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ANTIOXIDANT ACTIVITY, PHENOLS AND FLAVONOID CONTENTS OF PLANT EXTRACTS

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ABSTRACT: The extracts of two different plant were screened for their phenols, flavonoid and antioxidant activity by DPPH method for two locations. In this study seed oil and defatted seed cake extracts of *M. azedarach* and *P. pinnata* from two locations indicate high phenolics (13.5-51.2 mgGAE/g) and flavonoids content (1.5-4.2 mgCAE/g). The IC_{50} value was (0.032-0.048 mg/ml).

INTRODUCTION: Antioxidants are intensified that can defer oxidation forms or restrain the spread phase of free radical responses with a specific end goal to shield the body cells from oxidation. They have capacities, for example, rummaging free radicals, diminishing movement, chelating master oxidant metals, restraining lipid peroxidation and extinguishing singlet oxygen [1]. Natural antioxidants are accessible in different structures, for example, phenolics, flavonoid, tocopherol, lycopene and β-carotene [2]. They are found in different parts of plants, for example, natural products, leaves, seeds and oils [3]. The investigation of cancer prevention agent mixes has drawn the enthusiasm of specialists since they are viable in hindering free radicals and in this way decelerating the development of degenerative malady.

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Past examinations demonstrate that the cancer prevention agent mixes enhance human wellbeing, for example, hindrance of growth cells, enhancing the state of cardiovascular illnesses and diabetes [4], recuperating human endless ulceration [5], hostile to allergenic, against atherogenic, mitigating, hostile to microbial, cancer prevention agent, against thrombotic, cardioprotective and vasodilatory impacts [6].

MATERIALS AND METHODS: The seeds of plants were collected from district Palwal and campus of CCS HAU, Hisar, Haryana, India.

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 [7].

Carotenoids: Determination of total carotenoids was done by method [8].

Total Phenolic Content: The phenolic substance was determined by the technique of Folin-Ciocalteu reagent [9].

Flavonoids: The aluminum chloride colorimetric measure [10] 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 tocopherol in the ethanol was added to a volumetric flask, and the volume was adjusted to 8ml with ethanol. Each of the solutions and 1.0 ml of 2, 2ʾ - 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 10.0ml 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 [11].

Determination of Antioxidant Activity: Antioxidant activity studied by (DPPH) free radical scavenging method [12]. The scavenging activity of the extract will be calculated as:

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

Data Analysis: The data were completed in triplicate and results were determined as the mean of three replicates ± standard deviation. The 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 sensitizer pigments having the ability to quench singlet oxygen and can also serve as antioxidants under conditions other than photosensitization [13]. 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 *M. azedarach* and *P. pinnata*. But there was a small locational variation **Table 1**.

Total Phenolics: Plant phenolics are optional metabolites which are naturally aromatic and are highly antioxidants given their capacity to inhibit the free radicals and active oxygen. It is wonderful that phenolic substances contribute to the antioxidant activity of plant materials. Phenolics show significant free radical-inhibition activity. In this way, the measure of aggregate phenolics in two areas (Palwal and Hisar) of *M. azedarach* and *P. pinnata* in crude oil and methanol extracts of defatted seed cake were determined.

TABLE 1: PHYTOCHEMICAL COMPONENTS AND ANTIOXIDANT ACTIVITY (IC_{50}) (mg/ml) OF PHENOLIC EXTRACT OF SEED OILS (PALWAL AND HISAR)

