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Antioxidant potential and physiochemical properties of seed kernels and oil of *Melia azedarach* of two locations

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In the present study the phenolic extracts of crude oil and methanol extracts of defatted seed cake were used to evaluate total phenolics content, flavonoids content, total tocopherol and free radical scavenging activity by DPPH method for two locations. Total phenolics content were highest in oil content 28.7±0.3 mg GAE/g (Hisar), flavonoids content and total tocopherol were highest in methanol extract of defatted seed cake 4.2±0.1 mg CAE/g and 26.5±0.4 mg/g in Palwal location. The antioxidant activity having IC_{50} value which is highest in oil content 0.045±0.0 mg/ml (Hisar). The fatty acid composition of oil were: palmitic acid (5.9±0.4%, 7.5±0.6%), stearic acid (2.9±0.4%, 0.8±0.3%), oleic acid (15.1±0.2%, 16.0±0.1%) and linoleic acid (75.5±0.3%, 74.0±0.6%).

Keywords: *Melia azedarach*, total phenolics, physiochemical properties.

Introduction

Plants are essential wellspring of pharmaceuticals and assume a key part in human wellbeing. All societies from old circumstances to today have utilized plant as meds. Today restorative plants are essential to the worldwide economy, as roughly 85% of conventional medication arrangements include the utilization of plants or plant extricates. The restorative properties of plant species have made an extraordinary commitment in the beginning and development of numerous convention home grown treatments. Many plants contain an assortment of pharmaceuticals, which have discovered vital applications in the field of agribusiness, human and veterinary medicine.

Melia is a small sort of two species i.e. *azedarach and azadirachta*. Writing study uncovers that in many parts of the world planning of *Melia azedarach* are being utilized locally and efficiently to cure numerous ailments[1,2]. The plant is considered as resolvent, deobstruent and alexipharmic. Locally blooms, leaves, natural products/berries and bark are utilized for curing numerous sick skin condition, for example, dermatitis, ulcerative injuries, syphilitic ulcers, disease, scrofula and so on as area, treatment or poultice. Methodicallly it is uesd as an emetic, cathartic, anthelmintic, antipyretic, expectorant and diuretic[3,4]. *Melia azedarach* L. have a place with the family (Meliaceae) privately known as Bakain or Drek (Hindi), is a deciduous tree local to north-eastern India. It is elaborate tree in India and Pakistan. Economically the oil of Bakain is utilized as a part of cleanser and restorative enterprises. Leaves extracts of this plant have insecticidal properties.

Materials and methods:

Dry pods of *Melia azedarach* were collected from CCS HAU, Hisar and district Palwal, Haryana, India. The seeds were removed from their pods and ground to powder form utilizing electric granulating machine. The powdered seed was utilized for analysis.

Chemicals:

The chemicals utilized for the analyses were from Ranbaxy Merck and Qualigens, of most elevated immaculateness. Oil substance will be determined by Soxhlet method utilizing petroleum ether (60–80°C) for 8 h. The compound attributes of seed oil will be resolved by AOAC standard method[5].

Mineral contents:

Reagents:

Diacid mixture: Nitric acid and perchloric acid was mixed in ratio 5:1 just before use.

Hydrochloric mixture (1%): 1 ml of conc. HCl was added

in 50 ml distilled water and total volume 100 ml was made with distilled water.

Method:

Two gram powdered sample of the seeds was processed with 15 ml of diacid blend ($5HNO_3$:$HClO_4$) in a conical flask by warming on hot plate in open space till clear white hastens settle down at base of conical flask. The encourages were broken up in 1% HCl arranged in double distilled water, shifted and last volume of filterate 50 ml made with double distilled water and examination was finished by (AAS) atomic absorption spectrometer.

Fatty acid spectrum:

Fatty acid profile were dictated by Fractination of methyl esters by GLC[6].

Total phenolic content:

The phenolic substance was determined by the technique of Folin-Ciocalteu reagent[7].

