IN-VITRO FREE RADICALS SCAVENGING ACTIVITY OF *MADHUCA INDICA* GMEL

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Summary

The methanol and aqueous extracts of bark of *Madhuca indica* J. F. Gmel were explored for it's antioxidant potential. In the present study antioxidant potential was studied by using DPPH radical scavenging assay and reducing power assay. Methanol and aqueous extract significantly inhibited the DPPH free radical at the concentrations ranging from 25-150 µg/ml showed highest inhibition i.e. 81.70% and 57.38% at 150µg/ml respectively. Methanol and aqueous extract of *Madhuca indica* showed a very powerful antioxidant activity in DPPH radical-scavenging. IC_{50} were found to be 92.85µg/ml and 137.5µg/ml respectively. Methanol and aqueous extract of *Madhuca indica* also showed significant reductive ability. All the concentrations of methanol extract of *Madhuca indica* (MEMI) and aqueous extract of *Madhuca indica* (AEMI) showed significant antioxidant activity when compared to control and these differences were statistically significant ($p < 0.001$). *Madhuca indica* could be considered as a potential source of natural antioxidants.

Keywords: *Madhuca indica*, antioxidant activity, DPPH assay, free radicals scavenging activity

Introduction

Madhuca indica Gmelin. (Family Sapotaceae) is a medium to large sized deciduous tree grows throughout central India, Andhrapradesh, and Madhyapradesh. It is commonly known as Mahua. In our Traditional System of Medicine bark of *Madhuca indica* is used for rheumatism, ulcer, itches, bleeding, spongy gum and tonsillitis [1-4]. *Madhuca indica* is reported to have, inhibitory activity on free radical release from phagocyte [5], pesticidal activity, nematicidal activity [6]. The methanolic extract of flower stem and stem bark has been reported to have antibacterial activity against B. anthracis, B. pumilus, B. subtilis, Sal. Paratyphi, Vib. Cholerae, Xanth. Campestris [7].Free radicals are capable of attacking the healthy cells of the body, causing them to lose their structure and function. Cell damage caused by free radicals have been implicated in the pathogenesis of many disease such as aging, cancer, cardiovascular disease, cataracts, immune system decline, and brain dysfunction and degenerative diseases.Many medicinal plants contain large amounts of antioxidants other than vitamin C, vitamin E, and carotenoids. Many herb species, especially those belonging to the *Lamiaceae* family, show strong antioxidant activity. A number of phenolic compounds with strong antioxidant activity have been identified in these plant extracts. *Madhuca indica* has reported for presence of phenolic compounds.

The present investigation involves the evaluation of antioxidant potential of methanol and aqueous extract by using DPPH radical scavenging assay and reducing power assay .

Material and method

Plant material

Madhuca indica bark was collected from Khandawa district, Madhya Pradesh. The plant specimens were authenticated by Dr. Harshad M. Pandit, Botany Department, Gurunanak Khalsa College, Mumbai.

Preparation of extracts

The bark is cut into small pieces and dries at room temperature. The dried barks were subjected to size reduction to coarse powder by using dry grinder. This powder is packed into soxhlet apparatus and extracted with methanol [8]. The aqueous extract was prepared with maceration technique. The extracts were evaporated to dryness at 40^{o}C (yield: 12% w/w of methanol extract and 9% yield of water extract).

Preliminary phytochemical screening

A phytochemical screening of residues revealed the presence of phenolic compounds and tannins, saponins, proteins [9,10].

Evaluation of antioxidant activity:

Chemicals

DPPH solution (200µM) Phosphate buffer (0.2M, pH 6.6) (Potassium dihydrogen phosphate 0.2M): (NaOH 0.2M): Potassium ferricyanide (1%), TCA (10%), Ferric chloride (0.1%).

1. DPPH Free Radical Scavenging Assay:

1 mg extract powder was dissolved in 1 ml of 90% methanol solution to obtain 1000 µg/ml sample solution. 1000 µg/ml solutions were series diluted into concentration ranging from 25-150µg/ml (i.e. 25, 50, 75, 100, 125 and 150µg/ml). 200µM solution of DPPH in methanol was prepared and 1.5 ml of this solution was added to 1.5 ml of methanol extract solution at different concentrations (25-150µg/ml). Ascorbic acid was used as the standard control, with concentrations ranging from 2-20µg/ml (i.e. 2, 5, 10, 15 and 20µg/ml). Thirty minutes later, the absorbance was measured at 517 nm. The absorbance of DPPH solution decreases when kept in contact with antioxidant test sample and free radical scavenging activity is inversely proportional to the absorbance of DPPH solution [11]. The same procedure was followed, to study DPPH free radical scavenging assay of aqueous extract. Percent inhibition of DPPH free radical scavenging activity was calculated using the following formula,

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

Where Acont is the absorbance of the control reaction.

