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Phenolic and antioxidant potential of sunflower meal

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ABSTRACT

Sunflower oil cake/meal was subjected to conventional liquid solvent extraction using various solvents such as Methanol, Ethanol, Ethanol:Water 1:1, Methanol:Water 1:1, Acetone, Isopropanol and Ethyl acetate. The optimum solvent extraction conditions of phenols were 180 min. using 7 different solvent systems, at a solvent to sample ratio 5:1 (v/w). Solvent extracts were tested for their antioxidant activity by the DPPH radical scavenging method and by determination of peroxide value on Soybean oil and Sunflower oil. The Ethanol extract exhibited the highest antiradical activity, and no correlation was found between antiradical activity and phenol content. Moreover, the Ethanol extract appeared to be a stronger antioxidant than BHT by the Rancimat method performed on sunflower oil and soybean oil. LCMS analysis of the extracts showed that the predominant phenolic compound was Chlorogenic acic and Caffeic acid. Various phenolic acids and flavonoids were also identified.

Keywords: Antioxidant, Rancimat method, Sunflower meal, Solvent Extraction, LCMS.

INTRODUCTION

Sunflower (Helianthus annuus L) is the second largest oilseed crop as a world source of vegetable oil. Sunflower (*Helianthus annuus*) is an important oil seed crop of the world and it ranks third in production next to groundnut and soybean [1]. Because of its high content of protein, sunflower meal is used primarily in ruminant feed, but its nutritional, sensory and functional properties also make a great interest for human food as a protein source [2]. It is considered to be safe feed among many feed for all species, its only limitations being its fiber content and amino acid deficiencies. However, residues and contaminants from sunflower cultivation, harvest and post harvest operations may be of concern. Sunflower meals can be made from whole or decorticated seeds, and can be mechanically and/or solvent extracted. The quality of sunflower meal depends on the plant characteristics (seed composition, hulls/kernels ratio, dehullings potential, growth and storage conditions) and also on the process (dehulling, mechanical and/or solvent extraction). Sunflower cake is one the major protein meals used for livestock feeding and particularly for ruminant diets.

The content of phenolic compounds in meal may vary depending upon the content of hulls in meal and variety (regions) cultivate of sunflower [3]. Chlorogenic and caffeic acids compose 70 % of phenolic compounds in sunflower flour [4]. Chlorogenic acid is described as major phenolic compounds in sunflower seeds while caffeic acid is present in lower concentration [5]. Sunflower meal (blended with wheat flour) can be used for human nutrition. Despite their dark color, sunflower protein (71 %) concentrates have excellent digestibility [6]. In many studies it has been demonstrated that sunflower meal has high antioxidant potential, which could be beneficial for further technological utilization [3]. Numerous sunflower polyphenols such as caffeic, chlorogenic and ferulic acids have shown in many studies to exert a high antioxidative potential [3]. They can be used as effective antioxidants for

stabilization of sunflower oil [7]. The By-products of sunflower oil's production such as meal and hulls are valuable sources of phenolic compounds that might be recovered and used as natural antioxidants [3].

Phenolic compounds are essential for the cellular physiology and metabolism, and are involved in several plant functions such as their sensorial and reproductive properties [8]. Recently, there is a great interest in these compounds due to their antioxidant activity and its probable beneficial effects on human health [9]. It is known that in live organisms extremely reactive species of oxygen (ROS) are continuously produced by endogenous and exogenous conditions [10]. A hyper-physiological load of free radicals causes unbalance in the homeostasis between oxidants and antioxidants in the organism, leading to the oxidative stress that is suggested as cause of several diseases such as AIDS [11], cataract [12], osteoporosis [13], hyperglycemia, hyperlipidemia, cerebral vascular accident [14], cardiovascular diseases, and cancer [15].

Sunflower oil cake/meal is an excellent source of natural antioxidants. Phenolic compounds, which are considered to be the main antioxidant compounds in sunflower oil cake, are able to donate a hydrogen atom to the lipid radical formed during the propagation phase of lipid oxidation. So, they could be added to fatty foods to prevent the formation of off flavor and toxic compounds resulting from lipid oxidation. Hence, this study focuses on evaluation of antioxidant potential of sunflower oil cake

MATERIALS AND METHODS

Sunflower oil cake/meal was provided by a sunflower oil industry located in Beed (Maharashtra). The sunflower oil cake was promptly analyzed for, moisture, total solids and ash using the APHA, AWWA, WPCF (1985) [16] methods.

