EVALUATION OF ANTIOXIDANT AND ANTIMICROBIAL ACTIVITY OF MADHUCA INDICA

Pawan Kaushik.[1], Dhirender Kaushik .[1*], Sukhbir.L Khokra.[1], Chetan Sharma.[2], K.R Aneja.[2], Shalia Khah.[1], Ravinder Mehra.[1] and Gurvirender Singh.[1]

[1]Faculty of Pharmaceutical Sciences, Kurukshetra University, Kurukshetra – 136119, Haryana, India.
[2]Department of Microbiology, Kurukshetra University, Kurukshetra- 136119, Haryana, India.

Summary

The present study was designed to evaluate the antioxidant and antimicrobial activities of alcoholic extract of bark of *Madhuca indica* belonging to Sapotaceae family. The extract was screened for free radical scavenging effects at various concentrations (100, 300 and 500 µg/ml) by superoxide free radical scavenging activity and DPPH free radical scavenging method. All these antioxidant activities were concentration dependent which were compared with standard antioxidants such as BHA and ascorbic acid. The antimicrobial activity was studied using the agar well diffusion method. Extract of *Madhuca indica* at concentration of 100mg/ml was found to be most effective against *Staphylococcus aureus* followed by *Bacillus subtilis* whereas in case of Gram negative bacteria, extract was found to be most effective against *Escherichia coli* followed by *Pseudomonas aeruginosa*. The ethanolic extract of *M. indica* did not showed any activity against *C. albicans*.

Keywords: *Madhuca indica*, Antioxidant activity, Antimicrobial activity, alcoholic extract.

* Corresponding author:
Assistant Professor
Institute of Pharmaceutical Sciences,
Kurukshetra University, Kurukshetra,
Haryana, India.
Email: -dkaushik.apti@gmail.com, pwn.kaushik1@gmail.com
Phone No.: +919416055522, +919467648830

Introduction

Madhuca indica Syn. *Madhuca latifolia* Sapotaceae), commonly known as 'mahua' in the India, is an important economic plant growing throughout the subtropical region of the Indo-Pak sub-continent [1]. Different parts of this plant are used as stimulants, demulcents, emollients, heating and astringents [2]. The bark is a good remedy for itching, swellings, fractures and snake bites, as well as for diabetes mellitus [3]. Mahua oil is used for the treatment of skin diseases, rheumatism, 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. The constituents reported from *M. indica* include fatty acids [4-5], sapogenins [6], sugars [3], triterpenoids steroids [7-9], saponins [10-11], flavonoids and glycosides ([8, 9, 12]. A new isoflavone, 30, 40-dihydroxy-5, 20-dimethoxy-6, 7-methylendioxy, has also been reported from the plant [13]. Keeping all these facts in view we evaluated the plant for antioxidant activity and antimicrobial activity.

Materials and methods

Plant material

Madhuca indica (Sapotaceae) was collected from surrounding local areas and identified by Department of Botany, Kurukshetra University, Kurukshetra, Haryana, India. A voucher specimen (Sr.No. KUK/IPS/2008/MI-108) was deposited in the herbarium of Institute of Pharmaceutical Sciences, Kurukshetra University, Kurukshetra, Haryana, India.

Preparation of Extract

The dried and coarsely powdered plant material was extracted with petroleum ether (60-80°) by hot percolation in soxhlet apparatus until it become colorless. The defatted plant material was then extracted with alcohol until it become colorless. The extract was concentrated under reduced pressure to yield a crude semi-solid mass.

Drugs

Chemicals used in this study were 2, 2-diphenyl-1-picrylhydrazyl (DPPH), nicotinamid-adenin-dinucleotidphosphate, reduced form (NADH), butylated hydroxy anisole (BHA), ascorbic acid, phosphoric acid, nitro blue tetrazolium(NBT), phenazine methosulfate(PMS). All reagents used for the study were of analytical grade. All the standard drugs (Ciprofloxacin, Amphotericin) were obtained from various chemical units – Hi-media India Ltd. and S.D.Fine Chem. Ltd. (India).

