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Research Paper

Variations in antioxidant activity and phenolic contents of extracts among various plant species

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Abstract
In our study, six medicinal plants Acacia nilotica*,* Albizia lebbeck*,* Melia azedarach*,* Pongamia pinnata*,* Cassia fistula*, and* Salvadora persica *were analyzed for phytochemical constituents using extracts of seed cake and seed oil. The findings of the medicinal plants revealed that the yield of oil content was maximum 42.5±0.4% in S. persica respectively. Total phenols, flavonoids, carotenoids and total tocopherol in the oil were found to bemaximum 87.2±0.2mg GAE/g and11.9±0.4mgCAE/g in (A. nilotica), 294.3±0.3 mg/kg and 69.7±0.2 mg/g in (A. lebbeck) respectively. Total phenols, flavonoids and total tocopherol in the defatted seed cake were found to maximum 51.2±0.2mg GAE/g in (P. pinnata), 9.5±0.3mgCAE/g in (A. lebbeck) and 185.6±0.5 mg/g in (C. fistula) respectively. The major fatty acid composition of the oil were found as oleic acid 58.7±0.7 % in (P. pinnata) and linoleic acid 75.5±0.3% in (M. azedarach). Antioxidant activity was determined by DPPH free radical scavenging method in the methanol extracts of seed oil and defatted seed cake and found to be maximum 79 % at conc. of 0.07 mg/ml in (A. lebbeck) and 87 % at conc. 0.06mg/ml in (A. nilotica) extract respectively. Antioxidant activity in terms of IC_{50} was maximum 0.045±0.0 mg/ml in (S. Persica) and 0.067±0.0 mg/ml in (A. lebbeck) for the seed oil and defatted seed cake extract respectively.*

Keywords: *Oil seeds, tocophereols, carotenoids, phenols, flavonoid contents, antioxidant activity*

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I. Introduction

In developing countries, the shortage of nutritious animal feed has underscored the need for alternative dietary sources. Seeds from numerous forest trees produce oils of economic significance, and the by-product seed cake is widely used as fodder. Concurrently, increasing consumption of vegetable oils for domestic and industrial purposes has intensified research into these plant-based resources. Consequently, research efforts have focused on investigating new plant sources of oil, especially from underexploited seeds.

Antioxidants play a critical role in protecting cells from damage caused by reactive oxygen species (ROS). Studies have shown an inverse correlation between the consumption of antioxidants and the progression of diseases. Therefore, researchers are increasingly seeking natural, non-toxic antioxidants from medicinal plants. In India and other Asian countries, medicinal plants have long been used in traditional medicine. These plants contain bioactive compounds, such as phenolics, that help reduce oxidative stress–associated diseases. Polyphenols, aromatic compounds synthesized by plants, constitute one of the largest groups of secondary metabolites and are recognized for their remarkable free-radical scavenging abilities. Research has highlighted their potential in preventing cancer and viral infections (Hertog et al. 1998), as well as reducing the risk of cardiovascular diseases (C.T. Ho 1992). To date, more than 8,000 naturally occurring phenolic compounds have been identified across various plant sources (Balasundram2006).

Acacia is a genus of the Fabaceae family comprising about 135 species of trees and shrubs, widely distributed throughout arid and semi-arid tropics. *A. nilotica*, belonging to the subfamily Mimosoideae and commonly known as the Gum Arabic tree, is a 5–20 m high tree with a dense spherical crown. Its leaves, locally called "Chesh," are used for feeding sheep and goats (Abbasian et al. 2016). Species such as *Acacia nilotica* and *Prosopis juliflora* are suitable for afforestation of dry and degraded lands due to their minimal water requirements, unlike non-edible oil crops like jatropha and pongamia, which have low yields and high nutrient

demands. Oil extracted using traditional mechanical presses contains impurities such as free fatty acids (FFA), pigments, and gums, reducing flavor and stability. Solvent extraction, commonly using hexane, raises safety concerns due to residual solvents and harmful vapors. Supercritical fluid extraction with CO_2 has been explored as a safer option, but the extracted oil may still contain high FFA and phospholipids, requiring further refining (Rajeshwaran et al. 2020).

Albizia lebbeck (family Mimosaceae) is a fast-growing, nitrogen-fixing tree widely used for reforestation and firewood. It grows up to 20 m tall, with a broad spreading crown and pale bark. Its leaves are alternate and bipinnate, bearing 2–5 pairs of pinnae with 3–10 pairs of elliptic leaflets. The tree produces cream to yellowish-white mimosa-like flowers and elongated pods containing numerous seeds (Zia-ul-Haq et al. 2013). Traditionally, its bark is used to treat toothache and gum disorders, while leaf and bark decoctions manage bronchial asthma and allergies. The seeds are consumed after boiling and used as aphrodisiac, brain tonic, and for treating gonorrhea. Seed oil is applied topically for leucoderma, and extracts of pods show anti-protozoal, antifertility, hypoglycemic, and anticancer activities (Bobby et al. 2012; Zia-ul-Haq et al. 2013). Phytochemically, the plant contains saponins, alkaloids, tannins, flavonoids, and terpenes.