Flavonoids:

The aluminum chloride colorimetric measure was used. The absorbance was examined at 510 nm using UV observable spectrophotometer. Mean flavonoid substance was imparted as mg catechinper gram of the concentrate (mg CAE/g).

Tocopherol:

Aliquots (10, 15, 20, 25, 30, 35 and 40 ppm of a reply of tocopherol in the ethanol were traded to a volumetric flask and the volume was adjusted to 8 ml with ethanol. Each of the plan and 1.0 ml of 2,2′-dipyridyl reagent were pipetted into 10.0 ml volumetric flask and mixed. A 1.0 ml fragment of ferric chloride reagent was added to the 10.0 ml volumetric flask and the mix shaken for 10 s. The absorbance of the sample was measure at 520 nm against ethanol as a blank. By then the standard graph was drawn.

Determination of antioxidant activity:

Antioxidant activity studied by (DPPH) free radical scavenging method[8].

The scavenging activity of the extract will be calculated as:

$$\text{Inhibition (\%)} = [(\text{Abs(control)} - \text{Abs(sample)}] \times 100 / \text{Abs(control)}$$

Measurable analysis:

The trial were completed in triplicate and results were determined as mean of three replicates ± standard deviation. Quantifiable was completed utilizing Microsoft Excel 2007.

Results and discussion

Total phenolics content:

Plant phenolics are optional metabolites which are naturally aromatic and are highly antioxidants in view of their capacity to inhibits the free radicals and active oxygen. It is wonderful that phenolic substances contribute to the antioxidant activity of plant materials. Really phenolics show significant free radical-inhibition activity. In this way, the measure of aggregate phenolics in two areas (Palwal and Hisar) of *Melia azedarach* were determined.

Our findings showed that the substance of aggregate phenolics of *Melia azedarach* of two areas were 26.5±0.4 mg GAE/g to 28.7±0.3 mg GAE/g in phenolic extract of seed oil and 18.5±0.4 mg GAE/g to 16.8±0.3 mg GAE/g in methanolic extract of defatted seed cake. The higher estimation of aggregate phenolics found in seed cake when contrasted with seed oil shown in Fig. 1.

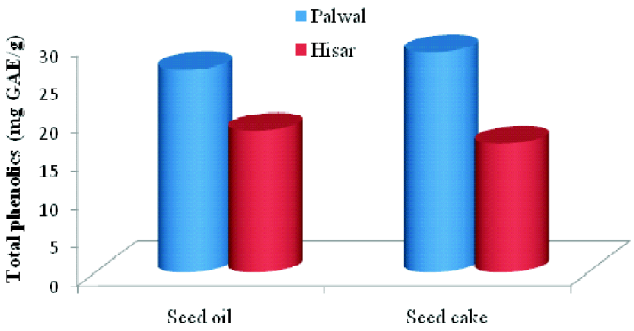

Fig. 1. Total phenolics (mg GAE/g) in phenolic extracts of seed oil and methanolic extracts of defatted seed cake.

Flavonoids content:

Flavonoids are presumably the most essential class of characteristic phenolics and can give electrons or hydrogen molecules promptly, so they can directly rummage responsive oxygen species. They are additionally antioxidants referred to go about as radical scavenger and as metal chelators. Along these lines, the flavonoid substance of *Melia azedarach* (two areas) were resolved. The TFCs are communicated as far as catechin equivalent (CE) and are introduced in Fig. 2.

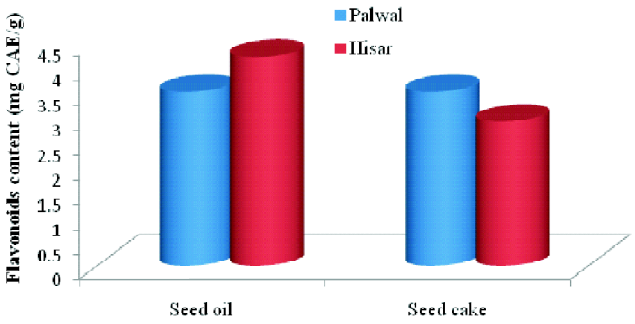

Fig. 2. Flavonoids content (mg CAE/g) in phenolic extracts of seed oil and methanolic extract of defatted seed cake.