Test microorganisms

A total of five microbial strains were selected on the basis of their clinical importance in causing diseases in human beings. Two Gram positive bacteria, *Staphylococcus aureus* (MTCC 96) and *Bacillus subtilis* (MTCC 121) and two Gram negative bacteria, *Escherichia coli* (MTCC 1652) and *Pseudomonas aeruginosa* (MTCC 741) and one yeast, *Candida albicans* (MTCC 227) were chosen for evaluation of antibacterial and antifungal activity of the extract of *M.indica.* All the strains used for these studies were procured from Microbial Type Culture Collection (MTCC), IMTECH, Chandigarh, India. All the bacterial strains were subcultured on Nutrient Agar and yeast on Malt yeast agar and incubated aerobically at 37^0C.

Determination of antimicrobial activity

Various concentrations (100mg/ml, 75mg/ml, 50mg/ml and 25mg/ml) of extract of *M.indica* were evaluated for antimicrobial activity by agar well diffusion method.[14] All the microbial strains were adjusted to 0.5 McFarland standard, which is visually comparable to a microbial suspension of approximately 1.5×10^8 cfu/ml.[15,16] 20ml of specific agar media was poured into each petri plate and plates were swabbed with 100 µl inocula of each test microbial strain and kept for 15 min for adsorption. Wells of 8mm diameter were punched into seeded agar plates and loaded with a 100 µl volume with different concentrations of leaf extract of *M.indica,* reconstituted in the dimethylsulphoxide (DMSO). All the plates were incubated at 37°C for 24 hrs. Antimicrobial activity was evaluated by measuring the diameter of inhibition zone with zone reader (Hi Antibiotic zone scale). DMSO served as the negative control and cipropfoxacin (for bacteria) and amphotericin-B (for fungi) served as the positive control. The experiment was carried out in triplicate and mean of the diameter of inhibition zones was calculated.

Antioxidant assay

DPPH free radical scavenging activity

The free-radical scavenging activity of extract was measured as decrease in the absorbance of methanol solution of DPPH [17]. A stock solution of DPPH (33 mg in 1 L) was prepared in methanol, which gave initial absorbance of 0.493, and 5 ml of this stock solution was added to 1 ml of extract solution at different concentrations (100, 300 and 500 µg/ml). After 30 min, absorbance was measured at 517 nm and compared with BHA and ascorbic acid taken as standards. Scavenging activity was expressed as the percentage inhibition calculated using the following formula:

$$\%\ Inhibition = \frac{Control\ absorbance - Sample\ absirbance}{Control\ absorbance}\ X\ 100$$

Superoxide radical scavenging assay

The reaction mixture consisting of 1ml of nitro blue tetrazolium (NBT) solution (156 mM NBT in phosphate buffer, pH 7.4), 1 ml NADH solution (468 mM NADH in phosphate buffer, pH 7.4), and 1ml of sample solution of extract was mixed. The reaction was started by adding 100 ml of phenazine methosulfate (PMS) solution (60 mM PMS in phosphate buffer, pH 7.4) to the mixture. The reaction mixture was incubated at 25oC for 5 min and the absorbance was measured at 560 nm against blank sample and compared with standards [18, 19]. Decreased absorbance of reaction mixture indicated increased superoxide anion scavenging activity. The percentage inhibition of superoxide anion generation was calculated using the following formula:

$$\%\ Inhibition = \frac{Control\ absorbance - Sample\ absirbance}{Control\ absorbance}\ X\ 100$$

Statistical analysis

All data were represented as mean ± S.E.M. and as percentage. Results were statistically evaluated using Dunnett's *t*- test. P<0.01 was considered significant.

Results

DPPH free radical scavenging activity

DPPH radical was used as a substrate to evaluate free radical scavenging activities of extract. It involves reaction of specific antioxidant with a stable free radical 2, 2-diphenyl-1- picryl-hydrazyl (DPPH). As a result, there is reduction of DPPH concentration by antioxidant, which decreases the optical absorbance of DPPH; this is detected by spectrophotometer at 517 nm. BHA and ascorbic acid were used as standards. The scavenging effect of *M.indica* extract on the DPPH radical was 87.6%, (Fig.1) at a concentration of 500 µg/ml. These results indicated that extract has a noticeable effect on scavenging the free radicals.

