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Assessment of Physicochemical Properties and Mineral Compositions of Almond (*Prunus amygdalus*) Gum of Libyan Origin

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ABSTRACT

Twenty-five authentic samples of *Prunus amygdalus* gum samples were collected from five different locations in northwest of Libya. Different physicochemical methods were used to characterize this type of gum samples. The mean values of the moisture ranged from 12.55 to 14.93%, ash mean values 4.03 to 4.62%, pH 3.89 to 4.40, W.H.C 52.85 to 60.0%, and O.H.C 136.2 to 156.3%. Solubility of *Prunus amygdalus* gum showed that it had low solubility in distilled water with the mean values ranged from 20.01 to 35.40%, but it dissolved perfectly in alkali media where solubility increased to 96.3% in 0.05 M Na_2CO_3. The study exhibited that the mean values of cationic concentration in %(w/w) such as calcium was ranged from 0.552 to 0.585, potassium 0.402 to 0.505, iron 0.0975 to 0.1125, and sodium 0.064 to 0.079, which indicates that Ca has the highest concentration of metallic ions present. Atomic absorption method indicate that all samples contain traces of elements, Li, Cr, Mn, Ni, Zn, Pb, Hg, and Cd. The main functional groups recorded from FTIR spectrum included bands at 3293 cm^{-1} (O-H in carboxylic group), 1601.77 cm^{-1} (C=O, amide N-H bend, and C=C), 1357.53 cm^{-1} (CH_3-, and C-O-H in- plane bending vibration), and 1025.74 cm^{-1} (C-O stretching).

1. Introduction

Almond trees are Mediterranean trees where they grow in Syria, Lebanon, Turkey, Palestine, Jordan, Tunisia, Algeria, Morocco and Libya [1,2]. It also grows in other countries such as the United States of America, which is one of the largest producers of almond fruits. Almond Gum, otherwise known in Libya as the almond tear, is an exudate of the *Prunus Amygdales* tree belonging to the family *Rosacea*. Furthermore, *Prunus Amygdales* Batsch is known by such different designations as *Prunus dulcis* (Miller), *Prunus communis* L. potoni, and recently designation as *Amygdalus communis* L. [3-5]. Verbeken et al. reported that, the natural exudate gums are produced as a result of microbial infection on the bark of *Acacia* trees, and this trees in turn exudate the viscous liquid as a defense mechanism to seal off the injure and block more invasion of the tissue [6], as *Acacia* gums, almond gum are defined as dried exudates obtained from the trunks and branches of almond trees. Polysaccharide gums obtained from varieties of *Acacia* species are widely used in food and pharmaceutical industries [6,7]. Recently, due to the extensive applications of almond gum in confectionary and beverage industries for flavor encapsulation. Almond gum was blended with plum, *Acacia senegal,* ghatti, and tragacanth gum to improving their emulsification properties in food industrial applications [3]. Also the recent studies reveled that, almond gum consist mainly arabinogalactan polysaccharides due to the high composition of arabinose (52.1%) and galactose (33.42%) sugars, with small proportions of xylose (4.8%), rhamnose (0.43%), glucose (0.15%), and mannose (0.18%) sugars [3,8,9]. Mahfoudhi et al. reported that almond gum consist high amounts of carbohydrates and proteins as well as a low amount of fatty acid were present [9]. Almond gum is also relatively rich in metal composition, especially calcium, potassium, iron, and magnesium [3,9,10]. The physicochemical and functional properties of the natural exudates gum are play and important role in determining their commercial value and their use. These properties differ with gums different, botanical source, and even a large differences in gums from the same species when collected from plants that growing under different climatic conditions, or even when collected from the same plant species at different seasons of the year [11]. On the other hand, these properties may also be affected by the way of gum treatment after collection such as extraction, purification, drying, and storage temperature, also these properties may be affected by the age of the tree, and type of soil [12]. About 95% of Libya is desert, and almost the cultivated soils in Libya are xerosols and yermosols, and usually are dunes or shifting sandy in texture, with low in organic matter content and water holding capacity [13]. This work aims to physicochemically characterize and evaluate the concentrations of metals in Almond gum of Libyan origin as a preliminary study to pave the way for its industrial and commercialization applications.

