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IN VITRO ANTIOXIDANT ACTIVITY OF *MADHUCA INDICA L.*

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ABSTRACT
The present investigation was aimed to evaluate the phytochemicals and *in vitro* antioxidant activity of different parts of *Madhuca indica*. Qualitative phytochemicals analysis confirmed the presence of carbohydrates, tannins, flavonoids, quinones, glycosides, terpenoids, phlobatannins, alkaloids, phlobatannins, anthraquinones, coumarins, steroids and phytosteroids and absence of phenol, saponins and triterpenoids. *In vitro* antioxidant property was also carried out at five different concentrations (50, 100, 150, 200 and 250 µg/ml) of wood, bark and leaf by total antioxidant capacity, DPPH scavenging activity, hydrogen peroxide scavenging activity and reducing power assay. All the results were compared with standard ascorbic acid. Results showed that dose dependent antioxidant activity in all models. Among the three different parts, bark extract exhibited highest antioxidant activity followed by leaf and wood extract. Our result suggests that bark extract of *Madhuca indica* is a potential source of antioxidant which can be used to treat many free radical mediated diseases.

KEYWORDS: Antioxidant, bark, leaf, *Madhuca indica*, phytochemicals, wood.

INTRODUCTION
Free radicals are defined as molecules having an unpaired electron in the outer orbit. They are generally unstable and very reactive.[1] Free radicals may play an important role in the origin of life and biological evolution, implicating their beneficial effects on the organisms.[2] Majority of diseases like atherosclerosis, hyper tension, ischemic disease, alzheimer's disease, parkinsonism, cancer, diabetes mellitus and inflammatory conditions are caused by free radicals which are being considered to be primarily due to the imbalance between pro oxidant and antioxidant homeostasis.[3] Oxidative stress is a harmful condition that occurs when there is an excess of ROS and a decrease in antioxidant levels, this may cause tissue damage by physical, chemical, psychological factors that lead to tissue injury in human and causes different diseases.[4]

Antioxidant means "Against oxidation," antioxidants work to protect lipids from peroxidation by free radicals. Antioxidants are effective because, they are willing to give up their own electrons to free radicals.[5] Antioxidants acts as a defense mechanism that protects against oxidative damage and include compounds to remove or repair damaged molecules. Medicinal plants are used worldwide in management of healthcare problems since time immermorial and approximately 60-80% of the world's population still depending on the traditional medicine. Currently, the global demand of herbal medicine is increasing rapidly because of their safety margin and low cost. With the help of medicinal plants still many of the disease are getting cured.

Madhuca Indica commonly known as *Madhuca* belongs to the family of *Sapotaceae*, is an important economic plant growing throughout the subtropical region of the Indo-Pakistan subcontinent. Phytochemicals like carbohydrates, flavonoids, alkaloids, proteins, lipids, anthaquinones, coumarins, quinines, glycosides, terpenoids, cardiac glycosides, steroids and tannins were reported in this plant. Various parts of this plant are used as stimulants, demulcents, emollients, heating and astringents**.** The bark is a good remedy for itching, swellings, fractures and snake bites, as well as for diabetes mellitus. Mahua oil is used for the treatment of skin diseases, rheumatism, and headache and as a laxative. Fruits are astringent and largely employed as a lotion for chronic ulcers, in acute and chronic tonsillitis, and in pharyngitis.[6,7]

Bark and leaves of *Madhuca indica* have reported to have antioxidant[8,12], anticancer[13], antimicrobial[9,12], cytotoxic[12], anti-inflammatory[14], antipyretic.[15] antidiabetic and antihyperglycemic[16,18,11], anti-epileptic[19], antinociceptive and antidiarrhoeal activities.[20] Antiulcer activity[21,22], hepatoprotective[23] activity. In the present study, three different parts such as

wood, park and leaves of *Madhuca indica* were selected to evaluate their antioxidant efficacy.

MATERIALS AND METHODS

Collection of plant materials

The plant materials used in the present study are wood, bark, leaf of *Madhuca indica.* The plants were collected from in and around Madhukkur, Thanjavur district, Tamil Nadu, India. The collected samples were carefully kept in polythene bags. They were dried in shade and stored in air tight containers until further studies.

