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*Original Article*

# Some Physicochemical and Rheological Properties of Zedo (Farsi) Gum Exudates from *Amygdalus scoparia*

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## ABSTRACT

**Background and Objectives:** Zedo gum is exuded in three colours (white, yellow, and red) from *Amygdalus scoparia*. It is used in both food and non-food industries, as well as in traditional medicine. This study aimed to determine the physicochemical and rheological properties of Zedo gum.

**Materials and Methods:** Zedo gum was collected in Sirjan and classified into three groups, based on colour (samples W, Y, and R). Their soluble parts were separated by centrifuging the dispersions and freeze-drying the supernatants. Surface tension was determined using Du Nouy ring method and NMR spectroscopy was carried out using a Varian Inova-500 MHz spectrometer. Gel Permeation chromatography was used for determining molecular weight and rheological data were obtained with a Physica MCR 301 rheometer.

**Results:** Surface tension decreased as the concentration decreased, except for sample R that had a maximum point at 1 %. The molecular weight of sample W was the highest, while its dispersity was the lowest. NMR spectroscopy indicated that Ara residues variously were linked to α-Araf residues, and Gal residues were determined as terminal α-Gal residues. Intrinsic viscosity values for samples W, Y, and R were 7.14±0.13, 6.45±0.11 and 5.40±0.15dl/gr, respectively. Rheological results showed that viscosity was inversely proportional to temperature and directly to concentration.

**Conclusions:** As three samples of Zedo gum had different characteristics, colour can be a good tool in trade.

**Keywords:** Zedo, Dispersity, GPC, *Amygdalus Scoparia*, NMR

## Introduction

The plant gum exudates such as gum arabic are complex carbohydrate polymers that are commonly used as a dietary fibre, thickening agent, foaming agent, emulsifier, stabilizer, and drug delivery agent (1). Zedo gum is also exuded from the stem and branches of mountain almond tree (*Amygdalus Scoparia* from Rosaceae family) in three colours (white, yellow and red) (2). Although the gum is generally called Farsi and Shirazi gum, because of some similarities in the appearance, it is sometimes misnamed as gum arabic or even Angum gum (Angum means gum in Persian) in the market and published papers (3, 4).

*Amygdalus Scoparia* is native to Iran and occupies large areas in many parts of the country. Besides the biological functions (e.g. as a protective agent against stress factors in the plant) Zedo gum is used in traditional medicine and in the textile, food and chemical industries. Surprisingly, Zedo gum has been traded on a commercial scale for more than a century; however, its characteristics are not well known and documented. For the first time, our previous study

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identified the gum as an arabinogalactan with a relationship between its colour and properties (5). Only few studies can be found on the properties of Zedo gum and other products of *Amygdalus Scoparia* including almond kernel oil (6).

Functional properties exhibited by polysaccharides are dependent on the structure, molecular weight (MW), and concentration. MW is a fundamental characteristic of molecules, which can be measured by techniques like gel electrophoresis, gel permeation chromatography (GPC), sedimentation, osmotic pressure, and light scattering (7). In recent years, many researchers have used one of the two latter methods or a combination of them to determine MW parameters (8). Intrinsic viscosity (IV) is a characteristic property of a single macromolecule in a given solvent, which is a measure of the hydrodynamic volume occupied by the polymer itself (9). The value of IV primarily depends on the molecular size and chain rigidity of the polymer, as well as on the solution quality. There is a relationship between IV and MW according to the Mark-Houwink relationship (10). Some polysaccharides exhibit surface activity by reducing the surface tension of water and stabilising emulsions; thereby, valuable studies have been focused on this property to introduce new applications for polysaccharides in the food industry as emulsion stabiliser (11).

Our objective was to study some properties of Zedo gum such as MW (by GPC-IR), structure, rheological behaviour, and surface activity.

## Materials and Methods

**Chemicals and Sample Preparation:** All chemicals were of analytical grade and were purchased from the Merck (Darmstadt, Germany). Dried Zedo gum samples were collected manually from mountain almond trees, growing in Sirjan city (Kerman Province, Iran) in summer. Then they were visually classified into three groups based on colour (white, W; yellow, Y; and red, R). The gums were powdered in a high-speed mechanical blender, sieved to obtain uniform particle size samples (less than 500 µm), and then kept in zip plastic bags at 25°C.