Parameter	Palwal		Hisar	
	Melia azedarach	***Pongamia pinnata***	***Melia azedarach***	***Pongamia pinnata***
Carotenoid content (mg/kg)	5.6 ± 0.3	110.3 ± 0.1	5.4 ± 0.2	115.3 ± 0.3
Total phenolics (mg GAE/g)	26.5 ± 0.4	13.5 ± 0.4	28.7 ± 0.3	15.2 ± 0.2
Flavonoids (mg CAE/g)	3.5 ± 0.4	1.5 ± 0.2	3.5 ± 0.3	2.0 ± 0.0
Total tocopherol (mg/g)	4.3 ± 0.2	38.0 ± 1.0	3.4 ± 0.2	40.0 ± 1.0
DPPH IC_{50} (mg/ml) of phenolic extract	0.038 ± 0.0	0.032 ± 0.0	0.045 ± 0.0	0.039 ± 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	
	Melia azedarach	***Pongamia pinnata***	***Melia azedarach***	***Pongamia pinnata***
Yield of methanol extract (%)	6.8 ± 0.2	10.5 ± 0.6	7.4 ± 0.3	9.8 ± 0.3
Total phenolics (mg GAE/g)	18.5 ± 0.4	48.2 ± 0.2	16.8 ± 0.1	51.2 ± 0.2
Flavonoids (mg CAE/g)	4.2 ± 0.1	2.5 ± 0.2	2.9 ± 0.0	1.8 ± 0.1
Total tocopherol (mg/g)	26.5 ± 0.4	100.0 ± 0.2	23.1 ± 0.2	110.0 ± 0.2
DPPH (IC_{50}) (mg/ml) of phenolic extract	0.040 ± 0.0	0.046 ± 0.0	0.031 ± 0.0	0.048 ± 0.0

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 *P. pinnata* (48.2 ± 0.2 - 51.2 ± 0.2 mg GAE/g) as compared to extracts of seed oil in Palwal and Hisar locations **Table 1** and **2**.

Flavonoids Content: Flavonoids are presumably the 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 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 a characteristic antioxidant, which is 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 period 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 [14, 15]. The tocopherol substance of nourishment is critical to ensure sustenance lipids against autoxidation and, in this way to build their capacity life and their esteem as wholesome nourishment. The tocopherol content in the seed oil of *M. azedarach* was very low (3.4 ± 0.2 - 26.5 ± 0.4 mg/g) as compared to *P. pinnata* in extracts of both seed oil and seed cake of two areas (38.0 ± 1.0 mg/g - 110.0 ± 0.2 mg/g). In comparison of total tocopherol in unrefined oil and methanol extracts of defatted seed cake, we found that there was large difference in aggregate tocopherol content between seed oil and seed cake **Table 1** and **2**.

TABLE 3: ANTIOXIDANT ACTIVITY (%) OF PHENOLIC EXTRACT OF OILS AT DIFFERENT CONCENTRATIONS (PALWAL AND HISAR)

Conc. mg/ml	Palwal		Hisar	
	Melia azedarach Activity (%)	*Pongamia pinnata* Activity (%)	*Melia azedarach* Activity (%)	*Pongamia pinnata* Activity (%)
0.01	10	14	11	12
0.02	23	28	26	24
0.03	38	45	37	36
0.04	51	58	48	50
0.05	62	72	53	63
0.06	69	74	55	66
0.07	69	74	55	66
0.08	69	74	55	66
0.09				

TABLE 4: ANTIOXIDANT ACTIVITY (%) OF METHANOLIC EXTRACT OF DEFATTED SEED CAKE AT DIFFERENT CONCENTRATIONS (PALWAL AND HISAR)

Conc. mg/ml	Palwal		Hisar	
	Melia azedarach Activity (%)	*Pongamia pinnata* Activity (%)	*Melia azedarach* Activity (%)	*Pongamia pinnata* Activity (%)
0.01	18	17	22	20
0.02	31	25	35	28
0.03	40	36	46	34
0.04	52	42	60	44
0.05	55	55	66	52
0.06	59	61	73	58
0.07	59	61	73	58
0.08	59	61	73	58
0.09				

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 the assessment of radical scavenging movement of natural and manmade antioxidants compounds [16], plant extracts [17] and foods [18]. 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 a 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 *M. azedarach* was 69% in seed oil, and 73% in seed cake and the case of *P. pinnata* were 74% in seed oil and seed cake 61% at a concentration of 0.06mg/ml which is higher in phenolic extract of oil than the methanolic extracts of defatted seed cake. 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 was highest in *P. pinnata* in an extract of seed oil and seed cake as compared to *M. azedarach*. 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.

