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TOTAL PHENOLICS, FLAVONOIDS AND ANTIOXIDANT EVALUATION IN THE LEAVES *ERIA ALBA*

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ABSTRACT

Eria alba (Orchidaceae) belongs to the tribe coelogyneae, It contain about 375 species in tropical Asia,Polynesia and Australia.50 species are found in india.Commonly found througout the Himalayan region at the altitude of 2200-3000m. Most plants of the genus *Eria* found in India grow as epiphytes. Some are also found growing on moist, moss covered rock structures on large, hilly slopes The determination of these characters will help further researches in their phytochemical as well as pharmacological analyses of this species. Total phenolics, flavonoids contents and antioxidant capacity were evaluated according to standard procedures. The results of this study showed that the water extract of *A. nervosa* leaf has significant amount of total phenolic (102.8 ± 0.12 mg GAE/g) and flavonoid contents (66.10 ± 0.29 mg RE/g) whereas total antioxidant capacity was found 1.08 ± 0.12 mg GAE/g when compared with gallic acid as standard. Hence, it is concluded that the *E.alba* leaf extract contain remarkable amount of total phenolic and flavonoid content can be used as a powerful herbal antioxidant.

KEYWORDS: *Eria alba*, Orchidaceae, Total antioxidant capacity, phytochemicals.

INTRODUCTION

The genus *Eria* (Orchidaceae) belongs to the tribe coelogyneae, It contain about 375 species in tropical Asia,Polynesia and Australia.50 species are found in india.Commonly found througout the Himalayan region at the altitude of 2200-3000m.[1] Most plants of the genus *Eria* found in India grow as epiphytes. Some are also found growing on moist, moss covered rock structures on large, hilly slopes.[2] On the earth, out of 4, 22,127 plant species, about 35,000 to 70,00 species are used as medicinal plants . In the third world countries, 20,000 plants species are believed to be used medicinally.[3] At present, the pharmaceutical sector in India is making use of 280 medicinal plant species, of which 175 are found in the IHR.[4] The plants of this genus have been studied extensively because of the traditional medicinal uses associated with them. The leaves, stems and flowers are used mostly in folk medicine for the treatment of dysentery ,treatment of asthma, coughs, bronchitis, eczema and wound healing. The plant leaves used as remedy for skin diseases to reduce swelling and pain. Plants are used medicinally in different countries and are a source of many powerful and potent drugs.[5] A wide range of medicinal plant parts is used for extract as raw drugs and they possess varied medicinal properties. The different parts of plants used include flower, root, stem, fruits and modified plant organs. While some of these raw drugs are collected in smaller quantities by the local communities and folk healers for local used, many other raw drugs are collected in larger quantities and traded in the market as the raw material for many herbal industries.[6]

Present research work has been made to study thequalitative, quantitative phytochemical parameters and antioxidant activity of the leaves of *eria alba*.

MATERIALS AND METHODS

Plant Material

Eria alba (Orchidaceae) whole plants were collected from the Ukhimath, Rudraprayag Uttrakhand, India in October 2014. The plant was identified from Department of Botany, HNB Garhwal University Uttrakhand. The leaves were separated, dried, coarsely powdered passed through sieve no 40 and stored in a closed container for further use. All chemicals and reagents used were of analytical grade obtained from S.D. Fine Chemicals Ltd., Mumbai.

Methods

The ash values and extractive values with various reagents were determined according to Indian Pharmacopoeia[7] Extractive values were performed with various solvents like petroleum ether, chloroform, ethyl acetate, alcohol and water. Preliminary phytochemical tests were carried out for ethanol, aqueous and powder with water type of extracts to identify the presence of various chemical constituents like alkaloids, phytosterols, carbohydrates, terpenoids, saponins,

flavonoids, phenolic compounds etc. using specific reagents through standard procedures.[8,9]

Extraction

Defined quantities of plant material were collected; shade dried at room temperature, pulverized and extracted different solvents like petroleum ether, chloroform, ethyl acetate, alcohol and water in a Soxhlet extractor. All extracts was concentrated and dried using rotary flash evaporator. It was kept in desiccators until further used.

Total Phenolic Content

The concentration of total phenolic compounds of the water extract was determined by the Folin-Ciocalteu method[10] using the extracts at a dilution of 1:100 in water. The absorbance of the samples was measured at 765 nm. The results are expressed as mg of gallic acid equivalent (GAE)/g of each sample.

Total Flavonoid Content

The total flavonoid contents of the *A. nervosa* (water extract) was measured using the aluminium chloride assay,[11] Briefly, ethanol extract (10 mg) of *A. nervosa* leaf was dissolved in H2O (1 mL) in a test tube, to which 5 % (w/v) NaNO2 (60 µL) was added. After 5 min, a 10 % (w/v) AlCl3 solution (60 µL) was added. After 6 min, 1 M NaOH (400 µL) was added and the total volume made up to 2 mL with H2O. The solution was mixed well and the absorbance measured at 510 nm against a reagent blank. Concentrations were determined using a rutin standard curve. Mean total flavonoid contents (n = 3) were expressed as milligrams rutin equivalents (RE) per g (mgRE/g dry).

Total Antioxidant Activity

Total antioxidant activity of *Eria alba* (water extract) was determined according to the method of Prieto[12] Briefly 2 mL of sample was taken at different concentrations (50, 10, 250, 500 and 1000 µg) and mixed with 1 mL of standard reagent 0.6 M Sulfuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate. Then Reaction mixture was incubated at 95° C for 90 min. Absorbance of all the sample was measured at 635 nm.

