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Study of Some Functional Properties and Antioxidant Activity of Two Types of Cherry Trees (*Prunus avium*) Gum Exudates Grown in Syria

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Abstract

The aim of this research is to study some functional properties and the antioxidant activity of cherry gum, collected from Serghaya and Suwayda in Syria, and to compare these features with those of Arabic gum. The values of the hydroxyl groups for the Arabic gum, Serghaya and Suwayda cherry gums were 757.1, 655.1 and 564.3 mg KOH/gm, respectively. The solubility of exudate gums ranged from 53.53 to 86.53% and was arranged as follows: Arabic gum>Serghaya cherry gum >Suwayda cherry gum. Gum solubility increased with rising the temperature. Water and oil holding capacities of cherry gums were significantly higher (p<0.05) than those of Arabic gum, while their emulsifying capacity was significantly lower than that of Arabic gum. The antioxidant activity of the gum was evaluated using the DPPH scavenging activity method and reducing power method. The inhibition percentages of DPPH were 51.52%, 23.23% and 21.19% for Serghaya, Suwayda and Arabic gums, respectively. The antioxidant activities of Serghaya and Suwayda gums using the reducing power method were 753.12 and 312.93 meq ascorbic acid/100gm, whereas Arabic gum didnot exhibit any Fe⁺³ reducing activity. All of gum types showed similar FT-IR spectra.

Keywords: Arabic gum, Suwayda, Serghaa, DPPH, FRAP, FTIR spectra

هدف هذا البحث إلى دراسة بعض الخصائص الوظيفية والنشاط المضاد للأكسدة لصمغ أشجار الكرز المجموع من منطقتي سرغايا والسويداء في سوريا ومقارنته بالصمغ العربي.بلغ عدد مجموعات الهيدروكسيل757.1 و 655.1 و 664.3 مغهيدروكسيد البوتاسيوم/غ للصمغ العربي و سرغايا والسويداء

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على التوالي. تزاوحت الإنحلالية بين 53.53 و 86.53% وكان مرتبةً كالتالي : الصمغ العربي> صمغ سرغايا> صمغ السويداء ،وازدادات انحلالية الصموغ بارتفاع درجات الحرارة.كانت القدرة على ربط الماء وربط الدهن أعلى معنوياً لصمغي الكرز (0.05<)مقارنةً بالصمغ العربي بينما كانت قدرتهما على الإستحلاب أقل بشكل معنوي لصمغي الكرز (0.05<)مقارنةً بالصمغ العربي بينما كانت قدرتهما على الإستحلاب أقل بشكل معنوي ملحوظ . تم تقدير النشاط المضاد للتأكمد باستعمال طريقة الملاطيقة الحديد أقل بشكل معنوي ملحوظ . تم تقدير النشاط المضاد للتأكمد باستعمال طريقة الدوريمة الحديد المرجع. بلغت النسبة المئوية لتثبيط جذر الـ51.53 DPPH% و 23.23% و 23.23% و 21.19% لكل من الصمغ العربي وصمغ سرغايا والسويداء على التوالي. وبلغ النشاط المضاد للأكسدة وفق طريقة الحديد المرجع العربي وصمغ مرغايا والسويداء على التوالي. وبلغ النشاط المضاد للأكسدة وفق طريقة الحديد المرجع العربي وصمغ مرغايا والسويداء على التوالي. وبلغ النشاط المضاد للأكسدة وفق طريقة الحديد المرجع العربي وصمغ مرغايا والسويداء على التوالي. وبلغ النشاط المضاد للمعاد للأكسدة وفق طريقة الحديد المرجع العربي وصمغ مرغايا والسويداء على التوالي. وبلغ النشاط المضاد للأكسدة وفق طريقة الحديد المرجع العربي وصمغ مرغايا والسويداء على التوالي. وبلغ النشاط المضاد للأكسدة وفق طريقة الحديد المرجع العربي وصمغ مرغايا والسويداء معلى المحونيك لكل من صمغ سرغايا والسويداء مينما لم يسجل المحمغ العربي قدرة على ارجاع شوارد الحديد. سجلت جميع الصموغ المدروسةتطابق في طيف المحالية.

