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Analysis of phenol and tannin in the bark extracts of medicinally important plant *Moringa oleifera* (Lam.) collected from different regions of Haryana

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Abstract

Moringa oleifera (Lam.), family Moringaceae is a plant that possesses high medicinal value and a good source of natural antioxidants. The present study was conducted to determine the content of tannin and phenol in the crude bark extracts of *Moringa oleifera*, collected from Rewari, Panchkula, Sirsa, Fatehabad and Hisar districts of Haryana state. Phenol was found maximum in Sirsa and tannin was found maximum in Panchkula district. The present study revealed the presence of tannin and phenol in the stem bark of *Moringa oleifera*.

Keywords: Moringa oleifera, phenol, tannin, antioxidant

Introduction

Now days, there is an upsurge of interest on phytochemical activities from antioxidants (Ou et al., 2002)^[7]. Various plant extracts have been utilized as natural antioxidant resources (Madhavi *et al.*, 1995)^[5]. Natural antioxidants are compounds originated from plant or animals. Natural antioxidant comprises of phenolic acid (phenols), flavonoid/ bioflavonoid and tannic acid (tannins) named as polyphenols. These compounds are normally found in significant quantities in plant seeds, fruits, vegetables and spices. Antioxidants are compounds responsible for preventing or delaying the oxidation of products by free radicals scavenging and reducing oxidative stress. Oxidative stress signifies the presence of reactive oxygen species (ROS) and free radicals. When substantial amount of ROS accumulated in the cells, oxidative stress may occur. This condition will cause macromolecules such as enzymes, protein and DNA to be oxidized hence induce significant damage to the intermediate metabolites (Dai and Mumper, 2010)^[3]. Natural antioxidants play a vital function against ROS for the defense system in human body, as well as having roles in food industry and in chemoprevention of diseases (Ou et al., 2002; Siddhuraju and Becker, 2003; Noguchi and Niki, 2000) ^[7, 10, 6]. For that reasons, there is a strong need for effective determination of antioxidants from plant source.

One such tree, which has natural antioxidants, is *Moringa oleifera*, popularly known as Drumstick, Sahjan or Sohanjana in India is a perennial tree, still considered as underutilized plant belongs to Moringaceae family. All plant parts possess remarkable range of some functional and nutraceutical properties (Singh *et al.*, 2012) ^[11]. In traditional Indian medicine, various parts of the tree are used therapeutically for treatment of ascites, rheumatism, venomous bites and as cardiac and circulatory stimulant. The leaves, roots, and seeds are of particular importance in Ayurveda. Uses of root bark, stem bark and exudates, leaves, flowers, and seeds in treating a wide range of ailments have been discussed in ancient Sanskrit texts on medicine (Ramachandran *et al.*, 1980) ^[8].

Natural antioxidants may provide many advantages over the synthetic ones, as they contain various essential bioactive constituents. The strong antioxidant properties of medicinal plants may improve the capability of plants to survive under polluted conditions, encourage smart utilization of the plant material and proper usage as medicine.

Therefore, it is significant to verify the presence of antioxidant compounds in *Moringa oleifera* bark extract. This research aims to determine the total phenol (TP) and total tannin (TT) content in *Moringa oleifera* bark.

Materials and Methods Selection of plant species

The plant material (bark) of *Moringa oleifera* was collected from different regions (Rewari, Panchkula, Sirsa, Fatehabad and Hisar) of Haryana, India. The plant's bark washed thoroughly with running tap water and once sterile with distilled water. Then the plant's bark were shade dried and coarsely powdered separately and stored in well-closed bottles for further analysis in laboratory.

Estimation of total phenols

Dry powdered plant sample (0.2 g) was refluxed with 25 ml of 80% methanol for one hour. The extracted material was centrifuged. The supernatant was taken and volume was made 25 ml with 80% methanol. Total phenol was estimated by the method of Swain and Hillis (1959)^[12].

One ml of the extract was taken in a test tube and diluted to 7.5 ml with distilled water. The contents were mixed well. To this, 0.5 ml of diluted Folin- ciocalteau reagent [diluted to 1:1 (v/v) with water before use] was added and tubes were shaken thoroughly. After 3 minutes, 1 ml of saturated sodium carbonate solution [The anhydrous sodium carbonate (35.0 g) was dissolved in 100 ml of distilled water by heating on a water bath at 70-80°C. The contents were cooled overnight and supernatant was used] was added and total volume was made to 10 ml with distilled water. The tubes were allowed to stand for 1 h and the absorbance was read at 725 nm in UV-Vis spectrophotometer using distilled water in place of sample extract as reagent blank. The amount of total phenols in the sample was determined from the standard curve prepared simultaneously by taking tannic acid as the standard phenol. The results have been expressed as mg g⁻¹ dry weight.