Atest is the absorbance in the presence of the sample of the extracts

2. Determination Of Reducing Power:

The total reducing power of Methanolic extract of *Madhuca indica* (MEMI) was determined according to the method of Oyaizu [12,13]. Different concentrations of MEMI (10, 20, 40, 60, 80 and100 µg/ml) in 1 ml of distilled water were mixed with phosphate buffer (2.5 ml, 0.2 M, pH 6.6) and potassium ferricyanide [$K_3Fe(CN)_6$] (2.5 ml, 1%). The mixture was incubated at 50ºC for 20 min. Trichloroacetic acid (2.5 ml, 10%) was added to the mixture, which was then centrifuged for 10 min at 3000 × g. The upper layer of solution (2.5 ml) was mixed with distilled water (2.5 ml) and FeCl3 (0.5 ml, 0.1%), and the absorbance was measured at 700 nm using a UV-Visible spectrophotometer. Increasing absorbance at 700 nm was interpreted as increasing reducing activity. The same procedure was followed, to study reducing power assay of aqueous extract of *Madhuca indica* (AEMI).

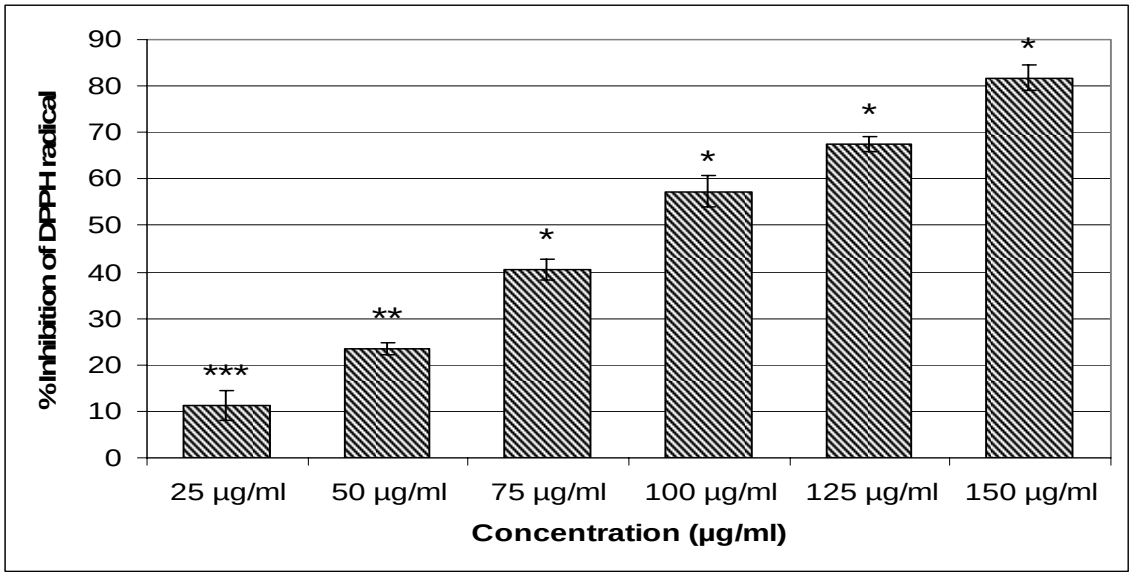
Ascorbic acid was used as the standard control with concentrations 10, 20, 40, 60, 80 and 100 µg / ml.

Result and discussion

DPPH Radical Scavenging Assay

MEMI and AEMI significantly inhibited the DPPH free radical at the concentrations ranging from 25-150 µg/ml showing highest inhibition i.e. 81.70% and 57.38% at 150µg/ml respectively (Figure No.1 and 2). The IC_{50} values of methanol and aqueous extracts were found to be 92.85µg/ml and 137.5µg/ml respectively. Ascorbic acid was used as reference standard for the DPPH free radical scavenging assay; it significantly inhibits DPPH free radical at the concentrations ranging from 2-20 µg/ml, showing highest % inhibition i.e. 91.26% at 20µg/ml (Figure No.3). The IC_{50} value obtained was found to be 9.22µg/ml. MEMI was showed significant inhibition of DPPH radicals as compared to AEMI.