Melia azedarach L. (Meliaceae), also known as Chinaberry or Persian Lilac, is widely used in traditional medicine to treat diseases such as diarrhea, malaria, skin disorders, intestinal obstruction, and leprosy (Rabbani et al. 2024). Its phytoconstituents include terpenoids, flavonoids, steroids, anthraquinones, alkaloids, saponins, and tannins. Leaf extracts are used for rheumatic pain and burns, while seed oil treats rheumatism and malarial fever (Rida et al. 2025).

Pongamia pinnata (L.) Pierre (Leguminosae, Papilionaceae; synonym *Pongamia glabra* Vent.), known as Karanj, is a medium-sized tree distributed throughout India and South-Eastern Asia. Seeds contain 28–34% oil rich in polyunsaturated fatty acids and have been traditionally used in Ayurvedic and Siddha medicine to treat tumors, skin diseases, rheumatic pain, ulcers, diarrhea, and more (Meera et al. 2003; Naik et al. 2008; Sarma et al. 2005; Shoba and Thomas 2001).

Cassia fistula Linn., or golden shower, is a moderate-sized deciduous tree belonging to Leguminosae. It is widely distributed in India and used as a folk remedy for burns, cancer, constipation, diarrhea, and various other ailments (Duke and Wain 1981; Rajput et al. 2022). Plant parts are rich in flavonoids, anthraquinones, polysaccharides, and other bioactive compounds (Farooq et al. 1956; Wati et al. 2017).

Salvadora persica L. (Salvadoraceae), the toothbrush tree, is a small branched evergreen distributed in tropical regions of Asia and Africa. It contains alkaloids, flavonoids, steroids, saponins, carbohydrates, and volatile oils. Traditionally, twigs are used for oral hygiene, leaves as mouthwash, and roots for asthma and cough treatment (Abdillahi et al. 2010; Al-Quran 2008; Kamil et al., 1999; Watt and Breyer-Brandwijk, 1962). Seeds, bark, and leaves are rich in fatty acids, tocopherols, sterols, and phenolic compounds (Ali et al. 1997; Almas et al. 2005; Malik et al. 1987; Siddiqui et al. 2006).

II. Experimental:

Material

Seeds of selected medicinal plants "*Acacia nilotica, Albizia lebbeck, Melia azedarach, Pongamia pinnata, Cassia fistula,* and *Salvadora persica"* The seeds were sourced from the fields of CCS HAU, Hisar, as well as from Palwal District. After being meticulously cleaned, they were ground into a fine powder by grinder.

Extraction of seed oil

In the first experiment, the seeds of all the six plants were collected from the forest area of CCS HAU Hisar and District Palwal were ground to powder form and oil content was determined by Soxhlet method using petroleum ether (60-80°C) for eight hours and condenser were attached for each sample in three replicates. The heating rate was adjusted to give a condensation rate of 2-3 drops/sec. Removed the thimble and retained petroleum ether. The excess of petroleum ether was evaporated from the solvent flask on a hot water bath and dried the flasks in a dessicator and weighed.

In the second experiment, the defatted seed cake were extracted with methanol as solvent by using Soxhlet extraction technique and the chemical composition of defatted seed cake of all the extracts were estimated. In the third experiment, DPPH free radical scavenging activity and phenolic compounds like phenols, flavonoids, tocopherol etc. were evaluated in all the methanolic extracts of oil and defatted seed cake.

The percentage yield of oil extract was estimated by

$$\text{Oil content in sample (\% dry wt. basis)} = \frac{(b-a)\times 100}{\text{Wt.of sample (g)}}$$

b = wt of powdered seed before extraction
a = wt of powdered seed after extraction

Fatty acid spectrum:
Preparation of methyl esters:
Methyl esters were prepared by the method of (Luddy et al.1968) as described below
Reagents:
(a) Sodium methoxide (0.5 N)
(b) Carbon disulphide
(c) Activated charcoal

Method:
A suitable amount of oil sample was taken in a test tube and 0.5 ml of 0.5 N sodium methoxide was added and covered with aluminium foil and then immersed in a water bath at 65ºC to a depth of half inch and was shaken vigorously for 2 - 3 min. The mixture became homogenous indicating the complete esterification of the oil sample. The test tube was removed from the water bath and cooled to room temperature. One ml of carbon disulphide was added and shaken for 1 - 2 min. Separated the lower layer and approximately 100 mg of activated charcoal was added mixed uniformly and filtered through Whatman No. 1 filter paper. The filtrate constituted all the methyl esters of fatty acids.

