

A Study on the *In Vitro* Antioxidant Activity of Aerial Parts of *Celsia coromandeliane* Vahl and Bark of *Mesua ferrea* Linn

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Summary

The *in vitro* antioxidant activity of aerial parts of *Celsia coromandeliane* Vahl and bark of *Mesua ferrea* Linn have been investigated by estimating degree of non-enzymatic haemoglobin glycosylation measured colorimetrically at 520 nm. It is found that chloroform extract of *C. coromandeliane* (CECC) and ethanol extract of *Mesua ferrea* (EEMF) has the highest antioxidant activity than other extracts of these plants, respectively. The antioxidant activity of the extracts is close and comparable to that of standard antioxidant compounds used.

Key words: antioxidant activity, *Celsia coromandeliane*, *Mesua ferrea*, non-enzymatic haemoglobin glycosylation.

Introduction

Celsia coromandeliane Vahl (Kukshima in Bengali, gadartambaku in Hindi, family: scrophulariaceae) is common throughout India, found widely in the plains of West Bengal, growing as shrubs [1]. Various parts of this plant were used in tribal medicine for diseases like insomnia, fever, diarrhoea, dysentery and syphilitic eruptions. The juice of the leaves is applied externally for relieving the burning sensation at the hands and feet and used as astringent, a drink in bleeding piles [2, 3]. The plant has been found also to possess antifertility and CNS depressant activities [1-5].

Mesua ferrea Linn (Nagakesara in Hindi, Nageswar in Bengali & Oriya, family: guttiferae), is very widely distributed tree found in India, Sri-Lanka, Himalayas and Andaman Islands. The roots of this plant are used as refrigerant, gentle laxative, antipyretic, tonic, an alternative in chronic rheumatic and venereal diseases. The leaves and flower in a combination with other drug are recommended for the treatment of snake bite and scorpion sting. Bark is astringent, aromatic, combined with ginger used as sudorific, cardiogenic, good in asthma, cure ulcers, piles and used in scabies, wounds [6-9].

The aerial parts of *C. coromandeliane* on preliminary chemical analysis are found to contain saponin and steroids [10-11]. Similarly, the bark of *M. ferrea* is found to contain saponin glycosides, flavonoids, tannins & phenolic compounds [10-11]. Recently, a great deal of interest has been directed towards the bioactivity of natural plants as sources of antioxidant [12-14]. Hence, the present communication deals with the *in-vitro* evaluation of the antioxidant activity of aerial parts of *C. coromandeliane* and barks of *M. ferrea*.

Evaluation of the antioxidant activity of any drug sample or herbal extract can be carried out either by *in vitro* or *in vivo* models. Various procedures are available in each model to determine the antioxidant capacity. Here, the evaluation is carried out by *in vitro* non-enzymatic glycosylation of haemoglobin method. Since non-enzymatic glycosylation of haemoglobin is an oxidation reaction, an antioxidant is expected to inhibit the reaction. The degree of haemoglycosylation *in vitro* in the presence of different concentration of extracts can be measured colorimetrically.

Material and Methods

Chemicals

Haemoglobin was purchased from Nice Chemicals Pvt. Ltd., Cochin. Glucose, phosphate buffer and D- α -tocopherol were procured from Merck, Mumbai. Ascorbic acid and gentamycin were obtained from Biokem International Pvt. Ltd., Bangalore and Nicholas Piramol India Ltd., Pithampur, respectively. All other reagents and solvents used were of analytical grade.

Preparation of extracts

Aerial parts of *C. coromandeliane* were collected from Panua, Bankura District, West Bengal, India in the month of June and barks of *M. ferrea* were collected from hill area near the Subarnarekha River in the District of Mayurbhanj, Orissa, India in the month of August. They were authenticated by Dr M. S. Mondal, Additional Director, Central National Herbarium, Botanical Survey of India, Howrah, and West Bengal, India. The voucher specimens have been preserved in our laboratory for future reference (DM1 and DS1). Shade-dried, powdered, sieved (40 mesh size) plant materials were exhaustively extracted successively with petroleum ether (40-60^o C), chloroform, ethyl acetate, ethanol and distilled water using a soxhlet extractor. The extracts were concentrated to dryness in vacuum. The yields of petroleum ether, chloroform, ethyl acetate, ethanol, water extracts of *C. coromandeliane* and *M. ferrea* were 4.2, 3.5, 1.9, 10.2 and 3.6 % w/w and 1.6, 0.7, 0.6, 3.6, 5.6 % w/w, respectively. The extracts were subjected to antioxidant studies.

Antioxidant studies

Non-enzymatic haemoglycosylation method: The antioxidant activities of different extracts were investigated by estimating degree of non-enzymatic haemoglobin glycosylation measured colorimetrically. Haemoglobin, 60 mg/100 mL in 0.01 M phosphate buffer (pH 7.4) was incubated in presence of 2 g/100 mL concentration of glucose for 72 h in order to find out the best condition for haemoglobin glycosylation. The assay was performed by adding 1 mL of glucose solution, 1 mL of haemoglobin solution and 1 mL of gentamycin (20 mg/ 100 mL) in 0.01 M phosphate buffer (pH 7.4). The mixture was incubated in dark at room temperature for 72 h. The degree of glycosylation of hemoglobin in the presence of different concentration of extracts and their absence were measured colorimetrically at 520 nm [15-19].

