Research Article

Comparision of Antioxidants in Phenol Extract and Methanol Extract of *Albizia lebbeck* from two Locations

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ABSTRACT

The present study analysed the antioxidant present in methanol extracts of defatted seed cake and phenolic extracts of seed oil of *Albizia lebbeck.* The extracts were used to evaluate total phenolics content, flavonoids content, total tocopherol, carotenoid content and free radical scavenging activity by DPPH method. Total phenolics, flavonoids and tocopherol content was highestin methanol extracts of defatted seed cake 22.6±0.3mgGAE/g, 9.5±0.3 mg CAE/g& 131.2±0.2 mg/g(Hisar) as compared to phenolic extracts of seed oil. Carotenoids content was highest 294.3±0.3mg/kg in Palwal location as compared to Hisar location. The antioxidant activity having IC_{50} value which was highest in methanol extract 0.067±0.0 (Palwal) as compared to seed oil extracts. The maximum antioxidant activity was found 79% (Hisar) in seed oil than seed cake. The seed composition of *Albizia lebeck* had a highest value of ash content 4.7±0.1%, protein 28.9±0.4% and fibre content 5.2±0.1%.

Keywords: *Albizia lebbeck,* antioxidant activity, proximate composition and total phenolics.

INTRODUCTION

Albizia lebbeck (L.)Benth. (Mimosaceae), referred to locally as shirish, is an unarmed deciduous woody tree. It is developed in many parts in farmlands, along roadsides, along rivers, and as an ornamental plant in gardens because of its wonderful appearance.[1, 2] The plant has a surprising reputation because of its food, feeds and medicinal values. It is considered a potent alexipharmic, and every part of it is prescribed for the treatment of bites and stings from venomous animals. Its leaves are reported to be good for ophthalmic diseases, night blindness, syphilis and ulcer[3,4] cool, hack, and respiratory issue.[5, 6] The leaves are likewise utilized as cows grain, mulch, and excrement because of high nitrogen substance.[7] The bark is intense, cooling, alexiteric, and anthelmintic, and cures maladies of blood, leucoderma, skin infection, heaps, over the top sweat, irritation, bronchitis, and toothache and reinforces the gums and teeth; it is utilized for uncleanliness, deafness, bubbles, scabies, syphilis and weakness.[8]

MATERIALS AND METHODS

Dry pods of *A. lebbeck* were collected from forest area of CCS HAU, Hisar and district Palwal, Haryana. The seeds were removed from their pods and ground to powder form using electric grinding machine. The powder seed was used for analysis.

Proximate Analysis

Proximate analysis done by the method of AOAC.[9]

Total Tocopherol

Aliquots (10, 15, 20 and 25 mg) of an answer of ∝tocopherol in the ethanol were exchanged to a volumetric flask and the volume was changed in accordance with 8ml with ethanol. Each of the arrangement and 1ml of 2,2̀ - dipyridyl reagent were pipetted into 10 ml volumetric flask and blended. A one ml part of ferric chloride reagent was added to the 10 ml volumetric flask and the blend shaken for 10 seconds. The absorbance of the blend was measured at 520 nm against ethanol as a blank. At that point the standard curve was drawn. The above portrayed strategy was trailed by utilizing 10, 20, 30, 40 mg test arrangements. The substance of ∝-tocopherol in the concentrate was determined by regression equation.

Total Carotenoids

Determination of total carotenoids was done by the method. [10]

Total Phenolic Content

The phenolic substance was determined by the technique of Folin-Ciocalteu reagent. [11]

Flavonoids

The aluminum chloride colorimetric measure[12] the absorbance was examined at 510 nm using UV observable spectrophotometer. Mean flavonoid substance was imparted as mg catechin reciprocals per gram of the concentrate (mg CAE/g).