Fractination of methyl esters by GLC:
Methyl esters of fatty acids were separated using Chemito 8610 HT Gas chromatograph equipped with FID and a BPX70, 0.25ml fused silica column (SGE Pvt. Ltd., Ringwood, Victoria, Australia) was used. The carrier gas was hydrogen and injection was operated in the split mode, the split ratio being approximately 50:1. Injector and detector temperature were 270ºC and 280ºC respectively. The oven temperature was held at 70ºC for 1 min. and then programmed at 30ºC/min. to 170ºC followed by further programming at 30ºC/ min. to 200ºC and held at this temperature for 6 min. Data was captured and analysed with, Chemito 5000 integrator (Tashniwal Instruments, India Ltd.).

Chemicals:
In the experiments, the chemicals used were procured from Qualigens, Merk and Ranbaxy, ensuring the highest purity.
Determination of total phenolics:
Folin-Ciocalteu reagent (Singleton and Rossi 1965) method used for the determination of total phenolics. Gallic acid using as standard.
Reagent: Methanol**,** Gallic acid**,** Sodium carbonate (20% w/v).
Estimation of total phenols in oil and seed cake of various seeds:
For estimation of total phenolics in oil and cake extract of various seeds "*Acacia nilotica, Albizia lebbeck, Melia azedarach, Pongamia pinnata, Cassia fistula* and *Salvadora persica*", firstly diluted extracts with methanol for calibration. Then took 1.0 ml extract and added 1.0 ml of 1.0 mol/L Folin-Ciocalteu reagent. After that added 2.0 ml of Na_2CO_3 (20%). Finally made total volume 10 ml by water and after 30minutes, centrifugation of the mixture was performed at 6000 rpm for a duration of 10 minutes. Then by using spectrophotometer Spectronic 20 (Milton Roy Company) the measurement of the supernatant's absorbance was taken at 730 nm, using a blank as a control. The values showed in mgGAE/g (milligrams of gallic acid equivalent per gram). The standard curve was used to find out the total phenol.
Determination of flavonoid content:
Colorimetric method had been used for flavonoids (Zhishen et al. 1999).
Reagents: Catechin**,** Sodium nitrite (5%)**,** Aluminium chloride (10%)**,** Sodium hydroxide (1M)
Determination of flavonoid content in oil and defatted seed cake of various seeds:
Firstly took a test tube and added 4ml of double distilled water in it. Then added 1.0 ml of extract (diluted) in it. After that added 5% $NaNO_2$ (sodium nitrite) 0.3 ml. 0.3 ml aluminium chloride 10% wasadded after 5 minutes. Instantly, 2.0 ml sodium hydroxide (1M) was added. Then made total volume of 10.0 ml by double distilled water. By using UV visible spectrophotometer the absorbance were recorded for both the blank and samples at 510 nm. The flavonoid content was expressed as mg CAE/g, representing milligrams of catechin equivalent per gram of the extract).

Determination of total tocopherol:
Solutions of α-tocopherol at concentrations of 10, 15, 20, 25, 30, 35, and 40 ppm were prepared in ethanol and transferred to separate volumetric flasks. The volume of each solution was adjusted to 8 ml using ethyl alcohol. Subsequently, 1.0 ml of 2,2'-dipyridyl reagent was added to a 10.0 ml volumetric flask, followed by the transfer of each prepared solution into the same flask. After adding 1.0 ml of ferric chloride ($FeCl_3$) reagent, shaking mixture for 10 seconds and measuring its absorbance at 520 nm, using a blank as control

described by Philip et al. (1954). A standard curve was then constructed based on the recorded absorbance values. Same methodology applied for sample solutions. To find out the value of tocopherol, the regression equation was used. Oil sample of 200±10 mg weighed exactly into a volumetric flask of 10.0 ml. Then 5.0 ml of toluene was added. 3.5 ml of 2,2′-bipyridine (0.07%) after that 0.5 ml of $FeCl_3 6H_2O$ (0.2%) added. Then made total volume 10.0 ml by ethyl alcohol (95% aqueous). After that at 520 nm the absorbance was recorded for the sample and the blank.

Total carotenoid content:
Total carotenoid content was estimated by using the method of (Vasconcellous et al. 1980).
Reagent: Cyclohexane
Method:
First of all took a 100 ml conical flask and 0.5 g oil was added to this flask. After that the The oil sample was dissolved in cyclohexane to a concentration of 2.5%. Absorbance at 417 nm was measured and total carotenoids were calculated by following formula:

$$\text{Carotenoid content (mg/kg)} = \frac{A \times V\ \text{ml}}{0.204 \times W\ \text{g}}$$

Where A= Absorbance at 417 nm
V= total volume of sample in mililitre
W= sample weight in gram

Estimation of antioxidant capacity:
Antioxidant capacity was estimated by using DPPH method (Hatano et al. 1988).
Reagent: DPPH

