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VARIATIONS IN PHENOLICS, ANTIOXIDANT AND PHYTOCHEMICAL COMPOSITION OF *SALVADORA PERSICA* IN TWO LOCATIONS

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Keywords:

Salvadora persica, Total phenolics, Antioxidant activity, Phytochemical composition, Minerals, Fatty acid composition

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ABSTRACT: Phenolic extracts of crude oil and methanol extracts of defatted seed cake were used to evaluate total phenolics, flavonoids content and antioxidant activity for two locations. The seeds were evaluated for their proximate composition while oils were subjected to chemical analysis. *Salvadora persica* had highest phenolics ranged from 12.3 ± 0.3 to 22.3 ± 0.3mg GAE/g and flavonoids content 3.7 ± 0.6 to 6.2 ± 0.2mg CAE/g. The antioxidant activity having IC_{50} value ranged from 0.024 ± 0.0 to 0.045mg/ml. The highest oil content as 42.5 ± 0.4% and highest protein content was 23.3 ± 0.3%. The oil content of the seeds recorded the highest iodine value 112.0 ± 0.8g/100g. The seeds were found to be rich in Ca, K, Na and P. The dominant fatty acids found in the oils were myristic and palmitic acid.

INTRODUCTION: *Salvadora persica* Linn. (family - Salvadoraceae), commonly known as tooth brush tree, is a branched evergreen small tree. In Hindi it is called as Pillu. It is distributed mainly in tropical regions of Asia and Africa. The *Salvadora persica* plant contains many biologically active compounds such as steroids, volatile oils, tarpenoides, saponins, and carbohydrates [1, 2]. Traditionally, the branches of this plant are widely used as toothbrush in the Middle East, Africa and India to relieve toothache. Leaves are used as mouthwash and also used as purgatives for curing asthma and cough. Roots of this plant were found to contain Salvadourea, which is a urea derivates [3].

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It contains large amount of alkaloidal compounds including trimethylamine [4], which has a motivating effect on the gums and sulphur like compounds and antibacterial activity [5]. The root and bark contains resin and traces of alkaloids called Salvadoricine [6]. Fruit contain a large amount of sugar fat and alkaloid. Leaves contain salvadoricinea indole alkaloid, flavanoids [7, 8, 9].

MATERIALS AND METHODS: Dry pods of *Salvadora persica* were gathered from field area of CCS HAU, Hisar and district Palwal, Haryana, India. The seeds were expelled 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 Merk and Qualigens, of most elevated immaculateness. Oil substance will be determined by Soxhlet strategy utilizing petroleum ether (60 - 80 °C) for 8 h.

The compound attributes of seed oil will be resolved by AOAC standard method [10].

Mineral Contents:
Reagents:

- **Di-acid Mixture:** Nitric acid and perchloric acid was mixed in ratio 5:1 just before use.
- **Hydrochloric Mixture (1%):** 1ml of conc. HCl was added in 50ml 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 di-acid 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, sifted 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 was dictated by Fractination of methyl esters by GLC [11].

Carotenoids: Determination of total carotenoids was done by the method [12].

Total Phenolic Content: The phenolic substance was determined by the technique of Folin-Ciocalteu reagent [13].

Flavonoids: The aluminum chloride colorimetric measure [14] was used. 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).

Tocopherol: Aliquots 10, 15, 20, 25, 30, 35 and 40 ppm of a reply of tcopherol in the ethanol were added to a volumetric flask and the volume was adjusted to 8ml with ethanol. Each of the solutions and 1.0 ml of 2, 2ˈ - 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 seconds. The absorbance of the mix was measure at 520 nm against ethanol as a blank. By then the standard graph was drawn [14].