Preparation of extracts

The dried wood, bark, leaf are powered using grinder, 60 gm of each powered was packed evenly in the sox let extractor and subjected to extraction with 210ml ethanol. After extraction, the solvent was distilled off and the extracts were concentrated on water bath to a dry residue and kept in a desiccator. The crude extract was used for phytochemical screening[24] and *in vitro* antioxidant activity using various models such as Total antioxidant capacity[25], DPPH scavenging activity[26], Hydrogen peroxide scavenging activity[27] and reducing power assay.[28]

RESULTS AND DISCUSSION

Plants have been the basis of traditional medicines throughout the world for thousands of years and continue to provide new remedies to humankind; a great deal of effort has therefore focused on using available experimental to identify natural antioxidants from plants.[29] In the proposed study, selected plant parts of wood, bark and leaf of *Madhuca indica* were collected and ethanol extracts were prepared and subjected to phytochemical screening and in *vitro* antioxidant activity. The obtained results were recorded and tabulated and discussed in this chapter.

Preliminary phytochemical screening

Plants constitute a source of novel chemical compounds which are of potential use in medicine and other applications. Plants contain many active compounds such as alkaloids, steroids, tannins, glycosides, volatile oils, fixed oils, resins, phenols and flavonoids. These active constituents are deposited in the specific parts of the plants such as leaves, flowers, bark, seeds, fruits, root, etc. These phytochemicals have much pharmacological potential including antioxidant, antibacterial[30], antifungal[31], antidiabetic[32], anti-inflammatory[33] and radio-protective activity[34] and due to these properties those medicinal plants are largely used for medicinal purpose.

In the present study, qualitative analysis of different parts of *Madhuca indica* was investigated and represented in table 1. Carbohydrate, coumarins, cardiac glycosides, flavonoids, glycosides, quinones, steroids, tannins were present in wood extract. In bark extract, phytocontituents of anthaquinones, alkaloids, cardiac glycosides, glycosides, quinones, steroids, phlobatannins and terpenoids were identified. Leaf extract showed the presence of phytocontituents of carbohydrates, flavonoids, tannins. Results revealed that ethanol wood extract of plant showed high amount of phytochemicals (8/15) followed by bark (7/15) and leaf extracts (3/15).

In vitro antioxidant activity

Under normal conditions, Reactive Oxygen Species (ROS) are continuously produced and converted to non reactive species by scavenging mechanism of different intracellular enzymatic and non-enzymatic antioxidant system.[35] It is possible to reduce the risks of chronic diseases and prevent disease progression by either enhancing the body's natural antioxidant defenses or by supplementing with proven dietary antioxidants.[36] Thus, a practical way to control these diseases is to increase the dietary intake of fruits and vegetables, which are rich sources of antioxidants.[37,38] Since, the plant kingdom offers a wide range of natural antioxidants; recently there has been considerable interest in finding natural antioxidants from plant materials to replace synthetic ones. Moreover, these natural antioxidant substances are accepted to be secure and sound from the time when they occur in plant foods, and more enviable than synthetic one.

Hence, in the current study, wood, bark and leaf extract of *Madhuca indica* were subjected to analyze their antioxidant activity by various *in vitro* model such as total antioxidant activity, DPPH free radical scavenging assay, hydrogen peroxide scavenging activity and reducing power ability**.** Five different concentrations of (50, 100, 150, 200 and 250 µg /ml) wood, bark and leaf were evaluated for antioxidant activity. Antioxidant assay was also performed for standard ascorbic acid in order to compare the efficacy of selected plant parts. All the experiments were performed thrice and the results were averaged.

