Research Article

Variations of Phytochemical Constituents of Plant Extracts of *Cassia Fistula* and *Salvadora Persica*

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Abstract

The extracts of two different plant namely (*Cassia fistula and Salvadora persica*) were evaluated for their total phenols, flavonoid, carotenoids, tocopherol and antioxidant activity by DPPH method for two locations. In this study seed oil and defatted seed cake extracts of *Cassia fistula and Salvadora persica* from two locations indicate high carotenoid (seed oil) and tocopherol contents (seed cake) in *Cassia fistula*. Total phenolics and flavonoids highest in *Salvadora persica*. The maximum antioxidant activity in *Cassia fistula* and IC_{50} value was highest in *Salvadora persica*.

Keywords: *Cassia fistula; Salvadora persica*; total phenolics; antioxidant activity

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Introduction

Plants synthesize very complex molecules with specific stereochemistry and can show biological activity with new modes of action. Several useful drugs have been developed from medicinal plants used in traditional medicine in the treatment of a variety of illnesses.

Cassia fistula Linn. also known as golden shower or Amaltash, Indian laburnum, belongs to the family Leguminoceae. The plant is a moderate sized deciduous tree, distributed throughout India and various countries including Mexico, Mauritius, South Africa, East Africa, West Indies, China. This plant is reported to be aperient, astringent, laxative, purgative and vermifuge, Indian laburnum is a folk remedy for burns, cancer, constipation, convulsions, delirium, diarrhea, dysuria, epilepsy, gravel, hematuria, pimples and glandular tumors [1].

Salvadora persica L. (family Salvadoraceae), commonly known as tooth brush tree or called as Pilu, is a branched evergreen small tree. It is distributed in tropical Africa and Asia extending up to India, Mascarene Island and China. The plant contains several biologically active compounds such as alkaloids, flavonoids, steroids, volatile oils, tarpenoides, saponins, and carbohydrates [2, 3]. Traditionally, the twigs 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 [4].

Materials and Methods

The seeds of plants were collected from district Palwal and campus of CCS HAU, Hisar, Haryana, India, **Figure 1** and **Figure 2**.

Figure 1 Cassia fistula plant

Figure 2 Salvadora persica plant

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 [5].

Carotenoids

Determination of total carotenoids was done by the method [6].

Total phenolic content

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

Flavonoids

The aluminum chloride colorimetric measure [8] 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, 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 seconds. The absorbance of the mix was measure at 520 nm against ethanol as a blank. By then the standard graph was drawn [9].

Determination of Antioxidant activity

Antioxidant activity studied by (DPPH) free radical scavenging method [10]. 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 data were completed in triplicate and results were determined as mean of three replicates ± standard deviation. Correlation was determined by Pearson's correlation coefficient by using OPSTAT CCS HAU, Hisar.

Results and Discussion

Carotenoids content:

Colour in oil is mainly due to the presence of carotenoid 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 [11]. Some crude oils can have unexpectedly high pigmentation caused by field damage, improper storage, or faulty handling during crushing, and extraction. There was a large difference between the carotenoid content determined in seed oils of *C. fistula* and *S. persica.* But there was a small locational variation (**Table 1**).

Total Phenolics

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 *C. fistula* and *S. persica* in crude oil and methanol extacts of defatted seed cake were determined.

Our findings showed that there was a significant difference between the extracts of seed oils and defatted seed cake of two plants. The total phenolics of two locations were highest in the methanol extract of defatted seed cake of *S. persica* (20.2±0.2-22.3±0.3 mgGAE/g) as compared to *C. fistula* extracts of seed oil in Palwal & Hisar locations (Table 1 and **Table 2**).