Determination of Antioxidant Activity

Antioxidant activity studied by (DPPH) free radical scavenging method.[13]

The scavenging activity of the extract will be calculated as: Inhibition (%) = [(Abs(control)-Abs(sample)] × 100 /Abs(control)

Data Analysis

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

RESULTS

Phytochemical Components and Antioxidant Activity

The results showed that the content of the phenolic extracts and methanolic extracts in crude oil and defatted seed cake of *A.lebbeck* in two locations (Palwal & Hisar) were found as total phenols 16.3±0.4mgGAE/g, 14.8±0.1mgGAE/g (in seed oil) and 20.2±0.2 mgGAE/g, 22.6±0.3 mg GAE/g (in seed cake). Flavonoids were 0.3±0.2 mgCAE/g,0.7±0.4 mg CAE/g (in oil) and 8.7±0.2 mg CAE/g, 9.5±0.3 mg CAE/g (in seed cake). The content of total tocopherol was 69.7±0.2 mg/g59.9±0.9 mg/g (in oil) and 130.3±0.3 mg/g, 131.2±0.2 mg/g (in seed cake). The carotenoids value determined were 294.3±0.3 mg/kg &230.3±0.4 mg/kg (in seed oil). The antioxidant activity (Inhibition concentration) exhibited by phenolic extracts of crude seed oil were 0.024±0.0, 0.023±0.0 and 0.067±0.0, 0.045±0.0 (in seed cake) at different concentration of mg/ml of the extract respectively, shown in (Table1).

Proximate Composition of Seeds of *Albizia lebbeck*

The compositions of seed powder of two locations were determined according to the standard methods of AOAC (1990) which were given in Table 2. The moisture content were 3.1±0.2% &3.5±0.2%, ash content were 4.7±0.1% & 4.5±0.1%, fiber content were 4.8±0.3% & 5.2±0.1%, protein content were 28.5±0.6% & 28.9±0.4%, Carbohydrates were 48.0±0.7% & 47.4±0.2% and energy values were 1688.4±1.4 kJ/100g& 1670.0±1.8 kJ/100g in both locations.

DISCUSSION

Total Phenol

Plants phenolics are the important secondary source in plants which contains a large number of antioxidants because these are capable to scavenge free radicals and active oxygen species like singlet, superoxide free radicals and hydroxyl radicals. Natural polyphenols have chain-breaking inhibitor activities. It's accepted that phenolic resin substances contribute on to the inhibitor activity of plant materials. In fact, phenolic resin compounds exhibit free radical-scavenging activities (through their reactivity as hydrogen-donating or electron-donating agents) and metal ion-chelating properties.[14] Therefore, the amount of total phenols in the phenolic extracts of crude oil and methanol extracts of defatted seed cake were determined. Our results showed that the content of total phenols varied in each plants. The highest value of total phenolics is 22.6±0.3 mgGAE/g(Hisar) in methanolic extract of defatted seed cake and lowest value was 14.8±0.1 (Hisar) in seed oil. From the data of two locations, it is inferred that phenolic content in the oil of the seeds are lower than the methanolic extracts of the seed cake collected from the two locations.

Flavonoid Content

Among synthetic resin compounds, flavonoids are the foremost necessary cluster of plant phenolics that have inhibitor potential. These flavonoids considerably to possess inhibitor, anticancer, anti-allergic, medicine and gastro protective properties. Flavones and flavonols are the subgroups of flavonoids. Flavonols are proverbial to act as inhibitor, each as radical scavengers [15]and as metal chelators.[16] The aglycones of those flavonols were rumored to be additional active than their glycosides.[17, 18] Flavonoids have the power to scavenge active element radical, superoxide and hydroperoxide by single electron transfer. Superoxide may be a biologically necessary substance which might be decomposed to make stronger oxidative species like singlet oxygen and hydroxyl radicals.[19] The extremely reactive chemical group radicals will cause oxidative damage to desoxyribonucleic acid, lipids and proteins.[20]

In the present study the flavonoid content in phenolic extracts of crude oil and methanol extracts of defatted seed cake were determined (Table 1). Our results showed that in *A. lebbeck*, the flavonoid content in the phenolic extract of oil were 0.3±0.2mgCAE/g and 0.7±0.4mgCAE/g much lower than in the methanolic extract of seed cake 8.7±0.2mgCAE/g and 9.5±0.3mgCAE/g in Palwal and Hisar locations respectively. The higher value of flavonoids content in methanol extract indicates that seed cake might contain higher levels of glycosylated flavonoids than the oil of seeds.

