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Antioxidant Activities of *Sesbania sesban* and *Moringa oleifera* Extractives

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Abstract

Extractives of two indigenous plants *Sesbania sesban* (Fabaceae) and *Moringa oleifera* (Moringaceae) were studied for evaluating antioxidant activity. The petroleum ether, carbon tetrachloride and chloroform soluble fractions of crude methanolic extract of the leaves of *Sesbania sesban* and n-hexane, carbon tetrachloride and dichloromethane soluble parts of crude methanolic extract of the stem bark of *Moringa oleifera* were subjected to measure the free radical scavenging capability with 1,1-Diphenyl-2-picrylhydrazyl (DPPH). The chloroform soluble fraction of *S. sesban* showed the highest free radical scavenging activity among it's extractives having IC_{50} value of 17.81 µg/ml and the IC_{50} value of aqueous soluble part was found 21.72 µg/ml. On the other hand, dichloromethane soluble fraction of *M. oleifera* exhibits the highest antioxidant (free radical scavenging) activity having IC_{50} value of 27.49 µg/ml. and the IC_{50} value of carbon tetrachloride soluble part was 35.78 µg/ml.

Key words: *Sesbania sesban*, *Moringa oleifera*, Antioxidant, Free radical.

I. Introduction

Antioxidants which scavenge free radicals are known to posses an important role in preventing these free radical induced-diseases. There is an increasing interest in the antioxidants effects of compounds derived from plants, which could be relevant in relations to their nutritional incidence and their role in health and diseases[1,2,3,4,5] A number of reports on the isolation and testing of plant derived antioxidants have been described during the past decade. Natural antioxidants constitute a broad range of substances including phenolic or nitrogen containing compounds and carotenoids[6,7,8]

Lipid peroxidation is one of the main reasons for deterioration of food products during processing and storage. Synthetic antioxidant such as *tert*-butyl-1-hydroxitoluene (TBHT), *tert*-butylhydroquinone (TBHQ) butylated hydroxianisole (BHA) and propyl gallate (PG) are widely used as food additives to increase shelf life, especially lipid and lipid containing products by retarding the process of lipid peroxidation. However, TBHT and BHA are known to have not only toxic and carcinogenic effects on humans[9] (Ito *et al.,* 1986), but abnormal effects on enzyme systems.[10]

Morever this, recent years have witnessed a renewed interest in plants as pharmaceuticals. This interest has been focused particularly on the adoption of extracts of plants, for self-medication by the general people. Within this context, considerable interest has arisen in the possibility that the impact of several major diseases may be either ameliorated or prevented by improving the dietary intake of natural nutrients with antioxidant properties, such as vitamin E, vitamin C, β-carotene and plant phenolics such as tannins and flavonoids.

The use of plant extracts in traditional medicine by old Indian and China people have been going on from ancient time. Herbalism and folk medicine, both ancient and modern, have been the source of much useful therapy.[11] Therefore, the demand for new and effective antioxidants from natural sources are increasing day by day. In order to identify plant species having potential free radical scavenging capability, in this paper, we report the preliminary antioxidant activity of methanolic extract of the aerial part of *S. sesbsn* and stem bark of *M. oleifera* growing in Bangladesh.

II. Materials and Methods

Sesbania sesban (local name-Jyonti, Daincha) is a small perennial tree with woody stems. Leaves are anthelmintic and also useful in diabetes, colic, catarrh and skin diseases. Seeds are stimulant, emmenagogue and astringent and also useful in diarrhoea. Paste of seeds is used to cure itches and other skin eruptions.[12,13] Stem bark *M. oleifera* (Sajne) is acrid, stimulant, diuretic and antiscorbutic, used as a cardiac stimulant, in asthma and cough. Root bark is also acrid, stimulant and diuretic. Fresh root of a young tree is given in intermittent fevers, epilepsy, hysteria, chronic rheumatism, gout, dropsy, dyspepsia and enlargement of liver and spleen[14].

