The Screening of Phytochemical and Antioxidant Activity of Two Endemic Ferns, *Mycodium Excertum* (Wall.Ex Hook) Copel and *Tectaria Zeilanica* (Holtt.) Sledge

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Summary

An ethanolic extract of *Mycodium excertum* (Wall.ex Hook) Copel and *Tectaria zeilanica* (Holtt.) Sledge leaves screened for phytochemicals and potential of antioxidant activity were studied by DPPH method. The results of the antioxidant activity of *Mycodium excertum* ($y= 55.9x + 27.9$; $r^2=0.96$) was the strongest, and *Tectaria zeylanica* ($y=38.155x+27.89$; $r^2=0.93$) showed minimum free radical scavenging activity was observed. The IC_{50} value of the ethanolic extract of *Mycodium excertum* (0.52mg/ml) and *Tectaria zeylanica* (0.78± 8.4mg/ml) were observed. An ethanolic extracts of *Mycodium excertum* and *Tectaria zeylanica* leaves shows the highly exhibited the potential of antioxidant activity. The preliminary phytochemical screened for the active constituents of alkaloids and flavonoids were more abundant in the both leaves of *Mycodium excertum* and *Tectaria zeilanica*. The active constituents of alkaloids and flavonoids may be acted as antioxidant activity of both fern leaves extract of *Mycodium excertum* and *Tectaria zeilanica*.

Keywords: Fern; *Mycodium excertum*; *Tectaria zeilanica*; extract; antioxidant

Introduction

Free radicals have significant role in the causation of certain diseases such as diabetes, cirrhosis, cancer, and cardiovascular diseases [1]. The antioxidant constituents are that protect cells against the damaging effects of reactive oxygen species, such as singlet oxygen, super oxide, peroxyl radicals, hydroxyl radicals and peroxynitrite which results in oxidative stress leading to cellular damage. Thus, compounds or antioxidants that can scavenge free radicals have vital role in the improvement of these diseased conditions [2]. Now available of the synthetic antioxidants such as butylated hydroxy anisole (BHA), butylated hydroxy toluene (BHT), tertiary butylated hydroquinon and gallic acid esters, have been prompt negative health effects. Therefore, best restrictions have been placed on their application and there is a trend to substitute them with naturally occurring plant constituents. Moreover, these synthetic antioxidants also show low solubility and moderate antioxidant activity [3-4].

Medicinal plants have been produced several different types of secondary metabolites, which have been subsequently exploited by humans for their beneficial role in a diverse array of applications [5]. Plants contains a wide variety of active constituents were phenols, flavonoids, vitamins, terpenoids that are rich in antioxidant activity [6-8]. Recently, the antioxidant activity was due to the active compounds like flavones, isoflavones, flavonoids, anthocyanin, coumarin lignans, catechins and isocatechins [8]. The first primary land vascular plants of ferns play an important role in traditional and folklore medicine. According to Chopra and his colleagues [9] and Kirtikar and his colleagues [10] worked on 44 and 27 species of ferns reported on the medicinal uses. Medicinal uses of several fern species were also described [11-12]. They also reported that 29 species of ferns were used in preparation of medicine. May (1978) published a detailed review of various ferns and their medicinal values [13]. Pourmorad et al., reported the free radical scavenging activity *Equisetum maximum* and *Adiantum capillus* – veneris [14]. To our knowledge, the present investigation is the first report on anti-oxidant activity of crude ethanolic extracts from two endemic ferns of *Mycodium excertum* (Wall.ex Hook) Copel and *Tectaria zeylanica* (Holtt.) Sledge leaves.

Materials and Methods

Collection of Plant Materials

Fresh leaves of *Mecodium exsertum* and *Tectaria zeilanica* (Holtt.) Sledge were collected from Upper Kothaiyar, and Periyamylor, Kalakad-Mundanthurai Tiger Reserve (KMTR), Tirunelveli. A voucher specimen was deposited in the Centre for Biodiversity and Biotechnology, St. Xavier's College (Autonomous), Palayamkottai, Tamil Nadu, India.

Extraction

100gm of powdered materials of *Mecodium exsertum* and *Tectaria zeilanica* (Holtt.) Sledge leaves were separately Soxhlet extracted with ethanol for 5h. The solvent was removed under reduced pressure.