Figure No: 1: DPPH radical scavenging assay of MEMI:



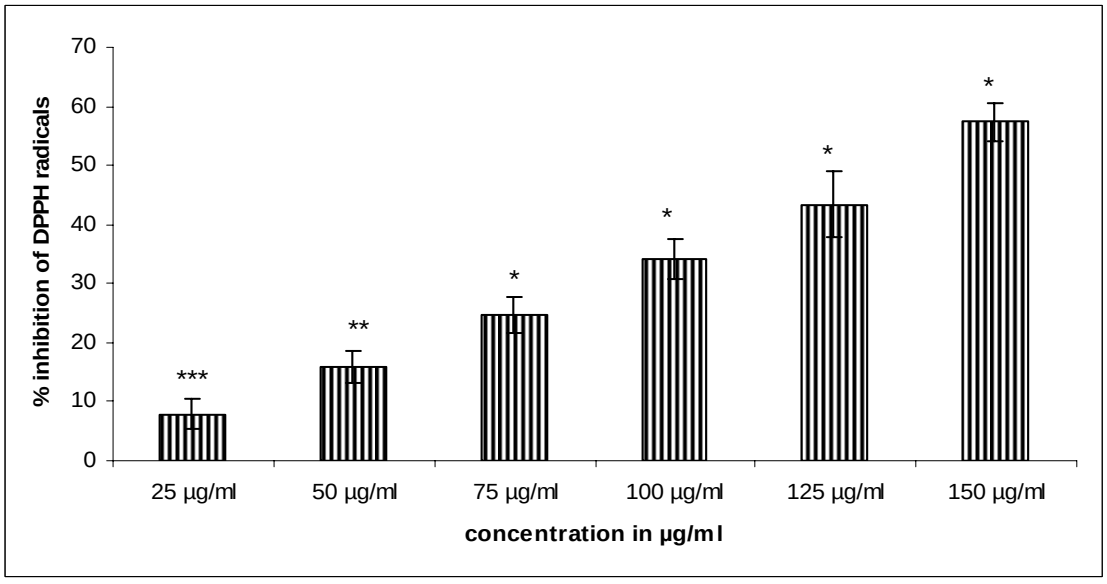
Values are expressed as a mean ± standard error of mean of 3 observations.

* Represents statistical significance: $p < 0.001$, when compared with control, n = 3

** Represents statistical significance: $p < 0.05$, n = 3.

*** Represents no statistical significance: $p > 0.05$, n = 3

Figure No: 2: DPPH radical scavenging assay of AEMI:



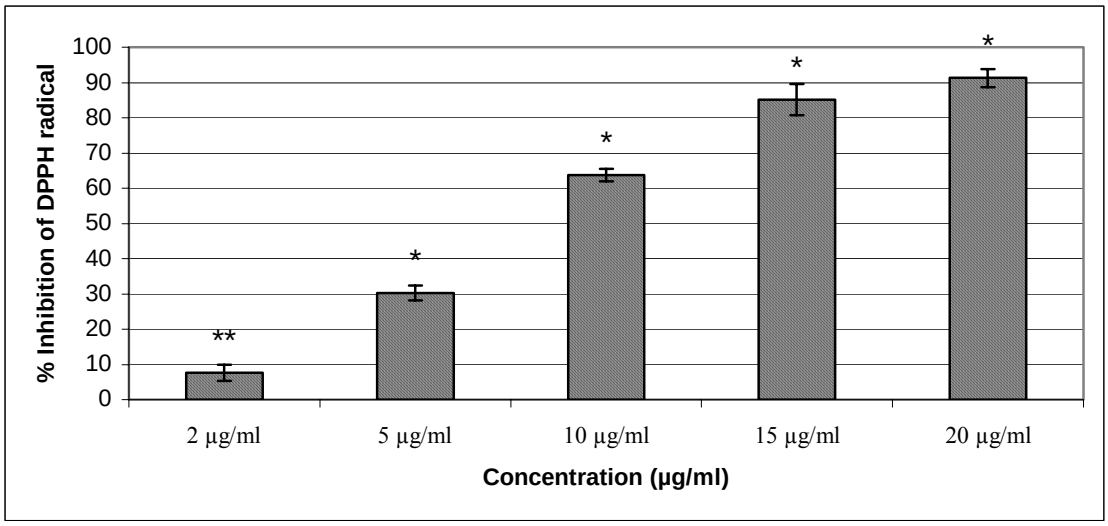
Values are expressed as a mean ± standard error of mean of 3 observations.

