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PHYSIOCHEMICAL CHARACTERISTICS AND FATTY ACID COMPOSITION OF *AMYGDALUS SPINOSISSIMA* SEED OIL FROM BALOCHISTAN

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Abstract:

Amygdalus spinosissima also known as Prunus spinosissma belongs to the genus Prunus, its subgenus is Amygdalus. A. spinosissima is a wild thorny almond found in approximately 600 to 1500 metres above sea level. Due to its medicinal value it is used as traditional medicine all over the world. The objective of present work was to evaluate the physiochemical characteristics, total phenolic content and fatty acid composition of crude oil extracted from A. spinosissima. In this study physicochemical properties and free fatty acid value of seed oil were studied to evaluate the compositional quality of oil. The phenolic content of oil extract was found 15mg/L Gallic acid. Fatty acid composition has shown that it has high monounsaturated fatty acid (MUFA) oleic acid, C18:1 (61.8%) followed by palmitoleic acid, C16:1 (0.36%). The content of polyunsaturated fatty acid (PUFA) linoleic acid C18:2 was (23.32%) and saturated fatty acids, palmitic acid C16:0 was (8.19%), stearic acid, C18:0(2.07%), erucic acid, C22:0(0.21%) and myristic acid C14:0 (0.20%) were respectively. The results of the present study demonstrated that the seed oil of A. spinosissima is rich in oleic acid which may be a good potential source for industrial applications.

Key words: *Amygdalus spinosissima, seed oil, physiochemical, phenolic compounds, GC-FID fatty acid.*

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INTRODUCTION:

In the family *Rosaceae* genus *Amygdalus* L. belongs to one amongst the most important genera. The family contains 29 genera having 243 species; out of this 58 are endemic taxonomy. Iran is the core centre of diversity for *Amygdalus*, species of this genus are found from the southern to northern slopes in the south of Iran. In addition to that other species of *Amygdalus* are also found in neighboring countries like Afghanistan, Pakistan, Tajikistan, Uzbekistan, Turkmenistan and Turkey [1, 2].

Amygdalus spinosissima belongs to the genus *Prunus* and sub-genus *Amygdalus*. *A. spinosissima* is a wild thorny almond found in approximately 600 to 1500 metre altitude on the sloppy hill of cold and dry valleys of Balochistan. It shares the similar terrain as in Iran and central Asia [3].The height of the plant is generally around 3-4 metres. The flowers of the herbs annual or perennial are light to dark pink with rounded petals, the leaves are somewhat curved while stem is grayish white having long spines. It produces a drupe-type fruit, initially producing a green pericarp becomes shiny yellow in color having a pink bluish coat due to a mild pubescence.

The flowering pattern, ripening date, kernel bitterness and self-incompatibility were evaluated in 18 wild almond species. The fruit size is generally small, has bitter taste, while the flavor are the most common obstacle for the consumption of this wild germplasm for cultivation purpose. Average leaf magnitude, it variances amongst and inside species are dependent on rainfall in the particular region [4].

Plant seeds are valuable source of vegetable oil having nutritional, industrial, pharmaceutical importance and also have relatively high oxidative stability [5]. The medicinal value of *A. spinosissima* with respect to human nutrition has been studied and reported that it has a valuable source of vegetable oil having a relative high oxidative stability that is good for human health. The seed oil has effective healing properties and is used to treat wounds [6].

The particular purpose and adequacy of oil is determined by its characteristics and composition of fatty acids. Due to different fatty acid composition any oil extracted from a single source has not been found to be acceptable for all purposes [7]. Therefore, in the present study physiochemical characteristics, total phenol content (TPC) and fatty acid composition of the seed oil of *A. spinosissisma* were investigated in order its potential use in pharmaceutical and industrial sector.

MATERIAL AND METHODS:

Chemicals

All the chemicals used in this present study such as n-hexane, ethyl acetate, hydrochloric acid , sodium hydroxide, potassium hydroxide, idobromine, sodium thiosulphate, potassium iodide (KI), acetic acid ,chloroform, butanol and methanol were analytical grade and purchased from Merck, Pakistan.