**Figure 1.** Zedo gum with different colours.

All the dispersions were prepared in deionised water and mixed on a magnetic stirrer for 30 min at 45°C. Then they were kept refrigerated overnight to hydrate completely. Centrifugation of the dispersions at 10,000 rpm for 60 min at 4°C allowed the separation of soluble parts from insoluble parts. The soluble fractions were freeze-dried in a Christ Alpha 2-4 LD freeze dryer under vacuum at -85°C for 16 hr, sealed in zip plastic bags, and stored at 25 °C. Whole samples were used for NMR spectroscopy, and the soluble parts were used for other tests.

**Surface Tension*:*** Zedo solutions were prepared with deionised water (3 %, w/w), and other concentrations (2, 1, and 0.5 %) were made by diluting the stock solution. A K100 tensiometer (Kruss, Germany) determined the surface tensions of the samples using the Du Nouy ring method at 25°C. Deionised water was used to calibrate the tensiometer. The platinum-iridium ring (diameter 4.85 mm), was washed with water and acetone, and cleaned by fire, each time it was used.

**Gel Permeation Chromatography (GPC):** Samples for GPC were prepared by mixing the soluble parts of Zedo gum (2.0 g/L - 3.0 g/L) with 0.1 M NaCl on a magnetic stirrer for 30 min at 45˚C and kept at room temperature for about 48 h. The solutions were filtered through a 1µm filter, and a 100 µl volume of the filtrate was injected into a guard column and three PSS-Suprema 10 µm columns (100, 1000 and 3000 A˚, Polymer Standards Service, Germany) with a refractive index detector (RID) of PSS SECcurity 1100 and flow rate of 1.0 ml/min at 23˚C. The eluant was 0.1 M NaCl, and 10 pullulan standards (P5 – P2500) from Polymer Laboratories were used for the calibration. MW and dispersity (PD = $M_w/M_n$) were calculated using the Polymer Standards Service software (PSS WinGPC Unity, ver. 8.1).

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**NMR Spectroscopy:** The samples (1-2 mg) were deuterium-exchanged by lyophilisation in $D_2O$. The dried samples were re-dissolved in 0.2 ml of $D_2O$. Then, 1-2 µl acetone (10 % solution in $D_2O$) was added to the samples. The solutions were transferred into a standard 3mm OD NMR tube. $^1H$ NMR and $^1H$-$^1H$ COSY spectra were acquired on a Varian Inova-500 MHz spectrometer at 25°C (analysed by Complex Carbohydrate Research Centre, University of Georgia, USA) with sufficient digital resolution to ensure errors ≤ 0.1 Hz in the measured *J*-couplings. 2D correlation spectroscopy (COSY) was used to confirm $^1H$ assignments. Chemical shifts were measured relative to the internal standard acetone peak ($\delta_H$ =2.218 ppm).

**Intrinsic Viscosity:** Dilute solution viscosities were measured at 25°C using a Lauda viscometer (Lauda, Lauda-König- shofen, Germany) made with an Ubbelohde capillary tube controlled by iVisc software and a Proline PV 15 thermostatic bath. Solutions of 0.5 % (w/w) of the soluble parts were made as stock solutions. Lower concentration solutions were prepared by diluting the stock solutions with deionised water. One of the concentrations (0.3 %) in the dilute region was chosen as its relative viscosity (ηr) fell in the range of the acceptable values of 1.2 – 2.5 in order to assure good accuracy. Each sample was introduced into the capillary tube, and was automatically measured four times by the viscometer (once for preconditioning the capillary tube and three times for the analyses), and the mean value was recorded. The single-point intrinsic viscosity was calculated from the relative viscosity by means of Ram Mohan Rao equation (Eq. 1):

$$[\eta] = \frac{1}{2C}[sp + Ln(r)] \qquad (1)$$

**Rheology:** Dispersions of 4 % (w/w) were prepared and the lower concentrations (3, 2, 1, 0.5, 0.3, and 0.1 %) were made by dilution with deionised water. Rheological measurements were performed by a Physica MCR 301 rheometer (Anton-Paar, GmbH, Graz, Austria) using a Concentric Cylinder CC27 system (26.665 mm in diameter). Temperature control was carried out using a Peltier system equipped with a fluid circulator. All the samples were maintained for 5 min to allow structural recovery and temperature equilibration. The samples were covered with a solvent trap to prevent evaporation. All the experiments were carried out at 25±0.1˚C. Viscosity was plotted against concentration at the constant shear rate of 10 1/s to analyse the sensitivity of the viscosity-concentration relationship. For temperature sweep experiments, the temperature of the 2% samples was raised at a ramp rate of 1˚C /min from 2 to 80˚C and at the constant shear rate of 5 1/s. Each sample's viscosity was determined as a function of temperature.