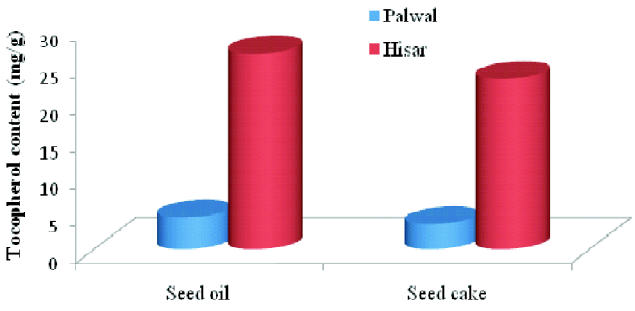

Fig. 3. Tocopherol content (mg/g) in phenolic extract of seed oil and methanolic extract of defatted seed cake.

The measure of flavonoids in phenolic extracts of *Melia azedarach* were (3.5±0.4 mg CAE/g to 3.5±0.3 mg CAE/g) and in methanolic extract of defatted seed cake were (4.2±0.1 mg CAE/g to 2.9±0.0 mg CAE/g). In this the flavonoids content comparative in seed oil yet had a huge diverse in seed cake in both areas. This is may be due to soil conditions, environmental and many others factors.

Total tocopherol:

Tocopherols are characteristic antioxidant, which are available in every vegetable oil in various sums that assume a key part in saving oil from rancidity amid capacity in this manner delaying its time span of usability. Tocopherols go about as natural criminals of free radicals and could counteract infections, other than having an imperative nutritious capacity for people as a wellspring of Vitamin E[9,10]. The tocopherol substance of nourishments is critical to ensure sustenance lipids against autoxidation and, in this way to build their capacity life and their esteem as wholesome nourishments. The tocopherol content in the phenolic extracts of seed oil of *Melia azedarach* two areas (Palwal and Hisar) were 3.4±0.2 mg/g to 4.3±0.2 mg/g and in methanolic extracts of defatted seed cake were 23.1±0.2 mg/g to 26.5±0.4 mg/g. In comparision of aggregate tocopherol in crude oil and methanol extract of defatted seed cake, we found that there were a large difference of total tocopherol content between seed oil and seed cake. The higher value of tocopherol was found in methanol extracts of seed cake as comparision to seed oil in both the locations. This might be because of various agroclimatic conditions.

DPPH free radical scavenging activity:

2,2′-Diphenyl-1-picrylhydrazyl radical is one of only a handful few stable and financially accessible natural free radical (DPPH•), regularly utilized as a part of assessment of radical scavenging movement of natural and manmade antioxidants compounds[11], plant extracts[12] and foods[13]. Alcoholic arrangements of DPPH• have a trademark absorption maximum at 517 nm. At the point when an electron or hydrogen ion giving cancer prevention agent (AH) is added to DPPH•, a diminishing in absorbance at 517 nm happens because of the arrangement of the non-radical shape DPPH-H which does not ingest at 517 nm. This response is measured by de-shading test where the diminishing in absorbance at 517 nm delivered by the expansion of the cancer prevention agent to the DPPH• in methanol or ethanol is measured.

$$DPPH^{\bullet} + AH \rightarrow DPPH\text{-}H + A^{\bullet}$$

All the phenolic concentrates were screened with the expectation of complimentary radical rummaging action against DPPH. The cell reinforcement movement (IC_{50}) displayed by phenolic extract of seed oil of *Melia azedarach* of two locations (Palwal and Hisar) were 0.038±0.0 mg/ml to 0.045±0.0 mg/ml and in methanolic extracts were 0.031±0.0 mg/ml to 0.040±0.0 mg/ml. Our perception practically similar in both the locations appeared in Fig. 4. These outcomes likewise associate with aggregate phenolic content which is additionally higher in acetone fraction. Higher polyphenolic content compares with higher cell reinforcement action which may be because of the joined activity of present substances in factor fixations and their hydrogen ion giving capacities[14].

