



## IN VITRO FREE RADICAL SCAVENGING ASSAY OF POD COAT AND POD SEED OF MORINGA OLEIFERA BY SPECTROPHOTOMETRIC METHOD

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### ABSTRACT

The present study was undertaken to find the antioxidant value of pod coat and pod seed of *M. oleifera* Lam. plant. Antioxidants have been reported to prevent oxidative damage caused by free radical and can be used in cardiovascular and anti-inflammatory diseases. The amount of total phenols, flavonoids and radical scavenging activity were studied. The total phenol in pod seed and pod coat varied from 0.40±0.01 to 0.55±0.06 mg g<sup>-1</sup> in the extracts. Total Flavonoid contents were between 0.5±0.01 and 0.52±0.01 mg g<sup>-1</sup>. Nitric oxide (NO) radical scavenging effect of the extracts was determined spectrophotometrically. The highest radical scavenging effect was observed in pod seed than pod coat with IC<sub>50</sub> 3.30 and 3.92 mg ml<sup>-1</sup>. The greater amount of phenolic compounds leads to more potent radical scavenging effect as shown by pods of *M. oleifera* extract.

**Keywords:** *M. oleifera*, Phenol, Flavonoid, Antioxidant, Nitric oxide scavenging.

### INTRODUCTION

Oxidation is essential to many living organisms for the production of energy to fuel biological processes. Reactive oxygen species (ROS) produced by ultraviolet, sunlight, ionizing radiation, chemical reactions and metabolic processes have a wide variety of pathological effects, such as carcinogenesis and cellular degeneration related to aging.<sup>1</sup> These radicals play important roles in degenerative processes such as ageing cardiovascular diseases, cancer, Alzheimer's disease and other neurodegenerative diseases.<sup>2-4</sup> Natural antioxidants are constituents of many fruits and vegetables, and they have attracted a great deal of public and scientific attention because of their anti carcinogenic potential and other health promoting effects.<sup>5</sup> The free radical neutralizing property of several plants was reported by previous studies. The extracts from number of medicinal plants which are known to have some biologically active principles are used in ayurvedic preparations and these extracts are prepared in bulk for commercial purpose.<sup>6</sup>

*Moringa oleifera* Lam. is the most widely cultivated species of a monogeneric family, Moringaceae which is native to the India, Pakistan, Bangladesh and Afghanistan. This rapidly-growing tree was utilized as a drug by the ancient Romans, Greeks and Egyptians, unani and ayurveda. Young unripe pods are cooked with curries as a preventive against intestinal worms and used as a folk.<sup>7</sup> It was predicted to have free radical scavenging activity. Hence, the aim of this study was to investigate the antioxidant profile of pod coat and pod seeds of *M. oleifera* against free radicals using specific *in vitro* mode.

### MATERIALS AND METHODS

#### Chemicals and Reagents

Methanol, sodium nitro prusside, sulphanilamide, aluminium chloride (HI Media), sodium nitrite, sodium carbonate, sodium hydroxide, Folin-Ciocalteu, N-1 naphthylethylenediamine hydrochloride, quercetin, orthophosphoric acid (Mearck), gallic acid and L-ascorbic acid (Sigma) were purchased. All other chemicals used were of analytical grade.

#### Preparation of extract

Fresh and healthy Pods of *M. oleifera* were collected from local garden of Bhopal and identify by Dr. Manish Mishra, IIFM, Bhopal. Pods were washed with tap water and sterilized with 2% hypo solution. Pods were peeled and remove the seeds. Both seeds and pod coat were shed dry. Dried plants (50 g) were crushed using a grinder. The powder was taken and extraction was carried out in soxhlet using hydroalcohol in 50:50 (Methanol: water). The concentrated extract was spray dried and the dried powder was taken to check the antioxidant activity.

#### Total phenolic compound assay

Total polyphenols were determined according to the spectrophotometer method with Folin-Ciocalteu's reagent. Gallic acid was used as calibration standard and results were expressed as gallic acid equivalents mg per g dry weight<sup>8</sup>. The extract was mixed with 1.5 mL of Folin-Ciocalteu reagent and allowed to stand at 22°C, for 5 min. A 1.5 mL sodium bicarbonate solution (20%) was added to the mixture. After 90 min at 22°C, absorbance was measured at 725 nm using a UV-Visible spectrophotometer.



### Total flavonoid assay

Total flavonoid content was measured by the aluminum chloride colorimetric assay<sup>9</sup> using quercetin as a standard. The plant extract and quercetin was added to 10 mL volumetric flask containing 4 mL of double distilled water. To the flask was added 0.3 mL 5 %NaNO<sub>2</sub>. After 5 min, 0.3 mL 10 % AlCl<sub>3</sub> was added, 2 mL 1M NaOH was added and the total volume was made up to 10 mL with double distilled water. The solution was mixed well and absorbance was measured against prepared reagent blank at 510 nm. Total flavonoid content was expressed as mg quercetin equivalents (QE)/g dry mass.

### Nitric oxide scavenging activity assay

Nitric oxide radical scavenging activity was determined according to the method reported by Garrat.<sup>10</sup> Sodium nitroprusside in aqueous solution at physiological pH spontaneously generates nitric oxide, which interacts with oxygen to produce nitrite ions, which can be determined by the use of the Griess Illosvoy reaction. 2 mL of 10 mM sodium nitroprusside in 0.5 mL phosphate buffer saline (pH 7.4) was mixed with 0.5 mL of extract at various concentrations and the mixture incubated at 25°C for 180 min. From the incubated mixture 0.5 mL was taken out and added into 1.0 mL sulfanilic acid reagent (33% in 20% glacial acetic acid) and incubated at room temperature for 5 min. finally, 1.0 mL naphthylethylenediaminedihydrochloride (0.1% w/v) was mixed and incubated at room temperature for 30 min before measuring the absorbance at 540 nm was measured with a spectrophotometer. The nitric oxide radicals scavenging activity was calculated.