Reagents and standards

Methanol, Ethanol, Isopropanol, Acetone and Ethyl acetate used were analytical reagent grade and purchased from 'Thomas and Baker pvt ltd'. The Folin–Ciocalteau phenol reagent and the free radical 1,1 Dipheny 2-2-picryl-hydrazyl (DPPH) were purchased from Sigma Chemical Co (Sigma–Aldrich Company Ltd). Soybean oil (SBO) and Sunflower oil (SFO) are gifted samples from Kamani oil industries Mumbai.

2.1. Extraction of phenolic compounds

The extraction of phenolic antioxidants was performed using various solvents such as Methanol, Ethanol, Ethanol:Water (1:1), Methanol:Water (1:1), Acetone, Isopropanol and Ethyl acetate under conditions of solvent to sample ratio 5:1 (v/w) and 3 hr extraction period, at ambient temperature. The sunflower meal (40gm) was acidified with HCl (pH 2.0) and was extracted for 1 h with n-hexane at a ratio of 5:1 (v/w) by continuous extraction, in a magnetic stirrer, at ambient temperature, for fat removal. The extract was filtered using Whatman filter paper no. 2 Buchner funnel, and the filtrate, which contained the lipids, was removed. The residue was re-extracted continuously or by steps with different extracting solvents (methanol, ethanol, mixture of ethanol: water 1:1, mixture of methanol: water 1:1, acetone, isopropanol and ethyl acetate), at proportions of solvent volume to sample mass 5:1 (v/w), for different extraction times (from 30 min to 6 h) in a magnetic stirrer, at ambient temperature. The new extract was filtrated using a Whatman filter paper no. 2, and the filtrate was obtained. The combined filtrates were evaporated to dryness in a rotary evaporator and the residue re-dissolved in methanol and kept at 20^{o} C until subsequent analyses.

2.2. Composition analysis of sunflower meal:

The total protein content of sunflower meal was determined by using the classical Kjeldahl method conducted at an automated testing machine (Gerhard Vapodest 50s and Kjeldahl Therm, Germany). The total protein contents (TPC) were calculated by using conversion factor of 6.25. Total lipid content and ash contents of sunflower meal were determined according to AOAC 948.22 and AOAC 950.49 standard methods [15], respectively. The sunflower meal was promptly analyzed for, moisture, and ash using the APHA, AWWA, WPCF methods [16]. Moisture content of sunflower meal was determined according to the ISO methods 665 by drying of the meal samples for 3 h at 103˚C [15]. The samples were then weighted after cooling down in a desiccators and the drying was continued with 1 h drying periods until difference between two successive weightings was equal to or less than 0.005 g. The average of three measurements was used for all compositional analysis tests. All results were given as percent in dry weight basis. The total carbohydrate content was calculated by subtracting percentages of total protein, lipid and ash contents from hundred.

2.3. Antioxidant assays

2.3.1. DPPH radical scavenging method:

The antioxidant activity of the phenol extracts was evaluated by using the stable 1,1-diphenyl-2-picryl-hydrazyl radical (DPPH) according to a modification of the method of APHA, AWWA, WPCF 1985 [16] Methanolic solutions of phenol extracts (0.1 ml) and 3.9 ml Methanolic solution of DPPH (0.0025 g/100 ml CH3OH) were added in a cuvette and the absorbance at 515 nm (till stabilization) was measured against methanol using a double-beam ultraviolet–visible spectrophotometer Hitachi U-3210 (Hitachi, Ltd., Tokyo, Japan). Simultaneously, the absorbance at 515 nm of the blank sample (0.1 ml Methanol + 3.9 ml Methanolic solution of DPPH) was measured against methanol. The radical scavenging activities of the tested samples, expressed as percentage inhibition of DPPH, were calculated according to the following formula proposed by Bandoniene, D et al [17]:

$$\% \text{ Inhibition} = 100 \times (A-A_0)/A_0$$

Where A_0 is the absorbance at 515 nm of the blank sample at time t=0 and A is the final absorbance of the test sample at 515 nm