Correlation Coefficient (r) Between Phytochemical Components and Antioxidant Activity (IC_{50}) of Phenolic Extract of Seed Oil of *Melia azedarach* (Palwal): The corresponding correlation values obtained for a phenolic extract of seed oil of *M. azedarach* (Palwal) shown in **Table 5**. The result of correlation analysis in phenolic extract indicate that there was a positive and highly significant correlation (r = 0.898**) between DPPH and flavonoids as well as total phenols (r = 0.876**).

Total phenolics showed a high positive correlation (r = 0.765*) with flavonoids. 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 *MELIA AZEDARACH* (PALWAL)

	DPPH	Phenolics	Flavonoids	Tocopherol	Carotenoids
DPPH	1.000				
Phenolics	0.876**	1.000			
Flavonoids	0.898**	0.765*	1.000		
Tocopherol	0.566	0.337	0.488	1.000	
Carotenoids	0.593	0.459	0.171	0.448	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 *Melia azedarach* (Hisar): The corresponding correlation values obtained for the phenolic extract of seed oil of *M. azedarach* (Hisar) shown in **Table 6**. The result of correlation analysis in phenolic extract indicate that there was a positive and highly significant correlation (r = 0.899**) between flavonoids and carotenoids. DPPH activity showed a high positive correlation with tocopherol (r = 0.886**). Total phenolics showed a high positive correlation (r = 0.758*) with flavonoids. Rest of the correlation values obtained for seed oil were found to be non- significant.

TABLE 6: CORRELATION COEFFICIENT (r) BETWEEN PHYTOCHEMICAL COMPONENTS AND ANTIOXIDANT ACTIVITY (IC_{50}) OF PHENOLIC EXTRACT OF SEED OIL OF *MELIA AZEDARACH* (HISAR)

	DPPH	Phenolics	Flavonoids	Tocopherol	Carotenoids
DPPH	1.000				
Phenolics	0.582	1.000			
Flavonoids	0.476	0.758*	1.000		
Tocopherol	0.886**	0.348	0.162	1.000	
Carotenoids	0.500	0.197	0.899**	0.500	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 *Melia azedarach* (Palwal): The corresponding correlation values obtained for a methanolic extract of defatted seed cake of *M. azedarach* (Palwal**)** shown in **Table 7**. The result of correlation analysis in phenolic extract indicate that there was a positive and highly significant correlation (r = 0.992**) between total phenols and tocopherol. Similarly DPPH showed a high positive correlation with total phenolics (r = 0.977**) as well as with tocopherol (r = 0.744*). 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 *MELIA AZEDARACH* (PALWAL)

	DPPH	Phenolics	Flavonoids	Tocopherol
DPPH	1.000			
Phenolics	0.977**	1.000		
Flavonoids	0.466	0.340	1.000	
Tocopherol	0.744*	0.992**	0.654	1.000

* Significant at 5%, ** Significant at 1%

Correlation Coefficient (r) Between Phytochemical Components and Antioxidant Activity (IC_{50}) of Methanol Extract of Defatted Seed Cake of *Melia azedarach* (Hisar): The corresponding correlation values obtained for **a** methanolic extract of defatted seed cake of *M. azedarach* Hisar shown in **Table 8**. The result of correlation analysis in methanolic extract of defatted seed cake indicates that there was a positive and highly significant correlation between total phenolics (r = 0.889**) with tocopherol. DPPH showed a high positive correlation (r = 0.681*) with total phenolics. Rest of the correlation values obtained for defatted seed cake 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 *MELIA AZEDARACH* (HISAR)

	DPPH	Phenolics	Flavonoids	Tocopherol
DPPH	1.000			
Phenolics	0.681*	1.000		
Flavonoids	0.506	0.555	1.000	
Tocopherol	0.444	0.889**	0.254	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 *Pongamia pinnata* (Palwal): The corresponding correlation values obtained for phenolic extracts of seed oil of *P. pinnata* (Palwal) shown in **Table 9**. The result of correlation analysis in phenolic extract indicate that there was a positive and highly significant correlation (r = 0.989**) between total phenolics with flavonoids. Similarly, DPPH activity showed a high positive correlation with carotenoids (r = 0.744*). Rest of the correlation values obtained for seed oil were found to be non-significant.