Method:
First of all (0.01, 0.02, 0.03, 0.04, 0.05, 0.06, 0.07, 0.08, 0.09) mg solutions of oil and seed cake were made. In each prepared solution, by adding 1.0 ml of DPPH· followed by methanol to bring total volume to 10 ml with methanol. The mixture was thoroughly mixed for 5 minutes, the sample's absorbance was recorded at 517nm by spectrophotometer. Measurements were taken at 10-minute intervals until a steady-state absorbance was achieved, indicating the completion of the reaction. In a similar manner, a control sample was also prepared. Here, using (BHA) butylated hydroxylanisole as standard. The quadratic regression equation ($y = ax^2+bx+c$) in Microsoft Excel was applied. In this equation, putting y = 50%. IC_{50} was calculated as:

$$X = \frac{-b \pm \sqrt{b^2-4ac}}{2a}$$

Where, x = IC_{50} (mg/ml)
Calculation:
Then using formula for percent DPPH scavenged:

$$\%\ B = \{(C_A - S_A)/C_A\} \times 100$$

Where B= % DPPH scavenged
Absorbance values for the control (C_A) and the sample (S_A).
A graph was plotted between inhibition percentage vs concentration.

III. Result and Discussion

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 6.4±0.1% to 42.5±0.4%. The high content of seed oil was found in *S. persica* (40.2±0.6% to 42.5±0.4%) followed by *P. pinnata* (39.2±0.7% to 40.3±0.4%) and *M. azedarach* (33.2±0.2% to 37.7±0.4%) in the locations Palwal and Hisar respectively shown in table 2 & 3. The result indicated that high yield of seed oil of *S. persica* which is similar with previous study (Reedy et al. 2008) and greater than chia seed oil(33.53-36.1%)(Ishak et al. 2020). The oil content in *S. persica* is very high as compared to other seed oils like *Sesame, Cotton, Groundnut, Caster bean*(Mariod et al. 2009).The application of co-solvents in supercritical carbon dioxide (SC-CO_2) extraction significantly influenced oil yield. The highest yield was achieved using methanol-modified SC-CO_2 extraction, with a result of 43% (w/w), followed by n-hexane-modified SC-CO_2 extraction, which yielded 38% (w/w). This indicates that the polarity of the co-solvent plays a crucial role in the oil extraction process (Pourhoseiniand Karimian2025).

Percentage yield of seed cake:
The yield of methanolic extracts of defatted seed cake were ranged from 1.3±0.2% to 10.5±0.5%. The highest yield was found in*S. persica* 11.6±0.2% (Hisar) and 10.4±0.5% (Palwal) while lowest in case of *A. lebbeck* 3.4±0.2% (Palwal) and 2.8±0.1% (Hisar) shown in table 2 & 3.

Fatty acids composition (%) of seed oils:

The fatty acid profiles of seed oils are presented in Table 1. In the present study the other unsaturated fatty acids linolenic acid and eicosenoic acids were present in low concentrations. Oils of all the seeds having oleic acid (57.4±1.2 to 58.7±0.7), linoleic acid(74.0±0.6 to 75.5±0.3)and myristic acid (54.0±0.3 to 55.5±0.8) in significant amount, which is similar to previous study(Kumari and Swer 2026; Rather et al. 2015). It was also observed that proportion of fatty acids were different among the oils of the seeds obtained from the two locations. The total unsaturated fatty acids in *A. nilotica* and *A. lebbeck* were higher in Hisar location which has hotter climate while it was found in lower concentration in the oil obtained from the seeds of Palwal location which has less harsh conditions than Hisar. In our study, *C. fistula* seed oil identified linoleic acid as the major constituent (54.3±0.7 to 53.6±0.7%), followed by oleic acid (19.2±0.4 to 18.9±0.4%) and palmitic acid (18.5±0.3 to 18.3±0.4%). The others were present as minor constituents, that's similar to previous study (Rajput et al. 2022).*S. persica* fatty acid composition was not comparable to the previous study (Mariod et al. 2009).

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 (Kalogeropoulos et al 2004). A MUFA rich- diet tends to decrease low density lipoprotein- cholesterol (Mensink and Katan 1987). The most predominant fatty acids in oil composition are oleic and linoleic acid which are monounsaturated and polyunsaturated respectively. Therefore, they are often referred to as predominantly unsaturated.

Table 1: Fatty acid composition of oils: (Palwal & Hisar)