Collection of Plant materials

The seeds of *A. spinosissima* were collected in the month of August 2016 from Sanjavee, Zairat and Shahban of district Zairat, Balochistan. The samples were transported to a bio-analytical laboratory, Institute of Bio-chemistry University of Balochistan, Quetta and kept at room temperature and thoroughly cleaned and dried.

Sample preparation

The seeds were taken from their shell and were crushed into powdered in a grinder. The powder sample (25 g) was extracted with n-hexane (350 ml) by using Soxhlet apparatus for 16 hours. After extraction, the hexane solvent was evaporated at 60˚C by using rotary evaporator (EYELA N-1110S-W Rikakikai Tokyo Japan). The oil obtained was stored in dark brown bottle and kept at 4˚C till further analysis.

Physiochemical characteristics of Oil

Determinations of boiling point, density, viscosity, saponification value, iodine value (IV), peroxide value (PV) and free fatty acid value of the extracted oil were carried out by AOCS (1993) official method.

Boiling point measurement

The boiling point of oil was measured by a thermometer ±1 ˚C. The boiling point depends upon the degree of unsaturation of fatty acids.

Density

Density of oil sample was measured by an R.D bottle with a capacity of 10 ml.

Viscosity measurement

The viscosity of oil sample was measured by an Ostwald Viscometer. The flow time of oil samples were recorded with a stop watch least count ± 0.01 s.

Saponification value measurement

1.0 g of oil was added into the conical flask. After that 15 ml 1.0 N KOH and 10 ml of distilled water

was added. The resulting mixture was heated under a reserved condenser for about 35–45 minutes. The solution was allowed to cool than 2-3 drops of phenolphthalein indicator was added and titrated with 0.5 mol dm^{-3} HCl to the end point pink. A blank was determined with the similar conditions.

Iodine value (IV) measurement

A known mass of oil was treated with iodobromine (excess) in glacial acetic acid. The remaining iodobromine reacts with potassium iodide to produce iodine. The iodine concentration was then calculated by titrating with standard sodium thiosulphate.

Iodine value = (b-v) x N x 126.9 x 100/w x 1000

Where 'N' is the normality of thiosulphate solution, 126.9 relative molecular mass of iodine, 'w' is the wt. of the oil sample. Whereas 'b' is the amount of sodium thiosulphate used for blank, 'v' is the amount of thiosulphate for sample [8].

Peroxide value (PV) Measurement

Peroxide value is measured by the iodine released from potassium iodide. A known mass of oil was dissolved in acetic acid followed by chloroform. To the sample saturated potassium iodide mixture was added and iodine liberated from potassium iodide by the oxidation of peroxides in the oil was determined by titrating with the standard sodium thiosulphate. The starch was used as an indicator. Titration was also performed for blanks.

Peroxide value (meq/kg oil) =(s-b) x W x N

Where 'N' is the normality of standard sodium thiosulphate, 'W' is the mass of sample, 'b' is blank volume of sodium thiosulphate, 's' is the titre volume of sodium thiosulphate for the oil sample [9].

Free fatty acid

About 27.0 g of oil sample was weighed and added into conical flask. The sample was mixed and dissolved completely in 50 ml of 99% isopropanol. The mixture was titrated with 0.1N sodium hydroxide solution with phenolphthalein as an indicator. At the end pink colour appears by a drop of sodium hydroxide and colour remains same for at least 30 seconds [10].

Evaluation of Total Phenol Contents (TPC)

Total phenolic content in seed oil was determined colorimetrically by the Folin-Ciocalteu method with slight modification [11]. The absorbance of the samples was detected at 725 nm using schizuma UV–Visible spectrophotometer. The content of total phenolics in the extract was determined using gallic acid as a standard at concentrations of 0-150mg /L.

Fatty acid analysis of oil

100 mg of oil sample was weighed and placed into universal bottle, 250 µl of sodium methoxide and 5ml of hexane was added. Mixture was vortexed for one minute in order to crumple the oil mixture. Subsequently, 5 ml of saturated NaCl was added into the bottle, caped and shaken. The bottle was incubated for ten minutes at room temperature to separate two layers. The upper layer containing hexane was separated and added into a vial containing Na_2SO_3. 0.5 µl of fatty acid methyl esters (FAMEs) was injected into GC (Agilent 7890B GC equipped with flame ionization detector (FID) in order to obtain standard and individual peaks of FAMEs. The identification and separation of fatty acids from oil was executed through GC by AOCS official method [12].