**Statistical analysis:** The reported data are means of triplicate observations. Analysis of Variance (ANOVA) was used for the data analysis (SPSS, 16). When F-values were significant ($p < 0.05$) in the ANOVA, Duncan's multiple range tests was used to compare the treatment means.

## Results

**Surface Tension:** Fig. 2 illustrates that the addition of the samples to water caused a fall in surface tension as compared with the reference value of water at 25°C (72.5 mN/m). A decrease in the concentration of W and Y samples from 3 to 0.5% (w/w) was followed by a decrease in the surface tension values while sample R had a maximum point at 1 %.

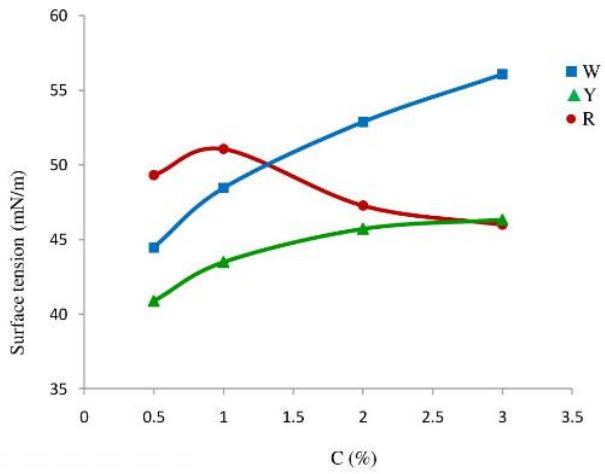



**Figure 2.** Surface tension of three Zedo samples as a function of concentration.

**Molecular Weight:** Table 1 exhibits the MW parameters of three Zedo samples, which were different in each column. The MW value of sample W was higher than the MW values for other samples. Also its dispersity was lower, which indicates having heavier and more homogenous molecules. GPC analysis showed that sample R consisted of very heterogeneous and low MW molecules.

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**Table 1.** Molecular weight values of three Zedo samples

| Sample | Mw ($\times 10^6$ Da) | PDI (Mw/Mn) |
|---|---|---|
| W | 3.14 | 6.61 |
| Y | 2.72 | 8.21 |
| R | 1.66 | 12.2 |
| GA* | 1.27 | 2.7 |

* Rreported by AC Stijnman, 2011

Figures 3-5 show the plots of molar mass distributions of the three samples. Sample Y had a similar, but wider, peak than W. In the plot of sample R, three fractions were recognised (the main peak in the middle and two shoulder peaks on both sides). The plot was wider and shorter than the other plots, which implied higher dispersity along with lower molecular weight polysaccharides.

**Intrinsic Viscosity:** The intrinsic viscosity of samples W, Y, and R was 7.14 ± 0.13, 6.45 ± 0.11, and 5.40 ± 0.15 dl/gr, respectively, which were statistically different.

**Proton NMR-Spectroscopy:** Proton NMR-spectra of all the samples were similar; therefore, proton and 2D COSY-spectra of sample W were analysed as representatives. In the anomeric region of the spectra, t-α-Araf and variously linked α -Araf residues, t- α -Galp, t-β-GlcA, and 4-OMe-β-GlcA resonances were detected. Anomeric signal of the α -L-Rha residue could not be distinguished due to signal overlap, but the chemical shift of methyl proton of Rha and its correlation with H5 of Ra were easily identified. Ara accounted for about 54 to 62 mol % of the Zedo samples, and the variously linked α-Araf residues could be identified from NMR-spectra (Fig. 6, Table 2). Gal residues that made up to 27-31 mol % of the samples were determined from the NMR-data as terminal α-Gal residues. Chemical shifts of t-β-GlcA and 4-O-Me-β-GlcA residues were tentatively assigned from the NMR-spectra.

