ISSN Print: 2394-7500
ISSN Online: 2394-5869
Impact Factor: 5.2
IJAR 2016; 2(9): 849-851
www.allresearchjournal.com
Received: 28-07-2016
Accepted: 29-08-2016

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Screening for antioxidant potential in methanolic leaf extract of *Madhuca Indica* L.

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Abstract
The antioxidant activity of the methanolic extract of the leaves of *Madhuca indica* was evaluated by free radical scavenging activity using 1, 1-diphenyl-2-picrylhydrazil (DPPH), reducing power assay. The results of the assay were then compared with a natural antioxidant ascorbic acid (vitamin C) and gallic acid. The Methanolic extract of the Leaves of *Madhuca indica* is a potent source of compounds with antioxidant properties while the extract also exhibited significant free radical scavenging activity. The methanolic extract of *Madhuca indica* was subjected to preliminary phytochemical studies. The results indicate the presence of alkaloids, flavonoids, proteins, and carbohydrates.

Keywords: DPPH, antioxidant, *Madhuca indica*, ascorbic acid

Introduction
Reactive oxygen species and free radicals play an important role in the initiation and evolution of numerous diseases. The use of compounds with antioxidant activity is expected to be useful for the treatment of these diseases. Therefore, there has been a growing interest in finding novel antioxidants in order to meet the requirements of pharmaceutical industries[1]. *Madhuca indica* belonging to family *Sapotaceae* is an important economic tree growing throughout India. Traditionally *Madhuca indica,* L. Leaves have been used as an Anti-diabetic, Rheumatism, Ulcers, Bleedings and Tonsillits[2]. The flowers, seeds and seed oil of *Madhuca* have great medicinal value. Externally, the seed oil massage is very effective to alleviate pain. In skin diseases, the juice of flowers is rubbed for oleation. It is also beneficial as a nasya (nasal drops) in diseases of the head due to pitta, like sinusitis. The external applications are in skin infections, analgesic, anti-pyretic, anti-oxidant and anti-diabetic. The purpose of the present study was to evaluate the antioxidant activity of the methanol leaves extract of *Madhuca indica.*

2. Material and Methods
2.1 Plant material
Disease free leaves were collected and identified by following the flora of Marathwada by V.N. Naik. The collected leaves were surface sterilized with 0.1% mercuric chloride & then washed with D/W 2-3 times separately & shade dried. Fine powder were made after complete drying and used for the experimental work.

2.2 Solvent Extraction of Leaves
Extracts were made in 80% methanol at room temperature by simple extraction method **(***Deshpande et al)***.** 10 gm dried powder of leaves mixed with 100ml solvent in 250 ml flask and were kept on shaker for 24 hrs. Then it was allowed to stand for the 30 min to stand the plant material. Thereafter it was filtered & centrifuged at 5000 rpm for 15 min.The supernatant was collected &solvent was evaporated at 45 ^{0}C in rotary evaporator to make the final volume 1/5 of the original volume.

2.3 DPPH free radical scavenging activity
The free radical scavenging activity was followed by the DPPH method. 0.1 mM solution of DPPH in methanol was prepared and 1.0 ml of this solution was added to 3.0 ml of extract solution in methanol at different concentration (50-250 µg/ml).

Thirty minutes later, the absorbance was measured at 517 nm. A blank was prepared without adding extract. Ascorbic acid at various concentrations (50 to250 µg/ml) was used as standard. Lower the absorbance of the reaction mixture indicates higher free radical scavenging activity. The capability to scavenge the DPPH radical was calculated using the following equation:

DPPH Scavenged (%) = [(A control- A test) / A control] × 100

Where A control is the absorbance of the control reaction and A test is the absorbance in the presence of the sample of the extracts. The antioxidant activity of the methanolic leaves extract was expressed as IC50 and compared with standard. The IC50 value was defined as the concentration (in µg/ml) of extracts. That scavenges the DPPH radicals by 50%.