2. Experimental Methods

2.1 Origin of the Gum Specimens

Twenty-five almond gum samples were collected during 2017-2018, from authenticated trunk of *Prunus Amygdales* trees growing in five location in the northwest region of Libya (Fig. 1), and collected five samples for each locality and labeled (A1, A2, A3, A4, and A5) for easy identification. The samples were hand cleaned to remove any foreign substance. Then few nodules of crude gum were ground finely using a pestle and mortar and stored in clean, labeled plastic bottles until used.

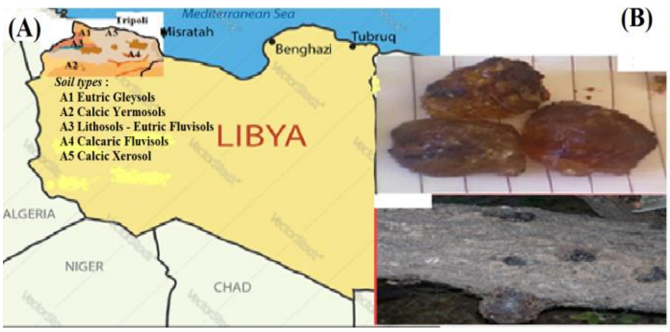

Fig. 1 (A) Soil map of northwest of Libya and (B) Crude *Prunus amygdalus* natural exudates gum

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2.2 Physicochemical Analysis

Solubility was determined by dissolving 1.0 g of dry sample in 100 cm^3 of distilled water and mixed in roller mixer overnight, then the solution centrifuged at 600 rpm for 30 minutes. 10 cm^3 of gum superannuated was placed in petri dish and dried at 105 °C for 1 hr, cooled and weighed [14,15]. Then the solubility was calculated as the percentage %(w/w). Moisture content was measured according to FAO paper No. 49 (1990) after incubation of fine powder gum samples at 105 °C for 24 hr [16, 17], Ash percentage was determined by incinerating dry powder samples in a muffle furnace at 550 °C for 6 hr [17]. The pH value of gum samples 1.0% (w/w) were determined using a glass electrode pH meter (Jenway- Model 3505) according to AOAC, 1990 method [18].

2.3 Water- and Oil -Holding Capacity (WHC, OHC)

1.0 g of almond gum was accurately weighed in a clean petri dish, then it was placed in a desiccator half filled with distilled water, and incubated for certain length of time: 24, 48, 96, 120, and 144 hr. The petri dish with sample was then reweighed. The increase in weight gave the W.H.C. of the sample per weight % (w/w) and finally expressed as percentage [19].

The oil holding capacity was also measured by dispersing 0.5 g of almond gum powders in 10 cm^3 of refined edible sunflower oil in centrifuge tube. The mixture was stirred for 4 min followed by centrifuge at 600 × g for 1 h. Finally, OHC was determined according to the following equation,

$$OHC\ \%(w/w) = \left(\frac{weight\ of\ swollen\ sample\ - weight\ of\ dry\ sample}{weight\ of\ dry\ sample}\right) \times 100$$

2.4 Intrinsic Viscosity Measurement

The intrinsic viscosity measurements were performed in a Ubbelohde Capillary Viscometer (Schott Kapillar – Viskosimeter Typ- Nr 530 -20, Germany) equipped in a water bath to maintain the temperature at 25 ± 0.1 °C, then intrinsic viscosity [η] was determined according to the following equations [9],

$$\frac{(\eta_{sp})}{C} = [\eta] + k_{SP}[\eta]^2\, C$$

$$\frac{(ln\, \eta_{inh})}{C} = [\eta] + k_{inh}[\eta]^2\, C$$

where k_{inh} and k_{sp} are constants that depend on the polysaccharides / solvent pair. The [η] was determined as the limit of the inherent viscosity or of the ratio between the specific viscosity and the gum concentration when the gum concentration approaches zero.

