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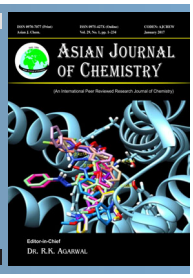

Compositional Analysis and Antioxidant Activity of *Acacia nilotica* from Two Locations

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In this study, the oils and defatted seed cake of *Acacia nilotica* from two locations were used to evaluate physico-chemical characteristics, fatty acids, mineral content, total phenols, flavonoids, total tocopherol, carotenoids and antioxidant activity by DPPH method. Peroxide value of fresh oil was higher (1.6 ± 0.1 meq/kg) in Hisar location, iodine value is higher (144.7 ± 0.3 g/100 g) in Hisar location, free fatty acid is higher (as % oleic acid) (0.9 ± 0.1 %) in Palwal location, saponification value is higher (219.4 ± 0.7 mg/g KOH) in Palwal location, unsaponifiable matter is higher (7.2 ± 0.1 %) in Hisar location. In different fatty acid highest composition found in linolenic acid (40.5 ± 0.4 %) in Palwal location. In mineral composition calcium found highest (203.0 ± 2.2 mg/100 g) in Hisar location and potassium and phosphorus found highest (110.0 ± 1.1 mg/100 g, 51.4 ± 1.5 mg/100 g) in Palwal location. From the data of two locations, it is inferred that phenolics and flavonoid content in the seed oil of *Acacia nilotica* was higher in the oil of the seeds collected from the Hisar (87.2 ± 0.2 mg GAE/g & 11.9 ± 0.4 mg CAE/g) than Palwal (80.1 ± 0.2 mg GAE/g & 11.3 ± 0.3 mg CAE/g), while in methanol extract of defatted seed cake of phenols higher in Palwal (18.2 ± 0.3 mg GAE/g) and flavonoid in Hisar (6.3 ± 0.5 mg CAE/g). The total tocopherol in methanol extract of defatted seed cake (15.5 ± 0.3 mg/g) and carotenoids in the oil (78.4 ± 0.1 mg/kg) were higher in Palwal location as compared to Hisar location. Antioxidant activity determined by DPPH method in which methanol extract of defatted seed cake had maximum antioxidant activity that is 87 % at a concentration of 0.06 mg/mL from Palwal.

Keywords: *Acacia nilotica*, Fatty acids, Mineral content, Total phenolics, Flavonoids, Total tocopherol, Carotenoids, Antioxidant activity.

INTRODUCTION

Acacia nilotica (L.) Del. belongs to the family Mimosaceae is generally circulated in tropical and sub-tropical nations. It is utilized as a part of Ayurvedic prescriptions. Plants give natural drugs which advance self-mending, great wellbeing and life span. This plant have restorative fixings to counteract, moderate or treat numerous ailments or conditions. Every part of the plants are useful for people. The bark, leaves, pods and flower of this plant are utilized against disease, sclerosis, blockage, smallpox, hack, loose bowels, draining heaps, fever, opthalmia, leucoderma, tuberculosis, sickness, draining heaps and menstrual problem [1,2]. Recently this plant used to toss of light on account of its higher biomass accessibility, so it can demonstrate compelling and less expensive medication for malignancy. Earlier studies revealed that the plant extract has antibacterial, molluscidal movement, antimalarial, antifungal, anti-toxin, anthelmentic, against hypertensive and anticancer property. The seeds of *Acacia nilotica* having nourishing qualities and physico-chemical arrangement.

EXPERIMENTAL

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

The chemicals used for the experiments were from Ranbaxy Merk and Qualigens, of highest purity. Oil content will be determined by Soxhlet method using petroleum ether (60-80 °C) for 8 h. The chemical characteristics of seed oil will be determined according to AOAC standard method [3].

Diacid mixture: Nitric acid and perchloric acid was mixed in ratio 5:1 just before use.

