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RESEARCH ARTICLE

INFLUENCE OF AGEING ON THE PHYTOCHEMISTRY AND ANTIOXIDANT PROPERTY OF THE LEAVES OF *ARTOCARPUS HETEROPHYLLUS* LAM

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ABSTRACT

The study analyses age related variations in the phytochemistry and antioxidant property of *Artocarpus heterophyllus* Lam. leaves. Aqueous and ethanolic extracts of tender, mature dark green and old yellow leaves were used for the analysis. Leaves of different ages were collected from the same plant. Qualitative phytochemical analysis revealed the presence of carbohydrate, protein, cardiac glycosides, terpenoids, phenols, saponin, tannin, steroids and coumarin in all extracts. Flavanoids were detected only in the old yellow leaves and the presence of amino acids was noticed only in the tender leaves. Comparatively higher concentrations of various secondary metabolites were detected in the older leaves. Antioxidant activity was higher in the alcoholic extract of older leaves followed by that of tender leaves. Results of the study indicate the importance of detailed investigation on the influence of ageing on the phytochemistry and bioactivities of *A.heterophyllus* for the proper utilization of this plant.

INTRODUCTION

More than three quarters of the world population relies mainly on plants and plant extract based drugs for health care needs. A plant can be considered as a biosynthetic laboratory, not only for their primary metabolites such as carbohydrates, proteins and lipids that are utilized as food by man, but also for a multitude of compounds like glycosides, alkaloids, volatile oils, tannins etc.. These compounds are used to perform specific functions in plants and found to mediate their effects on the human body through processes identical to those already well understood for the chemical compounds in conventional drugs. The genus *Artocarpus* (Moraceae) comprises about 50 species of evergreen and deciduous trees. Economically, the genus is of appreciable importance as a source of edible fruit, yield fairly good timber and is widely used in folk medicines. *Artocarpus heterophyllus* (Jack fruit) is perhaps the most widespread and economically important *Artocarpus* species, both providing fruit and functioning as a visual screen and ornamental (Elevitch and Manner, 2006). Several pharmacological studies of the natural products from *Artocarpus* have conclusively established their mode of action in treatment of various diseases and their health benefits. Since green and old yellow leaves and petioles of this plant are used differently in traditional medicine preparation, a detailed investigation is needed into the age related variations in the phytochemical properties of these leaves. With this view, the current project has been undertaken to analyze the influence of ageing on the

phytochemical and antioxidant properties of the tender, mature and old yellow leaves of *A.heterophyllus*.

MATERIALS AND METHODS

The leaves of three different ages viz: terminal tender, mature dark green and freshly fallen old yellow leaves of *Artocarpus heterophyllus* were collected from the same plant and subjected to microwave oven drying at 50°C after removing the petioles. The dried materials were ground to fine powder by a domestic grinder and stored in containers till further use. The extracts of the leaves were prepared with distilled water and ethanol.

Ethanol extraction: 10 gm each of the fine powder was extracted with 100ml of ethanol by keeping the solution for a period of two days in a magnetic stirrer. After two days, ethanol extracts were obtained by sieving to separate the extract from the residue. Residue was then rinsed three times with 5ml ethanol followed by filtration for complete separation of the extract from the residue

Aqueous extraction: 10 gm each of the fine powder was weighed out and transferred into separate conical flasks each containing 100 ml of distilled water. These were left to stand for 24 hrs. in a magnetic stirrer. After 24 hrs, the aqueous extracts were obtained by sieving to separate the extract from the residue. It was followed by rinsing the extracts with 5ml distilled water each time followed by filtration to complete the separation of the extract from the residue.

Preliminary Phytochemical Analysis: The ethanolic and aqueous extracts were subjected to preliminary phytochemical analysis by adopting standard methods given by Harborne (1973) and Sofowora (1982). Primary metabolites analyzed were carbohydrate (Molisch Test), sugar (Benedict's Test), ketose (Seliwanoff's Test), proteins (Lowry's Test), aminoacids (Ninhydrin Test), and fats (Filter Paper Test). Secondary metabolites tested were quinone (H₂SO₄ Test), cardiac glycosides (Keller-Killani Test), steroids (Salkowski Test), flavonoids (Shinoda Test), alkaloids (Mayer's Test), phenols (Folin Test), saponin (Foam Test), tannin (Lead Acetate Test), coumarin, and terpenoids (Salkowski Test).

DPPH based free radical scavenging activity: The antioxidant activity of the plant extracts was estimated using DPPH radical scavenging protocol (Blois, 1958). The assay was carried out in triplicate. The decrease in optical density of DPPH solution read at 523 nm on addition of test samples in relation to the control was used to calculate the antioxidant activity, as percentage inhibition of DPPH radical. The capability of scavenging DPPH radical was calculated using the following equation:

$$\text{DPPH Scavenged (\%)} = \frac{(\text{A control} - \text{A test}) \times 100}{(\text{A control})}$$

Where "A control" is the absorbance of the control reaction and "A test" is the absorbance of the sample containing plant extracts.