Table 1 Phytochemical components and Antioxidant activity (IC_{50}) (mg/ml) of phenolic extract of seed oils (Palwal and Hisar)

Parameter	***Palwal***		***Hisar***	
	Cassia fistula	***Salvadora persica***	***Cassia fistula***	***Salvadora persica***
Total phenolics (mg GAE/g)	7.0±0.0	14.0±0.2	11.5±0.6	12.3±0.3
Flavonoids (mg CAE/g)	1.3±0.1	4.0±0.1	1.1±0.3	6.2±0.2
Total tocopherol (mg/g)	8.4±0.1	18.8±0.5	10.2±0.3	19.2±0.2
Carotenoid content (mg/kg)	131.0±0.2	67.3±0.4	112.6±2.0	65.3±0.3
DPPH IC_{50} (mg/ml) of phenolic extract	0.040±0.0	0.045±0.0	0.041±0.0	0.035±0.0

Table 2 Phytochemical components and Antioxidant activity (IC_{50}) (mg/ml) of methanolic extract of defatted seed cake (Palwal and Hisar)

Parameter	***Palwal***		***Hisar***	
	Cassia fistula	***Salvadora persica***	***Cassia fistula***	***Salvadora persica***
Yield of methanol extract (%)	1.3±0.2	10.4±0.5	1.1±0.1	11.6±0.2
Total phenolics (mg GAE/g)	10.3±0.1	22.3±0.3	12.0±0.0	20.2±0.2
Flavonoids (mg CAE/g)	2.9±0.0	3.7±0.6	2.1±0.2	4.0±0.1
Total tocopherol (mg/g)	176.3±0.2	18.9±0.3	185.6±0.5	16.3±0.4
DPPH (IC_{50}) (mg/ml) of methanolic extract	0.028±0.0	0.027±0.0	0.033±0.0	0.024±0.0

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. There was a significant difference between phenols and flavonoids of seed oil and methanolic extracts. The flavonoid content higher in *S. persica* (seed oil). The difference may be due to the intrinsic properties of both plants. But there is a small variation in both the locations, this may be due to both location are hot and semi-arid region (Table 1 and 2).

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 [12, 13]. 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 was highest in methanol extract of *C. fistula* (176.3±0.2-185.6±0.5 mg/g) as compared to *S. persica* in extracts of both seed oil and seed cake of two area. In comparision of total tocopherol in unrefined oil and methanol extracts of defatted seed cake, we found that there were large difference of aggregate tocopherol content between seed oil and seed cake (Table 1 and 2).

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 [14], plant extracts [15] and foods [16]. 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 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 *C. fistula* were 65% in seed oil and 83% in seed cake and in case of *S. persica* were 76% at a concentration of 0.07 in seed oil and in seed cake 80% at a concentration of 0.06 mg/ml. There is a small variation in maximum antioxidant activity of both plant extracts with two locations.

The antioxidant activity in terms of (IC_{50}) displayed by plant extracts were highest in *S. persica (*0.045±0.0) in extract of seed oil and seed cake was lowest as compared to *C. fistula*. 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.

Table 3 Antioxidant activity (%) of phenolic extract of oils at different concentrations (Palwal and Hisar)

Conc. mg/ml	*Palwal*		*Hisar*	
	Cassia fistula	*Salvadora persica*	*Cassia fistula*	*Salvadora persica*
	Activity (%)	Activity (%)	Activity (%)	Activity (%)
0.01	18	22	21	26
0.02	28	28	30	34
0.03	36	36	37	43
0.04	50	45	49	55
0.05	59	54	57	62
0.06	65	60	62	68
0.07	65	69	62	76
0.08	65	69	62	76
0.09		69		76

Table 4 Antioxidant activity (%) of methanolic extract of defatted seed cake at different concentrations (Palwal and Hisar)

Conc. mg/ml	*Palwal*		*Hisar*	
	Cassia fistula	*Salvadora persica*	*Cassia fistula*	*Salvadora persica*
	Activity (%)	Activity (%)	Activity (%)	Activity (%)
0.01	28	32	24	35
0.02	38	40	34	45
0.03	54	52	46	55
0.04	60	59	58	61
0.05	71	70	62	71
0.06	83	78	71	80
0.07	83	78	71	80
0.08	83	78	71	80
0.09				

Statistical analysis

Correlation coefficient (r) between phytochemical components and antioxidant activity (IC_{50}) of phenolic extract of seed oil of Cassia fistula (Palwal)

The corresponding correlation values obtained for phenolic extract of seed oil of *C. fistula* (Palwal) shown in **Table 5**. The result of correlation analysis in phenolic extract indicate that there was a positive and highly significant correlation (r = 0.981**) between DPPH and flavonoids as well as with tocopherol (r = 0.928**). Similarly flavonoids showed a high positive correlation with tocopherol (r = 0.696*). Rest of the correlation values obtained for seed oil were found to be non- significant.