Qualitative phytochemical identification tests

The qualitative phytochemical tests were done to find the presence of the active principles such as alkaloid, glycoside, terpenoid and steroid, flavonoid, reducing sugar and tannin by the following the standard methods [15-16]:

Alkaloid

Alkaloids are basic nitrogenous compounds with definite physiological and pharmacological activity. Alkaloid solution produces white yellowish precipitate when a few drops of Mayer's reagents are added. Most alkaloids are precipitated from neutral or slightly acidic solution by Mayer's reagent. The alcoholic extract was evaporated to dryness and the residue was heated on a boiling water bath with 2% hydrochloric acid. After cooling, the mixture was filtered and treated with a few drops of Mayer's reagent. The samples were then observed for the presence of turbidity or yellow precipitation[15].

Glycoside

Glycosides are compounds which upon hydrolysis give rise to one or more sugars (glycones) and a compound which is not a sugar (aglycone or genine). To the solution of the extract in glacial acetic acid, few drops of ferric chloride and concentrated sulphuric acid are added, and observed for a reddish brown coloration at the junction of two layers and the bluish green color in the upper layer [15].

Terpenoid and steroid

10 mg of extract was treated with 0.5ml of acetic anhydride and 0.5ml of chloroform. Then concentrated solution of was added slowly and red violet color was observed for terpenoids and green bluish color for steroids [15].

Flavonoid

5ml of ethanolic extracts were added with metal of magnesium warmed in slowly. To this solution, 5-6 drops of Conc HCL was added and red color was observed for flavonoids and orange color for flavones [15].

Tannins

To 0.5 ml of extract solution 1ml of water and 1-2 drops of ferric chloride solution was added. Blue color was observed for gallic tannins and green black for catecholic tannins [16].

Reducing Sugar

To 0.5 ml of extract solution, 1 ml of water and 5-8 drops of Fehling's solution was added at hot and observed for brick red precipitate.

DPPH free radical scavenging activity

The DPPH free radical scavenging activity was assessed according to Okada & Okada method [17]. 0.05mM DPPH solution of 300µl was added to 40µl of extract solution with different concentrations (0.02-2mg/ml). The DPPH solution was freshly prepared and kept in the dark at 4°C. Ethanol 96% (2.7ml) was added and the mixture was shaken vigorously. The mixture was left to stand for 5min and the absorbance was measured using a spectrophotometer at 517nm. Ethanol was used to zero the spectrophotometer. A blank sample containing the same amount of ethanol and DPPH was also prepared. All determinations were performed in triplicate. The radical scavenging activities of the tested samples, expressed as percentage of inhibition were calculated according to the following equation [18]):

$$\text{Percent of DPPH inhibition} = \frac{(A0-A1)}{(A1)} \times 100$$

A0 = Absorption of control

A1 = Absorption of extract/standard

Where A_0 and A_1 are the absorbance values of the test and of the blank sample, respectively. Percentage of inhibition versus concentration curve was plotted and the concentration of sample required for 50% inhibition was determined the IC_{50} value.

Statistical analysis

The results were presented as the mean ± SEM. Regression analysis was used to analyzed the statistical significance at $P<0.05\%$.

Results and Discussion

The results of the extractive values of maximum content in *Tectaria zeilanica* (12.58%) and *Mecodium exsertum* (6.24%) were observed. From the qualitative phytochemical analysis, it is observed that alkaloids glycosides and flavonoids could be the more abundant group of chemicals found to be in the leaves of *Mecodium exsertum* Active constituents of alkaloids and flavonoids were more abundant observed in the *Tectaria zeilanica* (Table-1). The ethanolic leaf extracts of *Tectaria zeilanica* and *Mecodium exsertum* shows the potential of free radical scavenging properties were represented in Table-2. antioxidant activity of *Mycodium excertum* ($y= 55.9x + 27.9$; $r^2=0.96$) was the strongest, and *Tectaria zeylanica* ($y=38.155x+27.89$; $r^2=0.93$) showed minimum free radical scavenging activity was observed the DPPH method.