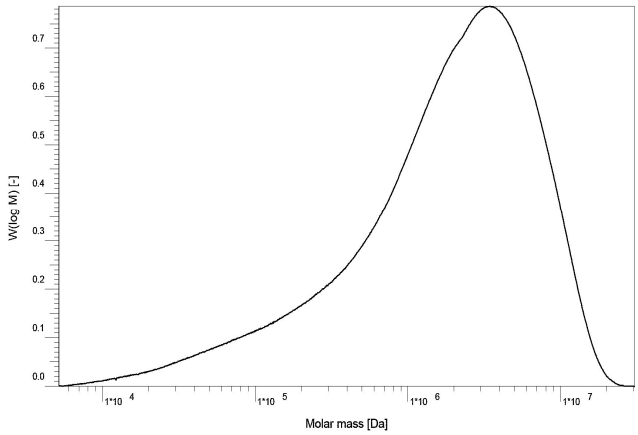



**Figure 3.** Molar mass distribution of sample W.

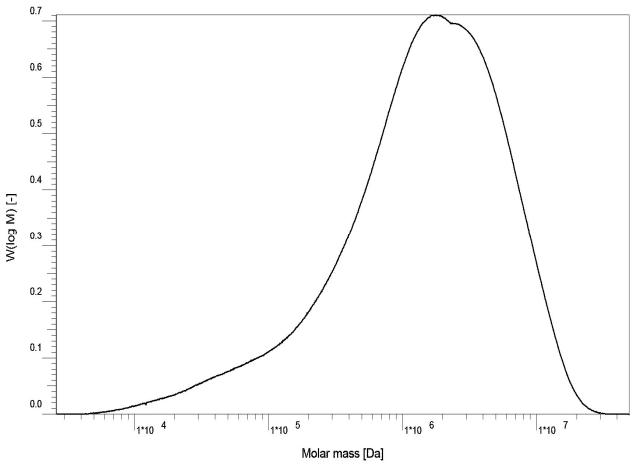



**Figure 4.** Molar mass distribution of sample Y.

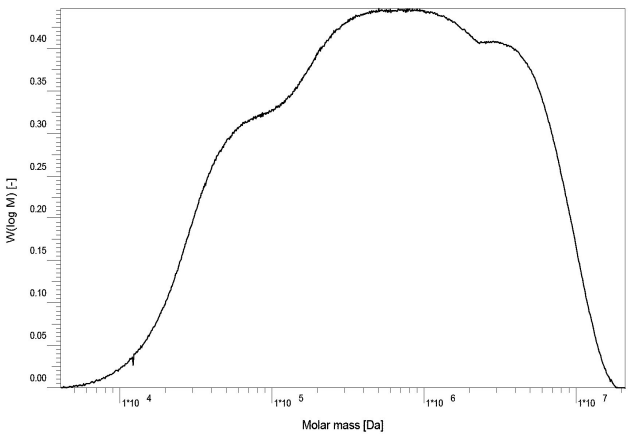



**Figure 5.** Molar mass distribution of sample R.

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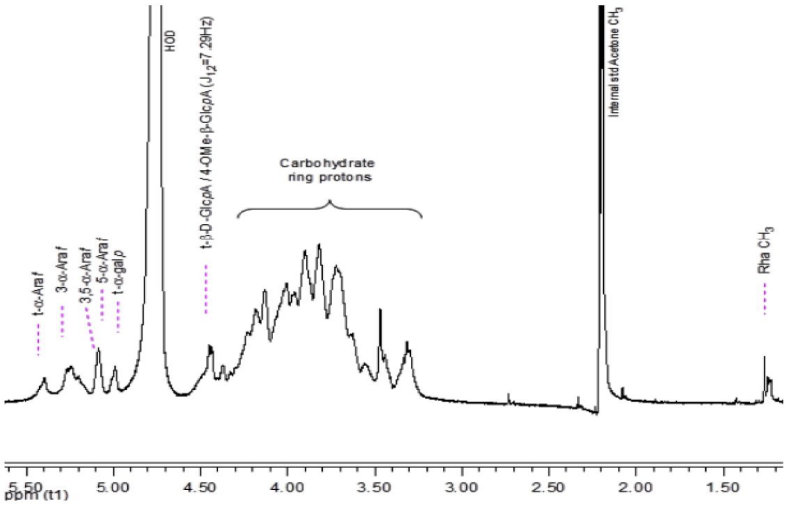