Correlation coefficient (r) between phytochemical components and antioxidant activity (IC_{50}) of phenolic extract of seed oil of Cassia fistula (Hisar)

The corresponding correlation values obtained for phenolic extract of seed oil of *C. fistula* (Hisar) shown in **Table 6**. The result of correlation analysis in phenolic extract indicate that there was a positive and highly significant

correlation (r = 0.898**) between DPPH and tocopherol as well as total phenolics (r = 0.782*). Rest of the correlation values obtained for seed oil were found to be non- significant.

Table 5 Correlation coefficient (r) between phytochemical components and antioxidant activity (IC_{50}) of phenolic extract of seed oil of *Cassia fistula* (Palwal)

	DPPH	Phenolics	Flavonoids	Tocopherol	Carotenoids
DPPH	1.000				
Phenolics	0.566	1.000			
Flavonoids	0.981**	0.494	1.000		
Tocopherol	0.928**	0.550	0.696*	1.000	
Carotenoids	0.466	0.500	0.167	0.244	1.000
*** Significant at 5%, ** Significant at 1%**					

Table 6 Correlation coefficient (r) between phytochemical components and antioxidant activity (IC_{50}) of phenolic extract of seed oil of *Cassia fistula* (Hisar)

	DPPH	Phenolics	Flavonoids	Tocopherol	Carotenoids
DPPH	1.000				
Phenolics	0.782*	1.000			
Flavonoids	0.335	0.479	1.000		
Tocopherol	0.898**	0.226	0.148	1.000	
Carotenoids	0.591	0.572	0.467	0.338	1.000
*** Significant at 5%, ** Significant at 1%**					

Correlation coefficient (r) between phytochemical components and antioxidant activity (IC_{50}) of methanolic extract of defatted seed cake of Cassia fistula (Palwal)

The corresponding correlation values obtained for methanolic extract of defatted seed cake of *C. fistula* (Palwal) shown in **Table 7**. The result of correlation analysis in phenolic extract indicate that there was a positive and highly significant correlation (r = 0.991**) between DPPH and total phenolics. Similarly total phenols showed a positive correlation with flavonoids (r = 0.684*). Rest of the correlation values obtained for defatted seed cake were found to be non- significant.

Table 7 Correlation coefficient (r) between phytochemical components and antioxidant activity (IC_{50}) of methanolic extract of defatted seed cake of *Cassia fistula* (Palwal)

	DPPH	Phenolics	Flavonoids	Tocopherol
DPPH	1.000			
Phenolics	0.991**	1.000		
Flavonoids	0.566	0.684*	1.000	
Tocopherol	0.444	0.389	0.481	1.000
*** Significant at 5%, ** Significant at 1%**				

Correlation coefficient (r) between phytochemical components and antioxidant activity (IC_{50}) of methanolic extract of defatted seed cake of Cassia fistula (Hisar)

The corresponding correlation values obtained for methanolic extract of defatted seed cake of *C. fistula* Hisar shown in **Table 8**. The result of correlation analysis in methanolic extract indicate that there was a positive and highly significant correlation (r = 0.890**) between total phenolics and flavonoids. Similarly DPPH showed a high positive correlation (r = 0.778*) with flavonoids. Rest of the correlation values obtained for defatted seed cake were found to be non- significant.

Correlation coefficient (r) between phytochemical components and antioxidant activity (IC_{50}) of phenolic extract of seed oil of Salvadora persica (Palwal)

The corresponding correlation values obtained for phenolic extract of crude oil of *S. persica* (Palwal) shown in **Table 9**. The result of correlation analysis in phenolic extract indicate that there was a positive and highly significant correlation (r = 0.999**) between DPPH and total tocopherol as well as with flavonoids (r = 0.901**) and carotenoids (r =

0.773*). Total phenolics showed a high positive correlation (r = 0.691*) with carotenoids. Rest of the correlation values obtained for seed oil were found to be non- significant.