* Represents statistical significance: $p < 0.001$, when compared with control, n = 3

** Represents statistical significance: $p < 0.05$, n = 3.

*** Represents no statistical significance: $p > 0.05$, n = 3

Figure no: 3: DPPH Free radical scavenging activity of L-ascorbic acid:



Values are expressed as a mean ± standard error of mean of the 3 observations.
. * Represents statistical significance: $p < 0.001$, when compared with control, n = 3
****** Represents no statistical significance: $p > 0.05$, n = 3.

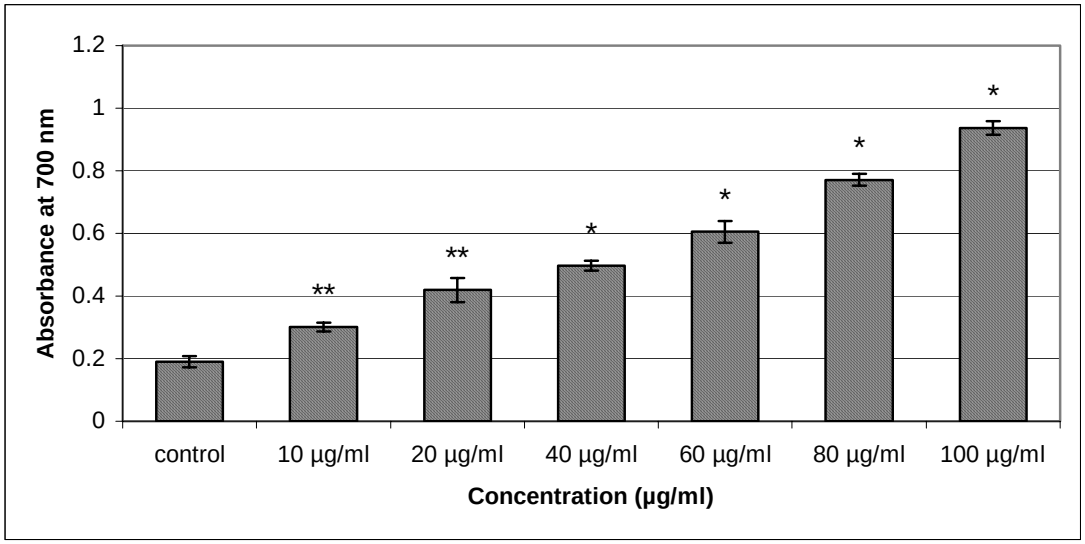
Reducing Power Assay

For the measurements of the reductive ability, we investigated the Fe^{3+}-Fe^{2+} transformation in the presence of methanol and aqueous extracts using the method of Oyaizu. The reducing capacity of a compound may serve as a significant indicator of its potential antioxidant. Reducing power of the selected diluted extract found to be significant ($p < 0.001$)(Figures 4-6). The antioxidant activity has been reported to be concomitant with development of reducing power. The reducing power of methanol and aqueous extracts were found to be increase with increasing amount of extracts concentration. All the concentrations of methanol and aqueous extracts were showed significant activity when compared to control and these differences were statistically significant ($p < 0.001$)

It has been studied that damages caused by free radical induced oxidative stress is the major causative agent of many disorders including cancer, tissue injury, and rheumatoid arthritis[14]. neurodegenerative diseases, aging[15]. Oxidative stress major pathological factor for many disease.

DPPH is a stable radical that has been used widely to evaluate the antioxidant activity of various natural products. In recent years, antioxidants derived from natural resources, mainly from plants, have been intensively used to prevent oxidative damages. Natural antioxidants have also some advantages over synthetic ones. They can be obtained easily and economically and have slight or negligible side effects Many plants have been announced to possess antioxidant activity etc [16-18]. Natural antioxidants have also some advantages over synthetic ones. The present study shows prominent antioxidant activity of *Madhuca indica*, the antioxidant activity may be due to phenolic compounds in *Madhuca indica* extract. However, the components responsible for the antioxidant activity of MEMI are not clear. Therefore, further work is necessary to isolate and characterize those constituents.

Figure No:4: Reducing power of MEMI. at 700 nm.