$$[\eta] = \lim_{c\to 0} \eta_{inh} = \lim_{c\to 0} \eta_{sp}$$

The intrinsic viscosity of soluble fraction gum was related to the molecular mass according to the Mark- Houwink equation,

$$[\eta] = K\, [M_v]^{\alpha}$$

where the constant parameters K and α depend on the nature of molecule and solvent, and on the temperature [20].

2.5 Salt Tolerance (S), and Chain Stiffness Parameter (B)

The value of salt tolerance (S) was determined from the Smidsrød and Haug equation:

$$[\eta]_0 = [\eta]_0^{\infty} + S \times 1/\sqrt{\mu}$$

where $[\eta]_0^{\infty}$ is the intrinsic viscosity at infinite ionic strength (μ), then the chain stiffness parameter (B) was obtained from the below equation: $S = B \times ([\eta]_0^{0.1})^{\upsilon}$, where the exponent υ used for this study equal to 1.3 [15].

2.6 Mineral Content

About 2.0 g of dry gum samples were ignited in muffle furnace at 550 °C for 6 hr. Then the ash was cooled and dissolved in 50 cm^3 of 0.5 M Nitric acid. The solution was warmed for 10 minutes to dissolve the ash, then the solution cooled, filtered and diluted to 100 cm^3 volume with deionized water and well mixed [17].

The essential elements as potassium, sodium, calcium, barium, and lithium were determined using a flame photometer (BWB Technologies, UK), while the heavy elements concentrations were measured using atomic absorption spectroscopy (Shimadzu AA 6701 F; A. A Spectrophotometer; Shimadzu Ltd, Japan) adapted with hollow cathode lamp.

2.7 Infra-Red Spectroscopy

The infra-red spectra of the crude gum sample in potassium bromide was recorded with FTIR spectrometer - Bruker, Model Tensor II, United States.

3. Results and Discussion

Spherically and irregular – shaped nodules of almond gum are deep brown color (Fig. 1). However, nodules when crushed and ground they give a clear white powder. Table 1 shows the results of physicochemical properties of almond gum samples.

The mean values of moisture content of the crude gum samples ranged from 12.55 to 14.93%, however, the mean values range reported here was slightly higher than the mean value of 10.3% indicated by Imtiyaz and Najeeb for *Prunes amygdalus* var *Dulcis* gum collected from Kashmir valley [21]. Results obtained were within the mean value 14.89% reported by Bouaziz et al. for almond gum collected from trunks of *Achaak's* variety trees in the suburb of Sfax City –Tunisia [10].

In the present investigation, the mean values range of solubility for natural almond gum samples were ranged from 20.01 to 35.40% in distilled water with a gel fraction ranged from 79.9 to 64.6%. Solubility of gum increased with removal calcium ions and H-bonds of the gum solution, in 0.05 M EDTA the solubility mean values were ranged from 37.2 to 45.6% and extended to 96.9% in 0.05 sodium carbonate at pH above 10. The behavior of almond gum solubility differs of ghatti gum, and similar to *Combretum glutinosum* gum [15].

The ash percentage indicates the presence of inorganic metals in gum samples, also ash content is a good parameter describe the purity, substitution, and adulteration of gums. Anderson and Dea reported that the type of the soil affects the ash content significantly, and usually heavy soil gave ash content higher than the sand soil [22], but this study showed that the location had no effect on the ash content of the Almond gum samples (insignificant differences). Table 1 shows that the mean values of the ash percentage were ranged from 4.03 to 4.62%, which are slightly higher than value 3.86 reported by Bashir and Haripriya for *Prunus dulcis* gum [23], which is less than the value 6.66% recommended by Imtiyaz and Najeeb [21].