Parameter	*A. nilotica*		*A. lebbeck*		*M. azedarach*		*P. pinnata*		*C. fistula*		*S. persica*	
Fatty acid	**Palwal**	**Hisar**	**Palwal**	**Hisar**	**Palwal**	**Hisar**	**Palwal**	**Hisar**	**Palwal**	**Hisar**	**Palwal**	**Hisar**
Myristic acid ($C_{14:0}$)	0.1±0.0	0.2±0.1	-	-	-	-	-	-	-	-	54.0±0.3	55.5±0.8
Palmitic acid ($C_{16:0}$)	13.7±0.4	13.4±0.2	13.5±0.3	14.3±0.4	5.9±0.4	7.5±0.6	9.2±0.6	9.1±0.3	18.5±0.3	18.3±0.4	20.2±0.4	19.3±0.2
Palmitoleic acid ($C_{16:1}$)	-	-	0.2±0.1	0.3±0.1	-	-	-	-	-	-	-	-
Stearic acid ($C_{18:0}$)	4.4±0.1	4.5±0.1	1.1±0.0	1.0±0.2	2.9±0.4	0.8±0.3	5.5±0.2	5.9±0.4	0.7±0.0	1.3±0.2	4.7±0.1	4.7±0.1
Oleic acid ($C_{18:1}$)	30.6±0.3	31.8±0.3	12.6±0.4	12.8±0.2	15.1±0.2	16.0±0.1	58.7±0.7	57.4±1.2	19.2±0.4	18.9±0.4	6.8±0.3	5.6±0.5
Linoleic acid ($C_{18:2}$)	40.5±0.4	39.6±0.2	62.7±0.8	64.8±0.5	75.5±0.3	74.0±0.6	16.8±0.1	17.6±0.1	54.3±0.7	53.6±0.7	3.4±0.3	2.3±0.0
Linolenic acid ($C_{18:3}$)	2.6±0.3	2.8±0.1	2.0±0.1	1.4±0.1	-	-	-	-	1.0±0.1	1.1±0.3	3.3±0.5	2.9±0.1
Arachidic acid ($C_{20:0}$)	0.1±0.1	0.1±0.0	0.9±0.1	0.6±0.0		-	2.4±0.4	2.4±0.1	0.3±0.1	0.1±0.1	2.1±0.1	3.1±0.4
Eicosenoic acid ($C_{20:1}$)	-	-	-	-	-	-	-	-	2.4±0.4	2.3±0.2	2.4±0.2	1.8±0.2
Behanic acid ($C_{22:0}$)	1.6±0.2	1.4±0.2	2.3±0.1	2.5±0.3	-	-	3.1±0.2	3.2±0.1	0.2±0.0	0.3±0.1	1.6±0.4	1.3±0.2

Values are mean of three replicates ± standard error

Phytochemical component of extract of crude oil and seed cake shown in Table 2, 3.
The values are presented as the mean of three replicates ± standard error.

TABLE 2: Phenolic compounds and IC_{50} value of seed oil& cake (Palwal)

Parameter	*A. nilotica*		*A. lebbeck*		*M. azedarach*		*P. pinnata*		*C. fistula*		*S. persica*		*Ascorbic acid*
	Seed oil	***Seed cake***	***Seed oil***	***Seed cake***	***Seed oil***	***Seed cake***	***Seed oil***	***Seed cake***	***Seed oil***	***Seed cake***	***Seed oil***	***Seed cake***	
Yield (%)	13.6±0.3	3.1±0.1	10.9±0.4	3.4±0.2	33.2±0.2	6.8±0.2	39.2±0.7	10.5±0.6	6.4±0.1	1.3±0.2	40.2±0.6	10.4±0.5	-
Phenolic content (mg GAE/g)	80.1±0.2	18.2±0.3	16.3±0.4	20.2±0.2	26.5±0.4	18.5±0.4	13.5±0.4	48.2±0.2	7.0±0.0	10.3±0.1	14.0±0.2	22.3±0.3	-
Flavonoid content (mg CAE/g)	11.3±0.3	4.9±0.1	0.3±0.2	8.7±0.2	3.5±0.4	4.2±0.1	1.5±0.2	2.5±0.2	1.3±0.1	2.9±0.0	4.0±0.1	3.7±0.6	-
Total tocopherol (mg/g)	12.2±0.6	15.5±0.3	69.7±0.2	130.3±0.3	4.3±0.2	26.5±0.4	38.0±1.0	100.0±0.2	8.4±0.1	176.3±0.2	18.8±0.5	18.9±0.3	-
β-Carotene (mg/kg)	78.4±0.1	-	294.3±0.3	-	5.6±0.3	-	110.3±0.1	-	131.0±0.2	-	67.3±0.4	-	-
IC_{50} (mg/ml)	0.037±0.04	0.020±0.05	0.024±0.03	0.067±0.01	0.038±0.08	0.040±0.06	0.032±0.09	0.046±0.05	0.040±0.02	0.028±0.07	0.045±0.01	0.027±0.09	0.1038±0.04

TABLE 3: Phenolic compounds and IC_{50} of seed oil and cake (Hisar)