2.3.2. Phenol content determination:

Total phenolic constituents of plant extracts were performed employing the literature methods involving Folin-Ciocalteu reagent and Gallic acid as standard [18]. Extract solution 0.1 ml was taken in a volumetric flask, 46 ml distilled water and 1 ml Folin-Ciocalteu reagent was added and flask was shaken thoroughly. After 3 min, 3 ml of solution 2% Na_2CO_3 was added and the mixture was allowed to stand for 2 hr with intermittent shaking. Absorbance was measured at 765 nm. The same procedure was repeated to all standard Gallic acid solutions (0–1000 mg, 0.1 ml^{-1}) and standard curve was obtained.

2.3.3. Rancimat test

The extract that presented the highest capacity in the *in vitro* method employed before, the antioxidant capacity of the sunflower meal solvent aqueous extract, as well as BHT at 0.2%, was also evaluated for a lipidic system. For that, a 743 Rancimat device from Metrohm was used with the PC: 743 Rancimat® 1.0 software, where the induction period of soybean oil containing the aqueous extract was measured by using the automatized Swift test. The extract volume was calculated in advance, based on its dry weight, adjusting its concentration in the substrate (antioxidant-free soybean oil gifted by Kamani oil industry Mumbai) was 0.2%. The aqueous extract volume was taken in triplicate and placed inside the Rancimat tubes. Then, 3 g of antioxidant free soybean oil was added to the content of each tube, and the mixture was homogenized for 15 min by using an ultrasound device. Right after that, with the temperature set to 110 °C, D*T* = 1.5 ℃, air flow of 20 L/h, the tubes were fitted to the Rancimat device until the conductivity curve *versus* the time of reaction was completed, in order to calculate the induction period (IP). A control was also prepared with antioxidant-free soybean oil. BHA and BHT at 0.2% were employed as standard.

The solvent extract of the sunflower cake was added into commercial sunflower oil and soybean without any added antioxidant at concentrations ranging from 0.2%, 0.4% and 0.6%. The antioxidant potential of this extract was investigated and the 0.2% exhibited highest antioxidant potential. The 7 different solvent extracted oleoresins were compared to the antioxidant potential of samples of and sunflower oil containing synthetic (TBHQ) antioxidants.

The results were expressed as percentage for the induction period increase in relation to the control, as follows.

$$\% \text{ IP} = 100 - [(\text{IPsample}/\text{IPcontrol}) \times 100]$$

2.3.4. Peroxide value determination.

All solvent extracts were added at concentrations 0.2% to commercial soybean oil and sunflower oil. Then, all the samples were put in an oven at 85 °C where thermal oxidation took place. Every 24 h the samples were analyzed for peroxide value in order to monitor the oxidation process. The peroxide value was determined according to the EEC method (EEC Regulation No. 2568/91, L-248/05-09-1991). In a stopper conical flask, 2 g of sample were weighed and 10 ml chloroform, 15 ml acetic acid and 1 ml potassium iodide 10% were added. The flask was shaken for 1 min and left in the dark for 5 min. Then, 75 ml of deionized water were added and the titration took place with a solution of sodium thiosulfate 0.01 N and 1% starch solution as index. Simultaneously, a black run was carried out.

The peroxide value expressed as moles of active oxygen per kg of sample, was calculated by the following formula:

PV (mmoles/kg) = [(V-Vo) x T x 1000]/m

Where V is the volume (ml) of the sodium thiosulfate solution for the blank, V_o is the volume (ml) of sodium thiosulfate solution for the sample, T is the normality of the sodium thiosulfate solution and m is the sample weight (g)

2.4. Analytical studies:

LCMS analysis: LC/MS of Ethanolic extract of sunflower meal were obtained using a Hewlett-Packard HP 1100 Series LC/MSD system equipped with an API source, model G1948A, working in negative mode. The interface settings were: nebuliser pressure, 40 psig; drying gas temperature and flow rate, 350°C and 6 l min^{-1} respectively; voltage at capillary entrance, 4000V; fragmentation voltage, 175V. A Zorbax SB-C18 (5mm, 150mm, and 4.6mm id) analytical column was used. The mobile phase was acetonitrile/ methanol/water/formic acid (5:5:85:5) at a flow rate of 0.4ml/min^{-1}. The injection volume and column temperature were 10ml and 30°C respectively. The scan range was set at m/z 105±650.