TABLE 9: CORRELATION COEFFICIENT (r) BETWEEN PHYTOCHEMICAL COMPONENTS AND ANTIOXIDANT ACTIVITY (IC_{50}) OF PHENOLIC EXTRACT OF SEED OIL OF *PONGAMIA PINNATA* (PALWAL)

	DPPH	Phenolics	Flavonoids	Tocopherol	Carotenoids
DPPH	1.000				
Phenolics	0.577	1.000			
Flavonoids	0.618	0.989**	1.000		
Tocopherol	0.540	0.452	0.355	1.000	
Carotenoids	0.744*	0.271	0.327	0.066	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 *Pongamia pinnata* (Hisar): The corresponding correlation values obtained for the phenolic extract of seed oil of *P. pinnata* (Hisar) shown in **Table 10**. The result of correlation analysis in phenolic extract indicate that there was a positive and highly significant correlation (r = 0.890**) between total phenols with flavonoids as well as tocopherol (r = 0.760*). Rest of the correlation values obtained for seed oil were found to be non- significant.

TABLE 10: CORRELATION COEFFICIENT (r) BETWEEN PHYTOCHEMICAL COMPONENTS AND ANTI-OXIDANT ACTIVITY (IC_{50}) OF PHENOLIC EXTRACT OF SEED OIL OF *PONGAMIA PINNATA* (HISAR)

	DPPH	Phenolics	Flavonoids	Tocopherol	Carotenoids
DPPH	1.000				
Phenolics	0.660	1.000			
Flavonoids	0.566	0.890**	1.000		
Tocopherol	0.500	0.760*	0.466	1.000	
Carotenoids	0.481	0.191	0.255	0.327	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 *Pongamia pinnata* (Palwal): The corresponding correlation values obtained for a methanolic extract of defatted seed cake of *P. pinnata* (Palwal) shown in **Table 11**. The result of correlation analysis in methanolic extract indicate that total phenolics showed a high positive correlation (r = 0.893**) with flavonoids as well as with tocopherol (r = 0.876**). Flavonoids showed a positive correlation with tocopherol (r = 0.764*). Rest of the correlation values obtained for defatted seed cake were found to be non- significant.

TABLE 11: CORRELATION COEFFICIENT (r) BETWEEN PHYTOCHEMICAL COMPONENTS AND ANTI-OXIDANT ACTIVITY (IC_{50}) OF METHANOLIC EXTRACT OF DEFATTED SEED CAKE OF *PONGAMIA PINNATA* (PALWAL)

	DPPH	Phenolics	Flavonoids	Tocopherol
DPPH	1.000			
Phenolics	0.603	1.000		
Flavonoids	0.581	0.893**	1.000	
Tocopherol	0.466	0.876**	0.764*	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 *Pongamia pinnata* (Hisar): The corresponding correlation values obtained for a methanolic extract of defatted seed cake of *P. pinnata* Hisar shown in **Table 12**. The result of correlation analysis in methanol extract indicate that there was a positive and highly significant correlation (r = 0.998**) between DPPH with total phenolics. Total phenolics showed a high positive correlation with flavonoids (r = 0.760*). Rest of the correlation values obtained for defatted seed cake were found to be non- significant.

TABLE 12: CORRELATION COEFFICIENT (r) BETWEEN PHYTOCHEMICAL COMPONENTS AND ANTI-OXIDANT ACTIVITY (IC_{50}) OF METHANOLIC EXTRACT OF DEFATTED SEED CAKE OF *PONGAMIA PINNATA* (HISAR)

	DPPH	Phenolics	Flavonoids	Tocopherol
DPPH	1.000			
Phenolics	0.998**	1.000		
Flavonoids	0.595	0.760*	1.000	
Tocopherol	0.553	0.393	0.522	1.000

* Significant at 5%, ** Significant at 1%

CONCLUSION: This study provides knowledge on phenol range for a majority of the common plant extracts and assists in identifying the most resourceful genera of the plant. These data are valuable additions to the database for the fertilizer, remedial, pharmaceutical and food processing

industries. 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 study concluded that these plants are 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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