In-vitro Estimation of Non-enzymatic Antioxidants from *Ficus racemosa* (Linn.) and *Caesalpinia bonducella* (Linn.)

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ABSTRACT

Introduction: Excessive formation of free radicals and reactive oxygen species can have deteriorating effects on the human body as it leads to oxidative stress. Non-enzymatic scavenging systems play a positive role by neutralizing these free radicals and reactive oxygen species. Thus identifying natural and safe sources like plants and herbs becomes necessary.

Aim/Objective: The present study was carried out to determine the non-enzymatic antioxidants of some medicinally important plants like *Ficus racemosa* Linn. And *Caesalpinia bonducella* (Linn.)

Methods: The non-enzymatic antioxidants were extracted from the plant parts like leaves, bark and seeds using different solvents and the non-enzymatic antioxidants like water soluble vitamins (Vitamin C, Thiamine, Riboflavin & Niacin), fat soluble vitamins (Vitamin A & Vitamin E) were estimated using standard methods.

Results: The study revealed that the leaf extract of *Ficus racemosa* Linn. showed better antioxidant activity as compared to *Ficus racemosa* Linn. bark extract. So also, extract of *Caesalpinia bonducella* Linn. seed kernels showed more potential antioxidant levels than the *Caesalpinia bonducella* seed extract.

Conclusion: Current study thus presents new natural sources of antioxidant that can replace the synthetic ones to be used in foods, pharmaceuticals and cosmetics industries.

Key Words: *Ficus racemosa* (Linn.), *Caesalpinia bonducella* (Linn.), Non-enzymatic antioxidants, Plant parts - leaves, Bark, Seeds and kernels

INTRODUCTION

Oxidative stress plays a major role in the development of several chronic and degenerative ailments like cancer, arthritis, aging, autoimmune disorders, cardiovascular and neurodegenerative diseases. One of the mechanism to counteract oxidative stress is production of antioxidants. These are either naturally produced in situ, or externally supplied through foods and/or supplements.¹ Antioxidants do the “mopping up” of free radicals contributing towards the safety of our body from the harmful effect of oxidative stress.² Antioxidants thus act as cell protectors, they bind to these free radicals, converting them into non-damaging compounds and repairing cellular damage.³

There are two major classes of antioxidants i.e., enzymatic and non-enzymatic. The enzymatic antioxidants are produced endogenously which include superoxide dismutase,

catalase and glutathione peroxidase etc. The non-enzymatic antioxidants include alpha-tocopherols, carotenoids, ascorbic acid, flavonoids and tannins etc., which are obtained from natural plant sources and form a part of regular diet.⁴ A wide range of antioxidants, both from natural and synthetic origin, have been suggested for use in the treatment of various human diseases.⁵

Ficus racemosa Linn. (Moraceae) or Cluster Fig Tree, also most popularly known as ‘Audumbar’ or ‘Umbar’ in Maharashtra. The tree is usually held sacred and associated with the Hindu deity, “Shri Guru Datta”. The fruits of the tree grow very close to the tree trunk and are called ‘Gular’ in north India. They are eaten as the vegetable by the villagers. The tree also serves as a food plant for the caterpillars. In Indian traditional system of medicine all parts of the tree viz. leaves, fruits, bark, latex, and sap of the root, are reported to be medicinally important.⁶

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Caesalpinia bonducella Linn. Commonly called as 'Karanja' or 'Sagargota' in Maharashtra. It is a large, irregular, very thorny hedging plant usually flowering in June and it fruits in November. Sagargota seeds are very hard, globular, with smooth and shiny surface and grey in colour. Seeds are composed of thick brittle shell with a yellowish white bitter fatty kernel.⁷ Plant is reported to have multiple therapeutic properties like antidiabetic, antibacterial, anti-anaphylactic and antidiarrhoeal, anti-asthmatic, antiviral, antiamoebic and antiestrogenic, antipyretic, antihelminthic activity.⁸

Researchers are in relentless pursuit for developing newer strategies to increase the overall quality and shelf life of the food products especially by reducing or impeding the oxidative damage. Inclusion of the edible medicinal plant powders with natural antioxidant properties can be used as one of the strategy to delay the oxidation process in biomolecules and hence has great potential as natural preservatives substituting the synthetic one. Many herbs have proven to have natural antioxidants and are being used in the formulation of ayurvedic and modern drug dosage forms.⁸

The present study was thus undertaken with the aim to extract and quantitatively estimate the non-enzymatic antioxidants in *Ficus racemosa* Linn. (leaves and bark) Linn. And *Caesalpinia bonducella* Linn. (seeds and kernels)

MATERIALS AND METHODS

Collection of the plant samples:

Leaves and bark sample of *Ficus racemosa* Linn. were collected from Mumbai region. *Caesalpinia bonducella* Linn. seeds were bought from the commercial market for herbal medicine in Mumbai. The leaves, bark and seeds were washed and cleaned. Kernels were separated from the seeds.