Total antioxidant activity

The total antioxidant capacity of the extracts were calculated based on the formation of the phosphomolybdenum complex which was spectrophotometrically measured at 695 nm. The total antioxidant activity of different concentrations (50, 100, 150, 200, 250 µg) of wood, bark and leaf extract of *Madhuca indica* were evaluated. Dose dependent antioxidant capacity was observed in all the selected parts and the percentage of antioxidant activity was found to be the range of 15.5% to 38.9%, 21.6% to 41.6% and 19.9% to 40.9% for wood, bark and leaf respectively. Percentage of antioxidant activity of ascorbic acid was found to be the range of 21.6% to 46.2% (table 2). Among the three different parts, bark extract exhibited maximum antioxidant activity but it was less than that of standard ascorbic acid. IC_{50} values were found to be 135µg/ml, 160µg/ml, 150 µg/ml and 140µg/ml for wood, bark, leaves and ascorbic acid respectively.

DPPH free radical scavenging assay

DPPH a stable free radical and any molecule that can donate an electron or hydrogen to DPPH, can react with it and thereby bleach the DPPH absorption. DPPH is a purple colour dye having absorption maxima of 517 nm and upon reaction with a hydrogen donor the purple colour fades or disappears due to conversion of it to 2, 2-diphenyl-1-picryl hydrazine resulting in decrease in absorbance.

In our study, wood, bark and leaf extracts showed the radical scavenging activity of 42.2%, 51.2% and 44.5% respectively at 250 µg /ml, whereas at the same concentration, ascorbic acid exhibited 46.2% inhibition (table 4). Bark extract exhibited highest radical scavenging activity followed by standard ascorbic acid, leaf and wood. IC_{50} of wood, bark and leaf extracts were found to be 140 µg/ml, 150µg/ml, 145µg/ml and 140µg/ml respectively which indicate the potency of scavenging activity of plant extracts. Standard ascorbic acid was found to have an IC_{50} 140 µg/ml.[39]

Hydrogen peroxide scavenging activity

Hydrogen peroxide itself is not particularly reactive with most biologically important molecules, but it is an intracellular precursor of hydroxyl radicals which is very toxic to the cell.[40] In the present study, different concentrations of wood, bark and leaf of *Madhuca indica* were subjected to hydrogen peroxide scavenging activity. The result showed the dose dependent response for all the extract. Wood extract showed the inhibition range of 15.6 -38.9%, bark extract showed the inhibition range of 21.6 - 41.6% and for the leaf extract the inhibition percentage were ranging from 19.9 to 40.9. Percentage of inhibition was found to be 21.6 - 46.2% for standard ascorbic acid (table 5).

IC_{50} value of wood, bark and leaf were 135µg/ml, 160µg/ml, 150µg/ml respectively. Standard showed the IC_{50} value 140µg/ml. Among the parts, bark showed the highest radical scavenging activity followed by leaf and wood but it was slightly less than that of standard ascorbic acid at 250 µg/ml. Plant extracts scavenged hydrogen peroxide, which might be attributed to the presence of phenolic group that donate electrons to hydrogen peroxide there by neutralizing it into water.[41]

Reducing power assay

Reducing power of the fractions was assessed using ferric to ferrous reducing activity as determined spectro photometrically from the formation of Perl's Prussian blue color complex.[42] Reducing power of different parts of *Madhuca indica* was evaluated in the current study which was compared with ascorbic acid. Highest reducing power of 0.25%, 0.51% and 0.42% were observed for wood, bark, leaf and for standard it was 46.2% at 250 µg/ml. The results confirmed the dose dependent response of all the parts of *Madhuca indica.* Among the parts, bark extract exhibited the potent reducing power. Moderate activity was observed in leaf extract and least activity was showed in wood extract of *Madhuca indica*. Plant extracts exhibited minimum activity than that of standard. IC_{50} value of wood, bark and leaf were 25µg/ml, 20µg/ml and 40µg/ml respectively. Standard showed the IC_{50} values 140µg/ml.

