koleva *et al*., 2002; Oke and Hamburger, 2002; Mantle *et al*., 2000; Chu *et al*., 2000; Schuler, 1990). Reported work shows that the antioxidant work of the plants is due to the phenolic compounds (Cook and Samman, 1996), flavonoids (Frankel, 1995). Koleva *et al*. (2002) have reported for a effective, rapid and easy method of exhibiting free radical elimination by DPPH (1,1-diphenyl-2-picrylhydrazyl). Phytochemical studies suggests a broad spectrum in understanding the compounds responsible for the antioxidant and antimicrobial activities along with other utilities (Khasawneh *et al*., 2011). Hence, in this study it have been investigated that the antioxidant and anti microbial potential of *Araucaria cookii* and *Brassaia actinophylla*.

MATERIALS AND METHODS

Leaves of *Araucaria cookie* and *Brassaia actinophylla* were collected from the Horticulture centre of VIT University, Vellore, Tamil Nadu. The specimens were identified and authenticated by the Technical Officer of the Horticulture centre of VIT University. The bacterial test pathogens *Pseudomonas* sp. (MTCC 4438), *Klebshiella* sp. and fungal test pathogens *Aspergillus flavus* (MTCC 277) and *Aspergillus niger* (MTCC 281) were purchased from MTCC, Chandigarh, India. The DPPH and other chemicals were purchsed from Sigma, India.

Preparation of crude extracts: Leaves of *Araucaria cookie* and *Brassaia actinophylla* were washed in running tap water, dried under sun light, powdered and weighed. Solvent extractions of the powdered samples were performed by the Soxhlet extraction method using different solvents (Methanol, Chloroform and Petroleum Ether) for 6-8 h at 10 degree below the respective boiling points of the solvents used. Condensation of the extracts was carried out by rotary evaporator apparatus where the extracts were maintained for 30 min at a temperature just below the boiling point of the solvents. The condensed extract obtained was then lyophilized by using freeze drier. The powdered crude extract was then preserved in the refrigerator at 4°C for until further experiments. Stock solutions were prepared by dissolving 1 mg mL^{-1} and working concentrations were prepared by diluting it for anti-oxidant and antimicrobial activity.

Phytochemical analysis: Preliminary phytochemical analysis of the extracts were performed to determine the presence of alkaloids, flavonoids, amino acids and proteins, oils and fats, carbohydrates, phenolic compounds, phytosterols, tannin, saponins and glycosides (Abd El-Baky *et al*., 2008).

Antioxidant assay: DPPH scanvenging method of analyzing antioxidant activity is quite easy, widely used and accepted. Antioxidant activity was done by preparing different concentration (1 mg mL^{-1}, 750 μg mL^{-1}, 500 μg mL^{-1}, 250 μg mL^{-1}) of the leaf extracts dissolved in 1 mL of Methanol. It was then mixed with 500 μL of DPPH (1,1-diphenly-2-picrylhydrazyl) solution and then, the sample was kept for 30 min at dark place. After 30 min the OD value was measured using UV-Visible spectrometer at 517 nm along with a positive control (BHT) and blank (Methanol and DPPH). All the experiments were preformed in triplicates.

Inhibition percentage was calculated by the following formula:

$$\text{Inhibition (\%)} = \left\{\frac{(A_C - A_{Test})}{A_C}\right\} \times 100$$

Where:
A = Absorbance
c = Control

Antimicrobial assay: The extracts were screened for antimicrobial activity by using both well diffusion and paper disc method. The test organisms (*Klebshiella* sp., *Pseudomona* sp., *Aspergillus flavus*, *Aspergillus niger*) were inoculated into freshly prepared Nutrient agar and Sabouraud Dextrose Agar and incubated at 37°C. The bacterial samples were then seeded into Mueller Hilton agar plates and the fungal samples were seeded in Sabouraud dextrose Agar. Wells of 6 mm diameter were made and then 100 μL of extracts of various

concentrations were added into the well. The plates were then kept for incubation for 24 h at 37°C for the bacterial samples and 28°C fungal samples. After incubation, the plates were checked for the presence of zone of inhibition. The antimicrobial activity of vector control was also determined and subtracted from the zone of inhibition of extracts.

RESULTS

Phytochemical analysis: The compounds determined as a result of the phytochemicals analysis of the crude extracts of methanol, petroleum ether and methanol are listed in Table 1 (*Araucaria cookii*) and Table 2 (*Brassaia actinophyla*). The phytochemical analysis shows that the methanol extracts showed better result than the other solvents. Phenolic compounds and flavonoids, carbohydrates and tannin were found in both the extracts with only an exception of amino acids and proteins in *Brassaia actinophyla*.

Antioxidant activity: The antioxidant activity of the four extracts of *Araucaria cookii* and *Brassaia actinophyla* were evaluated by using DPPH assay. BHT was taken as the positive control. The maximum percentage of inhibition was observed in 1 mg mL^{-1} concentration for both the plant extracts. Among the various solvents used the methanol extracts showed more activity. The leaf extract of *Araucaria cookie* showed more inhibition percentage than *Brassaia actinophyla*. The inhibition percentage of *Araucaria cookii* was 63% which suggests it to have good antioxidant activity. In case of *Brassaia actinophyla* the maximum inhibition percentage at the maximum concentration (1 mg mL^{-1}) was 41% which is below the normal level (Fig. 1).