RESULT AND DISCUSSION

3.1. Chemical composition of sunflower cake:

In Table 1, data on the chemical analysis of the sunflower cake and, especially, the total phenol content of the sunflower cake extracts is shown. The chemical characterization of the sunflower cake was a prior necessity in order to evaluate its potential, to determine the extraction yield, and to be controlled qualitatively. The phenol content of the extracts varied in response to the different solvents used (Table 1). The phenol content, estimated as Gallic acid equivalents on dry basis, varied from 0.43% to 1.29% (Table 1). The solubility and the response of phenolics in the Folin–Ciocalteau assay are governed by their chemical nature that may vary from simple to very highly polymerised substances.

Table 1. Chemical composition of sunflower meal/cake

Parameters	value
Total phenol content	
Protein content	36.338%
Moisture (% w/w)	9.92%
Ash (% w/w)	11.202%
Fat (% w/w)	2.4gm/40 gm of sunflower meal
Carbohydrate content	52.47%
Methanol extract	653.923 mg/gm GAE
Ethanol extract	727.76 mg/gm GAE
Methanol:water 1:1 extract	640 mg/gm GAE
Ethanol:water 1:1 extract	719 mg/gm GAE
Acetone extract	598 mg/gm GAE
Isopropanol extract	711.61 mg/gm GAE
Ethyl acetate extract	604 mg/gm GAE

Solvent extracts of sunflower cake collected after the extraction was performed for 4 h showed the highest antioxidant activity at 3 h and 3.1/2 h, whereas the extract exhibited the decrease in antioxidant activity as the extraction time increases. From above tabulation it is clear that Ethanolic extract exhibited highest TPC 727.76 mg/gm GAE whereas Acetone extract exhibited lowest TPC 598 mg/gm GAE.

3.2. Antioxidant activity of phenols extracts

The results of % inhibition of DPPH radical from the sunflower meal extracts are given in Table 2. Ethanol, methanol and IPA extracts showed equally the highest antioxidant activity, whereas the Acetone and Ethylacetate extract exhibited the lowest antioxidant activity (table 2 and fig 2). The radical scavenging activity of methanol and ethanol sunflower meal extracts was significantly higher than the antioxidant activity of the other solvent extracts (table 2 and fig 2). The different antioxidant activities of the phenolic extracts can be attributed to the different extracting solvents, as the antioxidant activity depends on the type and polarity of the extracting solvent the isolation procedures, the purity of the active compounds, as well as the test system. Some authors found a correlation between the polyphenol content and antioxidant activity; others found no such a relationship. The induction periods of SBO subjected to accelerated oxidation conditions without antioxidant and with added sunflower meal solvent extracts, BHT and BHA, are reported in table 4.