Hydrochloric acid mixture (1 %): 1 mL of conc. HCl was added in 50 mL distilled water and total volume 100 mL was made with distilled water.

Method: Powdered sample (2 g) of the seeds was digested with 15 mL of diacid mixture ($5HNO_3$:$HClO_4$) in a conical flask by heating on hot plate in open space till clear white precipitates settle down at bottom of conical flask. The

precipitates were dissolved in 1 % HCl prepared in double distilled water, filtered and final volume of filterate 50 mL made with double distilled water and analysis was done by atomic absorption spectrometer (AAS).

Fatty acid spectrum: Fatty acids were determined by fractination of methyl esters by GLC [4].

Total phenolics: The phenolic content was determined by the method of Folin-Ciocalteu reagent [5].

Methodology: All phenolic compounds present in the plant extracts are oxidized by Folin-Ciocalteu reagent. This reagent is a mixture of phosphotungstic acid ($H_3PW_{12}O_{40}$) and phosphomolybdic acid ($H_3PMo_{12}O_{40}$) which, after oxidation of the phenols, is reduced to a mixture of blue oxides of tungsten (W_8O_{23}) and molybdenum (Mo_8O_{23}). The blue colouration produced has a maximum absorption in the region of 730 nm and is directly proportional to the quantity of phenolic compounds present. The results are expressed as milligrams of gallic acid equivalent per gram (mg GAE/g).

Flavonoids: The aluminum chloride colorimetric measure was utilized. The absorbance was visualize at 510 nm utilizing UV noticeable spectrophotometer. Add up to flavonoid substance was communicated as mg catechin reciprocals per gram of the concentrate (mg CAE/g).

Tocopherol: Aliquots (10, 15, 20, 25,30,35 and 40 ppm of tocopherol in the ethanol were exchanged to a volumetric flask and the volume was acclimated to 8 mL with ethanol. Each of the arrangement and 1.0 mL of 2,2'-dipyridyl reagent were pipetted into 10 mL volumetric flask and blended. A 1 mL segment of ferric chloride reagent was added to the 10 mL volumetric flask and the blend shaken for 10 s. The absorbance of the blend was measured at 520 nm against ethanol as a blank. At that point the standard curve was drawn.

Carotenoid content: Carotenoid content was determined by the known method [6].

Determination of antioxidant activity: Antioxidant activity was studied by 2,2'-diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging method [7].

The scavenging activity of the extract will be calculated as:

$$\text{Inhibition (\%)} = \frac{(\text{Abs}_{\text{control}} - \text{Abs}_{\text{sample}})}{\text{Abs}_{\text{control}}} \times 100$$

Measurable analysis: The trial estimations were completed in triplicate and results were displayed as mean of three recreates ± standard deviation. Measurable examination was completed utilizing Microsoft Excel 2007.

RESULTS AND DISCUSSION

Physico-chemical characteristics of seed oil of *Acacia nilotica* (Palwal) and *Acacia nilotica* (Hisar): The physico-chemical characteristics of oil of *Acacia nilotica* (Palwal) and *Acacia nilotica* (Hisar) were resolved by standard techniques of AOAC (1990), which were given in Table-1. The free fatty were 0.9 ± 0.1 % & 0.7 ± 0.1 %, peroxide value of fresh oil were 1.4 ± 0.1 meq/kg & 1.6 ± 0.1 meq/kg, saponification values were 219.4 ± 0.7 mg KOH/g & 189 ± 0.4 mg KOH/g, unsaponifiable matter were 6.1 ± 0.2 % & 7.2 ± 0.1 %, iodine values were 140.5 ± 0.1 g/100 g & 144.7 ± 0.3 g/100 g and the yield of methanol extract 3.1 ± 0.1 g/100 g and 3.3 ± 0.1 g/100 g (in two locations).