Table - 1: Identification of active principle of fern

Constituents	**Present/Absent**	
	Mecodium exsertum	***Tectaria zeilanica***
Alkaloids	+++	+++
Glycosides	+++	++
Terpenoids	+	++
Tannins	+	+
Reducing Sugar	++	+
Flavonoids	+++	+++

+ trace level; ++minor level; +++ major level

Table-2: The potential of free radical scavenging properties of *Mecodium exsertum* and *Tectaria zeilanica* leaves

Concentration	Anti-oxidant activity(O.D)	
	Mecodium exsertum	***Tectaria zeilanica***
0.2	**41.60±1.33**	**36.54±0.86**
0.4	**44.40±0.75**	**42.56±1.46**
0.6	**64.80±1.02**	**47.12±0.45**
0.8	**73.40±0.51**	**63.45±0.74**
1.0	**83.00±0.45**	**64.25±1.33**
BHT	**84.40±0.74**	**84.40±0.74**
Qucertin	**86.00±0.63**	**86.00±0.63**

*All values are expressed as five replications = Average ± SE

All the ethanolic extracts exhibited antioxidant activity significantly. The IC_{50} value of the ethanolic extract of *Mycodium excertum* (0.52mg/ml) and *Tectaria zeilanica* (0.78mg/ml) were observed. Antioxidants have been defined as substances that, when present at low concentrations compared with oxidizable compounds (e.g. DNA, protein, lipid, or carbohydrate), delay or prevent oxidative damage due to the presence of reactive oxygen species (ROS). These ROS undergo redox reactions with phenolics, resulting in inhibition of antioxidant activity in a concentration dependant manner [19]. The present results observed that ferns extracts show satisfactory effect in inhibiting DPPH.

Previously, DPPH methods have been used by several authors to evaluate the free radical scavenging activity of antioxidant molecules and plant extracts [18,20,21]. Germano *et al*., [20] have been reported the hepatoprotective properties of root decoction of *Trichilia roka* Chiov. (Meliaceae), a plant used in Mali folk medicine, against carbon tetrachloride-induced hepatotoxicity and correlated this effect to the polyphenol antioxidant component of the fraction. In another study involving the screening of 78 other extracts from 20 Malian medicinal plants belonging to 14 families, Diallo *et al*., demonstrated with DPPH spray that 20% of the plants, including *Cussonia barteri* (Araliaceae), *Glinus oppositofolius*, *Lannea velutina* (Anacardiaceae) possessed potent antioxidant activity [21]. The same results using different tests on plant materials were observed in some other studies [22-25]. Different antioxidant and radical scavenging activity may partly be due to wide variety of antioxidant constituents such as phenolics,

ascorbate and carotenoids [26-27]. The present study was identification of active constituents of alkaloids and flavonoids may be acted as antioxidant activity of both fern leaves extract of *Mycodium excertum* and *Tectaria zeilanica*. The present study was first time reported for *Tectaria zeilanica* and *Mecodium exsertum* shows the anti-oxidant activity should be the basis for its therapeutic efficacy in traditional medicine.

Conclusion

The conclusion of the present study of two endemic fern, *Tectaria zeilanica* and *Mecodium exsertum* are hopeful sources of potential antioxidant and may be efficient as preventive agents in various diseases.

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References

1. Hertog MGL., Feskens EJM., *et al*. Dietary antioxidant flavonoids and risk of coronary heart disease: The Zutphen Elderly study. *Lancet*,1993**;** 342:1007-1011.
2. Wilson RL. Free radicals and tissue damage, mechanistic evidence from radiation studies. In: Biochemical Mechanisms of Liver Injury. New York, Academic Press pp: 123;1988.
3. Barlow SM. Toxicological aspects of antioxidants used as food additives. In Food Antioxidants, Hudson BJF (ed.) Elsevier, London, pp. 253-307;1988.
4. Branen AL. Toxicology and biochemistry of butylated hydroxyanisol and butylated hydroxytoluene. *J. American Oil Chemists Society*,1975;5:59- 63.
5. Balandrin MF., Klocke JA., Wurtele ES., Bollinger WH. Natural plant chemicals: Sources of industrial and medicinal materials. *Science*, 1985; 228:1154-1160.
6. Cai YZ., Sun M., *et al*. Antioxidant activity of betalins from plants of the Amaranthacea. *J . Agric. Food Chem.* 2003; 51: 2288-2294.
7. Rice-Evans C., Miller N., *et al*. Antioxidant properties of phenolic compounds. *Trends Plant Sci.* 1997; 2:152-159.
8. Aqil F., Ahmed I., Mehmood Z. Antioxidant and free radical scavenging properties of twelve traditionally used Indian medicinal plants. *Turk.J.Biol*., 2006;30:177-183,
9. Chopra RN. *Indigenous drugs of India and their economicaspects.* Calcutta. Art Press, Calcutta;1993.
10. Kirtikar KT, Basu BD, An ICS. Indian Medicinal Plants. Vol.4. (2nd edn.) Bishen Singh Mahendra Pal Singh, Dehra Dun, India;1975.