Estimation of tannin Reagents

i) Hydrochloric acid (8% v/v) in methanol.ii) Vanillin (4% w/v) in methanol.

These solutions were prepared daily and mixed in 1:1 ratio

just before use and avoided after a trace colour appear. 0.2 g ground bark sample (20 meshes or fine) was taken in a 50 ml test tube. Then 25 ml of methanol was added to each test tube, stopper with wax coated cork, swirled. Incubated at 300 °C for 20 hours, swirled 6 times during incubation and then it was allowed to settle. One ml of supernatant was pipetted in each of two test tubes, 5 ml of vanillin – HCl reagent was added, and test tubes were shaked and incubated for 20 minutes at 250 °C. A blank was run using only methanol. Optical density of solution at 525 nm using spectrophotometer was recorded. Standard curve was prepared using catechin as standard compound and tannin was calculated.

Result and discussion

Phenol ranged from 30.22 mg g⁻¹ to 44.22 mg g⁻¹ of dry weight (Table 1). The general mean was 37.09 mg g⁻¹ of dry weight. The bark extract from trees grown in Sirsa had highest phenol content (0.45 mg g⁻¹ of dry weight). The phenol content in the bark extract of the trees (43.44 mg g⁻¹ of dry weight) grown in Fatehabad was at par with the maximum phenol content (44.22 mg g⁻¹ of dry weight). The bark extract from trees grown in Rewari had lowest phenol content (30.22 mg g⁻¹ of dry weight).

Seed source	Phenol (mg g ⁻¹)
Rewari	30.22
Panchkula	33.00
Sirsa	44.22
Fatehabad	43.44
Hisar	34.56
Mean	37.09
CD	1.62

Table 1: Total phenol content in the stem bark of Moringa oleifera collected from different regions of Haryana

Phenolics have been found as strong antioxidants towards hindering the influence of free radicals and ROS, which is the basis of several chronic human infections (Yu *et al.*, 2003) ^[13], thus Moringa bark could be used in traditional systems of medicine. Various reports also exist that indicate *Moringa oleifera* as a rich source of phenolic compounds. Currently there has been an increased interest worldwide to identify antioxidant compounds from plant sources, which are pharmacologically potent and have small or no side effects for use in protective medicine and the food industry.

The result revealed that *Moringa oleifera* bark contained tannin ranged from 1.70 mg g⁻¹ to 1.89 mg g⁻¹ of dry weight (Table 2). The general mean was 1.78 mg g⁻¹ of dry weight. The maximum tannin content was found in the bark extract of trees (1.89 mg g⁻¹ of dry weight) grown in Panchkula. The tannin content in the bark extract of the trees (1.86 mg g⁻¹ of dry weight) grown in Hisar was at par with the maximum phenol content (1.89 mg g⁻¹ of dry weight). The lowest tannin content was found in the bark extract of the trees (1.70 mg g⁻¹ of dry weight) grown in Sirsa.

Table 2: Total tannin content in the stem bark of Moringa oleifera collected from different regions of Haryana

Seed source	Tannin (mg g ⁻¹)
Rewari	1.86 (1.73)
Panchkula	1.89
Sirsa	1.73 (1.70)
Fatehabad	1.72
Hisar	167 (1.86)
Mean	1.78
CD	0.07 (0.06)

In the present study, tannin was found to be 1.89 mg g⁻¹. Tannins are water-soluble antioxidant with molecular weight of 500-3000 g/mol. Tannins are natural polyphenols ubiquitously distributed in plants. Tannins are widely used in wine industry for colour stabilizer; balancing the complexity in wines, inhibit certain enzymes in infected fruits and act as wine fining agents (Sanz *et al.*, 2008) ^[9]. It also has the ability to precipitate proteins and alkaloids (Hagerman *et al.*, 1997 and Amarowicz, 2007) ^[4, 1]. The quantity of this compound is important in justifying the antioxidative properties of the Moringa bark.

Conclusion

From the present study, we conclude that the stem bark extract of *Moringa oleifera* Lam. contains phenol and tannin, both exhibit antioxidant and phytochemical potential. The study showed that the plants are a source of significant natural antioxidants, which play an important role in curing and even prevent oxidative damages caused by free radicals.

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