Washing, drying and preparing plant powder:

The fresh plant samples were brought to the Biochemistry laboratory of the Somaiya College and were washed thoroughly for two to three times under running tap water and then once with distilled water to remove all the possible impurities like dust, dirt, etc. Then the washed samples were kept in between the filter paper padding to remove the maximum amount of water and moisture and then shade dried till the samples were perfectly dry viz. contained no moisture.

In case of *Caesalpinia bonducella* Linn., kernels were separated from the seeds. Then all the plant samples were transferred to the incubator set at 37°-40°C for drying. The samples were kept there for 3-4 days until the samples were completely dried. Then the dried samples were crushed to fine pieces and pulverised in the grinder mixer to fine powder. The fine powder so obtained was sieved and stored in a neatly labelled air-tight container and refrigerated. The sam-

ples thus prepared, were used as and when required.

Preparation of the sample for estimation of water soluble vitamins:

Extraction of Thiamine from plant sample.^{9,10}

2.0gms of finely ground plant sample was weighed accurately into a 100ml conical flask in duplicate. 50ml of 0.1N H₂SO₄ was slowly added without shaking, stopper the flask and allowed it stand to overnight. Next morning the flask was shaken vigorously and the contents were filtered through Whatman No. 1 filter paper, discarding the first 10-15ml of the filtrate.

Extraction of Riboflavin from plant sample.^{9,10}

2.0gms of ground plant sample was weighed into a conical flask. 15ml of 0.1N H₂SO₄ was added to it and mixed. The flask was kept in boiling water bath for 30mins constant shaking after every 5mins. The flask was then allowed to cool down at room temperature. 1ml of 2.5M sodium acetate solution was added and mixed and allowed to stand for at least 1hr. The volume was made to 20ml with distilled water and filtered through Whatman filter paper No.1. The collected filtrate was used for the assay.

Extraction of Niacin from plant sample.^{9,15}

6.0gms of dried plant sample was weighed in a 500ml conical flask. 40ml of 0.5M H₂SO₄ was added to it and was autoclaved at 1 bar for 30mins. It was cooled down at R.T. and 50% NaOH was added to adjust pH to 4.5. The volume was made to 50ml with distilled water and filtered through Whatman filter paper No.1. The filtrate was then used for the assay.

Extraction of Vitamin C from plant sample for colorimetrically estimation^{9,10}

2.5gms of plant sample was ground either mechanically or using a mortar and pestle in 50.0ml of 4% Oxalic acid. It was then centrifuge or filtered and the filtrate was collected. 10ml aliquot of the filtrate was then transferred to a conical flask to which bromine water was added drop wise with constant stirring. Bromine removes the enolichydrogen atoms in ascorbic acid. When the extract turned orange yellow due to excess of bromine, it was expelled out by blowing in air. The final volume of was made to 25ml with 4% Oxalic acid. Similarly, 10ml of stock ascorbic acid was converted into its dehydro form by bromination.

Preparation of the sample for estimation of fat soluble vitamins:

Extraction of Retinol from plant sample.^{9,16}

2gms of the dried plant powder was weighed and was kept in Soxhlet extraction in petroleum benzene (40-60) for 2 hrs.

The petroleum benzene was distilled out and the residue was obtained. The residue was reconstituted in 5ml of n-Heptane and used.

Extraction of Tocopherol from plant sample^{9,16}

2gms of the dried plant powder was weighed and kept for soxhlet extraction in petroleum benzene (40-60) for 2 hrs. The petroleum benzene was distilled out and the residue was obtained. The residue was reconstituted in 5ml of ethanol and used.

Estimation of Non-enzymatic Antioxidants

The estimations of the non-enzymatic antioxidants were done as follows -

- i) Estimation of Thiamine by Thiochrome method^{9,10}
- ii) Estimation of Riboflavin Spectrophotometrically^{9,10}
- iii) Estimation of Niacin Spectrophotometrically by Cyanogen bromide method^{9,10}
- iv) Estimation of Ascorbic acid (Vitamin C) colorimetrically by DNPH method^{9,10}
- v) Estimation of Retinol (Vitamin A) colorimetrically by Carr-Price method^{9,11, 12, 13}
- vi) Estimation of Tocopherol colorimetrically (Vitamin E)^{9,11, 14}

RESULTS

The non-enzymatic antioxidants levels were studied from the leaves and bark samples of *Ficus racemosa* Linn. plant and seed and kernel samples of *Caesalpinia bonducella* Linn. either by the spectrophotometric method or the colorimetric method methods and all the results are shown in Table 1.