Parameter	*A. nilotica*		*A. lebbeck*		*M. azedarach*		*P. pinnata*		*C. fistula*		*S. persica*		*Ascorbic acid*
	Seed oil	***Seed cake***	***Seed oil***	***Seed cake***	***Seed oil***	***Seed cake***	***Seed oil***	***Seed cake***	***Seed oil***	***Seed cake***	***Seed oil***	***Seed cake***	
Yield (%)	12.4±0.1	3.3±0.1	10.5±0.1	2.8±0.1	37.7±0.4	7.4±0.3	40.3±0.4	9.8±0.3	6.9±0.3	1.1±0.1	42.5±0.4	11.6±0.2	-
Phenolics content (mgGAE/g)	87.2±0.2	17.5±0.4	14.8±0.1	22.6±0.3	28.7±0.3	16.8±0.1	15.2±0.2	51.2±0.2	11.5±0.6	12.0±0.0	12.3±0.3	20.2±0.2	-
Flavonoid content (mg CAE/g)	11.9±0.4	6.3±0.5	0.7±0.4	9.5±0.3	3.5±0.3	2.9±0.0	2.0±0.0	1.8±0.1	1.1±0.3	2.1±0.2	6.2±0.2	4.0±0.1	-
Total tocopherol (mg/g)	10.4±0.8	14.2±0.2	59.9±0.9	131.2±0.2	3.4±0.2	23.1±0.2	40.0±1.0	110.0±0.2	10.2±0.3	185.6±0.5	19.2±0.2	16.3±0.4	-
β-Carotene (mg/kg)	67.4±0.4	-	230.3±0.4	-	5.4±0.2	-	115.3±0.3	-	112.6±2.0	-	65.3±0.3	-	-
IC_{50} (mg/ml)	0.034±0.02	0.021±0.01	0.023±0.05	0.045±0.07	0.045±0.03	0.031±0.01	0.039±0.02	0.048±0.08	0.041±0.01	0.033±0.09	0.035±0.05	0.024±0.04	0.1038±0.04

Carotenoids content:

Carotenoids are secondary metabolites. They save cells from the possession of air and light. Carotenoids have capability of inhibiting singlet oxygen. The carotenoid is the main reason of colour in oils (Krinsky 1989). Due to improper storage, field damage and faulty handling during extraction crude oils show excessive colour. The value of carotenoid found in the seed oils varied between ranged from 5.4 and 294.3mg/kg. "The largest value found in *Albizia lebbeck* (Palwal) whereas lowest in *Melia azedarach*(Hisar) which is shown in table 2 & 3. Carotenoid is a kind of antioxidant, if they have larger value means they have high antioxidant activity. According to previous study tomato seed oil (765.7 mg/kg), flaxseed oil (76.9 mg/kg), sea buckthorn seed oil (55.3 mg/kg), and olive oil (50.3 mg/kg) have relatively high levels of β-carotene, which is typically the dominant carotenoid in vegetable oils (VOs). As for lutein, rapeseed oil contains up to 95 mg/kg, making it a better source than sunflower oil (12.4 mg/kg) and flaxseed oil (11.6 mg/kg) and an excellent source of vitamin A (Tian et al. 2023).

Tocopherol content:

Tocopherols are source of secondary metabolites. All types of oils contains tocopherol. Tocopherols are very important and during storage they help in preserving oil from rancidity and increase its shelf-life (Ruiz-Lopez 1995). (Brigelius- Flohe 2002 and Monahan 1993) revealed that tocopherols are biological inhibitors and a very good source of Vitamin E for human beings. The tocopherol content varied between 3.4mg/g and 69.7mg/g in the seed oil and 14.2mg/g to 185.6mg/g (defatted seed cake) of the seeds studied. *Albizia lebbeck* seed oil exhibited the highest tocopherol content 69.7±0.2mg/g and 59.9±0.9mg/gwhile lower in case of *Melia azedarach* 4.3±0.2mg/g 3.4±0.4mg/g in the two locations. The results indicate that tocopherol content was almost similar in the two locations. Soybean oil is a significant source of natural tocopherols, with a notably higher total tocopherol content compared to other vegetable oils like canola (Brassica napus L.), sunflower (Helianthus annuus L.), maize (Zea mays L.), or flax (Linum usitatissimum L.) (Carreraand Seguin 2016).

In case of methanol extract highest value of total tocopherol in *Cassia fistula* 185.6±0.5mg/g and 176.3±0.2mg/g and lower in *A. nilotica* 15.5±0.3mg/g and 14.2±0.2mg/g in the two locations shown in Fig. 3.There were significant differences of total tocopherol content between oil extract and seed cake of different plants. *C. fistula* seed cake contained a higher tocopherol content compared to its seed oil in both locations. Our observation of total tocopherol in *A. nilotica* seed oil is lower from (Tissouras et al 2013).

Total phenolic content:

Phenolics are most important natural antioxidants and they have very good scavenging activity. In fact, polyphenolics having metal chelating properties as well as scavenging activities (as electron-donating or hydrogen-donating agents) (Rice-Evanset al 1996). Therefore, total phenolics in oil and the seed cake was analyzed. Our findings revealed that the in seed oil total phenolics ranged from 7.0 to 87.2mgGAE/g and 10.3 to 51.2mgGAE/g in seed cake. The highest value of total phenolics in oil were found in *A. nilotica* 87.2±0.2mgGAE/g (Hisar) and 80.1±0.2mgGAE/g (Palwal) while lowest in case of *C. fistula* 11.5±0.1mgGAE/g (Hisar) and 7.0±0.0mgGAE/g (Palwal). Total phenolics in methanolic extract of defatted

seed had highest value in *P. pinnata* 51.2±0.2mgGAE/g (Hisar) and 48.2±0.2mgGAE/g (Palwal) while lowest in *C. fistula* 12.0±0.0mgGAE/g (Hisar) and 10.3±0.1mgGAE/g (Palwal) shown in table 2 & 3.