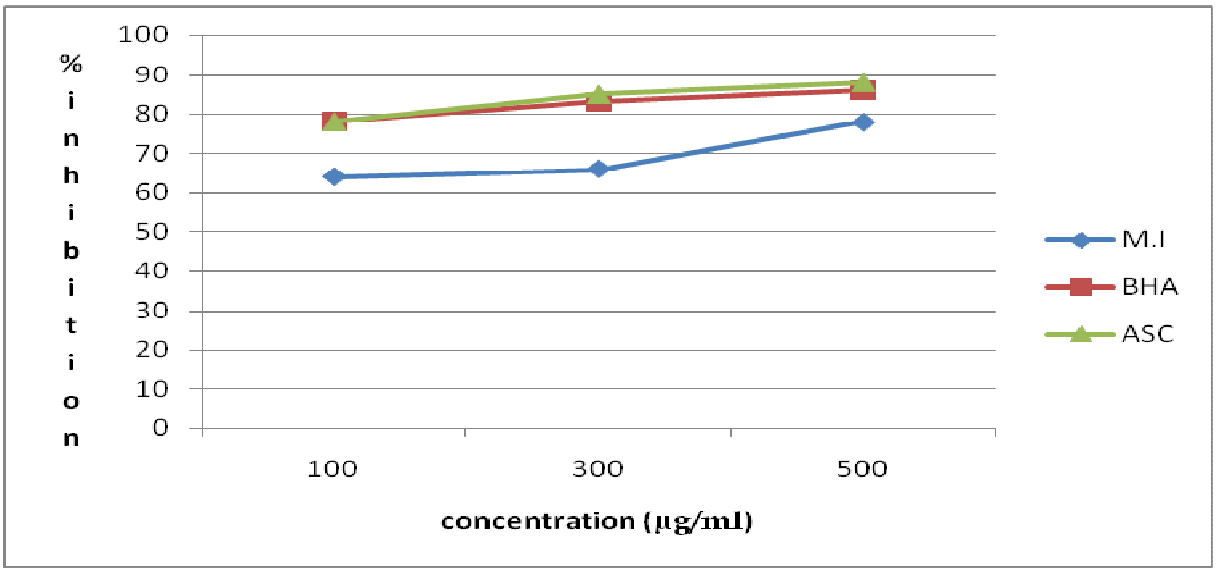

Fig.1. Anti-radical activity of MI extract, Ascorbic acid and BHA against 1,1-diphenyl-2-picrylhydrazyl free radicals

Superoxide radical scavenging assay

The obtained results have emphasized the capacity of MI extracts to annihilate the superoxide anions generated in PMS-NADH-NBT system. Superoxide anion scavenging activity of MI was 70% at a conc of 500 µg/ml. Superoxide anion scavenging activity of synthetic antioxidants ASC and BHA was 88% and 90%, respectively. (Fig. 2).