Thiamine content of the *Ficus racemosa* Linn. leaf extract was found to be 1.52 ± 0.057 gm%w/w which was twice the amount of thiamine content in the *Ficus racemosa* bark (0.89 ± 0.063 gm% w/w). Riboflavin was found to be equal (~ 0.02 gm%w/w) in both the leaf and bark samples of *Ficus racemosa*. Linn. Niacin content was found to be the least of all the other non-enzymatic antioxidants of *Ficus racemosa* Linn. samples tested. Niacin was found to be equal in both the samples of *Ficus racemosa* Linn. $\sim 0.7 \times 10^{-3}$ gm%w/w. The results obtained showed more amount of vitamin C in *Ficus racemosa* Linn. leaf extract (0.361 ± 0.024) gm% w/w sample as compared to *Ficus racemosa* Linn. bark (0.278 ± 0.028 gm% w/w). Retinol content was found to be more in *Ficus racemosa* leaf sample (0.040 ± 0.054 gm%w/w) than the bark sample (0.029 ± 0.048 gm%w/w).

Tocopherol content was observed to be 0.282 ± 0.047 gm % w/w in *Ficus racemosa* Linn. leaf sample and 0.153 ± 0.039 gm %w/w in the bark sample.

Amount of thiamine in the *Caesalpinia bonducella* Linn. seed Kernels was found to be 1.03 ± 0.051 gm%w/w and in *Caesal-*

pinia bonducella Linn. seed was 0.85 ± 0.023 gm%w/w. Riboflavin content was high in *Caesalpinia bonducella* Linn. seed sample 0.153 ± 0.062 gm%w/w; whereas it was only 0.049 ± 0.0028 gm%w/w in the seed kernel sample. Niacin content was found to be the least of all the other non-enzymatic antioxidant of *Caesalpinia bonducella* Linn. samples tested. Niacin level was more in seed kernels of *Caesalpinia bonducella* Linn. $2.290 \pm 0.069 \times 10^{-3}$ gm%w/w) and it was $0.899 \pm 0.033 \times 10^{-3}$ gm%w/w in seed sample. Vitamin C content estimated colorimetrically was found to be 0.488 ± 0.065 gm% w/w in *Caesalpinia bonducella* Linn. seed sample and 0.806 ± 0.037 gm%w/w in seed kernels. Retinol content was found to be almost same in both the samples (i.e. 0.026 ± 0.032 gm% w/w and 0.029 ± 0.061 gm%w/w respectively). Tocopherol content was found to be more in *Caesalpinia bonducella* Linn. seed kernels 0.071 ± 0.026 gm%w/w than the seed sample 0.047 ± 0.025 gm%w/w.

DISCUSSION

In a report by Kumar et al. 2010¹⁷, it is reported that the methanol extract of leaves of *Caesalpinia bonducella* Linn. showed the presence of vitamin C which is a determined factor in controlling and potentiating many aspects of host resistance to cancer. Also, vitamin C can protect cell membranes and lipoprotein particles from oxidative damage by regenerating the antioxidant form of vitamin E. Thus it can be said that vitamin C and E act synergistically in scavenging a wide variety of reactive oxygen species. Similar kind of antioxidant effect may be expected from the seed and seed kernel extracts of *Caesalpinia bonducella* Linn.

Overall when searched not much data was available on such kind of non-enzymatic antioxidant studies from leaf and bark extract of *Ficus racemosa* Linn. And seed kernel and seed extract of *Caesalpinia bonducella* Linn. Hence our study may be considered as the first to report the same and may serve the purpose of reference material for the other researchers in future.

CONCLUSION

Thus, it was observed that *Ficus racemosa* Linn. (leaf) and *Caesalpinia bonducella* Linn. (seed kernels) showed good antioxidant activity as compared to *Ficus racemosa* Linn. (bark) and *Caesalpinia bonducella* Linn. (seeds) samples. In future, more work on antioxidant content and its mechanism can be aimed to determine the efficacy of these non-enzymatic antioxidants. Current study thus presents new natural sources of antioxidant that can replace the synthetic ones to be used in foods, pharmaceuticals and cosmetics industries.

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Table 1: The non-enzymatic antioxidants levels of *Ficus racemosa* Linn. plant (leaves and bark) and *Caesalpinia bonducella* Linn. (seeds and kernels)

Sample	<i>Ficus racemosa</i> Linn. Leaves	<i>Ficus racemosa</i> Linn. Bark	<i>Caesalpinia Bonducella</i> Linn. Seed	<i>Caesalpinia Bonducella</i> Linn. Kernel
Thiamine	1.52 ± 0.057	0.89 ± 0.063	0.85 ± 0.023	1.03 ± 0.051
Riboflavin	0.028 ± 0.034	0.022 ± 0.044	0.153 ± 0.062	0.049 ± 0.0028
Niacin	0.723 ± 0.042 X 10 ⁻³	0.788 ± 0.021 X 10 ⁻³	0.899 ± 0.033 X 10 ⁻³	2.290 ± 0.069 X 10 ⁻³
Vit. C	0.361 ± 0.024	0.278 ± 0.028	0.488 ± 0.065	0.806 ± 0.037
Retinol	0.040 ± 0.054	0.029 ± 0.048	0.026 ± 0.032	0.029 ± 0.061
Vit. E	0.282 ± 0.047	0.153 ± 0.039	0.047 ± 0.025	0.071 ± 0.026

Note:

- All reading are expressed in gm% (W/W)
- All values are expressed as mean ± SD for three determinations