Phenolic content of methanolic seed extract of *A. niloticа*reported by (Naseer et al. 2014) differ from our findings for both the locations. But our findings are in partial concurrence with the previous study (Vadivel and Biesalski 2012). Antioxidant activity is closely linked to the total phenolic content in plants.The polarity of extraction solvents impacts both the composition and antioxidant activity of bioactive compounds in plant sources. Additionally, the recovery of polyphenols is influenced by their solubility in the solvent used during the extraction process. Chia seed oil extracted by acetone for 4 hrs, by acetone for 8 hrs, by hexane for 8 hrs having total phenolic content75.13±2.22, 61.88±1.96 and 14.44±2.67 respectively (Ishak et al. 2020).

Flavonoid content:

Flavonols and flavones are the subgroups of flavonoids. Flavonols having prominent antioxidant. They are also have metal chelating and radical scavenging activity (Bors and Saran 1987) (Afanaslejev et al 1989). Flavonols were found to exhibit greater activity as glycones compared to their glycosides (Hopia and Heinonen 1999). Flavonoids having the capability to inhibit active oxygen radical (Korycka-Dahl and Richardson 1978). The hydroxyl radicals which is highly reactive can cause oxidative damage to DNA, proteins and lipids (Grootveld and Jain 1989). In this finding the flavonoid content was analyzed in both oil extracts and seed cake extracts. Our analysis demonstrated that total flavonoid content varied from in the seeds from two locations under study. The highest value of flavonoids in seed oil was found in *A. niotica* 11.9±0.4mgCAE/g (Hisar) and 11.3±0.3mgCAE/g (Palwal) while least in case of *A. lebbeck* 0.7±0.4mgCAE/g (Hisar) and 0.3±0.2mgCAE/g (Palwal) and in seed cake extract highest flavonoids content was found in *A. lebbeck*9.5±0.3mgCAE/g (Hisar) and 8.7±0.2mgCAE/g (Palwal) while the lowest was observed *P. pinnata* 2.5±0.2mgCAE/g (Palwal) & 1.8±0.1mg CAE/g) in (Hisar) shown in table 2 & 3.

In *A. nilotica* flavonoid content in seed cake extracts were 4.9±0.1mgCAE/g (Palwal) & 6.3±0.5mgCAE/g (Hisar) which was lower than reported by (Naseer et al 2014). The lower value in the cake extract may be explained as the oil has higher flavonoids content and therefore, in the seed cake its concentration has been decreased. Further its higher value in the oil indicates esterified or methylated flavonoids.

The flavonoids in oil extract and seed cake extracts, we found that *A. nilotica* had higher flavonoids content (in oil extract) than seed cake extract.

Evaluation of antioxidant activity:

Antioxidant activity in oil and methanolic- based extracts was determined by DPPH method.Oxidation of lipids generates different radicals that are cause of oxidative damage in human health (Williams, 1993). 2,2'-diphenyl-1-picrylhydrazyl is a chemically stable free radical (DPPH•) and having inhibiting activity of bioactive compounds (Brand-Williams et al.1995; Yen and Duh 1994).

The maximum antioxidant activity exhibited by oil extract and seed cake of different plants of two locationsand in terms of IC_{50} values in (Table 2, 3, 4, 5). The findings showed that the maximum antioxidant activity of oil extract and seed cake of two locations ranged from 55% to 79% (seed oil) and 55% to 87% (defatted seed). Their IC_{50} values (mg/ml) varied from 0.023mg/ml to 0.045mg/ml (phenolic extract of the oils) and 0.020mg/ml to 0.067mg/ml (seed cake extract). The oil extracts displayed the highest level of antioxidant activity of both locations (Palwal and Hisar) were 73 (0.07) & 78 (0.06), 74 (0.06) & 79 (0.06), 69 (0.06) & 55 (0.06), 74 (0.06) & 66 (0.06), 65 (0.06) & 62 (0.06), 69 (0.07) & 76 (0.07) % at specified concentration (in mg/ml) respectively in Table 4 and 5. The higher antioxidant activity/capacity at different concentration of the phenolic extract of the oil may be attributed to the higher concentration of lower phenols extracted from the oils. The highest antioxidant activity showed by seed cake extracts were 87% & 80% at conc. 0.06mg/ml, 55 & 68% at conc. 0.07mg/ml, 59% & 73% at conc. 0.06mg/ml, 61% & 58% at conc. 0.06mg/ml, 83% & 71% at conc. 0.06mg/ml and 78% & 80% at the conc. of 0.06mg/ml from the seeds of Palwal & Hisar, respectively. The antioxidant activity demonstrated by oil and seed cake extracts in terms of their IC_{50} values.