Table 8 Correlation coefficient (r) between phytochemical components and antioxidant activity (IC_{50}) of methanolic extract of defatted seed cake of *Cassia fistula* (Hisar)

	DPPH	Phenolics	Flavonoids	Tocopherol
DPPH	1.000			
Phenolics	0.444	1.000		
Flavonoids	0.778*	0.890**	1.000	
Tocopherol	0.155	0.500	0.277	1.000
*** Significant at 5%, ** Significant at 1%**				

Table 9 Correlation coefficient (r) between phytochemical components and antioxidant activity (IC_{50}) of phenolic extract of seed oil of *Salvadora persica* (Palwal)

	DPPH	Phenolics	Flavonoids	Tocopherol	Carotenoids
DPPH	1.000				
Phenolics	0.538	1.000			
Flavonoids	0.901**	0.120	1.000		
Tocopherol	0.999**	0.458	0.274	1.000	
Carotenoids	0.773*	0.691*	0.012	0.481	1.000
* Significant at 5%, ** Significant at 1%					

Correlation coefficient (r) between phytochemical components and antioxidant activity (IC_{50}) of phenolic extract of seed oil of Salvadora persica (Hisar)

The corresponding correlation values obtained for phenolic extract of seed oil of *S. persica* (Hisar) shown in **Table 10**. The result of correlation analysis in phenolic extract indicate that there was a positive and highly significant correlation (r = 0.999**) between flavonoids and carotenoids and also with total tocopherol (r = 0.976**). Similarly DPPH showed a high positive correlation with carotenoids (r = 0.773*). Tocopherol showed a high positive correlation with carotenoids (r = 0.766*). Rest of the correlation values obtained for seed oil were found to be non-significant.

Table 10 Correlation coefficient (r) between phytochemical components and antioxidant activity (IC_{50}) of phenolic extract of seed oil of *Salvadora persica* (Hisar)

	DPPH	Phenolics	Flavonoids	Tocopherol	Carotenoids
DPPH	1.000				
Phenolics	0.593	1.000			
Flavonoids	0.553	0.121	1.000		
Tocopherol	0.366	0.304	0.976**	1.000	
Carotenoids	0.773*	0.273	0.999**	0.766*	1.000
* Significant at 5%, ** Significant at 1%					

Correlation coefficient (r) between phytochemical components and antioxidant activity (IC_{50}) of methanolic extract of defatted seed cake of Salvadora persica (Palwal)

The corresponding correlation values obtained for methanolic extract of defatted seed cake of *S. persica* (Palwal) shown in **Table 11**. The result of correlation analysis in methanolic extract indicate that there was a highly positive correlation (r = 0.994**) between DPPH and total phenolics as well as with flavonoids (r = 0.975**). Similarly flavonoids showed a high positive correlation with tocopherol (r = 0.776*). Rest of the correlation values obtained for methanolic extracts were found to be non- significant.

Correlation coefficient (r) between phytochemical components and antioxidant activity (IC_{50}) (IC_{50}) of methanolic extract of defatted seed cake of Salvadora persica (Hisar)

The corresponding correlation values obtained for methanolic extract of defatted seed cake of *S. persica* Hisar shown in **Table 12**. The result of correlation analysis in methanolic extract indicate that there was a positive and highly

significant correlation (r = 0.891**) between DPPH and total phenols as well as with tocopherol (r =0.875**). Similarly flavonoids showed high positive correlation (r = 0.782*) with tocopherol. Rest of the correlation values obtained for methanolic extract of defatted seed cake were found to be non- significant.