TABLE-1
PHYSICO-CHEMICAL CHARACTERISTICS OF SEED OIL OF *Acacia nilotica* (PALWAL) AND *Acacia nilotica* (HISAR)

Parameters	Composition: *Acacia nilotica*	
	Palwal	Hisar
Peroxide value (meq/kg)	1.4 ± 0.1	1.6 ± 0.1
Iodine value (g/100 g)	140.5 ± 0.1	144.7 ± 0.3
Saponification value (mg KOH/g)	219.4 ± 0.7	189.0 ± 0.4
Unsaponifiable matter (%)	6.1 ± 0.2	7.2 ± 0.1
Free fatty acid (%)	0.9 ± 0.1	0.7 ± 0.1

Values are mean of three replicates ± standard error.

Fatty acid profile of *Acacia nilotica* (Palwal) and *Acacia nilotica* (Hisar): Fatty acid profile of *Acacia nilotica* (Palwal) and *Acacia nilotica* (Hisar) having the following composition profile: myristic acid 0.1 ± 0.0 % and 0.2 ± 0.1 %, palmitic acid 13.7 ± 0.4 % and 13.4 ± 0.2 %, stearic acid 4.4 ± 0.1 % and 4.5 ± 0.1 %, oleic acid 30.6 ± 0.3 % and 31.8 ± 0.3 %, linoleic acid 40.5 ± 0.4 % and 39.6 ± 0.2 %, linolenic acid 2.6 ± 0.3 % and 2.8 ± 0.1 %, arachidic acid 0.1 ± 0.1 % and 0.1 ± 0.0 % and behanic acid 1.6 ± 0.2 % and 1.4 ± 0.2 % in the two locations (Palwal & Hisar) shown in Table-2.

TABLE-2
FATTY ACID PROFILE OF *Acacia nilotica* (PALWAL) AND *Acacia nilotica* (HISAR)

Parameters	Composition (%): *Acacia nilotica*	
	Palwal	Hisar
Myristic acid ($C_{14:0}$)	0.1 ± 0.0	0.2 ± 0.1
Palmitic acid ($C_{16:0}$)	13.7 ± 0.4	13.4 ± 0.2
Stearic acid ($C_{18:0}$)	4.4 ± 0.1	4.5 ± 0.1
Oleic acid ($C_{18:1}$)	30.6 ± 0.3	31.8 ± 0.3
Linoleic acid ($C_{18:2}$)	40.5 ± 0.4	39.6 ± 0.2
Linolenic acid ($C_{18:3}$)	2.6 ± 0.3	2.8 ± 0.1
Arachidic acid ($C_{20:0}$)	0.1 ± 0.1	0.1 ± 0.0
Behanic acid ($C_{22:0}$)	1.6 ± 0.2	1.4 ± 0.2

Values are mean of three replicates ± standard error.

Mineral Composition of seeds of *Acacia nilotica* (Palwal) and *Acacia nilotica* (Hisar): The seeds of *Acacia nilotica* (Palwal) and *Acacia nilotica* (Hisar) contained significant amount of important minerals shown in Table-3. Calcium 198.0 ± 1.8 mg/100 g & 203.0 ± 2.2 mg/100 g, potassium 110.0 ± 1.1 mg/100 g & 100.0 ± 1.4 mg/100 g, sodium 25.0 ± 0.3 mg/100 g & 27.0 ± 0.1 mg/100 g, magnesium 2.5 ± 0.1 mg/100 g & 2.4 ± 0.1 mg/100 g, iron 18.0 ± 0.3 mg/100 g & 20.0 ± 0.2 mg/100 g, zinc 2.4 ± 0.0 mg/100 g & 2.5 ± 0.0 mg/100 g, cobalt 1.9 ± 0.1 mg/100 g & 1.4 ± 0.1 mg/100 g, manganese 3.0 ± 0.2 & 3.2 ± 0.0, copper 0.3 ± 0.0 mg/100 g & 0.2 ± 0.1 mg/100 g and phosphorus 51.4 ± 1.5 mg/100 g & 50.7 ± 1.3 mg/100 g in the two locations (Palwal & Hisar).