Antimicrobial activity: The antimicrobial activity are investigated using two bacterial (*Pseudomonas* sp. and *Klebshiela* sp.) and two fungal pathogens (*Aspergillus flavus* and *Aspergillus niger*). After incubation with the plant extracts the plates were checked for formation of zone of inhibition. The zone formed for *klebshiella*. sp. was measured as 5 and 7 mm for *Araucaria cookii* and *Brassaia actinophyla*, respectively and for the *Pseudomonas* sp the zone was 2 mm for *Araucaria cookii* and for *Brassaia actinophyla* no activity was found. The zone of inhibition for *Aspergillus flavus* was 7 and 2 mm for *Araucaria cookii* and *Brassaia actinophyla*, respectively and no activity was found for *Aspergillus niger* in both the cases (Table 3).

Table 1: Phytochemical analysis of *Araucaria cookii*

	Extracts of *Araucaria cookii*		
Compounds	Methanol	Chloroform	Petroleum ether
Amino acid and protein	-	-	-
Carbohydrates	+	-	-
Alkaloids	+	+	-
Phenolic compounds	+	-	+
Flavonoids	+	+	+
Oil and fats	-	-	-
Glycosides	-	-	-
Tannins	+	+	+
Phytosterols	-	-	-
Saponins	-	-	-

Table 2: Phytochemical analysis of *Brassaia actinophyla*

	Extracts of *Brassaia actinophyla*		
Compounds	Methanol	Chloroform	Petroleum ether
Alkaloids	+	-	-
Carbohydrates	+	+	+
Amino acids and protein	+	-	-
Phenolic compounds	+	+	-
Oils and fats	-	-	-
Flavonoids	+	+	+
Saponins	-	-	-
Phytosterols	-	-	-
Glycosides	-	-	-
Tannins	+	+	+

Table 3: Antimicrobial activity of *Araucaria cookii* and *Brassaia actinophyla* using *Pseudomonas* sp., *Klebshiella* sp., *Aspergillus niger* and *Aspergillus flavus* as test organisms

Test pathogens	*Araucaria cookii* (mm)	*Brassaia actinophyla* (mm)
Klebshiella.sp.	5	7
Pseudomonas.sp.	2	-
Aspergillus flavus	7	2
Aspergillus niger	-	-

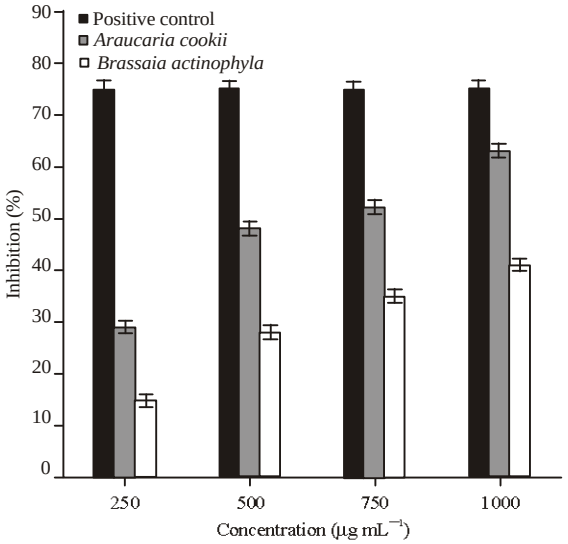

Fig. 1: Antioxidant activity of *Araucaria cookii* and *Brassaia actinophyla* using DPPH Assay method with BHT as positive control

DISCUSSION

The current investigation shows that both *Araucaria cookii* and *Brassaia actinophyla* possess good antioxidant activity and moderate antimicrobial activity against the tested pathogens. Initially the leaf was extracted with 6 different solvents and water. Among the various solvents used for extraction only methanol, chloroform and petroleum ether have shown activity and chosen for further studies. The leaves of both the plants were then extracted with the three solvents and used for antioxidant and antimicrobial activity. The methanolic extract of both the plants have shown significant activity compared to other two solvents used. Methanolic extract of *Araucaria cookie* found to have 63% inhibition when compared to *Brassaia actinophyla* which has 41% inhibition even at maximum concentration (1 mg mL^{-1}) used in this study. The antioxidant activities displayed by both plant extracts are found to be dose dependent. The antimicrobial activities of the extracts were checked against two bacterial and two fungal pathogens. The zone of inhibition was seen against *Klebsiella* sp. with both the extracts. *Araucaria cookie* extract inhibited the tested bacteria which showed 7 mm zone of inhibition where as *Brassaia actinophyla* extract produced 5 mm zone of inhibition. Both the plant extracts have no activity against *A. niger*. But there was no significant activity found against other pathogens. As reported by McLaughlin *et al*. (1993) the plant extracts exhibiting antioxidant activities are rich in phenolic and flavanoid compounds. Even some of the killer diseases like cancer are a cause of oxidative stress (Bandyopadhyay *et al*., 1999; Gulcin, 2009). Phenolics and flavonoids are very much helpful in avoiding the attack of ROS (Owen *et al*., 2003; Rebiai *et al*., 2011). From the phytochemical study it is suggested that the presence of the flavonoids and phenolic compounds might be responsible for the antioxidant activity. The phytochemical analysis of both the plants demonstrates that the presence of phenols, flavanoids, alkaloids and tannins in methanolic extracts plays a vital role in antioxidant and antimicrobial activity. Since, our study was carried out using crude extracts, it is necessary to purify the active principles and evaluate the bioactivities to take these plants for further research on therapeutic applications.

CONCLUSION

From the above study we can conclude that presence of the phenolics, alkaloids and flavonoids might be responsible for the antioxidant activities. Also, the methanolic extracts of both the plants shows a moderate activity towards microbial pathogens. Therefore, *Araucaria cookii* can be used as a potential source in antioxidant drug discovery. Also, *Araucaria cookii* and *Brassaia actinophyla* can be evaluated for antimicrobial activity against other pathogenic microoragnisms. Further investigations are necessary to assess the novelty of the phytochemicals and their biomedical applications.

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