RESULTS AND DISCUSION:

Physiochemical Characteristics

The quality of oil was investigated by determining the physicochemical properties such as boiling point, density, viscosity, saponification value, iodine, peroxide, and free fatty acid values. Results are presented in Table 1. Low levels of density and viscosity of oils are very significant to the consumers. The nature and arrangement of fatty acid on the glycerol backbone changes the viscosity value of the oil. Hence, viscosity is linked with the chemical properties of the oils such saturation: unsaturation ratio and chain length. The density and viscosity decreases with the rise in unsaturation and increases with saturation and polymerization [13]. Temperature and sheer stress also affects the viscosity. The edible oils are affected by temperature while sheer stress does not affect the storage capability of oil. As the temperature increases the average kinetic energy increases the molecules move apart and the intermolecular forces decreases. Due to reduction in the intermolecular forces the layers in the oil can easily slide over one another, reducing the viscosity of oil. Not only viscosity, the density of oil also decreases with rise in temperature. The standard range of density as approved by Standard Organization of Nigeria (SON) is 0.898–0.907 g/ mL [14]. However, in this study the density of *A. spinosismia* oil was found 0.921 g/mL which is slightly higher Table 1.

Oil qualities can be deteriorated during frying as result oxidation of lipid or thermo-oxidative and hydrolytic reactions occurs in oil [15-17]. The major deteriorating process during lipid oxidation leads to the off-flavors as a result of autoxidation. Primary oxidation in triacylglycerol also leads to

hydroperoxides formation, which may breakdown to produce numerous molecules, such as free fatty acids, aldehydes, ketones and alcohols, finally causing rancidity [18, 19].

During storage process of oil, peroxide value (PV) is an important parameter to check the stability and quality of fats and oils. The peroxide value increases with rise in temperature, storage time and contact with air of the oil sample. In the present study peroxide value obtained was 2.7meq/kg, which is in agreement with the standard value as given by Standard Organization of Nigeria (SON) and Nigerian Industrial Standard (NIS) [14, 20, 21].

In the oil sample saponification value (SV) is use to determine the average molecular mass of fatty acid. The SV value obtained for the oil samples was in 180.9 mg KOH/g (Table 1). The result of the study revealed that values are not in agreement with the values set by SON and NIS for the edible oil [14, 21]. The low saponification value suggests that the average molecular weight of fatty acids is low or contains less number of ester bonds, suggesting that the fat molecules did not interact with each other and may not be suitable for soap making [22].

Iodine value (IV) measures the extent of unsaturation in oil. It was observed that measured iodine value for *A. spinosissmia* seed oil is 90.3 g, which is less than 100g which ranked the oil in non-drying groups as the drying oil have high iodine value more than 100g. Therefore, the oil may be used as a raw material in the industrial sector [23].

Free fatty acid value (FFA) can be determined by the amount of alkakli required to neutralize the fatty acid in the oil. The conversion of glycerol to fatty acid is referred as hydrolytic rancidity [24]. The free fatty acid value obtained during the study was 0.27 which is not in agreement with the other reported seed oil values as *C.albidum*3.0, *D.edulis* 2.78, *Eguinensis* 7.06, *L.owaronsis* 7.70 and *N. imperialis* 2.60 [25].

TPC

The result of total phenol content of oil extracted from the seeds of *A. spinosissima* was 11.2-22.2 mg/L gallic acid.

The standard curve was constructed using standard solution of gallic acid within the range of 0-150 mg/L gallic acid as shown in figure1.