11. Nadkarni BK. Indian Materia Medica with ayurvedic, unantibbi, siddha, allopathic, homeopathic, naturopathic and home remedies.3rd edition, Popular Book Depot, Bombay;1954.
12. Nayar BK. Medicinal ferns of India. *Bull. Nat. Bot Gard*. Lucknow, 1959; 29: 1-36.
13. May IW. The economic uses and associated folklore of ferns and fern allies. *Bot. Rev*., 1978*;* 44: 191-528.
14. Pourmorad F., Hosseinimehr SJ., Shahabimajd, N. Antioxidant activity, phenol and flavonoid contents of some selected Iranian medicinal plants. *African Journal of Biotechnology*, 2006; 5 (11):1142-1145.
15. Siddiqui, AA., Ali, M. Practical Pharmaceutical chemistry. Ist ed., CBS Publishers and Distributors, New Delhi, pp.126-131;1997.
16. Iyengar, MA. Study of Crude Drugs. 8th ed., Manipal Power Press, Manipal, India. Pp.2;1995.
17. Okada Y., Okada M. Scavenging effect of soluble proteins in broad beans on free radicals and active oxygen species. *J. Agric. Food Chem*.,1998; 46: 401-406.
18. Yen GC., Duh PD. Scavenging effect of methanolic extracts of peanut hulls on free-radical and active-oxygen species. *J. Agric. Food Chem*., 1994; 42: 629-632.
19. Halliwell B., Gutteridge JMC. Role of free radicals and catalytic metal ions in human disease. *Methods Enzymol*.,1990; 186: 1-85.
20. Germano MP., D'Angelo V., Sanogo R., Morabito A., Pergolizzi S., De Pasquale R. Hepatoprotective activity of *Trichilia roka* on carbon tetrachloride-induced liver damage in rats. *J. Pharm. Pharmacol*., 2001; 53(11):1569 - 1574.
21. Dufall KG., Ngadjui BT., Simeon KF., Abegaz BM., Croft KD. Antioxidant activity of prenylated flavonoids from the West African medicinal plant *Dorstenia mannii*. *J. Ethnopharmacol*. 2003; 87(1):67-72.
22. Kim BJ., Kim JH., Kim HP., Heo MY. Biological screening of 100 plant extracts for cosmetic use (II): antioxidative activity and free radical scavenging activity. *Int. J. Cosmetic Sci*.,1997; 19: 299-307.
23. Deighton N., Brennan R., Finn Ch., Davies HV. Antioxidant properties of domesticated and wild Rubus species. *J. Sci. Food Agric*.2000; 80: 1307-1313.
24. Jadhav HR., Bhutani KK.Antioxidant properties of Indian medicinal plants. *Phytother. Res*., 2002;16: 771-773.
25. Lee SE., Hwang HJ., Ha JS., Jeong HS., Kim JH. Screening of medicinal plants for antioxidant activity. *Life Sci*., 2003; 73: 167-179.
26. Kaur C., Kapoor HC. Antioxidant activity and total phenolic content of some Asian vegetables. *Int. J. Food Sci. Tech.,* 2002;37:153 -162.
27. Velioglu YS, Mazza G, Gao L, Oomah BD. Antioxidant activity and total phenolics in selected fruits, vegetables and grain products. *J. Agric. Food Chem.,* 1998;46:4113 - 4117.