Proximate composition of seed kernels:

Proximate composition of seed kernels of *Melia azedarach* (Palwal and Hisar) demonstrated moisture

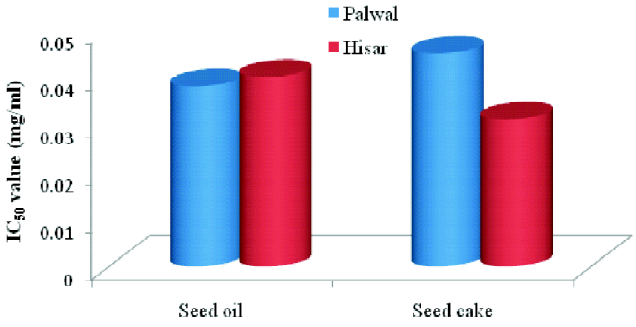

Fig. 4. IC_{50} Values (mg/ml) in phenolic extract of seed oil and methanolic extract of defatted seed cake.

6.3±0.1% to 6.6±0.1%, yield of oil 33.2±0.2% to 37.7±0.4%, crude proteins 22.7±0.2% to 23.1±0.1%, crude fiber 3.4±0.3% to 3.7±0.1%, ash content 7.3±0.1% to 7.1±0.4%, carbohydrates 46.2±0.7% to 27.1±0.7%, 21.8±0.5 and energy value 2081.6±2.2, 2171.1±2.8 kJ/100 g (Table1).

Table 1. Proximate composition of seeds of *Melia azedarach*

Parameters	Composition	
	Palwal	Hisar
Moisture (%)	6.3±0.1	6.6±0.1
Ash (%)	7.3±0.1	7.1±0.4
Fibre (%)	3.4±0.3	3.7±0.1
Protein (%)	22.7±0.2	23.1±0.1
Yield of oil (%)	33.2±0.2	37.7±0.4
Carbohydrates (%)	27.1±0.7	21.8±0.5
Energy (kJ/100 g)	2081.6±2.2	2171.1±2.8

Values are mean of three replicates ± standard error.

Mineral composition of seeds:

Minerals are required to initiate many enzymic responses inside the body. Life is needy upon the body's capacity to keep up harmony between the minerals[15]. The mineral contained in these seeds contemplated may assume imperative part in sustenance. Magnesium, calcium and potassium in the human were required for building red platelet and for body instrument[16]. The seeds of *Melia azedarach* (Palwal and Hisar) contained critical measure of vital minerals as calcium 208.0±2.5 mg/100 g and 201.0±2.1 mg/100 g, potassium 182.0±2.0 mg/100 g and 188.0±1.7 mg/100 g, sodium 110.0±1.2 mg/100 g and 108.0±1.0 mg/100 g, iron 50.0±0.5 mg/100 g and 63.0±0.2 mg/100 g and remaining are in lower fixation which is appeared in Table 2. Calcium

Table 2. Mineral composition of seeds of *Melia azedarach*

Parameters	Composition	
(mg/100 g)	Palwal	Hisar
Ca	208.0±2.5	201.0±2.1
K	182.0±2.0	188.0±1.7
Na	110.0±1.2	108.0±1.0
Mg	3.4±0.2	3.7±0.4
Fe	50.0±0.5	63.0±0.2
Zn	20.0±0.4	16.0±0.2
Co	Traces	Traces
Mn	0.4±0.1	0.5±0.1
Cu	0.1±0.1	0.2±0.1
P	50.0±0.3	41.0±0.5

Values are mean of three replicates ± standard error.

