International Research Journal of Pharmaceutical and Applied Sciences (IRJPAS)
Available online at **www.irjpas.com**
Int. Res J Pharm. App Sci., 2014; 4(3):4-8

Research Article

EVALUATION OF *IN VITRO* ANTIOXIDANT AND ANTIMICROBIAL ACTIVITY OF *MORINGA OLEIFERA L.* LEAVES

Neelima Routhu, Bandaru V Rao, Bhavani Y and Monika Singh
Department of Botany, Andhra University, Visakhapatnam 530 003, India

*Corresponding Author: Bandaru V Rao; Email: bandaruvrao@yahoo.co.in

Abstract: Natural plants have been receiving wide attention as source of biological active substances. In the present study, *Moringa oleifera L.* leaves are tested for their *in vitro* antioxidant and antimicrobial activities. Chloroform, ethanol and aqueous extracts of *Moringa* leaves were tested for scavenging of DPPH, Superoxide and FRAP radicals. Antimicrobial activity of the leaf extracts was evaluated against five bacterial strains by using cylinder plate assay. Concentration dependent antioxidant activity was observed by all the tested extracts, The extracts also induced significant zone of inhibition at 500µg/ml against tested bacterial strains. The highest zone of inhibition was shown by ethanolic extract against *Klebsiella pneumonia* at 500µg/ml dose.

Key words: *Moringa oleifera L.*, leaves, *in vitro* antioxidant, antimicrobial activity

INTRODUCTION

Plants have the major advantage of being the most effective and cheaper alternative source of drugs[1]. Historically, pharmacological screening of compounds of natural origin has been the source of innumerable therapeutic agents[2-4]. The problem of bacterial resistance to antibiotics has become a growing concern worldwide[5-9]. This led to the resurgence in search of the antimicrobial role of herbs against resistant strains[10-12]. In search of new, safe and effective antimicrobial agents from natural products, the present investigation was carried out to evaluate *in vitro* antioxidant and antimicrobial activity of *Moringa oleifera* leaves extracts[13-14]. *Moringa oleifera L.* is commonly known as the drum stick plant, belongs to family Moringaceae. Earlier studies on *Moringa oleifera* have been reported to contain different phytochemical compounds like lupeol, moriginine, and a bacteriocide, spirochin, β-sitosterol, daucosterol, stigmasterol[15].

MATERIALS AND METHODS

Preparation of extracts from leaves of *Moringa leifera*

The leaves of *Moringa oleifera.* were collected, shade dried, powdered and separately extracted in a Soxhlet apparatus for 6 hrs successively with three different solvents viz., hexane, chloroform and methanol. Each extract dried at 45°C under vacuum by using rotary evaporator (Buchi, Switzerland. And the samples were weighed in order to make concentrations of 100µg, 250µg, 500µg and 1000 µg each of water, chloroform and ethanol samples. These samples were further stored in air tight bottles for their use in antioxidant studies.

IN VITRO ANTIOXIDANT ACTIVITY

The alcoholic extracts of *Moringa* leaves were screened for free radical scavenging activity against DPPH, Superoxide and FRAP radicals at different concentrations. The Percentage Inhibition was calculated. All experiments were performed thrice and the mean values are summarized. DPPH radical scavenging activity was measured according to the method of Braca et al[16]. An aliquot of 3ml of 0.004% DPPH solution in ethanol and 0.1ml of plant extract at different concentrations were mixed and incubated at 37˚c for 30 min. and absorbance of the test mixture was read at 517nm. Positive controls are treated in the same way as test sample except sample replaced by positive standard. Superoxide radical scavenging activity of the extracts was measured according to McCord and Fridovich method[17]. All the solutions were prepared in phosphate buffer (pH 7.8). FRAP working reagent (3 ml) was taken in a test tube and added 100µl of plant extract. The solution is vortex mixed and the absorbance was read at 593nm against a reagent blank at a predetermined time after sample–reagent mixture. The results are expressed as ascorbic acid equivalents (µ moles/ml) or FRAP units[18].

Calculation of percentage of inhibition

The percentage inhibition of superoxide production by the extract was calculated using the formula:

$$\text{Inhibitory ratio} = \frac{(A_o - A_1) \times 100}{A_0}$$

Where, A_0 is the absorbance of control;

A_1 is the absorbance with addition of plant extract/ascorbic acid.

