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Assessment of proximate analysis, phytochemical and antioxidant activity of olive cake (*Olea europaea* L.)

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Abstract
Olive cake (*Olea europaea* L.) is a soild by product of olive oil extraction which have been used for feed application to poultry, fish and swine as well as biomass generators. In the present study, Proximate analysis, qualitative phytochemical screening, antioxidant activities and flavonoid content of olive oil cakes were evaluated. The chemical composition showed that olive cake is rich in crude fiber and carbohydrates and contains moderate amounts of crude protein, fat, ash and energy value. Total Phenolic content of the fruit was determined according to the standard analytical methods recommended by API 2008. Qualitative phytochemical testing of the studied oil cake showed the presence of carbohydrate, glycoside, protein, tannin, phenolic compounds, flavonoids, steroid, terpenoids. However the sample of the given oil cake lacked Alkaloids, starch and saponins. Olive oil cake samples also contain little amounts of antioxidants.

Keywords: olive cake, proximate, phytochemical, antioxidant, flavonoid

Introduction
Oil cakes have been in use for feed applications to poultry, fish and swine industry. Some of these have also been considered ideal for food supplementation owing to their rich protein content. With increasing emphasis on cost reduction of industrial processes and value addition to agro-industrial residues, oil cakes could be ideal source of proteinaceous nutrients and as support matrix for various biotechnological processes. Several oil cakes, in particular edible oil cakes offer potential benefits when utilized as substrate for bioprocesses and these have been utilized for fermentative production of enzymes, antibiotics, mushrooms, etc. Biotechnological applications of oil cakes also include their usages for vitamins and antioxidants production (Ramachandran *et al* 2007) [16].
Olive cake (*Olea europaea* L.) is a solid by-product of olive oil extraction composed of a mixture of skins, pulp, woody endocarps, seeds and which represents 35% of the weight of olives pressed (Dal Bosco *et al*., 2007) [8] is available locally in large quantities. The amounts used as a fuel are low and olive cake is usually deposited around the oil mills or thrown into rivers. According to Rupić *et al*. (1999), due to the long period required for its degradation, olive cakes present an environmental pollution source. Olive cake can be used in animal feed (Heuzé *et al*., 2014) [12]. However, it contains high amounts of crude fibre (220 to 350g / kg), several studies have reported the use of olive cake in animal's diet. Furthermore, the olive cake is a potentially useful source of indigestible fibre (Carraro *et al*., 2005) [5]. Besides its use as animal fodder Olive oil-cake (OC), which is a waste of olive oil-mill, also serves as an important biomass generator in Mediterranean countries. OC is available in large amounts at a very low cost. Vegetable oils and OC can be considered as an alternative source of fuel, which do not contain sulfur. The cake still has a commercial value owing to the oil it contains. Up to 40 kg of cake is obtained from 100 kg of olives. Oil cakes provides a clean, renewable source of energy that could dramatically improve the environment, economy and energy security (Hani *et al* 2016) [11].
There are 3 different types of olive cake according to the oil extraction method used: crude olive cake obtained from traditional oil mills which use hydraulic press system and the woven mats, the exhausted olive oil cake obtained after extracting oil from the crude olive cake, and the residue derived from modern mills using a continuous string extraction process (Chaabane *et al*., 1997) [6]. There is very little data on the nutritional value of olive cake and application as feed for animals. The aim of this study is to determine the nutritive value of sun dried crude olive cake that can be used as fodder.

Methodology
Collection of olive cake
Olive (*Olea europaea L*.) oil cakes were obtained from the ROCL, Lunkaransar, Rajasthan that uses cold-pressed technology based on screw presses for the production of oil from seeds. The oil cakes used in all experiments were from the same batch of oil production. Olive cake was brought to the laboratory and sun-dried to reduce to constant moisture level for 3 days. It was finely ground and sieved through a mesh (1 mm).
Ground olive cake was treated with 6% lime with little modification as per Ashraf (2011) [2]. Briefly slaked lime solution was prepared by dissolving 6 g of slaked lime in 20 ml distilled water and was sprinkled over 100 g of olive cake spread out on a polythene sheet. The samples were dried in sunlight for 4 h and packed in sealed polythene bags for a week's incubation in the laboratory.

Nutritional Analysis

Proximate analysis

Proximate analysis was carried out according to the procedure of FSSAI Lab Manual- Fruit and Vegetables, (FSSAI 2016) to determine the moisture content, crude fiber and ash content, protein, crude fat, estimation was done at CEG Test House and Research Centre Pvt. Ltd. (CEGTH), Jaipur, Rajasthan, India. carbohydrate and energy was determined as per BIS specification (BIS 2007) [4].

Estimation of Total Phenolics.