Table 1 Analytical data of physicochemical characterization of almond gum

Location	Moisture%	Solubility (%W/W)			Ash content%	pH	WHC%	OHC%	Tannins
		D.W.	0.05M EDTA	0.05M Na_2CO_3					
A1	12.55±0.95	20.1±7.11	37.6±2.21	95.3±3.87	4.62±0.23	4.32±0.13	52.85±6.72	136.2±8.72	-
A_2	14.38±0.35	29.0±7.23	37.2±2.03	95.7±3.66	4.22±0.28	4.14±0.17	55.56±6.56	142.0±6.51	-
A_3	13.52±0.68	35.4±8.32	45.6±3.32	96.2±2.96	4.43±0.13	4.40±0.16	56.00±6.63	137.6±8.37	-
A_4	12.63±0.85	31.6±7.43	43.1±2.73	95.6±3.37	4.19±0.22	3.98±0.22	57.14±6.33	145.0±9.94	-
A_5	14.93±0.98	29.6±6.91	43.7±3.02	96.9±2.24	4.03±0.16	3.89±0.23	60.00±6.79	156.3±5.68	-

All values are expressed as: mean ± standard deviation ; - = absent

Table 2 Mineral composition of almond gum

Location	Ca%	K%	Fe%	Na%	Li%	Ni%	Cr%	Mn%	Zn%	Pb%	Hg% × 10^{-7}	Cd%× 10^{-4}
A1	0.552	0.420	0.1125	0.075	0.003	0.00025	0.0035	0.0075	0.00225	0.00228	4.77	2.50
A2	0.554	0.430	0.1050	0.070	0.005	0.00025	0.0083	0.0080	0.0025	0.00225	5.31	2.52
A3	0.585	0.467	0.0975	0.067	0.003	0.00025	0.0019	0.0065	0.00025	0.00225	2.75	2.55
A4	0.567	0.505	0.1075	0.079	0.004	0.00025	0.0083	0.0076	0.00275	0.0075	4.00	2.53
A5	0.575	0.402	0.1200	0.064	0.004	0.00025	0.0035	0.0091	0.002278	0.0025	4.03	2.50

All values are expressed as mean

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It also seen in Table 1 that the pH of almond gum solution was found to be acidic due to the presence of few free carboxyl group of its constituent acidic residues such as D-glucouronic acid. The mean values of pH of almond gum solution were ranged from 3.89 to 4.40, which are slightly lower than value of 4.87 given by Rezaei et al. [3], and value 5.82 reported by Imtiyaz and Najeeb [21].

The ability of almond gum to hold water against gravity was recorded as water holding capacity, and studies have shown that the mean values of WHC of gum samples had been found to range between 52.85 to 60.0%. This range disagrees with the value (91%) obtained by Grasso et al., [24]. As shown in Table 1, the mean value of OHC of gum samples ranged from 136.6 to 156.3 %(w/w), which are higher than value 90.0 %(w/w) reported by Bashir and Haripriya of *Prunus dulcis* gum in mustard oil [23], and lower than the value 163 %(w/w) reported by Bouaziz et al. for Almond (*Achaak's* variety) gum collected from suburb of Sfax city (Tunisia) [14].

The above results show that the location has no significant effect on the physicochemical properties of natural *Prunus amygdalus* gum, these may be due to the similarity between the all locations in climate condition, rainfall, and other ecological factors. These results also support the findings of Siddig [25] who reported that the soil type (and time of picking), rainfall and temperature had no significant effect in *Acacia senegal* gum physicochemical properties. Ballal et al. found that yield of *Acacia senegal* gum was affected by the temperature, rainfall, and time of tapping [26].