Table 1: Preliminary phytochemical screening of *Madhuca indica.*

S.No.	Phytochemicals	Results		
		Wood	Bark	Leaf
1	Anthaquinones	_	+	_
2	Alkaloids	_	+	_
3	Carbohydrates	+	_	+
4	Coumarins	+	_	_
5	Cardiac glycosides	+	+	_
6	Flavonoids	+	_	+
7	Glycosides	+	+	_
8	Phlobatannins	_	+	_
9	Phenols	_	_	_
10	Quinones	+	+	_
11	Saponins	_	_	_
12	Steroids	+	+	_
13	Triterpenoids	_	_	_
14	Terpenoids	_	+	_
15	Tannins	+	_	+

+ indicates presence
- indicates absence

Table 2: Total antioxidant activity of *Madhuca indica.*

S. No.	Concentration (µg/ml)	Total antioxidant activity in %			
		Plant extracts			Standard Ascorbic acid
		Wood	Bark	Leaf	
1	50	15.6±0.8	21.6±2.5	19.9±0.5	21.6±0.8
2	100	22.2±0.5	25.5±0.9	22.2±0.5	30.5±0.3
3	150	30.3±1.05	29.8±0.7	30.3±1.0	35.7±1.2
4	200	34.16±1.5	35.6±0.7	35.1±1.5	40.4±2.3
5	250	38.9±1.7	41.6±1.7	40.9±1.7	46.2±0.1
IC_{50} value (µg/ml)		**135**	**160**	**150**	**140**

All values are expressed as mean ±S.D for three determinations.

Table 3: DPPH scavenging activity of *Madhuca indica.*

S.No.	Concentration (µg/ml)	Reducing power assay in %			
		Plant extracts			Standard Ascorbic acid
		Wood	Bark	Leaf	
1	50	0.11±0.007	0.23±0.02	0.12±0.007	21.6±0.8
2	100	0.14±0.10	0.27±0.007	0.22±0.007	30.5±0.3
3	150	0.18±0.007	0.33±0.02	0.28±0.007	35.7±1.2
4	200	0.22±0.03	0.43±0.06	0.37±0.002	40.4±2.3
5	250	0.25±0.01	0.51±0.007	0.42±0.004	46.2±0.1
IC_{50} value (µg/ml)		**25**	**20**	**40**	**140**

All values are expressed as mean ±S.D for three determinations.

Table 4: Hydrogen peroxide scavenging activity of *Madhuca indica.*

S.No.	Concentration (µg/ml)	DPPH scavenging activity in %			
		Plant extracts			Standard Ascorbic acid
		Wood	Bark	Leaf	
1	50	16.9±0.51	18.60±2.8	19.3±5.1	21.6±0.8
2	100	23.2±1.2	26.20±3.1	24.8±2.4	30.5±0.3
3	150	31.3±1.2	34.03±1.45	31.1±1.3	35.7±1.2
4	200	33.±1.10	40.10±4.9	37.5±3.6	40.4±2.3
5	250	42.20±0.6	51.20±4.8	44.5±0.92	46.2±0.1
IC_{50} value (µg/ml)		**140**	**150**	**145**	**140**

All values are expressed as mean ±S.D for three determinations.

Table 5: Reducing power assay of ethanol extract of *Madhuca indica.*

S.No.	Concentration (µg/ml)	Hydrogen peroxide scavenging activity in %			
		Plant extracts			Standard Ascorbic acid
		Wood	Bark	Leaf	
1	50	15.6±0.8	21.6±2.5	19.9±0.5	21.6±0.8
2	100	22.2±0.5	25.5±0.9	22.2±0.5	30.5±0.3
3	150	30.3±1.05	29.8±0.7	30.3±1.0	35.7±1.2
4	200	34.16±1.5	35.6±0.7	35.1±1.5	40.4±2.3
5	250	38.9±1.7	41.6±1.7	40.9±1.7	46.2±0.1
IC_{50} value (µg/ml)		**135**	**160**	**150**	**140**

All values are expressed as mean ±S.D for three determinations.

CONCLUSION

From the result, it can be concluded that bark extract of *Madhuca indica* showed the highest antioxidant activity than leaf and wood extracts due to the presence of high number of phytochemicals. Further studies are needed to elucidate the *in vivo* antioxidant potential of the bark extract of *Madhuca indica* in the treatment of free radical induced human disease.

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