Total phenolics: Phenolics are fragrant auxiliary plant metabolites are abnormal state cancer prevention agents as a result of their capacity to rummage free radicals and dynamic oxygen species, for example, superoxide free radicals and hydroxyl radicals. It is notable that phenolic substances contribute specifically to the cancer prevention agent action of plant materials. Therefore, phenolics mixes display significant

TABLE-3
MINERAL COMPOSITION OF SEEDS OF *Acacia nilotica* (PALWAL) AND *Acacia nilotica* (HISAR)

Parameters	Composition: *Acacia nilotica*	
	Palwal	Hisar
Ca (mg/100 g)	198.0 ± 1.8	203.0 ± 2.2
K (mg/100 g)	110.0 ± 1.1	100.0 ± 1.4
Na (mg/100 g)	25.0 ± 0.3	27.0 ± 0.1
Mg (mg/100 g)	2.5 ± 0.1	2.4 ± 0.1
Fe (mg/100 g)	18.0 ± 0.3	20.0 ± 0.2
Zn (mg/100 g)	2.4 ± 0.0	2.5 ± 0.0
Co (mg/100 g)	1.9 ± 0.1	1.4 ± 0.1
Mn (mg/100 g)	3.0 ± 0.2	3.2 ± 0.1
Cu (mg/100 g)	0.3 ± 0.0	0.2 ± 0.1
P (mg/100 g)	51.4 ± 1.5	50.7 ± 1.3

Values are mean of three replicates ± standard error.

free radical-rummaging exercises (through their reactivity as hydrogen-giving or electron-giving specialists) and metal particle chelating properties [8]. Our outcomes demonstrated that the substance of aggregate phenols ranged from 80.1 ± 0.2 mg GAE/g (Palwal) to 87.2 ± 0.2 mg GAE/g (Hisar) in seed oil of *Acacia nilotica* and 17.5 ± 0.4 mg GAE/g (Hisar) to18.2 ± 0.3 mg GAE/g (Palwal) in methanol extract of defatted seed cake of *Acacia nilotica* as appeared in Fig. 1. The variation in our findings of the phenolic content in *Acacia nilotica* from two unique areas might be ascribed because of hereditary components, level of development and natural conditions. Also, extractability of various phenolic substance is administered by the sort of dissolvable (extremity) utilized, level of polymerization of phenolics. These all factors affect the phytochemical compositions; further total phenol content in *Acacia nilotica* higher in methanol extract of seed oil (Hisar) as compared to methanol extract of defatted seed cake.

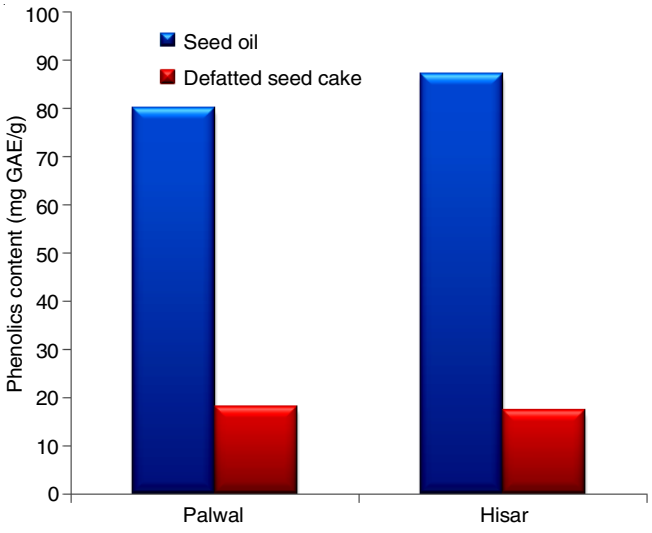

Fig. 1. Total phenolics content of phenolic extract of seed oil and methanolic extract of defatted seed cake of *Acacia nilotica* (Palwal) and *Acacia nilotica* (Hisar)