Tocopherol Content

Tocopherols are natural antioxidants, that are gift all told vegetable oils in several amounts that play a key role in c onserving oil from rancidity throughout storage so prolonging its shelf-life.[21] Tocopherols act as biological kidnappers of free radicals and could prevent diseases, besides possessing a very important biological process operate for groups of people as a supply of vitamin E.[22 23] The vitamin E content of foods is very important to safeguard food lipids against auto oxidation and, thereby to extend their storage life and their worth as wholesome foods. The tocopherol content ranged from 59.9 mg/g to 131.2 mg/g. The highest tocopherol content was found in *Albizia lebbeck* 131.2±0.2 mg/g in methanol extract than seed oil. There are a difference in tocopherol content in both locations in seed oil may be due to agro climatic conditions.

Carotenoids Content

The colour in oils is principally owing to the presence of pigment or chlorophyll pigments. Carotenoids shield cells against the impact of light, air and sensitizer pigments having the power to quench singlet oxygen and

may conjointly function antioxidants beneath conditions apart from photosensitization.[24] Some crude oils will have unexpectedly high pigmentation caused by field injury, improper storage, or faulty handling throughout crushing, and extraction. Pigment amount determined in oils of the seeds studied ranged from 230.3 to 294.3 mg/kg the very best was in Palwal location.

Evaluation of Antioxidant Activity by DPPH Free Radical Scavenging Method

Various radicals fashioned throughout lipoid oxidization are among the most causes for aerobic injury to human health.[25] Antioxidants will exercise their protecting perform by scavenging free radicals, that are the most propagators of lipoid oxidization. 2, 2'-diphenyl-1-picrylhydrazyl radical is one amongst the few stable and commercially offered organic element radical (DPPH•), usually employed in the analysis of radical scavenging activity of antioxidants-natural and artificial pure compounds.[26, 27] Methanolic solutions of DPPH• have a characteristic absorption most at 517nm. When an electron or hydron atom donating inhibitor (AH) is additional to DPPH• a decrease in absorbance at 517nm takes place due to the formation of the non-radical form DPPH-H, that doesn't absorb at 517nm. Originally, it had been monitored by ESR qualitative analysis and relied on the signal intensity of DPPH• being reciprocally associated with the inhibitor concentration and also the time interval. Additional recently, this reaction has been measured by the de-coloration assay wherever the decrease in absorbance at 517nm made by the addition of the inhibitor to the DPPH• in methanol or ethanol is measured.

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

All the above described extracts were screened for radical scavenging activity against DPPH•. The maximum antioxidant activity/capacity exhibited by phenolic extracts of crude seed oil and methanol extracts of defatted seed cake. The results obtained by DPPH method showed that the maximum antioxidant was found 79% (Hisar) at a concentration of 0.07 mg/ml in seed oil and minimum value found in methanol extracts that is 55% at a concentration of 0.07 mg/ml.

The maximum antioxidant activity in terms of IC_{50} was found 0.067±0.0mg/ml (Palwal) and minimum value was found 0.023±0.0 mg/ml (Hisar) shown in Table 1.At last in overall comparison, it was found that the antioxidant activity of methanol extracts of defatted seed cake is higher than that the phenolic extracts of the oil which might be due to some other extractable antioxidants from the defatted seed cake.