Total Phenolic content of the fruit was determined according to the standard analytical methods recommended by (API 2008) [3].

Prepare a stock solution (1 mg/ml) of the extract in methanol. From the stock solution, take suitable quantity of the extract into 25-ml volumetric flask and add 10 ml of water and 1.5 ml of Folin Ciocalteau reagent. Keep the mixture for 5 min, and then add 4 ml of 20 per cent sodium carbonate solution and make up to 25 ml with double distilled water. Keep the mixture for 30 min and record absorbance at 765 nm. Calculate percentage of total phenolics from calibration curve of gallic acid prepared by using the above procedure and express total phenolics as percentage of gallic acid.

Qualitative Phytochemical Screening.

The qualitative phytochemical analysis gives information on the presence or absence of different classes of secondary metabolites. The sample under investigation were subjected to a range of phytochemical tests (Table 3) for the detection of the following compounds: alkaloids, tannins, saponins, flavonoids, glycosides, terpenoids, steroids, carbohydrates, reducing sugars proteins etc) with minor modifications. Qualitative tests for various phytochemicals present in the methanolic extracts of *olive fruit* were carried out using standard phytochemical screening procedures given by (CCRAS, 2010). Visual examination of the appearance of colour or frothing was used as an indicator for the presence or absence of a given phytochemical group.

Antioxidant activity DPPH Assay and total flavonoids

Extraction

The oilcake extract was pulverized and about 100 gms of powdered oilcake were extracted with increasing order of polarity solvents series starting from Pet. ether, Chloroform, Ethanol via soxhlet apparatus by successive hot continuous percolation method. At last, the extract was concentrated in a rotary flash evaporator and the residue were dried in a desiccator over Sodium sulphite. After this, practical yield was weighed and calculated as 2.5 gm, 1.5 gm and 3 gm for pet. ether, chloroform and ethanol extracts respectively as performed by (Shekhar and Anju 2014) [18].

Evaluation of antioxidant activity by DPPH radical scavenging method

Free radical scavenging activity of olive oilcake extract was measured by 1, 1- diphenyl-2-picryl hydrazyl (DPPH). In brief, 0.1 mM solution of DPPH in ethanol was prepared. This solution (1 ml) was added to 3 ml. of different extracts in ethanol at different concentration (5, 10, 15, 20, 25, 30 µg/ml). Here, only those extracts are used which are solubilised in ethanol and their various concentrations were prepared by dilution method. The mixture was shaken vigorously and allowed to stand at room temp for 30 min. then, absorbance was measured at 517 nm. by using spectrophotometer (UV-VIS Shimadzu). Reference standard compound being used was ascorbic acid and experiment was done in triplicate. The IC 50 value of the sample, which is the concentration of sample required to inhibit 50% of the DPPH free radical, was calculated using Log dose inhibition curve. Lower absorbance of the reaction mixture indicated higher free radical activity. The percent DPPH scavenging effect was calculated by using following equation: DPPH scavenging effect (%) or Percent inhibition = A0 - A 1 / A0 × 100.

Where A0 was the Absorbance of control reaction and A1 was the Absorbance in presence of test or standard sample.

Total Flavonoids

The effects of (1) the herbal material: Solvent ratio (0.02, 0.03, 0.05, 0.07, and 0.08 g/mL), (2) stock solution volume (0.8, 2.3, 4.4, 6.5, and 8.0 mL) and (3) $AlCl_3$ volume (0.8, 1.0, 1.2, 1.4, and 1.6 mL) on the TFC were evaluated (Silva, *et al* 2015).

Result and Discussion

Analysis of Olive oil cake: Oil cake were collected and physiochemically, phytochemically analyzed also antioxidant activity was found following table depicts the detailed analysis of olive oil cake. Seeing its nutritional values it can be used for fodder and therapeutic purpose.

Proximate analysis

Table 1: Physico-chemical parameters

Sl No	Test Parameters	Method of Test	Test Results	Unit
1	Moisture	FSSAI lab manual- Fruits and Vegetables, 2016	7.50	%
2	Fat	CEGTH/STP/C/203	1.08	%
3	Protein	FSSAI lab manual- Fruits and Vegetables, 2016	9.40	%
4	Crude Fibre	FSSAI lab manual- Fruits and Vegetables, 2016	53.59	%
5	Ash Content	FSSAI lab manual- Fruits and Vegetables, 2016	4.04	%
6	Carbohydrate	IS 1656-2007, RA 2012	77.98	g/100g
7	Energy	IS 14433-2007, RA 2012	359.24	Kcal/100g
8	Total Phenolics	API, Part-I, volume-VI, 2008	42.96	Mg/100g

Proximate analysis was carried out on the olive-oil cake and elemental composition of bio-oils Table 1. Moisture content was estimated as 7.50% which was higher than that reported by (Hani *et al* 2016) [11] where the moisture content was 6.8%.