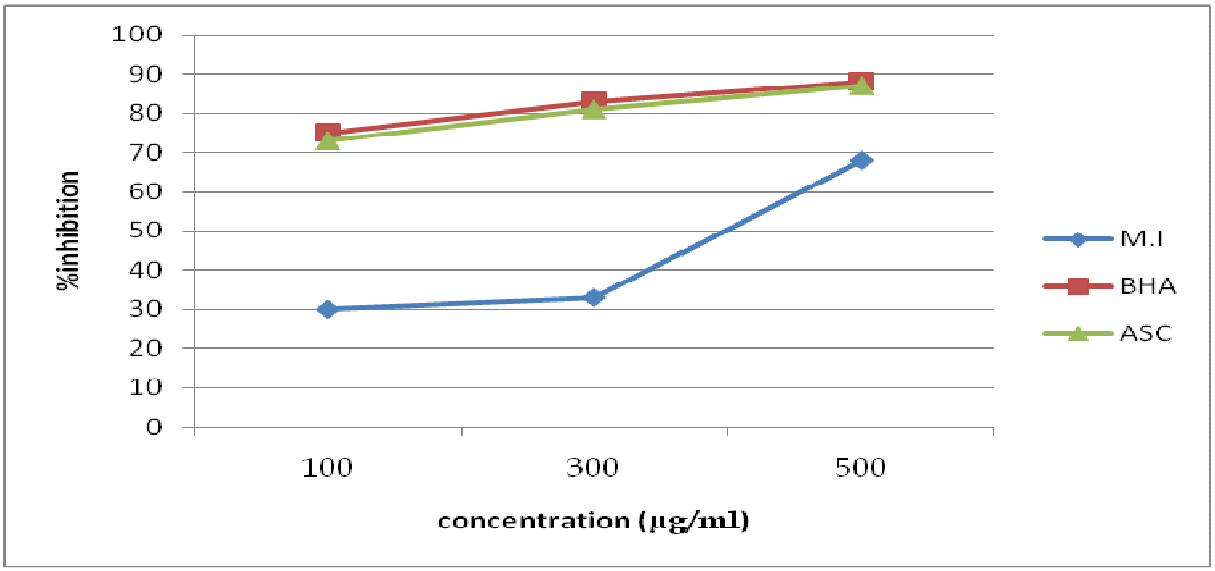

Fig. 2 Superoxide assay of MI extract, Ascorbic acid and BHA

In vitro antimicrobial activity

The extract of *M. indica* inhibited all the tested bacterial strains as shown in Table 1. The results showed that the increase in concentration of extract increased the zone of inhibition against all the tested bacterial strains. From the results, extract of *M. indica* was found to be best in inhibiting the growth of Gram positive bacteria at all the tested concentrations (100mg, 75mg, 50mg and 25mg) whereas Gram negative bacteria did not showed any activity at an concentration of 25mg/ml. The extract of *M. indica* showed good activity against the tested microorganisms except for *P. aeruginosa*, which showed low activity as compare to other tested microorganisms. Extract of *M. indica* was found to be most effective against *S. aureus* showing the maximum zone of inhibition (20.6mm) followed by *B. sublitis* (18.3mm) whereas in case of Gram negative bacteria, ethanolic extract was found to be most effective against *E. coli* showing the maximum zone of inhibition (16.6mm) followed by *P. aeruginosa* (15.6mm). However, *M. indica* extract didn't exhibit any activity against *C. albicans*.

Table-1. Antimicrobial activity of *M.indica* extract

Organisms	Diameter of growth of inhibition zone (mm)[a]						
	Extract concentration (mg/ml)				Control		
	100	75	50	25	Ciprofloxacin (20 µg/ml)	Amphotericin B (100 µg /ml)	DMSO
Staphylococcus aureus	20.6	18.3	17.3	13.6	26.3	ns	-
Bacillus subtilis	18.3	16	15.6	12.3	25.6	ns	-
Escherichia coli	16.6	14.3	12	-	25	ns	-
Pseudomonas aeruginosa	15.6	14	13.3	-	23.3	ns	-
Candida albicans	-	-	-	-	ns	13.6	-

- No activity, ns –not studied

[a] Values, including diameter of the well (8mm), are means of three replicates

Discussion

The MI extract was able to reduce the stable radical DPPH to the yellow coloured diphenylpicrylhydrazine, it was also found to be an efficient scavenger of superoxide anions – generated by the phenazin methosulfate (PMS) / nicotinamid-adenin-dinucleotide, reduced form (NADH) system – was detected within by the reaction with chloride of 2,2'-di-p-nitrophenyl)-5,5'-diphenyl-(3,3'-dimethoxy-4,4'-difphenylene) ditetrazolium chloride (nitro blue tetrazolium – NBT) in-vitro and their activity are in comparable to that of ascorbic acid and Butylated hydroxyl toluene These assay provided useful information on the reactivity of the compounds with stable free radicals, because of the odd number of electrons. The reducing properties are generally associated with the presence of reductones, which have been shown to exert antioxidant action by breaking the free radical chain by donating a hydrogen atom [20]. Therefore, antioxidants with free radical scavenging activities may have great relevance in the prevention and treatment of diseases associated with oxidants or free radicals [21]. Extract of *Madhuca indica* showed good potency in terms of inhibition zones against all the tested bacterial strains. It was observed that the zone of inhibition varies from one organism to another at different concentrations. According to Prescott [22], the activity of antimicrobial agent is concentration dependent. Among the Gram positive and negative bacteria tested, Gram positive bacteria were found to be more susceptible to the *M. indica* extracts. These results are in

accordance with the earlier reports indicating that plant extract are most active against Gram positive bacteria than that of Gram negative bacteria [23].

In conclusion, *M. indica* extracts possess a broad spectrum activity so these extracts open the possibility of finding new clinically effective antibacterial compounds. Further purification of the active compounds and *in vivo* evaluation of antimicrobial activity along with toxicity studies of the extracts should be carried out.

Acknowledgement

The authors are grateful to the Director, Institute of Pharmaceutical Sciences, Kurukshetra University, Kurukshetra for providing necessary facilities.