### Statistical analysis

Values were represented as mean±SD of three parallel measurements.

## RESULTS

### Total phenol estimation

The results of the Folin-Ciocalteu total phenol assay with hydroalcoholic extract are reported in table 1. The pod coat was show high phenol compound in compare to pod seed of *M. oleifera*. The pod coat and seed contained the considerable amount of phenolic content of 0.55±0.06 and 0.40±0.01 mg GAE/g.

**Table 1:** Total Phenol Estimation with Standard of Gallic Acid

Plant parts	Total phenol GAEmg/g
Pod Seed	0.40±0.01
Pod Coat	0.55±0.06

### Total flavonoid assay

Flavonoids are regarded as one of the most widespread groups of natural constituents found in plants. The values of flavonoid content varied from 0.52±0.01mg/gm to 0.50±0.01 mg QE/gm (table 2). High amount of

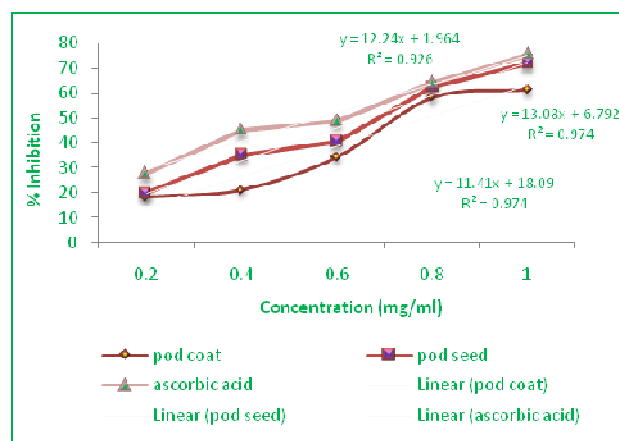
flavonoid content was observed in the pod seed in compared to that of the pod coat.

**Table 2:** Total Flavonoid Estimation with Standard of Quercetin

Plant part	Total flavonoid QE mg/g
Pod seed	0.52±0.01
Pod coat	0.50±0.01

### Nitric oxide scavenging assay

The antioxidant capacity of extracts of *M. oleifera* pods part was measured as ascorbic acid equivalent gm<sup>-1</sup> dry wt. (AAE) using nitric oxide free radicals. The highest antioxidant capacity was exhibited by extracts of pod seed as compare to pod coat (Fig. 1) at 1mg ml<sup>-1</sup>. The percentage inhibition/discoloration of free radicals by different extracts was investigated against NO. The highest percentage of discoloration (71.93%) of NO was observed in the extract of seed and (61.3 %) in pod coat. Regression equations to derive the IC50 values (concentration of extracts required to scavenge 50% NO free radicals.) showed inverse relationship between IC50 value and percentage scavenging potential of a sample. The strongest NO radical scavenging activity was exhibited by extracts of pod coat with IC50 = 3.92mg.ml<sup>-1</sup> while the pod seed with IC50 = 3.30mg.ml<sup>-1</sup>. The work is significant.



**Figure 1:** Nitric oxide scavenging assay

## DISCUSSION

The present investigation provides a comprehensive profile of the antioxidant activity of extracts of pod seed and pod coat of *M. Oleifera* plant with respect to its phenols and flavonoids content. Many reports of natural antioxidants of plant origin have been published and their importance in health, food and preventive medicine has been well documented.<sup>11</sup> The antioxidant activity of Moringa leaves have reported previously. The extract of *M. oleifera* has characterized by the higher value of the TAA. For the extracts of *M. oleifera* also the stronger antiradical activity against DPPH radical and reducing power has noted. Phenolic acids were the dominant phenolic constituents of *M. oleifera* leaves extract.<sup>12</sup> Literature also revealed a significant no. of reports regarding the antioxidant potential of *M. oleifera* leaves.<sup>13</sup>



Nitric oxide (NO) is a potent pleiotropic mediator of physiological processes such as smooth muscle relaxation, neuronal signaling, inhibition of platelet aggregation and regulation of cell mediated toxicity. It is a diffusible free radical which plays many roles as an effector molecule in diverse biological systems including neuronal messenger, vasodilation and antimicrobial and antitumor activities<sup>14</sup>. Figure 1 illustrates the percentage inhibition of nitric oxide generation by *M. oleifera* pod extract. Ascorbic acid was used as a reference compound. The components responsible for the antioxidant activity of MO extracts are currently unclear. Therefore, it is suggested that further work be performed on the isolation and identification of the antioxidant components of *M. oleifera*.

### CONCLUSION

The result of the present study showed that the extract of *M. oleifera*, which contain highest amount of flavonoid and phenolic compounds, exhibited the greatest antioxidant activity. The high scavenging property of *M. oleifera*, may be due to hydroxyl groups existing in the phenolic compounds' chemical structure that can provide the necessary component as a radical scavenger. All of the extracts of different part in this research exhibited different extent of antioxidant activity. *M. oleifera*, extract showed a higher potency in scavenging of NO free radical. This may be related to the high amount of flavonoid and phenolic compounds in this plant extract.

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