The leaves of *S. sesban* were collected from plantation area of the BCSIR Laboratories, Chittagong and was identified at the Plant Taxonomy Division of BCSIR and Bangladesh National Herbarium, Mirpur, Dhaka (Accession no: DACB 32889). It was dried at room temperature for several days to make crude extract by conventional method. A portion of the concentrated methanol extract (MSLE, 5 gm) was fractionated by the modified Kupchan partitioning method[15] into *n*-hexane, carbon tetrachloride, chloroform and aqueous soluble fractions. The stem bark of *M. oleifera* was collected from Rajbari and was identified at department of Botany, University of Dhaka. The fractions were prepared by the above processes.

Only 2 mg of each fraction of the both plant were taken as test sample (Table 1)

Table. 1. Test samples of Experimental Plants

Plant	Test samples	Code	Measured amount (mg)
Sesbania sesban	Methanol soluble leaves extract	MSLE	2.00
	Pet. ether soluble partitionate of MSLE	PESP	2.00
	Carbon tetrachloride soluble partitionate of MSLE	CTSP	2.00
	Chloroform soluble partitionate of MSLE	CFSP	2.00
	Aqueous soluble partitionate of MSLE	AQSP	2.00
Moringa oleifera	Methanol soluble bark extract (crude)	MSBE	2.00
	n-Hexane soluble partitionate of MSBE	HXSP	2.00
	Carbon tetrachloride soluble partitionate of MSBE	CTSP	2.00
	Dichloromethane soluble partitionate of MSBE	DMSP	2.00
	Aqueous soluble partitionate of MSBE	AQSP	2.00

The free radical scavenging activities (antioxidant capability) of the plant extracts on the persistent radical 1,1-diphenyl-2-picrylhydrazyl (DPPH) were estimated by the method of Brand-Williams *et al.*, 1995.[16] 2.0 ml of a methanol solution of the extract at different concentration were mixed with 3.0 ml of a DPPH methanol solution (20 µg/ml). The antioxidant potential was assayed from the bleaching of purple colored methanol solution of DPPH radical by the plant extract as compared to that of *tert*-butyl-1-hydroxytoluene (TBHT) by UV spectrophotometer.

Choi *et al.,* 2000[17] and Desmarchelier *et al.,* 1997[18] also used the following method to evaluate the free radical scavenging activity (antioxidant potential) of various compounds and medicinal plants

- 2.0 ml of a methanol solution of the extract at different concentration (500 to 0.977 µg/ml) were mixed with 3.0 ml of a DPPH methanol solution (20 µg/ml).
- After 30 min reaction period at room temperature in dark place absorbance was measured against at 517 nm against methanol as blank by spectrophotometer.
- Inhibition free radical DPPH in percent (I%) was calculated as follows: (I%) = $\{(A_{blank} - A_{sample})/ A_{blank}\} \times 100$

  Where A_{blank} is the absorbance of the control reaction (containing all reagents except the test material).
- Extract concentration providing 50% inhibition (IC_{50}) was calculated from the graph plotted inhibition percentage against extract concentration.
- TBHT was used as positive control.
- Tests carried out in triplicate and average value was taken.

III. Results and Discussion

We have work with ten extractives of two plants of two families those are enlisted in table 2 and 3. Among all five fractions of *S. sesban*, CFSP and AQSP showed the highest free radical scavenging activity having IC_{50} value 17.81 µg/ml and 21.72 µg/ml, respectively.. At the same time PESP and CTSP of MSLE also exhibit moderate antioxidant potential having IC_{50} values 25.73 and 48.5 µg/ml, respectively (table 2, fiq. 1) .