RESULTS AND DISCUSSION

The various physical constants such as total cash value (5.2%), acid insoluble ash (2.0 %) and water soluble ash (4.12%) were determined as shown in (Figure 1). The extractive values of the powder with different solvents were determined and its result was reported in Table 2 which indicates the nature of constituents present.

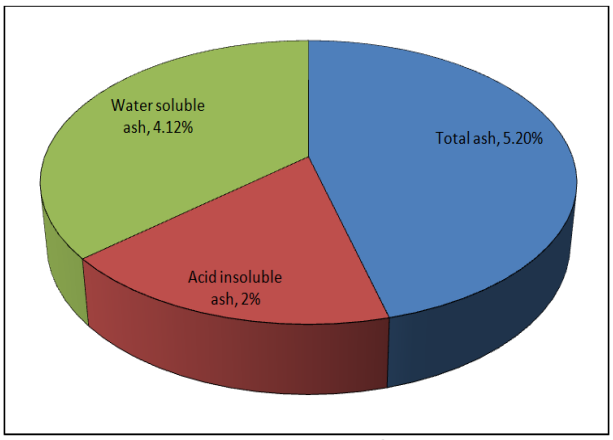

Figure 1: Ash Values of *Eria alba*.

Table 1: Determination of Ash Values of *Eria alba*.

S. No.	Ash type	Percentage of Ash
1.	Total ash	4.2% w/w
2.	Acid insoluble ash	1.30% w/w
3.	Water soluble ash	5.02% w/w

Table 2: Determination of Extractive Values *of Eria alba*.

S. No.	Extracts	Percentage of extractive
1.	Petroleum ether	1.29% w/w
2.	Chloroform	0.50% w/w
3.	Ethyl acetate	0.97% w/w
4.	Ethanol	1.45% w/w
5.	Water	8.23% w/w

Table 3: Preliminary phytochemical screening of *Eria alba*.

S. No.	Tests	Powder + Water	Ethanol extract	Water extract
1.	**Alkaloids**			
	Dragendroff's test	+ve	-ve	-ve
	Mayer's test	+ve	+ve	+ve
	Hager's test	+ve	-ve	+ve
	Wagner's test	-ve	-ve	-ve
2.	**Carbohydrates**			
	Fehling's test	+ve	+ve	+ve
	Molish test	+ve	+ve	+ve
3.	**Gums/Mucilage**			
	Water	+ve	-ve	-ve
4.	**Tannins**			
	Aq. FeCl3 Test	+ve	-ve	+ve
	Alc. FeCl3 Test	-ve	+ve	-ve
5.	**Flavonoids**			

	Lead acetate test	+ve	-ve	-ve
	Shinoda test	-ve	-ve	-ve
	Mg/Hcl	+ve	-ve	-ve

The various qualitative chemical tests (Table 3) have shown the presence of triterpenoids, saponins, sterols, flavanoids, carbohydrates phenols and tannins in large amount whereas aromatic acids, gums, mucilage and volatile oils were totally absent in the water extract of this plant part. Qualitative chemical test confirms that the water extract showed maximum phytoconstituents (including flavonoids mostly responsible for antioxidant activity) in the leaves of *Eria alba*. Hence, water extract was used for the estimation of total phenolic, flavonoid and antioxidant capacity,

Total Phenolics, Flavonoids Contents and Antioxidant Capacity

The results showed that total phenolic content of *Eria alba* water extract was 102.8 ± 0.12 mg GAE/g whereas the concentrations of total flavonoids in the same extract was found to be 66.10 ± 0.29 mg RE/g. It is well known that the qualitative and quantitative composition of polyphenolic compounds depends onnumerous factors, particularly plant chemotype and growth conditions (rain, soil, temperature, etc.). Theprogressive increase of total polyphenolic compounds found discards the probable presence of chemotypes.

However the influence of ecological factors may explain the wide variation of concentrations detected in thewater extracts. The concentration of flavonoids in plant extracts depends on the polarity of solvents used in the extract preparation. More polar solvent contain more flavonoid content.19 The water extracts of *A. nervosa* leaf showed significant total antioxidant capacity which was found to be 1.08 ± 0.12 mg GAE/g when compared to gallic acid as standard.

CONCLUSION

In this research, we have made an attempt to provide the antioxidant potential and phytochemicals present in the *A. nervosa*, a medicinal plant found in tropical and subtropica countries. All the results indicate that flavanoids presented in the plant part could be an important source of antioxidant molecules. The antioxidant capacity of flavanoids is based on their molecular structure. The hydroxyl group position and other characteristics in the chemical structure of flavanoids are more important for their antioxidant and free radical scavenging actions. Plant phenolics in general are effective free radical scavengers and antioxidants. In conclusion the remarkably strong *A. nervosa* leaf extract can be used as a powerful herbal antioxidant. The antioxidant activity should be regarded as an additional health promoting value for use as phytonutrients. This plant has diversified pharmacological potential and was used since ancient times. It has a strong future in the field of herbal medicine, thus the plant should be cultivated in a large scale particularly in unutilized and wasteland which will helpful the financial upliftment of the farmers along with the development of research in the field of herbal medicine. Furthermore, systemic and scientific research is required to explore the maximum pharmacological potential of the plant.

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