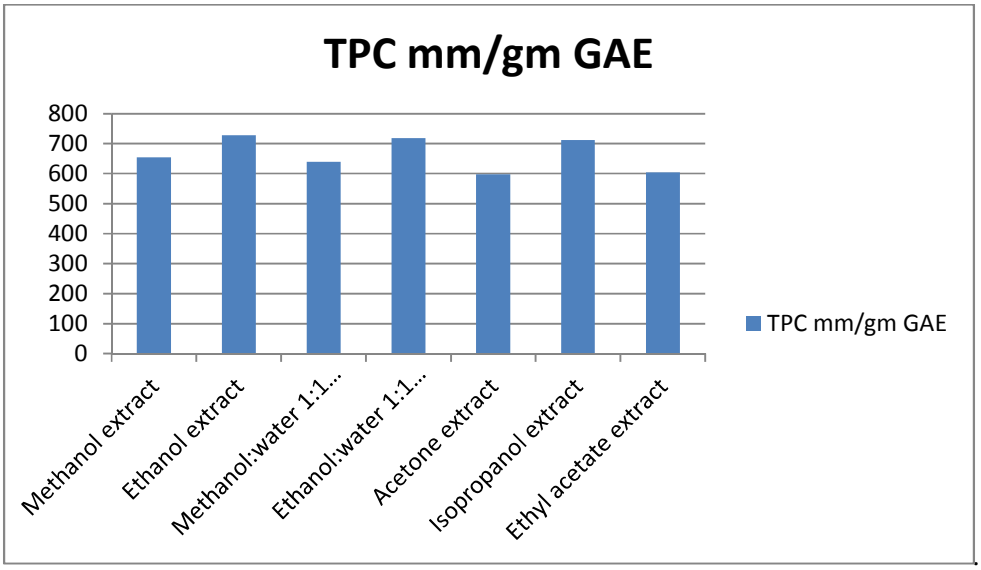

Fig 1. Total phenol content of sunflower meal solvent extract

Table 2 Antioxidant activity of sunflower meal

Sunflower oil cake extract	Antioxidant activity as % inhibition
Methanol extract	3176.92
Ethanol extract	3264.102
Ethanol/water 1:1 extract	3108.97
Methanol/water 1:1 extract	3024.102
Acetone extract	1848.717
Isopropanol extract	3152.864
Ethyl acetate extract	2848.35

Solvent extracts of sunflower cake collected after the extraction was performed for 4 h showed the highest antioxidant activity at 3 h and 3.1/2 h, whereas the extract exhibited the decrease in antioxidant activity as the extraction time increases. From above tabulation it is clear that Ethanolic extract exhibited highest DPPH 3264.1 % inhibition whereas Acetone extract exhibited lowest DPPH 1848.1 % inhibition

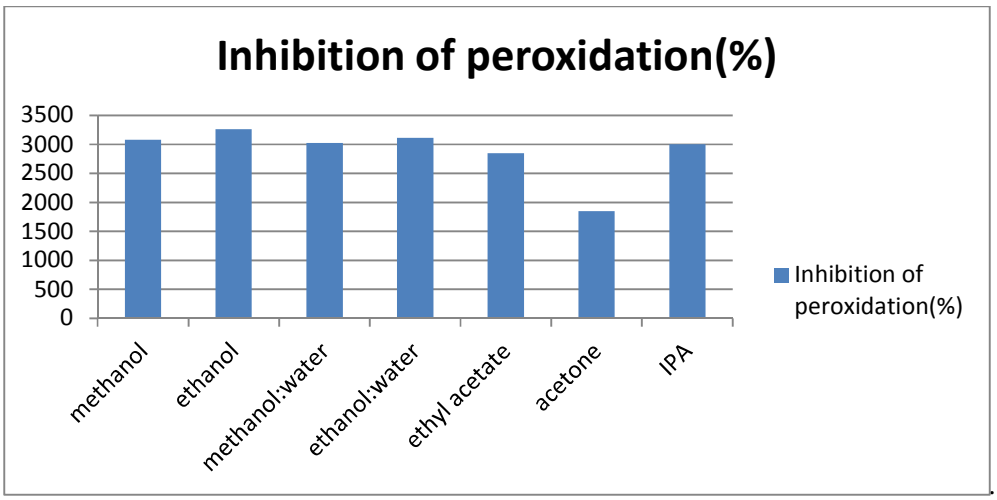

Figure 2 Inhibition of peroxidation of extracts of sunflower meal as measured by the DPPH method

3.3. Rancimat method:
By taking into account the results shown by the aqueous extract, the previously employed trial, and by seeking a better application of this extract concerning the food and cosmetic industry, its antioxidant capacity was tested for a lipidic system. Among the methods available, the Rancimat lipidic system is the one that is more likely to meet the needs of the industrial evaluation of the oil and fat stability. In Table 4, the results of the percentage of increase of the induction period (% IP) are shown for the aqueous extract of the studied sunflower meal and for BHT added to the refined soybean oil, compared to BHA as a standard for the Rancimat method at the temperature of 110˚C. As it may be seen in Table 4, the induction period of the aqueous extract at 0.2% was comparable to that of the BHA standard ant BHT standard with Ethanolic extract and IPA extract.