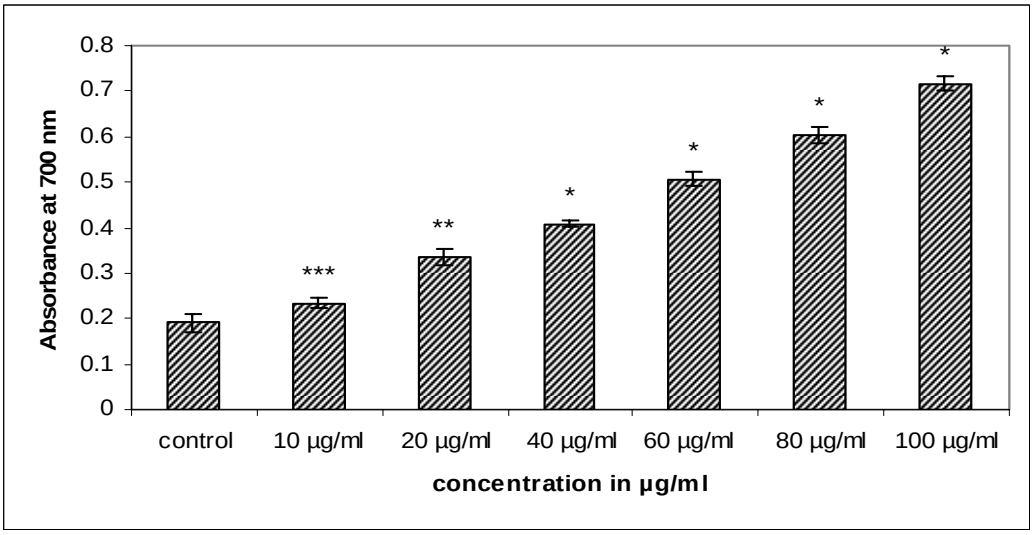


Values are expressed as a mean ± standard error of mean of the 3 observations.

* Represents statistical significance: $p < 0.001$, $n = 3$

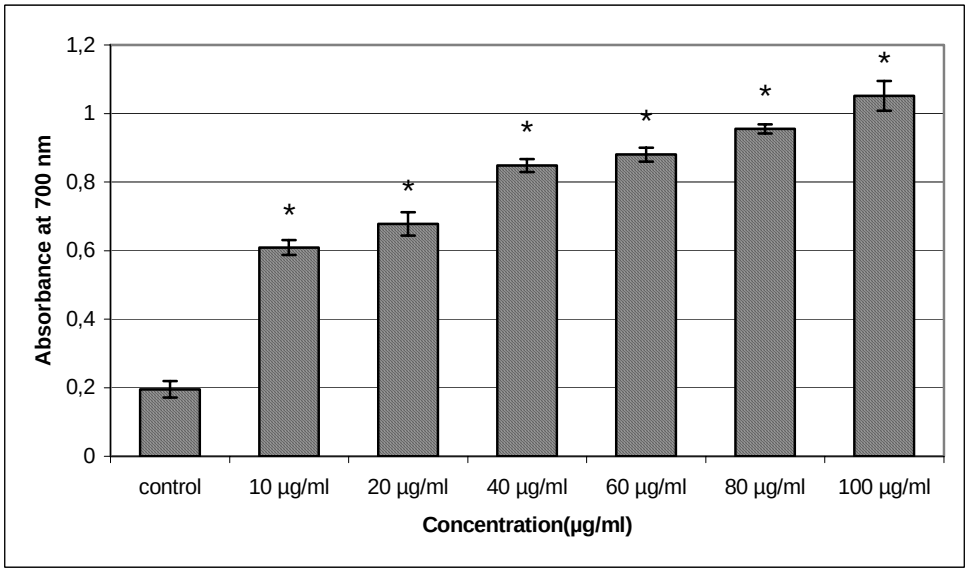
** Represent statistical significance: $p < 0.05$, $n = 3$

Figure No:5: Reducing power of AEMI. at 700 nm.



Values are expressed as a mean ± standard error of mean of 3 observations.
* Represents statistical significance: $p < 0.001$, when compared with control, $n = 3$
** Represents statistical significance: $p < 0.05$, $n = 3$.
*** Represents no statistical significance: $p > 0.05$, $n = 3$

Figure No: 6: Reducing power of L-ascorbic acid at 700 nm:



Values are expressed as a mean ± standard error of mean of the 3 observations.
* Represents statistical significance: $p < 0.001$, $n = 3$

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