Table1: Phytochemical components and antioxidant activity in phenolic extract of seed oil and methanolic extract of defatted seed cake of *Albizia lebbeck*

lebbeck	Composition			
	Phenolic extract of seed oil		Methanolic extract of seed cake	
	Palwal	Hisar	Palwal	Hisar
Total phenolics (mgGAE/g)	16.3±0.4	14.8±0.1	20.2±0.2	22.6±0.3
Flavonoids (mgCAE/g)	0.3±0.2	0.7±0.4	8.7±0.2	9.5±0.3
Total tocopherol (mg/g)	69.7±0.2	59.9±0.9	130.3±0.3	131.2±0.2
Carotenoid content (mg/kg)	294.3±0.3	230.3±0.4	-	-
DPPH IC_{50} (mg/ml)	0.024±0.0	0.023±0.0	0.067±0.0	0.045±0.0
Antioxidant activity (%)	74	79	55	68

Values are mean of three replicates ± standard error

Table 2: Proximate Composition of Seeds of *Albizia lebbeck*

Parameters	Composition	
	Palwal	Hisar
Moisture (%)	3.1±0.2	3.5±0.2
Ash (%)	4.7±0.1	4.5±0.1
Fibre (%)	4.8±0.3	5.2±0.1
Protein (%)	28.5±0.6	28.9±0.4
Yield of oil (%)	10.9±0.4	10.5±0.1
Carbohydrates (%)	48.0±0.7	47.4±0.2
Energy (kJ/100g)	1688.4±1.4	1670.0±1.8

Values are mean of three replicates ± standard error

Proximate Composition of Seeds

Proximate composition of the seeds from Palwal and Hisar locations are presented in the Table 2.

Moisture Content

The moisture content varied from 3.1±0.2% to 3.5±0.3%. The low moisture content in the seeds of *Albizia lebeck* in both the locations. Low moisture content in the seeds informed that they will have a long life, 28 since the low moisture content may prevent microbial spoilage and pest attack during storage.

Ash Content

Ash content assurance is huge in nourishment and encourage for different reasons. Among others, it is records of the nature of nourishing materials utilized by creature encourage compounders for poultry and cows bolstering. It has been built up by,[29] that a plant material proposed for sustain detailing ought to have fiery remains substance of < 2.5%. The highest value of ash content was 4.7±0.1% in Palwal location as compared to Hisar.

Fibre Content

Fiber is a vital dietry part for bowl development. The fiber substance is additionally utilized as a measure of nutritive estimation of poultry and animals encourages. In the present review it varied from 4.8±0.3% to 5.2±0.1% of seeds. Nearness of high fiber content in eating regimen may bring about intestinal aggravation, bring down edibility and general reduction supplement usage.[30]

Crude Protein

Protein content is the most imperative encourage part that ought to be taken into thought while selecting any material for nourish definition. In this study the protein content differed from 28.5±0.6% to 28.9±0.4%. Generally higher measures of crude protein were found in seeds of *Albizia lebbeck*. There show up no locational variety in the protein content. The protein content in *Albizia lebbeck* comparable and.[31]The crude protein content in seeds considered sufficiently satisfactory for meeting the support prerequisites of sheep, goat, for poultry and rabbits.

Percentage Yield of Seed Oil

In this study, petroleum-ether (60-80°C) was used for the extraction of oil using Soxhlet technique. The yield of extractable seed oil were ranged from 10.5±0.1% (Hisar)to 10.9±0.4% (Palwal). There is no location variation in the yield of oil.

The carbohydrate and energy values of seeds were found to maximum in Palwal location 48.0±0.7% and 1688.4±1.4kj/100g.

CONCLUSION

This study indicates *Albizia lebbeck* to be a cheap, reliable and safe plant-based resource to fulfill the demand for protein-rich foods. It may provide healthy and useful food by providing several of the nutrients the human body needs, as it is high in protein, high in dietary fibers, and low in oil content. On the basis of the results of this study, it is concluded that extracts of *Albizia lebbeck*have significant antioxidant activity along with nutritional values. This study highlights the maximum IC_{50}value, total phenolics, flavonoids and tocopherol content in methanol extracts of defatted seed cake as compared to phenolic extracts of seed oil.

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