3.3.1. The comparative study of methanolic sunflower meal extract:
The methanolic sunflower meal extract of 0.2%, 0.4%, 0.6% concentration added to SFO compared with TBHQ + SFO sample is given in table 3. Increased proxidation activity was found with increasing extract concentration, but the concentration leading to maximum antioxidant activity is closely dependent on the extracts and, for the same extract, it is dependent on the antioxidant activity test. Moreover, the phenol antioxidant concentration of 0.2% led to highest inhibition of oxidation (Table 3). The phenol extracts of the sunflower meal acted as antioxidants in a narrow range of concentrations, from 0.2%-0.4%; outside the above range they acted as prooxidants. As a general rule, the antioxidants extracted from plants can show prooxidant activity at high concentration and antioxidant activity over certain critical value.

Table 3 The comparative study of Induction period at 100˚C of SFO without or with the addition of synthetic and natural antioxidant at 0.2%, 0.4%, 0.6% concentration

Sample	Induction period (h)
SFO + TBHQ 0.2%	14.57 h
SFO + Methanolic extract at 0.2%	7.21 h
SFO + Methanolic extract at 0.4%	3 h
SFO + Methanolic extract at 0.6%	40 min

The antioxidant potential decreased according to the following sequence:

BHT>BHA>Ethanolic extract>IPA>Methanolic extract>E: W>M: W>EA>A

The ethanol extract of the sunflower cake increased the induction time of sunflower oil from 1.7 to 6.6 h. BHA and BHT were proven comparable protectors against oil oxidation, with induction times which did not differ significantly, and were to than that of ethanol extract (Table 4). The high antioxidant activity of the ethanol extract can be attributed to its component chlorogenic acid.

Table 4 Induction period at 110˚C of soybean oil without or with the addition of synthetic and natural antioxidant

Sample	Induction period (h)
Blank SBO	1.7
Soybean oil + BHT	6.91
Soybean oil + BHA	6.87
Soybean oil + Methanol sunflower oil cake extract	5.9
Soybean oil + Ethanol sunflower oil cake extract	6.6
Soybean oil + Ethanol/water(1:1) sunflower oil cake extract	6.1
Soybean oil + Methanol/water(1:1) sunflower oil cake extract	5.2
Soybean oil + Acetone sunflower oil cake extract	2.9
Soybean oil + Isopropanol sunflower oil cake extract	6.3
Soybean oil + Ethyl acetate sunflower oil cake extract	3.4

3.4. Stability study of oil by using sunflower cake extract:
The evaluation of the oxidative stability of commercial Sunflower oil and Soybean oil enriched with phenol extracts of sunflower cake, by the determination of peroxide value, is presented in (Table 5) The presence of unsaturated fatty acids together with low concentrations of antioxidants promotes the susceptibility of oils to oxidation. Soy bean and sunflower oil show the highest antioxidant capacity compared to other commercial oils. The increase of heating time resulted to increased peroxide values in all samples .The antioxidant capacity of the phenolic compounds was reduced because of the thermal reduction of phenolic molecules and/or because they were used in the protection of

oil against oxidation. The oxidation rate of soybean and sunflower oil after phenol extract addition, decreased markedly, even after three days.

Table 5 Stability study of sunflower solvent extract in SFO and SBO

samples	Peroxide value (mmoles/kg)					
	24 hr		48hr		72 hr	
Oil	SFO	SBO	SFO	SBO	SFO	SBO
Blank	7.89	9.25	11	13.55	24	26.77
Oil+ BHT	3.56	3.60	3.98	4.55	4.37	5.34
Oil + Methanol extract	3.99	4.45	4.76	4.65	5.11	5.87
Oil + Ethanol extract	3.5	3.41	4	3.68	4.47	4.56
Oil + Methanol: Water extract(1:1)	5.32	5.38	6.13	5.59	7.65	6.43
Oil + Ethanol: Water extract(1:1)	3.87	4.71	5.5	5.21	6.00	6.87
Oil + Isopropanol extract	3.78	3.90	3.99	4.23	4.33	4.77
Oil + Ethylacetate extract	7.45	8.75	8.98	9.21	13.43	12.43
Oil + Acetone extract	7	8.17	8.56	9.02	14	13

Above tabulation it is clear that the sunflower oil cake solvent extracted sample exhibits good antioxidant activity. When shall oven test was performed the blank oil sample readings showed tremendous increase in oxidation. The Ethanol extract exhibited the highest antiradical activity, followed by Methanol extract. On the contrary, the Ethyl acetate and Acetone sunflower cake extract showed the lowest antioxidant activity. The above results are in agreement with the results obtained by the DPPH method (table 2).The study of PV of sunflower meal extract.0.2% concentration added to soybean and sunflower oil with bank oil sample for 6 day observation by shall oven test.