The highest antioxidant activity (IC_{50}) was found in *M. azedarach* 0.038±0.0mg/ml (Palwal) & 0.045±0.0mg/ml (Hisar) and the lowest value was observed in *A. lebbeck* 0.024±0.0mg/ml (Palwal) & 0.023±0.0mg/ml (Hisar) that differ from the previous study , may be due to solvent and extraction method (Mariod et al. 2009; Varshney et al. 2025).

In overall comparision, it was found that IC_{50} value in seed cake is higher than that extracts of the oil because of methanol, being a highly polar protic solvent, proved to be the most effective in solubilizing flavonoids and oxygenated aromatic compounds. The success of methanol extraction in delivering maximum yield(Likhar and Fegade1. 2025). Leaf extract of Cassia fistula shows the second highest position with approx. 90% antioxidant activity. The phenolic content of the leaf extract deals with the peroxidation process of lipids. This reveals the antioxidant behavior of Cassia fistula leaves(Singh et al, 2023)

In general there is increase in total phenol content in the defatted seed of*P. pinnata* in the two locations (Palwal and Hisar). The difference in our results may be due to some environmental conditions.

TABLE 4: Percent antioxidant activity of seed oil & cake at various concentrations (Palwal)

Conc. mg/ml	*A. nilotica* (%) Activity		*A. lebbeck* (%) Activity		*M.azedarach* (%) Activity		*P. pinnata* (%) Activity		*C. fistula* (%) Activity		*S. persica* (%) Activity		*Ascorbic acid* (%) Activity
	Seed oil	*Seed cake*	*Seed oil*	*Seed cake*	*Seed oil*	*Seed cake*	*Seed oil*	*Seed cake*	*Seed oil*	*Seed cake*	*Seed oil*	*Seed cake*	
0.01	20	25	30	13	10	18	14	17	18	28	22	32	30
0.02	32	47	44	20	23	31	28	25	28	38	28	40	36
0.03	44	72	58	27	38	40	45	36	36	54	36	52	40
0.04	50	83	65	34	51	52	58	42	50	60	45	59	44
0.05	58	86	70	40	62	55	72	55	59	71	54	70	47
0.06	68	87	74	47	69	59	74	61	65	83	60	78	49
0.07	73	87	74	55	69	59	74	61	65	83	69	78	52
0.08	73	87	74	55	69	59	74	61	65	83	69	78	55
0.09	73			55							69		57

TABLE 5: Percent antioxidant activity of seed oil& cake at various concentrations (Hisar)

Conc. mg/ml	*A. nilotica* (%) Activity		*A. lebbeck* (%) Activity		*M.azedarach* (%) Activity		*P. pinnata* (%) Activity		*C. fistula* (%) Activity		*S. persica* (%) Activity		*Ascorbic acid*
	Seed oil	*Seed cake*	*Seed oil*	*Seed cake*	*Seed oil*	*Seed cake*	*Seed oil*	*Seed cake*	*Seed oil*	*Seed cake*	*Seed oil*	*Seed cake*	
0.01	17	28	33	15	11	22	12	20	21	24	26	35	30
0.02	35	42	47	23	26	35	24	28	30	34	34	45	36
0.03	47	54	56	35	37	46	36	34	37	46	43	55	40
0.04	53	68	68	42	48	60	50	44	49	58	55	61	44
0.05	61	74	72	54	53	66	63	52	57	62	62	71	47
0.06	71	80	76	62	55	73	66	58	62	71	68	80	49
0.07	78	80	79	68	55	73	66	58	62	71	76	80	52
0.08	78	80	79	68	55	73	66	58	62	71	76	80	55
0.09	78			68							76		57

IV. Conclusion:

The present study evaluated the phytochemical profiles, oil content, and antioxidant potential of six selected medicinal plants. Among them, *Salvadora persica* exhibited the highest oil yield ($40.2 \pm 0.6\%$ to $42.5 \pm 0.4\%$), while *Cassia fistula* showed the lowest ($6.4 \pm 0.1\%$ to $6.9 \pm 0.3\%$). Total phenolic content was greatest in the seed oil of *Acacia nilotica* (87.2 ± 0.2 mg GAE/g) from Hisar, and fatty acid analysis of *S. persica* revealed that myristic and palmitic acids together accounted for over 74%. Antioxidant activity was generally higher in methanol extracts of defatted seed cake compared to seed oil, with *A. nilotica* showing maximum activity (80%) and *A. lebbeck* exhibiting the lowest IC_{50} value (0.067 mg/ml). Overall, all six plant*A. nilotica, A. lebbeck, M. azedarach, P. pinnata, C. fistula,* and *S. persica*are rich sources of oil, phenolics, and flavonoids, demonstrating significant antioxidant potential and supporting their use as valuable resources for medicinal and nutritional application.

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