Determination of Antioxidant activity: Antioxidant activity studied by (DPPH) free radical scavenging method [15]. The scavenging activity of the extract will be calculated as:

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

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

RESULTS AND DISCUSSION:

Carotenoids Content: Colour in oil is mainly due to the presence of carotenoid pigments and/or chlorophyll pigments. Carotenoids protect cells against the effect of light, air and sensitiser pigments having the ability to quench singlet oxygen and can also serve as antioxidants under conditions other than photosensitization [16]. Some crude oils can have unexpectedly high pigmentation caused by field damage, improper storage, or faulty handling during crushing, and extraction. Carotenoid value determined in oils of the seeds studied in Palwal and Hisar locations were 67.3 ± 0.4 and 65.3 ± 0.3 mg/kg. The highest was in Palwal while lowest in case of Hisar shown in **Fig. 1**. Carotenoid is a type of antioxidant, higher value of this indicates higher antioxidant activity.

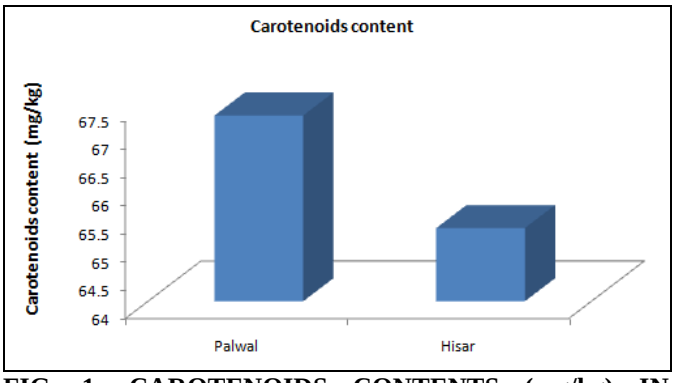

FIG. 1: CAROTENOIDS CONTENTS (mg/kg) IN PHENOLIC EXTRACT OF SEED OILS

Total Phenolics Content: Plant phenolics are optional metabolites which are naturally aromatic and are highly antioxidants in view of their capacity to inhibit 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 *Salvadora persica* was determined. Our findings showed that the substance of aggregate phenolics of *Salvadora persica* of two areas were highest in the methanol extract of defatted seed cake 20.2 ± 0.2mg GAE/g to 22.3 ± 0.3mg GAE/g as compared to phenolic extract of seed oil 12.3 ± 0.3mg GAE/g to 14.0 ± 0.2mg GAE/g, this might be because of variou s agroclimatic conditions shown in **Fig. 2**.

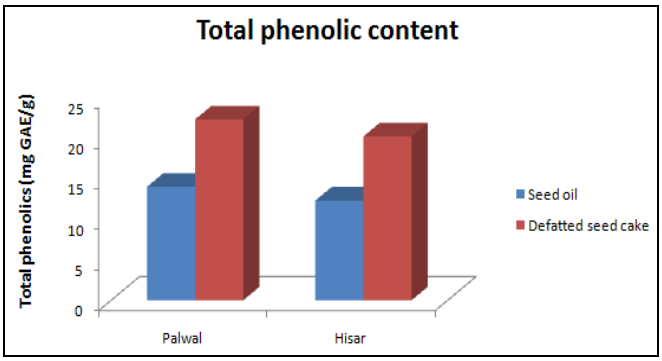

FIG. 2: TOTAL PHENOLICS (mgGAE/g) IN PHENOLIC EXTRACTS OF SEED OIL AND METHANOLIC EXTRACTS OF DEFATTED SEED CAKE

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 flavonoids of *Salvadora persica* (two locations) were resolved. The TFCs are communicated as far as catechin equivalent (CE) and are introduced in **Fig. 3**.