**Figure 6.** H NMR spectrum of the sample W in $D_2O$.

**Table 2.** Glycosyl-linkage analysis of Zedo gum

| Residue | | 1 | 2 | 3 | 4 | 5 | 6 |
|---|---|---|---|---|---|---|---|
| t-α-L-Araf | $^1H$ | 5.4 | 4.17 | n.d.*** | n.d. | n.d. | n.d. |
| 3- α -L-Araf | $^1H$ | 5.28 | 4.33 | n.d. | n.d. | n.d. | n.d. |
| 3,5- α -L-Araf | $^1H$ | 5.12 | 4.22 | n.d. | n.d. | n.d. | n.d. |
| 5- α -L-Araf | $^1H$ | 5.11 | 4.14 | n.d. | n.d. | n.d. | n.d. |
| t- α -L-Galp | $^1H$ | 5 | 3.88 | n.d. | n.d. | n.d. | n.d. |
| t-β-D-GlcpA | $^1H$ | 4.48 | 3.33** | n.d. | n.d. | n.d. | n.d. |
| 4-OMe- β -GlcA | $^1H$ | 4.46* | 3.38** | n.d. | n.d. | n.d. | n.d. |
| t- α -L-Rhap | $^1H$ | n.d. | n.d. | n.d. | n.d. | 4.04 | 1.26 |

*doublet, $J_{1,2}$=7.29Hz; ** assignments may be interchangeable; tentative assignments, *** Non detected

**Rheological Behaviour :** As shown in Fig. 7, the viscosity of samples W and Y increased continuously with the increase of their concentration.

Fig. 8 shows the viscosity as a function of temperature. According to the figure, an increase in temperature leads to a reduction in the viscosity of the samples, confirming the well-established fact that the viscosity is inversely proportional to temperature. Although a similar downward trend was observed for all the samples, the viscosity of sample W was higher than that of the other two samples during the tested temperature range (2–80°C).