Fig. 2 shows that the intrinsic viscosity (cm^3/g) of water-soluble fraction of almond gum in 1.0 M sodium chloride was found to be 20.05 cm^3/g, and the values of Huggins constant (K_{sp}), and Kraemer constant (K_{inh}) equal 3.63, and -1.51 dL/g, respectively of water-soluble fraction of almond gum. The viscosity average molar mass (Mv) dependence of [η] for soluble fraction gum could be represented by The Mark–Houwink equation bellow,

$$20.04 = 1.31 \times 10^{-3} [M_v]^{0.58} \text{ (g /mol)}$$

The M_v of water soluble fraction of almond gum equal 16.42 × 10^6 g/mol. This result slightly higher than the value 15 .9× 10^6 g/mol of weight average molecular weight (Mw) of water soluble fraction of almond gum reported by Rezaei et al. [3].

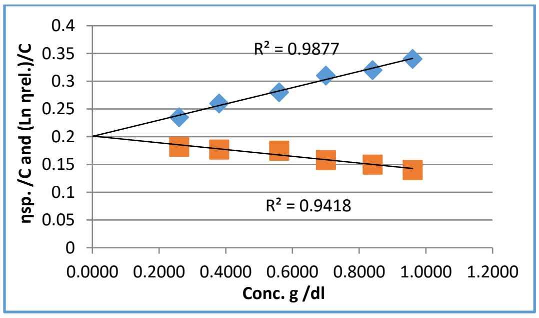

Fig. 2 Combined Huggins (♦) and Kraemer, (■) Extrapolation to zero concentration for soluble fraction of almond gum

Fig. 3 shows that the values of constant salt tolerance (S), and the intrinsic viscosity at infinite ionic strength (η_0^∞) were found to be 0.054 dL × $M^{1/2}$/g, and 0.143 dL/g respectively, the constant (S) is strongly dependent on polymer molecular mass (Mw) and the nature of counter ions. The constant (S) value of several polymer backbone can be developed only when the comparison was expressed at a specific Mw. According to this fact, Smidsrod and Haug introduced a new parameter (B) as an empirical chain stiffness which is independent on the polymer Mw. The value of B for the water-soluble fraction of gum Almond gum was found to be 0.649 by using the υ constant equal to 1.3.

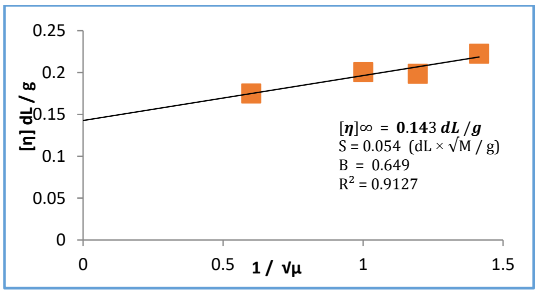

Fig. 3 [η] vs. inverse square root of ionic strength plot for water soluble fraction of almond gum with sodium chloride

3.1 Mineral Composition

Mineral composition is a measure of the amount of specific inorganic elements present within a gum samples. Table 2 shows that the Ca, K, and Fe are the three most abundant elements, and other of lower concentration are present in almond gum samples. Fig. 4 shows that the Ca, K, Fe, and Na are found in the same amount in all almond gum samples in spite of the typically differences between the five locations in soil nature. The mean values of calcium content ranged from 0.552 to 0.585 % (w/w). These range values are higher than that reported by Mahfoudhi et al. (0.1425%) [9], and lower than that observed by Bouaziz et al. (0.98%) [10]. The values of potassium content ranged from 0.402 to 0.505 %(w/w), these values lower than that presented by Mahfoudhi et al. (1.1123%), and this indicates that almond gum is a good source of Ca and K ions. The sequence of arrangement is Ca > K > Na. Similar observation has been reported previously by Rezaei et al. [3]. This sequence is different from (K > Ca > Na) that reported by Mahfoudhi et al. [9]. Table 2 shows that the mean values of Li, Ni, Cr, Mn, Zn elements were found as traces value in almond gum samples, and all the values falls within the range of the limits specified for heavy elements in food grade gums additives. Pb, Hg, and Cd are high toxic elements at certain concentration. However, concentrations of Cd, Hg, and Pb obtained in this study are below the standard limit in British Pharmacopoeia [27]. As mentioned above, Table 2 shows that the effect of location on values of Li, Ni, Cr, Mn, Zn, Pb, Hg, and Cd was not significant, and there is no significant differences among the values of elements in almond gum samples due to the locations.