Results and Conclusions

Results of antioxidant activity of aerial parts of *C. coromandeliane* Vahl and bark of *M. ferrea* Linn extracts are summarized in Table- 1 and Figure-1, respectively. The results obtained indicate that chloroform extract of aerial parts of *C. coromandeliane* has better antioxidant activity than petroleum ether, ethyl acetate, ethanol and aqueous extract.

Again, ethanol extract of barks of *M. ferrea* has better antioxidant activity than petroleum ether, chloroform, ethyl acetate and aqueous extract. It was also found that the antioxidant activities of both the extract are concentration dependent. The activities were compared with D- α - tocopherol (vitamin E) and ascorbic acid (vitamin C) that were used as standard antioxidant compounds. Preliminary phytochemical investigations indicate the presence of saponin glycosides, flavonoids, tannins & phenolic compounds in ethanol extract of bark of *M. ferrea* which might be responsible for antioxidant activity of this plant [13, 14, 20, 21]. However, the detailed chemical nature of the active principle(s) responsible for antioxidant activity and their mode of action are under investigation.

Table- 1. Antioxidant activity of different extracts of *C. coromandeliane* Vahl.

Samples	Final concentration of the tested compound (mg/mL)	
	0.5	1.0
Petroleum ether extract	15.0 \pm 0.42	32.2 \pm 0.45
Chloroform extract	29.1 \pm 0.45	56.3 \pm 0.65
Ethyl acetate extract	19.6 \pm 0.35	40.4 \pm 0.50
Ethanol extract	10.5 \pm 0.30	19.5 \pm 0.31
Aqueous extract	13.0 \pm 0.28	23.7 \pm 0.30
D- α - tocopherol	11.3 \pm 0.16	18.8 \pm 0.40
Ascorbic acid	5.4 \pm 0.10	10.2 \pm 0.23

Percent inhibition of haemoglobin glycosylation was measured at two concentrations of petroleum ether extract, chloroform extract, ethyl acetate extract, ethanol extract and aqueous extract. The activities were compared with those of D- α - tocopherol and ascorbic acid. Values are mean \pm S.E.M. of three replicates.

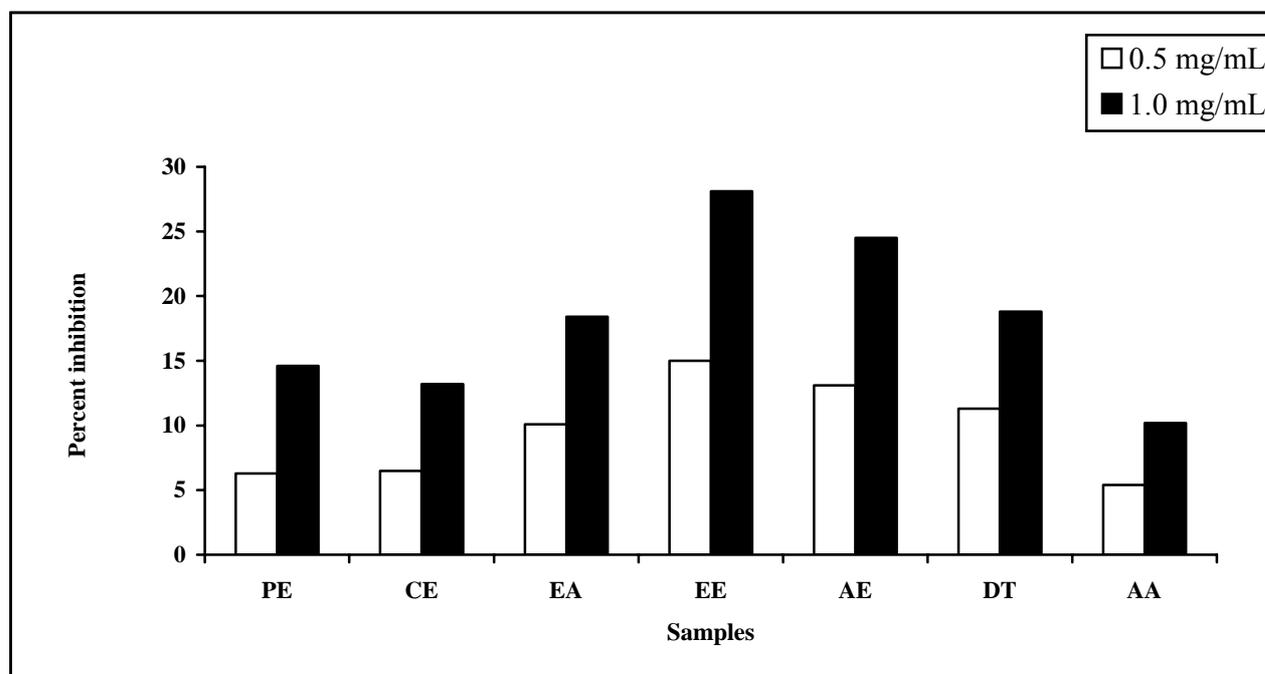


Figure-1. The antioxidant activity of different extracts of bark of *M. ferrea* Linn

Percent inhibition of the glycosylation of haemoglobin was measured at two concentrations of the petroleum ether extract (PE), chloroform extract (CE), ethyl acetate extract (EA), ethanol extract (EE), aqueous extract (AE), D- α - tocopherol (DT) and ascorbic acid (AA).

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