Test organisms

The microorganisms used in the experiment were procured from MTCC, IMTECH-Chandigarh. The Gram-positive bacterial strains employed in the present study are *Staphylococcus aureus* and *Bacillus subtilis* and the Gram-negative strains are *Escherichia coli, Klebsiella pneumonia* and *Proteus vulgaris.*

Evaluation of *in vitro* antimicrobial activity

The cylinder plate assay of drug potency is based on measurement of the diameter of zone of inhibition of microbial growth surrounding cylinders (cups), containing various dilutions of test extracts. A sterile borer was used to prepare the cups of 6 mm diameter in the agar medium spread with the micro-organisms and 0.1 ml of inoculums. These cups were spread on the agar plate by spread plate technique. Accurately measured (0.05 ml) solution of each concentration and reference standards were added to the cups with a micropipette. All the plates were kept in a refrigerator at 2 to 8°C for a period of 2 hours for effective diffusion of test compounds and the standards. Later, they were incubated at 37°C for 24 hours. The presence of definite zone of inhibition of any size around the cup was measured to assess the antimicrobial activity[19].

RESULTS

Antioxidant activity

The results of *in-vitro* antioxidant activity of methanolic leaf extract clearly indicate the presence of free radical scavenging activity and it produced dose dependent inhibition of free radical generation of DPPH, Superoxide and FRAP radicals. Graphs were plotted from the observed values to find the percentage inhibition of the leaf extracts (Tables 1-3 &Figs 1- 3).

Evaluation of antimicrobial activity

Among all the tested extracts, ethanol extracts have shown significant antimicrobial activity when compared to that of chloroform and aqueous extracts. All the extracts had produced a minimum zone of inhibition against tested bacterial strains at 500µg/ml dose. However, aqueous extract did not show significant effect on both *S.aureus* and *B.subtilis (*Diameter of Zone of Inhibition 1mm) and *E.coli (*Dia 2mm) and completely inactive on *P. vugaris.* The highest zone of inhibition was shown by ethanolic extract against *Klebsiella pneumonia(*Dia 22mm) at 500µg/ml. Chloform extracts generated a minimum zone of inhibition against *B.subtilis(*Dia 10mm) and *P.vulgaris (*Dia 14mm). The results are summarized in Table 4.

Table-1. Percentage inhibition of DPPH radical by leaf extracts of *M. oliefera.*

Conc. of plant	% inhibition of DPPH radical		
	Aqueous extract	Chloroform extract	Ethanol extract
100µg	22.002±0.02	27.046±0.12	29.140±0.15
250 µg	25.225±0.11	27.605±0.18	31.324±0.11
500 µg	26.232±0.13	28.571±0.14	34.598±0.13
1000 µg	26.800±0.11	32.011±0.15	37.102±0.12

Values are means ± SD (n=3)

Table 2: Percentage inhibition of Superoxide radical by leaf extracts of *M.oliefera.*

Conc. of plant extract	% inhibition of Superoxide radical		
	Aqueous extract	Chloroform extract	Ethanol extract
100µg	11.195±0.08	19.191±0.02	39.487±0.06
250 µg	18.705±0.02	38.461±0.08	64.487±0.02
500 µg	20.261±0.05	69.465±0.04	83.462±0.06
1000 µg	24.421±0.05	71.830±0.06	90.642±0.06

Values are means ± SD (n=3)

Table 3: Total Antioxidant Assay-FRAP Method

Plant Name	FRAP units in µM
Moringa oleifera	780±0.06

Values are means ± SD (n=3).

Table 4: Antimicrobial activity of *M. oleifera L.* leaf extracts

	Diameters of zones of inhibition in mm			
	(E)	(C)	(A)	Rifampicin
E.coli	19	9	2	24
B.subtilis	9	10	1	22
S.aureus	21	14	1	24
K. pneumonia	22	9	21	25
P.vulgaris	20	14	0	23