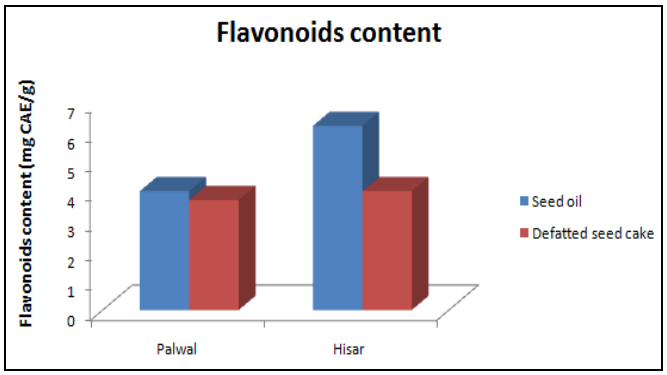

FIG. 3: FLAVONOIDS CONTENT (mgCAE/g) IN PHENOLIC EXTRACTS OF SEED OIL AND METHANOLIC EXTRACT OF DEFATTED SEED CAKE

The measure of flavonoids in phenolic extracts of seed oil of *Salvadora persica* was (4.0 ± 0.1 mg CAE/g to 6.2 ± 0.2mg CAE/g) and in methanolic extract of defatted seed cake were (3.7 ± 0.6mg CAE/g to 4.0 ± 0.1mg CAE/g). In this the flavonoids content in seed oil have higher value in comparison to seed cake.

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 [17, 18]. 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 *Salvadora persica* two areas (Palwal and Hisar) were 18.8 ± 0.5mg/g to 19.2 ± 0.2mg/g and in methanolic extracts of defatted seed cake were 16.3 ± 0.4mg/g to 18.9 ± 0.3mg/g. In comparision of total tocopherol in unrefined oil and methanol extracts of defatted seed cake, we found that there were small difference of aggregate tocopherol content between seed oil and seed cake (**Fig. 4**).

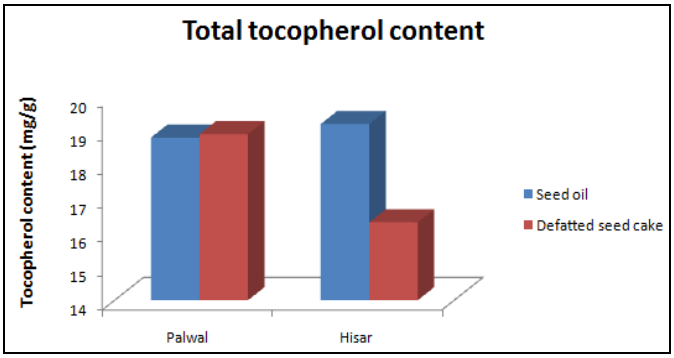

FIG. 4: TOCOPHEROL CONTENT (mg/g) IN PHENOLIC EXTRACT OF SEED OIL AND METHANOLIC EXTRACT OF DEFATTED SEED CAKE

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

[19], plant extracts [20] and foods [21]. Alcoholic arrangements of DPPH• have a trademark absorption maximum at 517nm. At the point when an electron or hydrogen ion giving cancer prevention agent (AH) is added to DPPH•, a diminishing in absorbance at 517nm happens because of the arrangement of the non-radical shape DPPH-H which does not ingest at 517nm. This response is measured by de-shading test where the diminishing in absorbance at 517nm 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 maximum antioxidant capacity of *Salvadora persica* in seed oil was from 69% to 76% at a concentration of 0.07mg/ml and in seed cake from 78% to 80% at a concentration of 0.06mg/ml which is higher in methanol extract of defatted seed cake shown in **Fig. 5**.

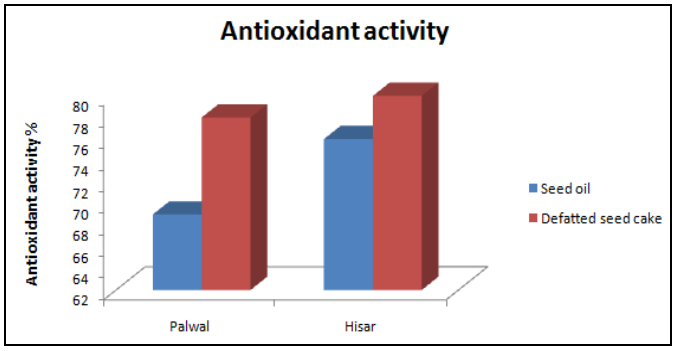

FIG. 5: MAXIMUM ANTIOXIDANT ACTIVITY (%) OF PHENOLIC EXTRACT OF SEED OIL AND METHANOLIC EXTRACT OF DEFATTED SEED CAKE AT DIFFERENT CONCENTRATION (mg/ml)