Introduction

The growing attention of consumers towards healthier and natural foods has led to an increasing global demand for natural gums[1].Plantgums have hydrophilic carbohydrates with high molecular weights that are generally composed of monosaccharide units linked by glucosidic bonds [2]. They have thickening or gelling effects, as well as specific interfacial properties.The composition of gum polysaccharides varies among species and cultivars [3].

Arabic gum is a natural arabinogalactan-protein type of polysaccharides derived from Acacia senegal[4](Figure-1), and it is one of the oldest commonly used exudate gums for food and non-food applications.

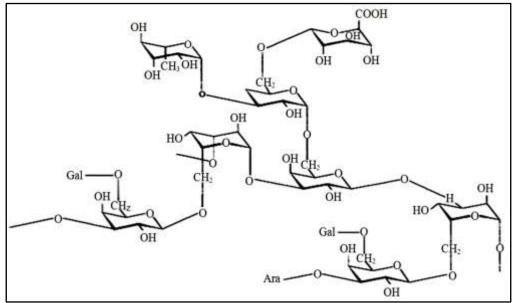


Figure 1-Chemical structure of Arabic gum

A wide range of families produce gums as *Rosaceae*. The exudates of the *Rosaceae* gum are caused by infection, insect attack, mechanical and chemical damage, water stress and other environmental stresses [5].

These polysaccharides have functional and rheological properties depending on their monosaccharide composition, physicochemical properties and interaction with water [2].Due to its wider application in the pharmaceutical, food supplements, printing and binding industries, the need to explore more natural sources of gums in addition to those already known is becoming more demanding. The aim of this research is to study some functional properties of cherry gums collected from two regions in Syria (Serghaya and Suwayda) and to compare them with Arabic gum in order to obtain information on their future use in the food industry.

2-Materials and methods

2-1-Plant and chemical material:

The cherry gum samples were collected from the trunks and branches of cherry trees in Serghaya and Suwayda (Syria, August 2017). Geographically, Serghaya is in south-western Syria. It is located

60 km from Damascus and at 1,446 m (4,744 ft) above sea level. Suwayda is located southwestern Syria, close to the border with Jordan, and it is situated at an elevation of 1068 meters above sea level.

Arabic gum was purchased from local markets in Damascus city.

All the chemicals and solvents used in this work were purchased from Sigma-Aldrich.

2-2-Purification of exudate

Purification of the exudate gum was carried out using Bhushette and Annapure's method [6] with some modifications. The gums were grounded with a hammer mill (Starmix) and sieved. Solution of powdered gum exudate (5% w/v) was stirred at 50° C for 3 hours and then filtered through a filter cloth for removing impurities. The filtrate was then concentrated twice in the oven at 50° C. The cherry gum exudate was precipitated by absolute ethanol (exudate solution: ethanol, 1:3 v/v). The precipitated exudate was separated by centrifugation at 4500rpm for 3 minutes. The centrifuged pellet was dried in the oven for 48 hours to have a constant weight and kept in an airtight container for further use. The solution of pure Arabic gum was dried in an oven at 105 °C until a cracked dry layer was obtained. Dry gum was then collected and grinded with an electricmill and the resulting soft powder was kept in an airtight container for further use.

2-3-Analysis of the hydroxyl value: The hydroxyl value corresponds to the milligrams of potassium hydroxide (KOH) required to neutralize an equivalent amount of acetic acid combined with hydroxyl groups in 1 gm of a sample. About 3 gm of the sample was weighed out into a round-bottom flask and 20 mL acetylating mixture (1 volume of acetic anhydride + 3 volumes of pyridine) was added. The flask was then connected to a water condenser and the contents were refluxed for 30 minutes. The reaction mixture was then cooled to room temperature and distilled H_2O (50 mL) was added through the reflux condenser. The free acid was determined by titration with standard KOH using phenolphthalein. A blank was also carried out using 20 ml acetylated mixture with the same method[7]. The hydroxyl value was calculated using Equation 1. The acid value of the sample must also be taken into consideration.

$$Hydroxyl value = \frac{5.61 \times (B-A) \times N}{M} + Acid value \qquad \dots \dots \dots (1)$$

Where A =Volume of KOH required for sample

- B = Volume of KOH required to titrate blank
- W = Weight of sample used
- N = Normality of alcoholic KOH

2-4-Solubility:Solubility of gum samples was measured at different temperature (20-50 and 100°C). The concentration of the gum solutions used was0.1% v/v. This was prepared in distilled water and stirred using a magnetic stirrer at different temperatures (20, 50 and 100 °C) for 30 minutes, then centrifuged under 1800g at room temperature for 30 minutes. The separated supernatant was dried in an electrical oven at105°C for 24 hours. The solubility of the gums was calculated using Equation 2 [8].