A- Aqueous extract; C- Chloroform extract; E- Ethanol extract of *M. oliefera.*

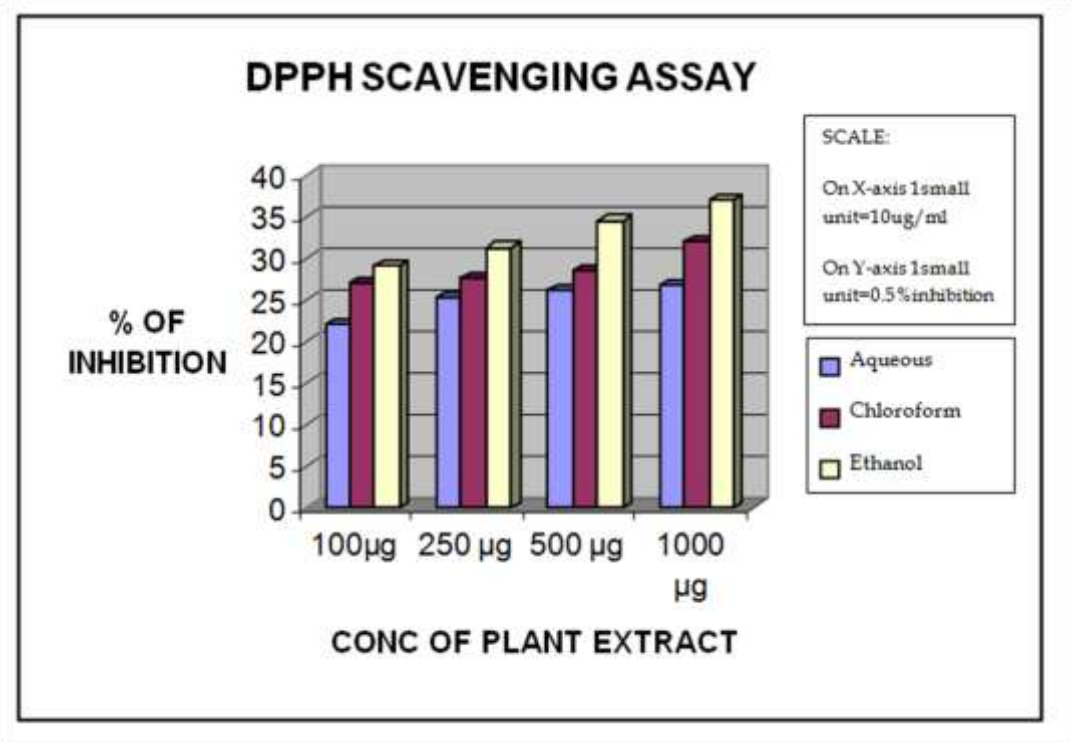

Fig-1 : Percentage inhibition of DPPH radical by leaf extracts of *M. oliefera*.

Positive Control:

Compound	% of Inhibition
Ascorbic acid	68.0±0.13

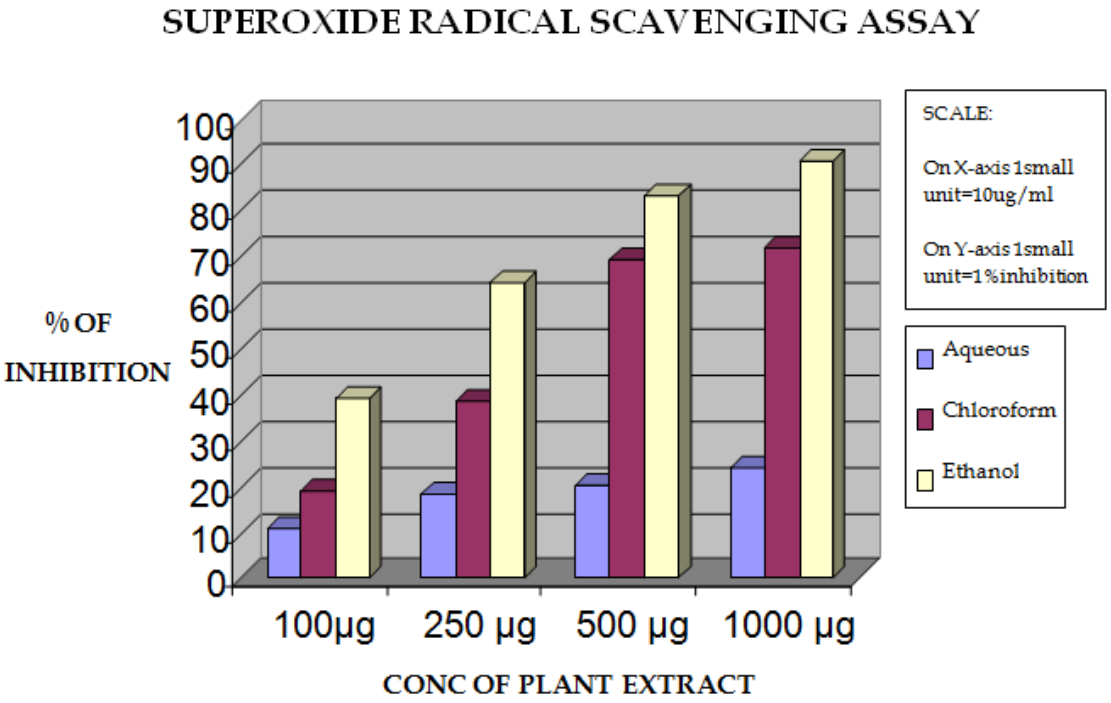

Fig 2: Percentage inhibition of Superoxide radical by leaf extracts of *M. oliefera.*

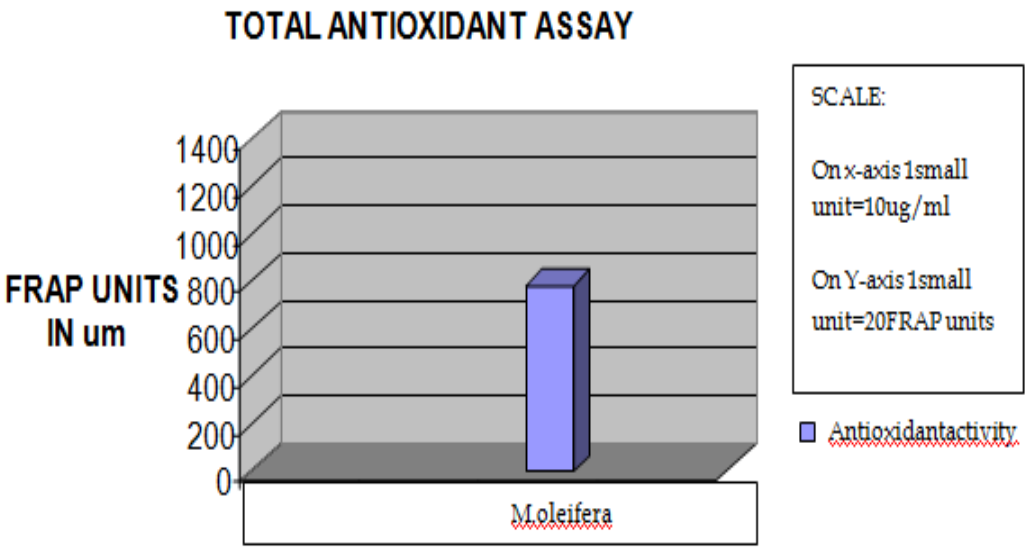

Fig 3: *In-vitro* total antioxidant assay (FRAP) by leaf extracts of *M. oliefera.*

DISCUSSION

By and large, the three tested extracts showed good percentage of inhibition of DPPH, Superoxide and FRAP radicals. Of the three tested extracts ethanolic extract exerted highest activity followed by chloroform and aqueous extracts. The activity of all the three extracts was dose dependent. Ethanol and aqueous extracts generated considerable size of zone of inhibition on *K. pneumoniae* . On the other hand aqueous extract did not show any effect on *E.coli*, *S.aureus* and *P.vulgaris* compared to the zone of inhibition generated by Rifampicin at 50 µg/ml (Table 4) at the current dose levels employed in the present study, the non-activity of aqueous extract on the test bacterial strains could be attributable to presence of active compound(s) in insufficient quantities[20]. Lack of activity can thus only be further confirmed with non- activity even with higher doses[21]. Alternatively, even if the active principle is present in high enough quantities, there could be other constituents exerting antagonistic effects or negating the positive effects of the bioactive agents[22].

CONCLUSION

All the extracts exhibited antioxidant activities in a dose dependent manner. The antimicrobial activity by the three different extracts was relatively low a when compared to that of control.

ACKNOWLEDGEMENT

The third and fourth authors are thankful to U.G.C for financial assistance under special assistance programme to the department of Botany, Andhra University, Visakhapatnam.

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