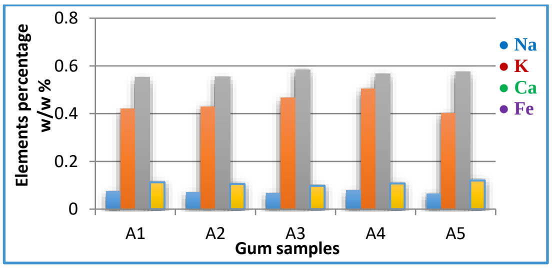

Fig. 4 Distribution of sodium, potassium, calcium, and iron in gum samples

3.2 Infrared Spectroscopy

Fig. 5 shows the IR spectra of almond gum depict a characteristic absorption broad peak in 3293 cm^{-1} representing the presence of hydrogen bonded O-H group. The bands at 2926 and 2899 cm^{-1} represent the presence of polysaccharides, and these values related to C-H Asymmetry and symmetry stretch vibration bond respectively. The other intense FTIR band at 1601.77 cm^{-1} represent the presence of C=C stretch, and amide N-H bend from aliphatic and aromatic galactoproteins, and COOH (carboxylic group) from amino acids. Carboxylic acids of glucuronic acid residues of gum polysaccharides show characteristic, C=O symmetric stretching, and O-H in-plane bands at 1601.77 and 1414.28, respectively [28,29]. Peaks at 1553.58, 1530.29, 1512.86, and 1500.27 represent of C-N-H bent of trans amide II. The band at 1357.53 cm^{-1} represent both C-O-H in- plane bending vibrations and deformations, and methyl ester CH_3-bending vibrational, which could come, for instance, from 4-O-Meglucuronic acids and proteins in polysaccharides backbone [30]. A high intensity band at 1025.74 cm^{-1} represents C-O stretching vibration. The peak at 893.74 cm^{-1} represent alkene C-H bend from polysaccharides backbone, also indicates the β-glycosidic linkages mainly present in galactose and mannose units in polysaccharides backbone [31], also the peak at 893.74 cm^{-1} represent of $-NH_2$ at about 836.17 cm^{-1}. Peaks observed at 668.38 cm^{-1} represent O-H out of plane bending band. Additionally, bands at 648.91 and 606.57 cm^{-1}, which are attributed to combination of CO vibrational of arabinose ring and various rings deformation modes. The bands at 575.15, 562.64, 553.28, 537.96, and 527.93 cm^{-1} represent the presence of β (C-C-C), β(C-O-C) in glycosidic linkage [32].

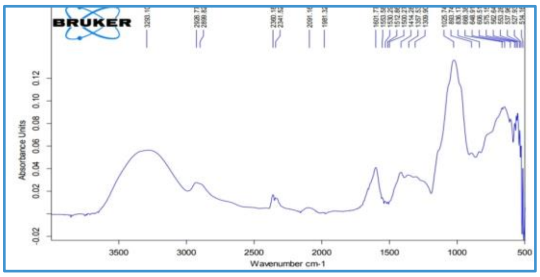

Fig. 5 FTIR Spectrum of almond gum

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4. Conclusion

The obtained results indicate that *Prunus amygdalus* gum is considered as acidic polysaccharide with low solubility in cold distilled water. Location has no significant effect on physicochemical properties and mineral composition of almond gum. The data analysis of this study clearly showed that almond gum have good physicochemical properties and good concentration of essential elements, which agree with the values reported by the FAO/WHO (JECFA) specification for Gum Arabic. Thus, *Prunus amygdalus* gum can be utilized in gum-based industries, most importantly in pharmaceuticals and food as emulsifier in food processing and effective binder in drug formulations.

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