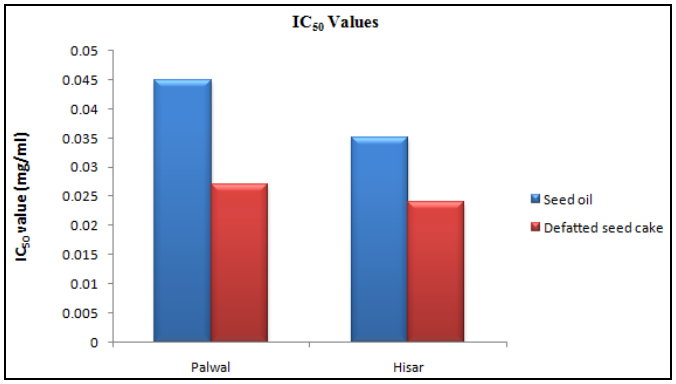

FIG. 6: IC_{50} VALUES (mg/ml) IN PHENOLIC EXTRACT OF SEED OIL AND METHANOLIC EXTRACT OF DEFATTED SEED CAKE

The antioxidant activity in terms of (IC_{50}) displayed by phenolic extract of seed oil of *Salvadora persica* of two locations were 0.035 ± 0.0mg/ml to 0.045 ± 0.0 mg/ml and in methanolic extracts were 0.024 ± 0.0mg/ml to 0.027 ± 0.0 mg/ml. Our perception in both the locations higher in seed oil as compared to seed cake appeared in **Fig. 6**. 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 iota giving capacities. In any case, the higher extremity dissolvable portions were not the most dynamic as the radical searching action of a specific cell reinforcement relies on upon structure and in addition on the kind of response kinetics [22].

Proximate Composition of Seed kernals: Proximate composition of seed kernals of *Salvadora persica* (Palwal and Hisar) determined as moisture 8.9 ± 0.4% and 10.3 ± 0.3%, ash content 3.8 ± 02% and 3.4 ± 0.1%, crude fiber 10.5 ± 0.1% and 11.1 ± 0.4%, crude proteins 23.3 ± 0.3% and 22.8 ± 0.2%, yield of oil 40.2 ± 0.6% and 42.5 ± 0.4. This plant contained a higher amount of oil as compared to some other like as *Acacia nilotica*, *Albizia lebbeck*, *Cassia fistula etc*. carbohydrates 13.3 ± 0.2% and 9.9 ± 0.5%, and energy value 2126.7 ± 2.1 and 2148.3 ± 2.4 kJ/100g individually (**Table1**).

TABLE 1: PROXIMATE COMPOSITION OF SEEDS OF *SALVADORA PERSICA*

Parameters	Composition	
	Palwal	Hisar
Moisture (%)	8.9 ± 0.4	10.3 ± 0.3
Ash (%)	3.8 ± 0.2	3.4 ± 0.1
Fibre (%)	10.5 ± 0.1	11.1 ± 0.4
Protein (%)	23.3 ± 0.3	22.8 ± 0.2
Yield of oil (%)	40.2 ± 0.6	42.5 ± 0.4
Carbohydrates (%)	13.3 ± 0.2	9.9 ± 0.5
Energy (KJ/100g)	2126.7 ± 2.1	2148.3 ± 2.4

Values are mean of three replicates ± standard error.

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 [23]. 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 [24].

The healthfully essential minerals of seeds are introduced in **Table 2**. The seeds of *Salvadora persica* (Palwal and Hisar) contained critical measure of vital minerals as Calcium 128.0 ± 1.6 mg/100g and 136.0 ± 1.9mg/100g, potassium 380.0 ± 2.4mg/100g and 135.0 ± 2.0mg/100g, sodium 215.0 ± 1.3mg/100g and 224.0 ± 1.0mg/100g, remaining are in lower concentration which is appeared in **Table 2**. Calcium assumes a noteworthy part in CNS work. Calcium is fundamental for nerve motivation conduction and enacts a few compounds which create neuro-transmitters. 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 [25].