Table 11 Correlation coefficient (r) between phytochemical components and antioxidant activity (IC_{50}) of methanolic extract of defatted seed cake of *Salvadora persica* (Palwal)

	DPPH	**Phenolics**	**Flavonoids**	**Tocopherol**
DPPH	1.000			
Phenolics	0.994**	1.000		
Flavonoids	0.975**	0.449	1.000	
Tocopherol	0.384	0.314	0.776*	1.000
* Significant at 5%, ** Significant at 1%				

Table 12 Correlation coefficient (r) between phytochemical components and antioxidant activity (IC_{50}) of methanolic extract of defatted seed cake of *Salvadora s persica* (Hisar)

	DPPH	**Phenolics**	**Flavonoids**	**Tocopherol**
DPPH	1.000			
Phenolics	0.891**	1.000		
Flavonoids	0.566	0.601	1.000	
Tocopherol	0.875**	0.430	0.782*	1.000
* Significant at 5%, ** Significant at 1%				

Conclusion

The selected medicinal plants are the source of the secondary metabolites i.e., alkaloids, flavonoids, terpenoids, phlobatannins and reducing sugars. Medicinal plants play a vital role in preventing various diseases. The antidiuretic, anti-inflammatory, antianalgesic, anticancer, anti-viral, anti-malarial, anti-bacterial and anti-fungal activities of the medicinal plants are due to the presence of the above mentioned secondary metabolites. Medicinal plants are used for discovering and screening of the phytochemical constituents which are very helpful for the manufacturing of new drugs. The previous phytochemical analysis and present studied show nearly the similar results due to the presence of the phytochemical constituents. The phytochemical analysis of the medicinal plants are also important and have commercial interest in both research institutes and pharmaceuticals companies for the manufacturing of the new drugs for treatment of various diseases. In this study the variation of phenols and flavonoids in seed oil and seed cake depend upon the type of seeds and the solvent used in extraction. The overall studied concluded that these plants are good source of natural antioxidant.

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References

[1] S. Al-Quran. Journal of Natural Products. 2008, 1: 10-26.
[2] H. S. Abdillahi, G. I. Stafford, J. F. Finnie and J. V. Staden. South African Journal of Botany. 2010, 76: 1-24.
[3] J. A. Duke and K. K. Wain. Medicinal plants of the world. Computer index with more than 85,000 entries. 3, 1981.
[4] M. Kamil, F. Ahmed, A. F. Jayaraj, C. Guna-sekhar, S. Thomas, M. Habibullah and K. Chan. Pharmacognostical and Phytochemical studies on Salvadora persica L. 1999, 42: 64-75.
[5] AOAC. Official methods of analyses. Association of Official Analytical Chemists:Washington, DC. 1990.
[6] J. A. Vasconcellous, J W Berry and CW Weber. J. Am.Oil Chem. Soc. 1980, 57: 310-313.
[7] V. L. Singleton and J A Rossi. Am. J. Enology Viticulture. 1965, 16:144-158.
[8] J. Zhishen, T. Mengcheng and W. Jjianming. Food Chemistry. 1999, 64:555- 559.
[9] B. Philip, L. Bernard and H. William. In: Practical Physiological Chemistry, McGraw- Hill company, INC. New York, Toronto, London, 1954, 1272-1274.
[10] T. Hatano, H. Kagawa, T. Yasahara and T. Okuda. Chem. Pharma. Bull. 1988, 36: 2090-2097.

[11] N. Krinsky. Free Radical Biology and Medicine. 1989; 7: 617-635.
[12] F. J. Monahan, J. I. Gray, A. Asghar, A. Haug, B. Shi and D. J. Bukley. Food Chem. 1993, 46: 1-6.
[13] R. Brigelius-Flohe, F. J. Kelly, J. T. Salonem, J. Neuzil, J. M. Zingg and A. Azzi. Am. J. Clin. Nutr. 2002, 76: 703-716.
[14] G. M. Williams & H. Sies. Princeton Scientific Publishing Company. 1993.
[15] W. Brand-Williams, M. E. Cuvelier and C. Berset. Lebensm. Wiss. Technol. 1995, 28: 25-30.
[16] G. C. Yen and P. D. Duh. J. Agric. Food. Chem., 1994, 42: 629-632.

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