The cancer prevention agent movement of divisions may not just be because of the presence of phenolics additionally identified with the presence of some individual dynamic parts in the extract. The hazy relationship between the cell reinforcement action and aggregate phenolic substance might be clarified by the way that the aggregate phenolic content does not join every one of the cancer prevention agents. Furthermore, the synergism between the cancer prevention agents in the blend makes the cell reinforcement action subject to the fixation as well as on the structure and cooperation between the cell reinforcements.

Flavonoid content: Among phenolic compound, flavonoids are the most important group of plant phenolics that have antioxidant potential. These flavonoids are known to posses antioxidant, anticancer, antiallergic, anti-inflammatory and gastroprotective properties. Flavones and flavonols are the subgroups of flavonoids. Flavonols are referred to go about as cell reinforcement, both as radical scavengers [9] and as metal chelators [10]. The aglycones of these flavonols were accounted for to be more active than their glycosides [11]. Flavonoids can search dynamic oxygen radical, superoxide and hydroperoxide by single electron exchange. Superoxide is a naturally vital substance which can be decayed to frame more grounded oxidative species, for example, singlet oxygen and hydroxyl radicals [12]. The profoundly responsive hydroxyl radicals can bring about oxidative harm to DNA, lipids and proteins [13]. In the present study the flavonoid content in *Acacia nilotica* differed from 11.3 ± 0.3 mg CAE/g (Palwal) to 11.9 ± 0.4 mg CAE/g (Hisar) in phenolic extract of seed oil. In the methanol extract of defatted seed cake of *Acacia nilotica* it shifted from 4.9 ± 0.1 mg CAE/g (Palwal) to 6.3 ± 0.5 mg CAE/g (Hisar) (Fig. 2). The flavonoid substance of *Acacia nilotica* were higher in phenolic extract of seed oil (Hisar) when compared with methanol extract of defatted seed cake.

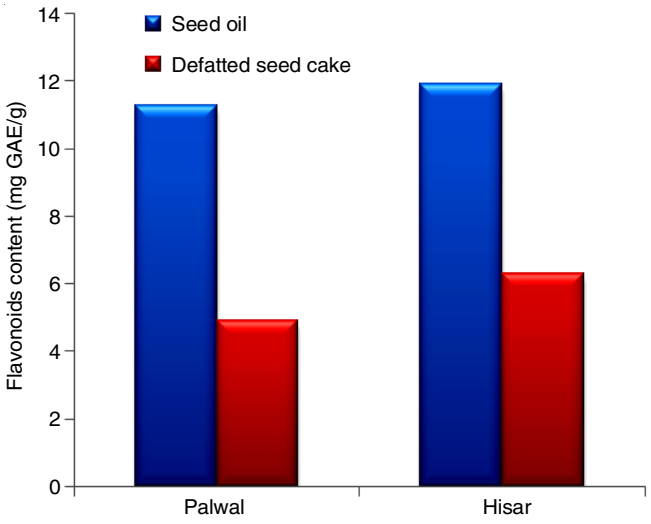

Fig. 2. Flavonoid content of phenolic extract of seed oil and methanolic extract of defatted seed cake of *Acacia nilotica* (Palwal) and *Acacia nilotica* (Hisar)