Table. 2. IC_{50} values of standard and test samples of *S. sesban*

Test sample /Code	Equation of regression line	R^2	IC_{50} (µg/ml)
TBHT	y = 14.776Ln(x) + 10.812	0.9351	14.18±1.01
MSLE	y = 8.6915Ln(x) + 16.257	0.9877	48.5±0.78
PESP	y = 6.2183Ln(x) + 29.801	0.9874	25.73±2.3
CTSP	y = 6.0195Ln(x) + 24.466	0.9834	69.49±1.71
CFSP	y = 8.8342Ln(x) + 24.555	0.9829	17.81±0.86
AQSP	y = 6.0164Ln(x) + 31.4780.8474	0.8474	21.72±1.45

[#]The values of IC_{50} are expressed as mean±SD (n=3)
(Code- as mentioned in table 1)

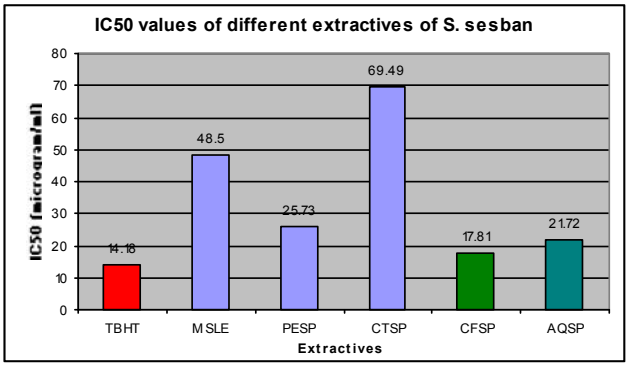

Fig. 1. IC_{50} value of the standard and partitionares of *S. sesban*

In the cases of *M. oleifera*, DMSP and CTSP showed the highest free radical scavenging activity having IC_{50} values of 27.49 µg/ml and 35.78 µg/ml, respectively.. At the same time, MSBE also exhibit moderate antioxidant potential having IC_{50} values of 44.3 µg/ml. MSBE, HXSP and AQSP showed low antioxidant potential (table 3, fiq.2).

Table 3. IC_{50} values of standard and partitionates of *M. oleifera*

Test samplesCode	Equation of regression line	R^2	IC_{50} (µg/ml)
TBHT	y = 14.776Ln(x) + 10.812	0.9351	14.18±1.01
MSBE	y = 11.156Ln(x) + 7.7007	0.9071	44.3±0.98
HXSP	y = 8.5434Ln(x) + 16.839	0.9684	48.47±2.41
CTSP	y = 8.6283Ln(x) + 19.128	0.9723	35.78±1.83
DMSP	y = 6.9879Ln(x) + 26.84	0.9556	27.49±0.87
AQSP	y = 7.4341Ln(x) + 17.628	0.9596	77.77±2.62

#The values of IC_{50} are expressed as mean±SD (n=3)
(Code- as mentioned in table 1.)

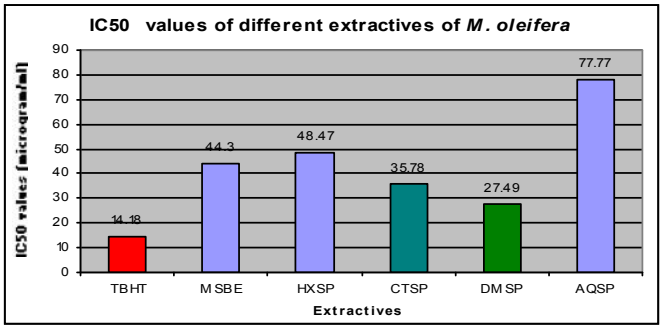

Fig. 2. IC_{50} value of the standard and partitionares of *M. oleifera*

IV. Conclusion

The results of chloroform and aqueous soluble fraction and ethanol extract of *S. sesban* showed potential antioxidant activities. Aqueous and n-hexane soluble fraction also showed moderate free radical scavenging activities. In the case of *M. oleifera,* the dichloromethane extract and carbon tetrachloride soluble part of methanol extract showed potential antioxidant activities. Further work especially bioassay-guided fractionation is warranted in order to isolate and characterize the free radical scavenging constituents responsible for the antioxidant property.

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