3. Results

DPPH free radical has strong electron attracting ability from antioxidant. The graph showed that the conc. of methanolic extract is directly proportional to Absorbance. The Maximum scavenging activity is shown at the lowest conc.200µl/ml which was 3.50 %. The order of five scavenging activity is 50>100>150>200<250 µl/ml. (Table1.) The percentage of scavenging activity for methanolic extract of *Madhuca indica* L. leaves is inversely proportional to absorbance. Ascorbic acid was used as control. The TLC analyzed result of methanolic extract of *Madhuca indica* L with mobile phase Chloroform: Methanol: ethyl acetate (8.0:1.5:1.0)**.**Mean of the R_f values of TLC analysis is 0.57**.**

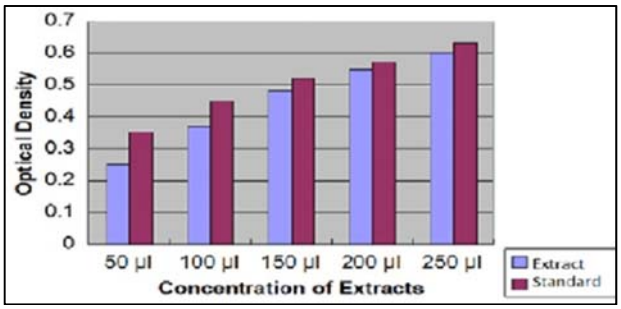
4. Discussion

Antioxidant assay revealed that the free radical scavenging activity showed by extract at different concentration. The minimum %scavenging activity at 200 µl/ml was 3.50% and highest activity at 50 µl/ml was 28.57% which was nearer to the standard activity of ascorbic acid. The reduction capability of DPPH radicals was determined by the decrease in its absorbance at 517 nm, which is induced by antioxidants. Table 1 shows the percentage of DPPH radical scavenged by ascorbic acid and methanolic extract of leaves at various concentrations (µg/ml). A substance may act as an antioxidant due to its ability to reduce reactive oxygen species by donating hydrogen atom.

The reducing property of methanolic leaves extract of *Madhuca indica* L. implies that it is capable of donating hydrogen atom in a dose dependent manner. The high content of phenolic compounds in the extract may be a contributing factor towards antioxidant activity because the phenolic compounds are known to have direct antioxidant property due to the presence of hydroxyl groups, which can function as hydrogen donor. Research in the direction of partial isolation and characterization of the constituents of methanolic leaves extract of *Madhuca indica,* L. in order to decipher the specific phytochemical constituent(s) responsible for the free radical scavenging activity of the plant. When this is done, extracts of *Madhuca indica,* L. could find important application in phytotherapy.

Table 1: Scavenging activity of MeoH leaf extracts

Sr.no.	Conc. of extract	Absorbance at 517 nm		Percent scavenging activity
		Plant Extract	Ascorbic acid (Std)	
1	50	0.25	0.35	28.57 %
2	100	0.37	0.45	17.77 %
3	150	0.48	0.52	7.69 %
4	200	0.55	0.57	3.50 %
5	250	0.60	0.63	4.76 %



Graph 1: Optical density at 517nm of MeoH leaf extracts

Table 2: TLC analysis of MeoH extract

Sr. No.	*Madhuca indica* (10%) (10µl)	
	R_f values in cm	Mean of R_f
1	0.52	0.57
2	0.63	
3	0.57	

5. Conclusion

Oxidative stress can arise from overproduction of ROS by metabolic reactions that use oxygen and shift the balance between oxidant/antioxidant statuses in favor of the oxidants. ROS are produced by cellular metabolic activities and environmental factors, such as air pollutants or cigarette smoke. ROS are highly reactive molecules because of unpaired electrons in their structure and react with several biological macromolecules in cell, such as carbohydrates, nucleic acids, lipids, and proteins, and alter their functions. ROS also affects the expression of several genes by upregulation of redox-sensitive transcription factors and chromatin remodeling via alteration in histone acetylation/ deacetylation. Regulation of redox state is critical for cell viability, activation, proliferation, and organ function. Experiments confirming these activities of the extract of *Madhuca indica* in an *in vivo* system would be necessary. However, herbal remedies often do not produce any side effects. Therefore, alternative medicine become popular remedy to various types of ailments In conclusion, *Madhuca indica* extracts have revealed significant antibacterial activities against test organisms used for the study.

6. References

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