Solubility %=
$$\frac{\text{supernatant concentration (mg/ml)}}{\text{Initial preparation concentration (mg/ml)}} \times 100$$
(2)

2-5-Water holding capacity (WHC) and oil holding capacity (OHC): The WHC and OHC of gums were determined according to the literature [9]. One gram of the gums was weighted in a centrifuge tube and distilled water (10 ml) or sunflower oil was added. Samples were vortexed for 5 minutes and allowed to stand for 30 minutes at room temperature before centrifuging at 5000 rpm for 25 minutes. The free water or oil was decanted and the water and oil absorbed by the samples was expressed as ml of water or oil absorbed per gm of gums.

2-6-Emulsifying capacity (EC): The emulsification properties were determined according to the literature [10]. Samples (1gm) were suspended in 50 mlof distilled water, then 50 ml of refined sunflower oil was added. The mixture was then emulsified with an ace homogenizer at 10,000 rpm for 1 minute. The emulsion obtained was divided equally into two 50-mlcentrifuge tubes and centrifuged at 4100 rpm for 5 minutes. The emulsifying capacity of the gums were calculated using Equation 3.

$$EC = \frac{\text{Height of emulsified layer}}{\text{Height of whole layer}} \times 100 \qquad \dots \dots (3)$$

2-7- Determination of total phenolic compounds: The methanolic extract was prepared by dissolving the gums (1 gm) in methanol(25 ml) and stirring using a magnetic stirrer at room temperature for 3 hours. The solution was then filtered through a filter paper and the extract was used

for determining the phenolic compounds and antioxidant activity. The amount of TPC in gums was determined according to the Folin-Ciocalteu method [11].

2-8-Antioxidant activity: The antioxidant activity of the gum exudates was evaluated by determination of DPPH activity and of reducing power.

2-8-1-DPPH free radical scavenging activity: DPPH activity was measured according to the literature[12]. DPPH solution was prepared by dissolving 2.4mg DPPH in methanol (100 ml) in a volumetric flask. Thereafter, extracted or standard ascorbic acid (1 ml) was added to the DPPH solution (1 ml). The mixture was shaken vigorously then left in the dark for 30 minutes. The absorbance of the resulting solution was measured using a UV-Visible Spectrophotometer (Optizim 3000 plus-Korea) at 517 nm. The control solution was prepared by adding methanol (1 ml) to the DPPH solution and methanol was used as a blank. The scavenging activity of the extract was calculated using the following formula:

% inhibition = $\frac{Absorbance of control - sample Absorbance of test sample}{2} \times 100$

Absorbance of control sample \land 100 The antioxidant activity was also expressed as IC₅₀. The IC₅₀ value was defined as the concentration

(mg/L) of gum that inhibits the formation of DPPH radicals by 50%. **2-8-2-Determination of reducing power:** A mixture of methanolic extract(200 μ L), sodium phosphate buffer (2.5 mL, pH 6.6) and potassium ferricyanide (2.5 mL, 1%) was prepared and incubated at 50°C for 20 minutes. After cooling to the room temperature, trichloroacetic acid (2.5 mL, 10%) was added. Following this, 2.5 ml of the mixture was mixed with water (2.5 ml) and freshly prepared ferric chloride solution (0.5 ml, 0.1%). After vortexing, the absorbance was measured at 700 nm. A blank solution was prepared under the same conditions. The standard curve was prepared from the ascorbic acid solutions at concentrations of 0.5 to 2.5 μ g/ml and the antioxidant activity was expressed as micrograms of ascorbic acid equivalents per 100gm [13].

2-9-Infrared spectrum analysis of gum

FT-IR spectra were recorded on a FTIR-4200 Jasco in the Chemistry Department-Faculty of Science-Damascus University. Spectrometry was applied in the wavelength range of 4000-400 cm⁻¹at a resolution of 4 cm⁻¹ using the KBr disc method[14].