References

1. Awasthi Y.C, Mitra C.R. Madhuca latifolia: Constituents of fruit pulp and nut shell. Phytochemistry. 1967; 6:121–125.
2. Kirtikar, K.R., & Basu, B.D. Indian medicinal plants Allahabad India: Sudhindra Nath Basu. 1918; p. 747.
3. Khaleque A, Wahed Miah, M.A, Huq M.S & Khan N.A. Madhuca latifolia. I. Constituents of the seeds. Science Research (Dacca, Pakistan). 1969; 6: 227–228.
4. Dhingra D.R, Seth G.L, Speers P.C. Indian seed fat-mowha (Bassia latifola) and tamal (Garcinia morella) fats. Journal of the Society of Chemical Industry. 1933; 52: 116–118.
5. Hilditch T.P, Ichaporia M.B. The fatty acids and glycerides of solid seed fats. III. The seed fat of madhuca (Bassia latifolia) (mowrah fat). Journal of the Society of Chemical Industry. 1938; 57: 44.
6. Heywood B.J, Kon, G.A.R, Ware L.L. Sapogenin. Part V. Bassic acid. Journal of the Chemical Society. 1939; 33: 1124–1129.
7. Awasthi Y.C, Mitra, C.R. Madhuca latifolia: Triterpenoid constituents of the trunk bark. Phytochemistry. 1968; 7: 1433–1434.
8. Bhargava P.N, Singh, R.P. Studies of mahua sterol from Bassia latifola. Journal of the Indian Chemical Society. 1958; 35: 763–767.
9. Bhatnagar S.C, Awasthi, Y.C, Mitra C.R. Steroidal and other constituents of Madhuca latifolia leaves. Phytochemistry. 1972; 11: 1533.
10. Banerji R, Mishra G, Nigam S.K. Madhuca indica leaf saponin and its biological activity. Fitoterapia. 1985; 56: 186–188.
11. Hariharan V, Rangaswami S, Sarangan S. Saponins of the seeds of Bassia latifolia. Phytochemistry. 1972; 11: 1791–1795.
12. Subramanian S.S, Nair A.G.R. Myricetin and myricetin-3-O-L-rhamnoside from the leaves of Madhuca indica and Achras sapota. Phytochemistry. 1972; 11: 3090–3091.
13. Bina S. Siddiqui, Shazia Khan, M. Nadeem Kardar. Natural Product Research2010; 24(1): 76–80.

14. Ahmad I, Beg AJ. Antimicrobial and phytochemical studies on 45 Indian medicinal plants against multidrug resistant human pathogens. J Ethno Pharmacol 2007; 74:113-23.
15. Andrews JM. Determination of minimum inhibitory concentrations. J Ant Chem 2001; 48: 5-16.
16. Aneja K R, Joshi R. And Sharma C. Antimicrobial activity of Dalchini (*Cinnamomum zeylanicum* bark) extracts on some dental caries pathogens. Journal of pharmacy research. 2009; 2(9): 1387-1390.
17. Sreejayan N, Rao MNA. Free radical scavenging activity of curcuminoids. Arzneimittelforschung. 1996; 46: 169-171.
18. Ilhami GI, Haci AA and C Mehmet Determination of in Vitro Antioxidant and Radical Scavenging Activities of Propofol. Chem. Pharm. Bull. 2005; 53(3): 281-285.
19. Nishikimi M, Rao NA and K Yagi The occurrence of superoxide anion in the reaction of reduced phenazine methosulfate and molecular oxygen. Biochem. Biophys. Res. Commun. 1972; 46: 849–853.
20. Duh P.D, Tu Y.Y, Yen G.C. Antioxidant activity of water extract of harn jyur (Chyrsanthemum morifolium Ramat). Lebensmittel-Wissenschaft und-. Technologie-Food Science and Technology. 1999; 32: 269–277.
21. Soares J R, Dinis T C, Cunha A P, Almeida L M. Antioxidant activities of some extracts of Thymus zygis. Free radical research. 1997; 26(5): 469-78.
22.Prescott, L.M., Harley, J.P., Klein, D.A. Microbiology. 5th edition. Mc Graw – Hill Companies, Inc. New York. 2002; P. 811.
23.Jigna, P. and Sumitra, C. In – vitro antimicrobial activities of extracts of Launaea procumbns Roxb. (Labiateae), Vitis vinifera L. (Vitaceae) and Cyperus rotundus L.(Cyperaceae). Afr J Biomed Res. 2006; 9(2): 89-93.