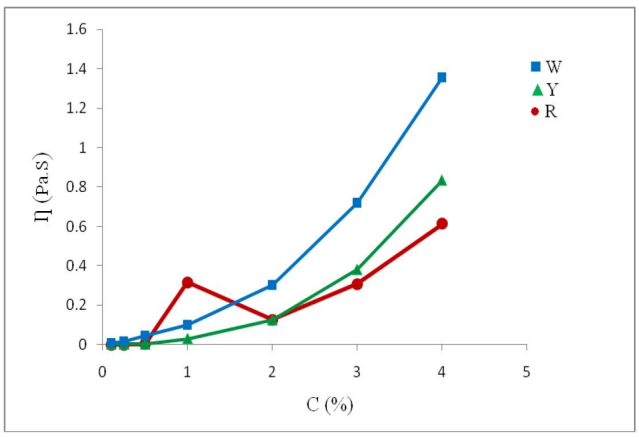



**Figure 7.** Viscosity as a function of Concentration (at $\dot{\gamma}$ =10 $s^{-1}$) in deionised water.

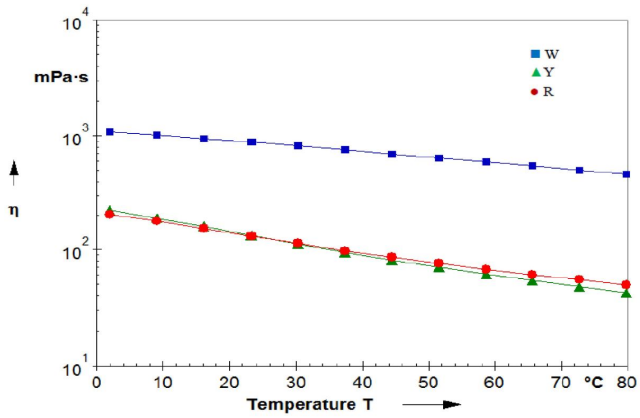



**Figure 8.** Viscosity as a function of Temperature of 2% (w/w) Zedo gum dispersions.

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## Discussion

The difference between surface tension of sample R and other samples could be related to typical polyelectrolyte behaviour (12). The presence of charged groups along the backbone of polyelectrolytes (polymers containing ionisable groups) plays a significant role in both the static structure and the dynamic behaviour of such systems (13). Coil expansion is one of the ways in which the unusual nature of polyelectrolytes affects the properties of the systems. A polyelectrolyte molecule expands in dilute aqueous solution because the like charges on the polymer chain repel each other, and the expansion allows these charges to be as far apart as possible (14). The surface tension values of W (44.6 mN/m) and Y (40.9 mN/m) at a given concentration (0.5% w/w) were below the values reported for gum arabic (46.9 mN/m) and other food gums (65–45 mN/m), while the value for R (49.3 mN/m) fell within the latter limits (15).

All samples were highly dispersed and quite different from the reported values for gum arabic (16). The reported values for MW of the samples determined by GPC-MALLs in our previous study (5) were higher than our new findings. However, similar observations have been published by other researchers who showed that GPC-RI underestimates the MW of the same materials by a factor of 1.5 – 1.8 compared with GPC-MALLs (17).

The dispersity is given by the ratio of the mass average molar mass to the number average molar mass and it is a general measure of the heterogeneity with regard to chain length (18). Dispersities out of range of 1.5-8 are called unusual (19) and higher dispersity is known to be the cause of lower thermal stability and more elasticity (20).

One of the most noticeable features of polysaccharides is the intense increment in viscosity that they produce in very dilute solutions. The viscosity intensifying effect, characterized by the intrinsic viscosity, [η], is used to analysis or characterize synthetic polymers, biological macromolecules, nanoparticles, and colloids. Indeed, IV provides information about fundamental properties of the solute and its interaction with the solvent. It can also be precisely related to the conformation of flexible (linear and nonlinear) and rigid particles of arbitrary shape (21). In some cases when determining intrinsic viscosity, extrapolations do not have a common value at their origin ordinates, which can be caused by inadequate lineal extrapolations. The procedure is laborious and consumes a considerable amount of time and reactive (22), so we used the single-point method. Although the IV of Zedo samples were much less than that of the xanthan gum, which was reported to be highly viscous with the IV of 55.2 dl/gr (23), Zedo samples' IV values were much higher than that value for gum arabic (1.4 dl/gr) (24). Researchers have demonstrated that the IV of flaxseed gum is between 4.3 and 6.6 dl/gr (25), which is similar to that of our samples. It is reported that intrinsic viscosity is affected by the composition, size, and shape of water-soluble polysaccharides, and can be related to MW by the Mark–Houwink–Sakurada equation (26).

Solution NMR can be applied effectively to identify the type, the number, and the proportions of different sugar residues in a polysaccharide as well as different linkage configurations and positions in the polymer (27). We used the technique and the results approved our sugar analysis (5) but the structure of the sample could not be fully determined because of some signal overlaps and low signal to noise ratio. Chemical shift assignments of the $^{1}H$ NMR and $^{1}H$-$^{1}H$ COSY spectra were made using the reported values (28).

A positive correlation was found between the viscosity and the gum concentration, which is correlated with other studied (29). A similar trend was observed for sample R in the concentration range of 4% to 2%, after which a maximum point at 1 % was revealed. This behaviour could be due to the polyelectrolyte character of the polymer as other researchers have reported (30). This intermolecular phenomenon, i.e. increasing the solution's viscosity with decreasing the concentration, is believed to be a reflection of a coil – rod transition, caused by electrostatic forces (31).

It has been reported that, under a given shear stress, the flow rate is determined by the polymer chain entanglement/disentanglement and slippage, when the chains pass each other (32). In most cases, an increased temperature promotes disentanglement of the chains, which, in turn, can cause a reduction in viscosity. However, our findings are in agreement with the common expectations and previously reported data for other polysaccharides (33). The

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viscosities of Zedo gum samples (2 %) at 20°C were comparable with that of gum arabic at 20% concentration or more (34).

**Conclusion:** We classified Zedo gum samples into three distinct groups based on their colour. This classification seems to provide a good tool for evaluating the overall quality of Zedo gum. Regarding the molecular weight, dispersity, thermal viscosity stability and colour, white Zedo sample showed better qualities to be used in food formulations. Although, in terms of viscosity, red sample was more efficient at concentration of 1%, compared to other samples, the colour can limit its application.

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