assumes a noteworthy part in CNS work. Calcium is fundamental for nerve motivation conduction and enacts a few compounds which create neurotransmitters. Phosphorus is fixing to calcium in bone structure and assumes a critical part in CNS work. Numerous proteins contain as a base phosphoproteins. Phospholipids are included in nerve conduction. Phosphate is the essential particle in additional and intracellular liquid. It helps absorption of dietary constituents, keep up the blood at a somewhat antacid levels, directs protein action and is included in the transmission of nerve driving forces[17]. Abundance of potassium can deliver neurological aggravations, for example, deadness of hand and feet. Zinc inadequacy is connected with mental retardation, passionate turmoil and crabbiness. Iron assumes critical part in oxygen transport in the body. Unsettling influence in mental capacity can be brought about by stream in the metabolic pathways that require iron. This is a direct result of too little oxygen achieving the cerebrum. Iron is required for DNA blend. Iron is additionally important for the initiation of chemicals required in cerebrum neurotransmitters.

Physiochemical analysis of seed oil:

Table 3 presents the results of the physicochemical analysis of the oils of *Melia azedarach* two locations (Palwal and Hisar). The free fatty acid were 2.0±0.1% to 2.7±0.1%, iodine value 122.4±0.5 to 124.6±0.7, peroxide value of fresh oil were 2.2±0.1 to 1.7±0.4 meq/kg, saponification values 184.7±0.6 to 188.0±0.3 mg/g KOH and unsaponifiable matter 2.2±0.0% to 2.1±0.1.

Table 3. Chemical characteristics of seed oil of *Melia azedarach*

Parameters	Composition	
	Palwal	Hisar
Peroxide value (meq/kg)	2.2±0.1	1.7±0.4
Iodine value (g/100 g)	122.4±0.5	124.6±0.7
Saponification value (mg KOH/g)	184.7±0.6	188.0±0.3
Unsaponifiable matter (%)	2.2±0.0	2.1±0.1
Free fatty acid (%)	2.0±0.1	2.7±0.1

Values are mean of three replicates ± standard error.

Fatty acid composition:

For fatty acids composition first converted into their respective volatile esters by transesterification i.e. by converting them from glycerol esters to methyl esters. The esters are then identified and quantified by injecting the processed samples into GLC and by comparing with chromatographic patterns of a set of standard esters. The important major fatty acid in *Melia azedarach* two locations (Palwal and Hisar) linoleic acid 75.5±0.3% and 74.0±0.6%, oleic acid 15.1±0.2% and 16.0±0.1% (Table 4). Oils having oleic acid and linoleic acid are useful in health promoting effect. Therefore the oils of the above seeds having high amount of oleic acid and linoleic acid are useful for food and feed formulations. It was also observed that proportion of fatty acids were different among the oils of the seeds obtained from the two locations. The fatty acid profile of the seed oil generally exhibited dominant of two class MUFAs and PUFAs. MUFAs have been paid attention during past decades due to the beneficial effects on cardiovascular heart disease[18]. A MUFA rich-diet tends to decrease low density lipoprotein-cholesterol[19].

Table 4. Fatty acid composition of seed oil of *Melia azedarach*

Parameters	Composition	
	Palwal	Hisar
Palmitic acid ($C_{16:0}$)	5.9±0.4	7.5±0.6
Stearic acid ($C_{18:0}$)	2.9±0.4	0.8±0.3
Oleic acid ($C_{18:1}$)	15.1±0.2	16.0±0.1
Linoleic acid ($C_{18:2}$)	75.5±0.3	74.0±0.6

Values are mean of three replicates ± standard error.

In this study the seeds and oils of *Melia azedarach* have been evaluated for proximate and chemical composition. The results of the proximate analysis revealed the presence of high amounts of protein and carbohydrate in the seeds. The oil of *Melia azedarach* had the highest iodine value. Linoleic acid was the dominant fatty acid in the studied oils. The overall studied concluded that it also acts as a good source of natural antioxidant.

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