TABLE 2: MINERAL COMPOSITION OF SEEDS OF *SALVADORA PERSICA*

Parameters	Composition	
	Palwal	Hisar
Ca (mg/100g)	128.0 ± 1.6	136.0 ± 1.9
K (mg/100g)	380.0 ± 2.4	350.0 ± 2.0
Na (mg/100g)	215.0 ± 1.3	224.0 ± 1.0
Mg (mg/100g)	2.4 ± 0.5	2.3 ± 0.2
Fe (mg/100g)	24.7 ± 0.3	28.9 ± 0.4
Zn (mg/100g)	3.5 ± 0.1	3.1 ± 0.1
Co (mg/100g)	0.3 ± 0.2	0.4 ± 0.1
Mn (mg/100g)	0.2 ± 0.1	0.1 ± 0.0
Cu (mg/100g)	Traces	Traces
P (mg/100g)	88.0 ± 0.4	92.0 ± 0.7

Values are mean of three replicates ± standard error.

TABLE 3: CHEMICAL CHARACTERISTICS OF SEED OIL OF *SALVADORA PERSICA*

Parameters	Composition	
	Palwal	Hisar
Peroxide value (meq/kg)	0.8 ± 0.1	1.3 ± 0.4
Iodine value (g/100g)	112.0 ± 0.8	108.0 ± 0.6
Saponification value (mg KOH/g)	190.0 ± 1.4	187.0 ± 0.9
Unsaponifiable matter (%)	6.9 ± 0.6	4.2 ± 0.5
Free fatty acid (%)	1.4 ± 0.3	1.2 ± 0.0

Values are mean of three replicates ± standard error.

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 to124.6 ± 0.7g/100g, peroxide value of fresh oil were 2.2 ± 0.1 to 1.7 ± 0.4 meq/kg, saponification values 184.7 ± 0.6 to188.0 ± 0.3mg/g KOH and unsaponifiable matter 2.2 ± 0.0% to 2.1 ± 0.1%.

Fatty Acid Composition: For fatty acids composition first converted into their respective volatile esters by trans- esterification, *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 *Salvadora persica* two locations (Palwal and Hisar) myristic acid 54.0 ± 0.3% and 55.5 ± 0.8%, palmitic acid 20.2 ± 0.4% and 19.3 ± 0.2% (**Table 4**). 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 [26]. A MUFA rich- diet tends to decrease low density lipoprotein-cholesterol [27].

TABLE 4: FATTY ACID COMPOSITION OF SEED OIL OF *SALVADORA PERSICA*

Parameters	Composition	
	Palwal	Hisar
Myristic acid ($C_{14:0}$)	54.0 ± 0.3	55.5 ± 0.8
Palmitic acid ($C_{16:0}$)	20.2 ± 0.4	19.3 ± 0.2
Stearic acid ($C_{18:0}$)	4.7 ± 0.1	4.7 ± 0.1
Oleic acid ($C_{18:1}$)	6.8 ± 0.3	5.6 ± 0.5
Linoleic acid ($C_{18:2}$)	3.4 ± 0.3	2.3 ± 0.0
Linolenic acid ($C_{18:3}$)	3.3 ± 0.5	2.9 ± 0.1
Arachidic acid ($C_{20:0}$)	2.1 ± 0.1	3.1 ± 0.4
Eicosenoic acid ($C_{20:1}$)	2.4 ± 0.2	1.8 ± 0.2
Behanic acid ($C_{22:0}$)	1.6 ± 0.4	1.3 ± 0.2

Values are mean of three replicates ± standard error.

In this study the seeds and oils of *Salvadora persica* have been evaluated for proximate and chemical composition. The results of the proximate analysis revealed the presence of high amounts of oil content, fibre and protein in the seeds. So it is used as feed and food formulations. In the seed oil of *Salvadora persica*, myristic acid and palmitic acid together constitute more than 74% in both locations. The oil will be very useful in soap industry as they augment leather forming property

as well as its stability. The overall studied concluded that it also acts as a good source of natural antioxidant.

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CONFLICT OF INTEREST: There is no area of conflict of interest.

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