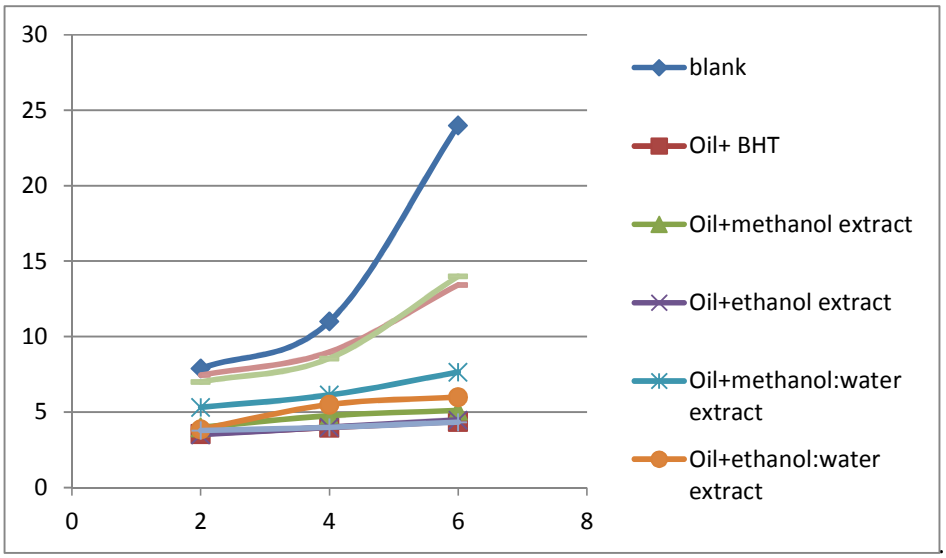

Fig 3 Represents the PV value graph of different solvent extract antioxidant added to SFO oil and its comparison with oil sample without antioxidant

3.5. Identification of phenolic compounds by LC/MS

A complex mixture of phenolic compounds in a wide range of polarities, which was difficult to resolve, was revealed by LC/MS analysis of the solvent extracts from sunflower cake. The LC/API-ES/MS spectrum, in the negative mode, of derivative Ethanolic extract (Fig 5) exhibits a deprotonated molecular ion [M\H] at m/z 515 corresponding to the molecular formula $C_{25}H_{24}O_{12}$, and peaks at m/z 354 which is characteristics of Chlorogenic acid. According to these data, derivative 1 could be a di-O-caffeoylquinic acid (di-CQA), 3, 4-Di-CQA, 3, 5-di-CQA and 4, 5-di-CQA, detected in all the samples in very low concentrations (Fig. 5), shows the LCMS profile.

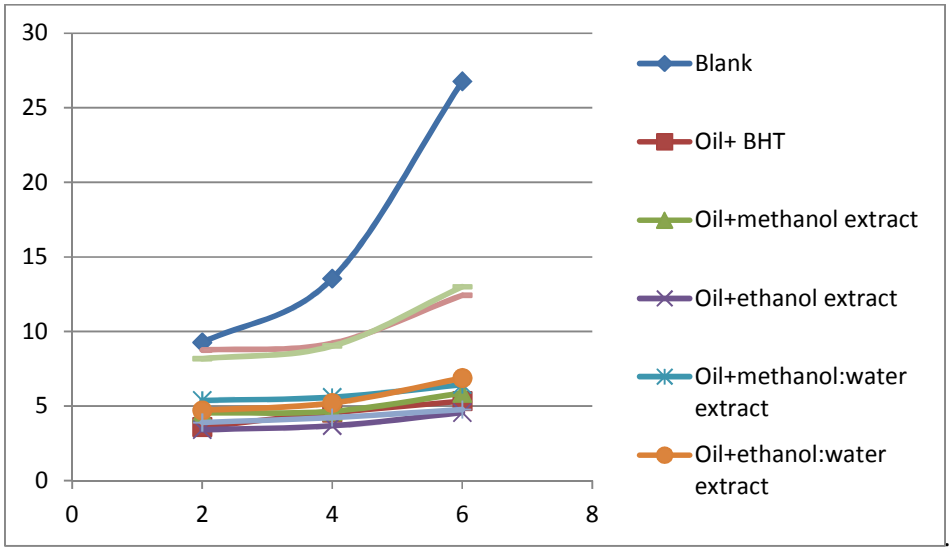

Fig 4 Represents the PV value graph of different solvent extract antioxidant added to SBO oil and its comparison with oil sample without antioxidant