Table 1: Physiochemical properties of A. spinosissmia seed oil

Name	*Value*
Boiling point (˚C)	180.0 ± 0.1
Density g/mL	0.921 ± 0.01
Viscousity (millipoise)	72.36 ± 0.01
Saponification Value (mg)	180.9 ± 0.1
Iodine value (g)	90.3 ± 0.1
Peroxidise (meq/Kg)	2.70 ± 0.01
Free fatty Acid (FFA)	0.27 ±0.01

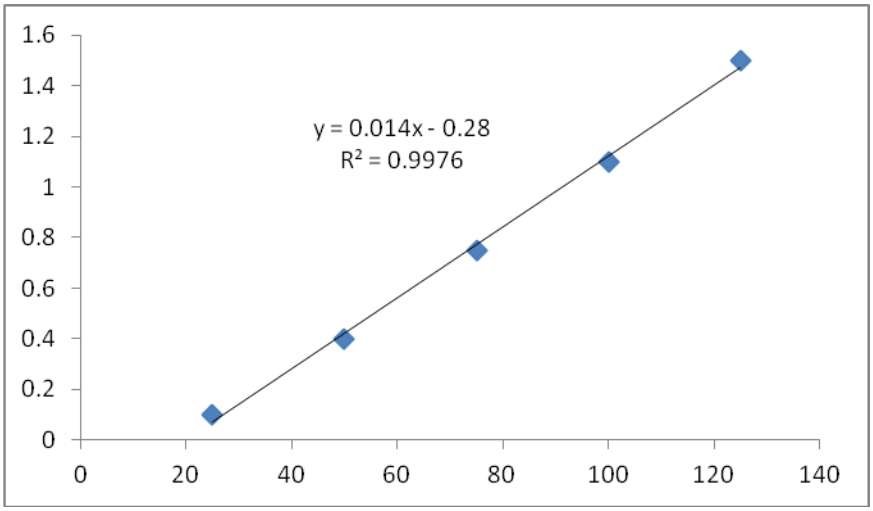

Fig 1: Standard curve for Total phenol content (TPC)

Fatty acid composition

The results of the study revealed with presence of 7 different types of fatty acids in the *A. spinosissima* seed oil. Gas chromatography (GC) analysis showed high oleic acid content having a percentage of 61.8% followed by linoleic acid (23.32%), palmitic acid (8.19%), stearic acid (2.07%), palmitoleic acid (0.36%), erucic Acid (0.21%) and myristic acid. The results of fatty acid compositions are shown in Figure- 2.

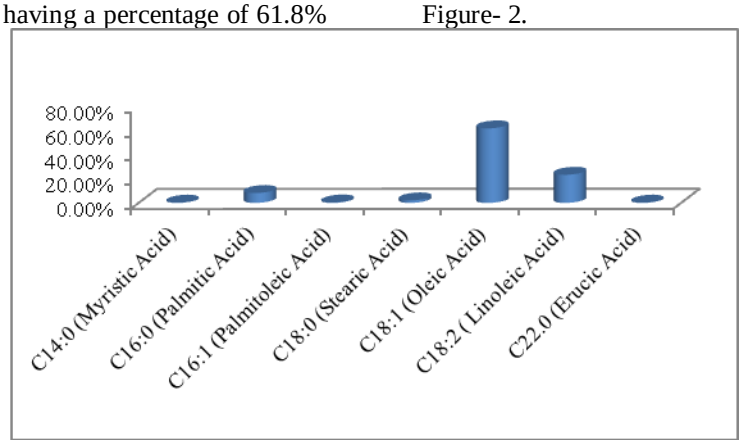

Fig.2: Fatty acid composition A. spinosissima seed oil

Table 2: Fatty acid composition, retention time, height, area and % area

Peak #	Name	RT [min]	Height	Area	Area %
1	C14:0 (Myristic Acid)	24.882	0.39	1.27	0.202
2	C16:0 (Palmitic Acid)	27.957	16.21	51.59	8.187
3	C16:1 (Palmitoleic Acid)	28.757	0.82	2.26	0.359
4	C18:0 (Stearic Acid)	30.704	4.39	13.05	2.071
5	C18:1 (Oleic Acid)	31.57	112.69	389.44	61.799
6	C18:2 (Linoleic Acid)	32.380	47.79	146.95	23.320
7	C22.0 (Erucic Acid)	36.795	0.58	1.32	0.210