The olive cake fats may constitute an important contribution of energy in animal feeds.they are rich in C16 and C18 unsaturated fatty acids (approximately 95%). Fat content of

the olive cake was 1.08 percent much lower than that obtained by (Ouaini *et al* 2010) which ranged from 7% to 17.5%for olive cakes obtained from various types of mills.

Olive oil cakes are relatively low in nitrogenous matter (3-5%) whatever the mill type they may be derived from. However, some differences in the result may come from the type of olive fruit. In the present study, the olive oilcake contained 9.40% protein. The protein content of olive oil cake considerably differs from the values (17.40-25.2%) reported by (Ramachandran *et al* 2007; Moftah *et al* 2011) [16, 13]

The content of protein in the present study was within the range found in literature (Contreras *et al* 2020) [7] which varied from 0.3 to 10.6 percent.

The crude fibre content of the oilcake was 53.59 percent which was fairly high as compared to the results obtained from studies by (Ramachandran *et al* 2007; Moftah *et al* 2011) [16, 13] which ranged from 40 to 60.1 percent.

Ash, which increases the nutritional values of food contains the total cations of carbonates and other mineral salts. Ash content was 4.04% which was almost equal with the result obtained by (Hani *et al* 2016) [11] at 4.4%.

The carbohydrates can influence microbial growth rate and hence, indirectly, modulate the activity of enzymes with growth rate-related activities. They can also influence enzyme activities through induction and catabolite repression (Williams *et al* 1989) [22]. Carbohydrate content of the olive oilcake was found to be much higher (77.98 percent) than those obtained by (Ramachandran *et al* 2007 [16]; Moftah *et al* 2011) [13] which ranged from 10.1 to 20.6 percent.

Energy/ Calorific value of the oil cake was obtained at 359.24 Kcal/100g. Similar study on calorific value estimation of crude olive oil cake by (Hani *et al* 2016) [11] showed 20.7 MJ/kg which translates to 494.11 Kcal/100g. Table 2 provides a critical review of the results of different oilcakes on their proximate analysis as summarised by Ramachandran *et al*. (2007) [16], Sivaramakrishnan and Gangadharan (2009) [19].

Result showed that the total phenolic content was 42.96 mg/100g. Total phenolic content found by Mohammad *et al* 2017, where the Total phenolics were determined by the folin–ciocalteuic method was much higher than the values found by investigated sample.

Table 2: A critical review on proximate analysis of edible and non-edible oil cakes as summarized by Ramachandran *et al*. (2007) [16], Sivarama krishnan and Gangadharan (2009) [19].

Oil cakes	Dry matter (%)	Protein (%)	Fibre (%)	Carbohydrate (%)	Fat (%)	Ash (%)
Soybean cakes	84.8–90.3	47.5–51.8	5.1–17.8	23 ± 0.6	0.8 ± 0.1	6.4–7.3
Rapeseed cakes	89.8–90.7	38.5–42.8	3.5–12.1	32 ± 0.2	4 ± 0.1	7–9.9
Cottonseed cakes	91.5–94.3	40.3–41.5	14.7–15.7	26.5 ± 0.5	5 ± 0.8	6.5–6.8
Groundnut cakes	90–92.6	45.6–49.5	5.3–8.3	14 ± 0.1	2 ± 0.5	4.5–5
Sunflower seed cakes	91–93	34.1–35.6	13.2–28.4	22.5 ± 0.5	1.5 ± 0.3	6.6–7.4
Palm kernel cakes	90.8–93	17.5–18.6	11.9–37	45 ± 0.5	7 ± 0.4	4.5–4.8
Copra cakes	88.8–89.9	20.9–25.2	10.8–11.5	42 ± 0.4	7.5 ± 0.5	5.5–6

Table 3: Qualitative Phytochemical composition of olive oil cake

Sl No	Test Parameters	Method of Test	Test Results
1.	Carbohydrate Test (Fehling solution)	Guide for ASU drugs, CCRAS, 2010	Present
2.	Glycoside (Borntrager's Test)	Guide for ASU drugs, CCRAS, 2010	Present
3.	Protein Test (Biuret Test)	Guide for ASU drugs, CCRAS, 2010	Present
4.	Tannin (Ferric chloride Test)	Guide for ASU drugs, CCRAS, 2010	Present
5.	Phenolic Compound Test	Guide for ASU drugs, CCRAS, 2010	Present
6.	Alkaloids Test (Mayer Reagent)	Guide for ASU drugs, CCRAS, 2010	Absent
7.	Starch (Iodine Test)	Guide for ASU drugs, CCRAS, 2010	Absent
8.	Flavonoid (Shinoda Test)	Guide for ASU drugs, CCRAS, 2010	Present
9.	Steroid (Salkowaski Test)	Guide for ASU drugs, CCRAS, 2010	Present
10.	Terpenoids Test	Guide for ASU drugs, CCRAS, 2010	Present
11.	Saponins (Foam Test)	Guide for ASU drugs, CCRAS, 2010	Absent