Tocopherols: Tocopherols are characteristic cancer prevention agents, which are available in every single vegetable oil in various sums that assume a key part in protecting oil from rancidity amid capacity in this way dragging out its self-life [14]. Tocopherols go about as natural hijackers of free radicals and could counteract illnesses, other than having an essential dietary capacity for people as a wellspring of vitamin E [15]. The tocopherol substance of nourishments is imperative to ensure sustenance lipids against autoxidation and, along

these lines to expand their capacity life and their esteem as wholesome nourishments. In the present study the aggregate tocopherol content in *Acacia nilotica* ranged from 10.4 ± 0.8 mg/g (Hisar) to 12.2 ± 0.6 mg/g (Palwal) in seed oil. In the methanol extract of defatted seed cake of *Acacia nilotica* it differed from 14.2 ± 0.2 mg/g (Hisar) to 15.5 ± 0.3 mg/g (Palwal) (Fig. 3). The aggregate tocopherol content highest in methanol extract of defatted seed cake (Palwal) when compared with seed oil. The seeds contain higher tocopherol than pods which is interesting for the creation of normally happening tocopherols for the adjustment of fats and oils against oxidative decay and for applications in dietary, pharmaceutical or biomedical products [16].

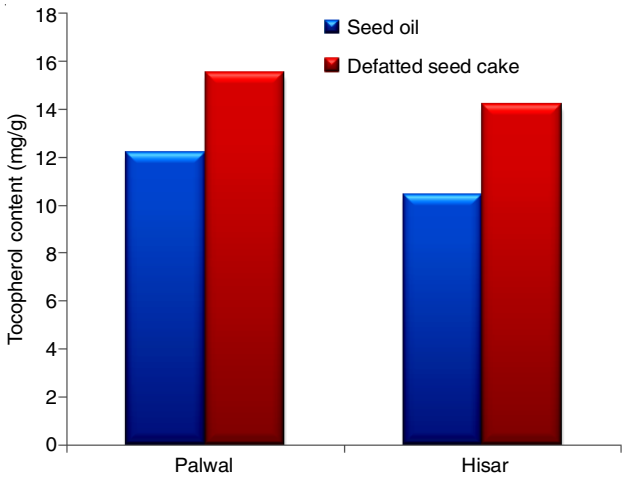

Fig. 3. Total tocopherol content of phenolic extract of seed oil and methanolic extract of defatted seed cake of *Acacia nilotica* (Palwal) and *Acacia nilotica* (Hisar)

Carotenoids content: The shading in oils is for the most part because of the presence of carotenoid colours and additionally chlorophyll colours. Carotenoids ensure cells against the impact of light, air and sensitiser shades being able to extinguish singlet oxygen and can likewise serve as cancer prevention agents under conditions other than photosensitization [17]. Some unrefined oils can have out of the blue high pigmentation brought on by field harm, shameful capacity, or flawed taking care of amid pounding and extraction. Carotenoid value determined in oil of *Acacia nilotica* which is varied from 67.4 ± 0.4 mg/kg (Hisar) to 78.4 ± 0.1 mg/kg (Palwal). Carotenoids is a type of antioxidant, higher value of this indicates higher antioxidant activity (Fig. 4).

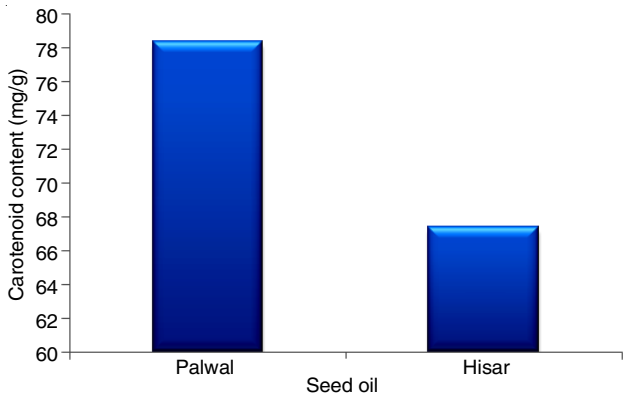

Fig. 4. Carotenoid content in seed oil of *Acacia nilotica* (Palwal) and *Acacia nilotica* (Hisar)

Antioxidant activity by DPPH: Antioxidant activity influence the procedure of lipid oxidation at various stages because of contrasts in their method of activity. Oxidation of lipids is an extremely complex process bringing about an extraordinary assortment of oxidation items. Many components especially temperature, light and the presence of initiators (metal chemicals), impact the oxidation procedure and coming about items. Along these lines, the antioxidant activity was measured in the phenolic extract of seed oil and methanolic extract of defatted seed cake. The technique utilized for the assurance of antioxidant activity was 2,2'-diphenyl-1-picryl-hydrazyl radical (DPPH) strategy.