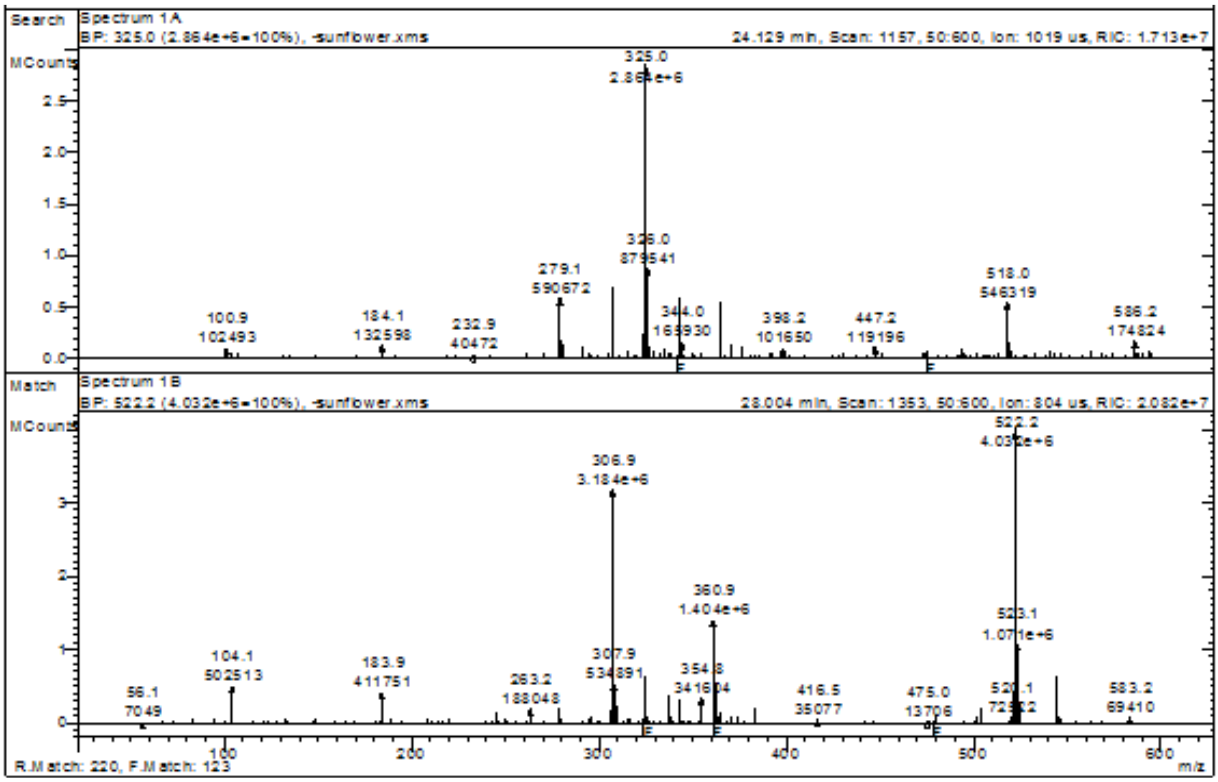

Fig 5 LC/MS profile of the Ethanolic extracts from sunflower cake

CONCLUSION

Extracts with different antioxidant (phenolics) concentrations and activities were obtained from sunflower meal by changing the conventional solvent extraction conditions, namely, time, solvent concentration and type. Ethanol was selected as the most appropriate solvent for the extraction of phenolic compounds from sunflower meal for the production of extracts with high phenol content and high antioxidant activity. The optimum time, solvent to sample

ratio were 180 min, 5:1 v/w, respectively. Solvent extracts exerted good hydrogen donating abilities, revealing their potent antioxidant capacity. Therefore, the sunflower meal is a low-cost, renewable and abundant source of phenolic antioxidants.

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