Qualitative Phytochemical composition of olive oil cake

Qualitative phytochemical testing of the studied oil cake showed the presence of carbohydrate, glycoside, protein, tannin, phenolic compounds, flavonoids, steroid, Terpenoids. However the sample of the given oil cake lacked Alkaloids, starch and saponins. Olive oil cake samples also contain little amounts of antioxidants (Table 3).

Anti-nutritional factors are substates of natural or synthetic origin, found in the human diet or animal feed, which can affect the health, and growth performance of livestock. These factors can have different effects on animals depending on their digestive process, for example, trypsin inhibitors have a negative effect on monogastric animals, but not in ruminants because it is degraded. The direct utilization of oilseed cakes in human or animal nutrition is limited by the presence of antinutrients, which influences the organoleptic properties, protein digestibility, and macro-/micro-elements bioavailability. In the gastrointestinal tract, during digestion cyanogenic glycosides form hydrogen cyanide, causing below 50 mg/Kg acute toxicity in adults.. The majority of oilcakes (rapeseed/canola, sesame, sunflower, soybean, groundnut, hempseed) contain antinutrients such as phytic acid and trypsin inhibitors, which can diminish protein and vitamin utilization. More precisely the second one (trypsin inhibitors) connects to the digestive enzyme trypsin, thus reducing proteins adsorption and digestion. Saponins are a group of steroidal glycosides (natural foam producers) that cause hemolysis and interference in bile acids, lipid-soluble vitamins, cholesterol,

and dietary lipids. Phytic acid can bind with minerals, proteins and amino acids forming phytates and insoluble complexes, thus reducing essential nutrient bioavailability, and digestibility. They can also lower the minerals availability and amylase activity (Ancuta and Sonia 2020).

Table 4: Antioxidant activity (DPPH Assay)

Sl. No	Antioxidant Activity (DPPH Assay)	Method of Testing	Test results	Unit
1	Free radical Scavenging Assay (IC_{50})- For Methanolic Extract	American Journal of Ethnomedicene, 2012, vol.1, No.4, 244-249	0.54	Mg/ml
2	Free radical Scavenging Assay (IC_{50})- For Std. Gallic Acid		0.0021	Mg/ml
3	Total Flavonoids (Equivalent to Quercrtin content)	Pharmacogn Mag. 2015 Jan – Mar; 11 (41:96-101)	84.32	Mg/100g

Antioxidant activity.

Table 4 indicates the Antioxidant activity and total flavonoid content of the olive fruit where, the methanol extract of olive fruit showed better antioxidant potential when compare to standard ascorbic acid by DPPH scavenging assay method. The absorbance at 517 nm by UV visible spectrophotometer were found to be as 0.54 and 0.0989 for standard ascorbic acid and the Free radical scavenging assay (IC_{50}) for Std. Gallic Acid obtained was as 0.0021 mg/ml. Flavonoids are one of the major groups of phenolic compounds present in olive fruits and in their by-products (Vlahov, 1992) [21]. Although only about 2% of the total phenols found in olive fruits are transferred to the extracted olive oil, remaining 98% are retained in the olive cake (Suarez *et al*., 2010).The total flavonoids (Equivalent to Quercitin content of the present study was estimated at 84.32 mg/100g.

Conclusion

Olea europaea fruit and oil consumption has been recognized as a key factor supporting the beneficial effects of the "Mediterranean diet" owing to the well-balanced oil composition (highly enriched in monounsaturated fatty acid) and rich minor components (such as polyphenols and phytosterols) in the fruits. The byproducts of olive oil production can be utilized for feed applications to poultry, fish and swine industry. Besides its use as animal fodder Olive oil-cake (OC), which is a waste of olive oil-mill, also serves as an important biomass generator in Mediterranean countries. From the obtained results, it was concluded that the olive (*Olea europaea* L) oil cake have appreciable antioxidant capacity and antioxidant-associated phytochemicals. The nutritional content of the oil cake makes it unique among oil by products and can be successfully recommended for the purposes of animal feeding and fertilizer. Also by using olive cake in different industrial sectors the environmental impact of olive cake (environmental pollution) can be reduced.

Conflicts of interest

The authors declare that there are no conflicts of interest regarding the publication of this paper.

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