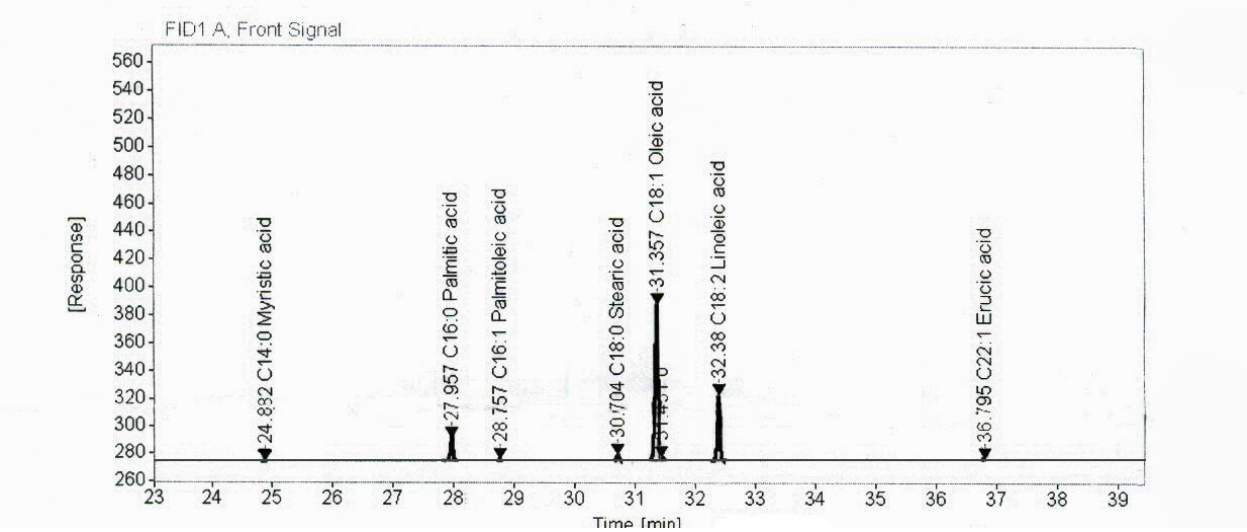

Fig.3: GC spectrum of fatty acid composition

Fatty acid composition of the oil extracted from *A. spinosissima* showed that it contain high percentage of monounsaturated fatty acid (MUFA) oleic acid (62.8%) followed by palmitoleic acid (0.36%).The polyunsaturated fatty acid (PUFA), linoleic acid was found (23.32%) while the saturated fatty acid (SFA) palmitic acid was (8.19%) stearic acid (2.07%), erucic acid (0.21%) and myristic acid (0.20%) were respectively. The stability of oil depends upon the ratio between MUFA to PUFA. In the present study, the ratio of MUFA to PUFA (2.67) is not in agreement with the other studies, which is due to richness of linoleic acid present in the seed oil [26]. Similar studies shows that oleic acid content of

cultivated almond species contains 69.2% - 69.9% while the oleic acid in wild species were ranges from 60.9% to 62.9% respectively which is in agreement with our reported value of 61.8% [27-30].

MUFA contents in the seed oil of *A. spinosissima* in the current sudy was 62.16% while saturated fatty acid (SFA) value *A. spinosissimais was* 8.5%. The monounsaturated fatty acid (MUFA) reported value were much higher than of soybean 21.3%, sunflower 20.5%, corn 29.9%, palm 37.1% and safflower 12.0% oil [31]. Therefore, due to the higher MUFA to PUFA ratio, the oil of *A. spinosissima* expected to be more stable than prevalent cooking oils with similar saturation levels.

Despite the fact that saturated fatty acid containing oils are more stable but have adverse affects on human health. Therefore, in the present study the oil of *A. spinosissima* contain much lower saturated fatty acid level which needs to be investigated further.

CONCLUSION:

The chemical and physical characterization of the *A. spinossima* seed oil is comparable with some other vegetable oils. On the basis of physiochemical properties, the seed oil may not be considered as potential sources of vegetable oil. Therefore, cannot be used as potential source in soap making industry. Further, extensive studies are required to find out more possible anti-nutritional components.

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