Different radicals formed during lipid oxidation are among the primary driver for oxidative harm to human health [18]. Antioxidants can practice their defensive capacity by rummaging free radicals, which are the principle propagators of lipid oxidation. 2,2'-Diphenyl-1-picrylhydrazyl radical is one of only a handful few stable and monetarily accessible natural nitrogen radical (DPPH$^{\bullet}$). Methanolic solution of DPPH$^{\bullet}$ have a trademark retention maximum at 517 nm. At the point when an electron or hydrogen molecule giving antioxidants (AH) is added to DPPH$^{\bullet}$ a diminishing in absorbance at 517 nm happens because of the development of the non-radical frame DPPH-H, which does not assimilate at 517 nm. Initially, it was observed by ESR spectroscopy and depended on the flag force of DPPH$^{\bullet}$ being contrarily identified with the cancer prevention agent fixation and the response time. All the more as of late, this response has been measured by the decolouration examine where the diminishing in absorbance at 517 nm created by the expansion of the cell reinforcement to the DPPH$^{\bullet}$ in methanol or ethanol is measured.

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

All the above depicted concentrates were screened for radical searching action against DPPH$^{\bullet}$.

Our outcomes demonstrated that the antioxidant activity of *Acacia nilotica* ranged from 73 % (Palwal) to 78 % (Hisar) in the methanol extract of seed oil and in defatted seed cake, antioxidant activity of *Acacia nilotica* varied from 80 % (Hisar) to 87 % (Palwal) (Fig. 5). The antioxidant activity higher in methanol extract of defatted seed cake (Palwal) as compared to phenolic extract of seed oil which might be due to some other extractable antioxidants from the seed cake.

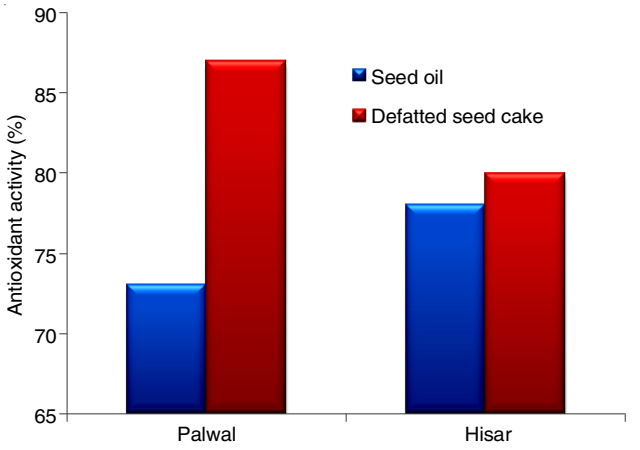

Fig. 5. % Antioxidant activity of phenolic extract of seed oil and methanolic extract of defatted seed cake of *Acacia nilotica* (Palwal) and *Acacia nilotica* (Hisar)

The Folin-Ciocalteu reagent recognize all phenolic compounds found in the extracts [19]. This shows figures other than aggregate phenolics assume a part in the cancer prevention agent movement. Additionally, every one of the phenolics don't have a similar cancer prevention agent movement, some are intense, others are feeble and they create adversarial or synergistic impacts with themselves or with alternate constituents of the extracts [20,21]. This reality could imply that either their segments don't have, great hydrogen giving properties or that some active elements affected their response with the radical, or that their segments meddle with the radical rummaging process [18] have found that the radical searching action of specific antioxidant relies on upon structure and in addition on the sort of response energy (Fig. 5).

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