RESULTS AND DISCUSSION

The results of qualitative phytochemical analysis of leaves of different ages revealed the presence of various metabolites like carbohydrate, protein, cardiac glycosides, terpenoids, phenols, saponin, tannin, steroids and coumarin (Table 1). Flavanoids were detected only in the old yellow leaves and the presence of amino acids was detected only in the tender leaves. All the secondary metabolites were present in comparatively higher concentration in older leaves as indicated by the high intensity color development in the test solution. Free radical scavenging assay revealed that aqueous extracts of all the leaves possessed significantly lesser antioxidant activity compared to alcoholic extracts (Table 2.a). Alcoholic extract of old yellow leaves possessed higher antioxidant activity with IC₅₀ of 58.64 µg/ml, followed by alcoholic extract of tender leaves (IC₅₀-76.7 µg/ml) (Table 2.b). Mature green leaves showed comparatively lesser activity both in aqueous and ethanolic extracts. Quantitative estimation of secondary metabolites like alkaloids, saponins, flavanoids and phenolics in jackfruit seeds was done by Deepika *et al.* (2011) and Sirisha *et al.* (2014) and their results established the correlation between polyphenolic content and antioxidant properties of the seeds. In the current study, presence of appreciable amount of flavanoids, a polyphenol was detected in old leaves, while it was found to be absent in mature leaves. This age wise variations in the flavanoid content of the leaves might have contributed to the corresponding changes in their antioxidant property. Results of the current study establishes the high potential of old yellow leaves of *Artocarpus heterophyllus* as an effective antioxidant agent that is valuable in the treatment of a number of diseases and disorders. No literature is available regarding the influence of ageing on the secondary metabolites content of *A. heterophyllus* leaves.

Table 1. Qualitative phytochemical analysis of *Artocarpus heterophyllus* leaves

| Primary/ Secondary metabolites | Tender | | Mature | | Old | |
|--------------------------------------|---------|---------|---------|---------|---------|---------|
| | Aqueous | Ethanol | Aqueous | Ethanol | Aqueous | Ethanol |
| Carbohydrate | +++ | +++ | +++ | +++ | +++ | +++ |
| Sugar | - | - | - | - | - | - |
| Ketose | - | - | - | - | - | - |
| Proteins | +++ | +++ | +++ | +++ | +++ | +++ |
| Fats | - | - | - | - | - | - |
| Quinone | - | - | - | - | - | ++ |
| Cardiac | - | + | +++ | +++ | +++ | +++ |
| Terpenoids | +++ | +++ | +++ | +++ | +++ | +++ |
| Phenols | +++ | +++ | +++ | +++ | ++ | +++ |
| Flavonoids | - | +++ | - | - | +++ | +++ |
| Saponins | + | - | +++ | - | +++ | - |
| Alkaloid | - | - | - | - | - | - |
| Tannin | +++ | +++ | +++ | - | +++ | +++ |
| Amino acid | +++ | +++ | - | - | - | - |
| Steroids | +++ | +++ | +++ | +++ | +++ | +++ |
| Coumarin | +++ | +++ | +++ | +++ | +++ | +++ |

Table 2.a. DPPH scavenging activity of aqueous extract of *A. heterophyllus* leaves

| Concentration (µg/ml) | % inhibition of DPPH | | |
|-----------------------|----------------------|--------------|--------------|
| | Tender | Mature | Old yellow |
| 20 | 9.35 ± 1.31 | 2.62 ± 1.44 | 14.75 ± 0.74 |
| 40 | 11.89 ± 0.93 | 7.86 ± 0.87 | 18.68 ± 0.94 |
| 60 | 30.99 ± 0.60 | 9.67 ± 0.41 | 27.21 ± 2.51 |
| 80 | 39.18 ± 0.87 | 10.49 ± 0.34 | 28.19 ± 0.19 |
| 100 | 46.00 ± 0.41 | 11.14 ± 1.14 | 29.50 ± 0.87 |

Table 2.b. DPPH scavenging activity of ethanolic extract of *A. heterophyllus* leaves

| Concentration (µg/ml) | % inhibition of DPPH | | |
|-----------------------|----------------------|--------------|--------------|
| | Tender | Mature | Old yellow |
| 20 | 25 ± 2.7 | 14.92 ± 0.67 | 26.39 ± 1.01 |
| 40 | 33.33 ± 2.14 | 23.5 ± 1.26 | 56.91 ± 1.15 |
| 60 | 45.45 ± 1.02 | 31.6 ± 2.3 | 77.05 ± 0.36 |
| 80 | 51.51 ± 1.06 | 33.48 ± 2.3 | 86.12 ± 0.97 |
| 100 | 60.60 ± 0.42 | 38.52 ± 1.84 | 90.31 ± 1.62 |

Hence a detailed exploration in this direction can open new possibilities in the utilization of this plant.

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