Statistical analysis:

All analyses were performed in triplicate for each treatment. Data were subjected to analysis of variance (ANOVA) to determine the significant differences among treatments at 5% level using Genstat software 12.

3-Results and discussion

3-1-Hydroxyl value: the hydroxyl value generally corresponds to the content of free hydroxyl groups in a compound, as shown in Figure-2. The hydroxyl value of Arabic gum was significantly higher(p<0.05) as compared to cherry gums.

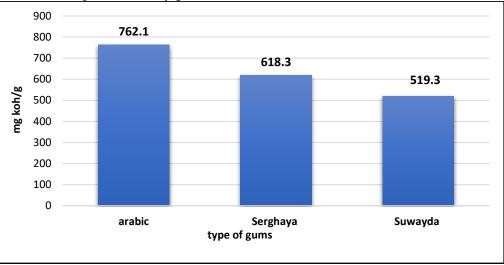


Figure 2-Hydroxyl value of gums

3-1-Solubility

The results in table 1 show that the Suwayda gum had relatively low solubility at 20 °C (53.53%) as

compared to the Serghayagum (82.26%) and Arabic gum (86.66%). The low solubility of Suwayda gum may be due to the large number of glycosidic linkages, highly branched structure and high molecular weight[15]. Rising temperature from 20 to 50°C led to improve the solubility of cherry gum to about 5.47 and 4.69% for Serghaya and Suwayda cherry gums, respectively, as shown in Table 1. Arabic gum did not show any significant increase in solubility at 50° C. On the other hand, increasing the temperature to 100 °C led to increase the solubility to around 11.46%,11.86% and 21.02% for Arabic, Serghaya and Suwayda gums, respectively. Increasing temperature leads to dissolve some high molecular weight molecules and galactomannan with a low galactose content [16]. In addition, hydrogen bonds between polysaccharide chains are broken at high temperatures, with OH groups being exposed to water and ready to form hydrogen bonds with water, thus enhancing solubility. Increasing the temperature may alter the soluble mass of galactose in high molecular weight polysaccharides present in the aqueous solution [17].

Gum	Temperature		
Guin	20 °C	50° C	100°C
Arabic gum	86.53±0.54 ^a	87.47 ± 0.81^{a}	96.45 ± 0.3^{b}
Suwayda gum	53.53±1.13 ^a	56.46±1.28 ^b	$64.78 \pm 0.67^{\circ}$
Serghaya gum	82.26±0.06 ^a	86.11±0.57 ^b	$92.02 \pm 0.08^{\circ}$

Table 1-Solubility of gums at different temperature

Mean \pm SD values followed by different letters within the same row are significantly different according to T-test at $P \le 0.05$.

3-2- Water holding capacity (WHC) and Oil holding capacity (OHC)

WHC is defined as a substance's ability to associate with water through hydrogen bonds under limited water conditions [18]. Gums are desirable in industrial applications to hold gels producing water or highly viscous solution [19], which is important for many food products such as bakery products, pastas and others. As shown in Figure-3, WHC of the cherry gum was significantly higher than the Arabic gum(p<0.05). However, the water holding capacity of gums did not only depend on the hydrophilic functional group of carbohydrate, but also on the proteins present in gums since they also contain functional groups that were able to bind with water molecules [20]. The greatest WHC of cherry gums could be due to the hydrophilic group in proteins, such as the polar side chain containing carboxyl, amino, hydroxyl, and sulfhydryl groups as well as the non-dissociable carboxyl and amino groups of the peptide bonds.

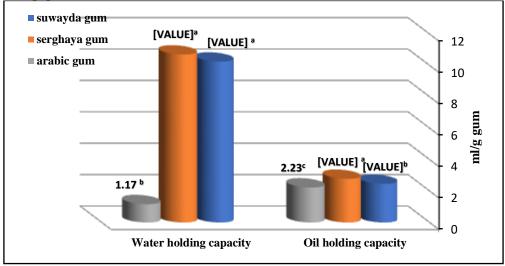


Figure 3-Water holding capacity and Oil holding capacity of gums.

OHC is the ability of the substance to absorb oil [21]. It is important in the meat industry (sausages, hamburgers, etc.) as well as in the bakery industry(doughnuts, etc). The results in Figure-3 show that OHC of Serghaya gum(2.78 ml oil/gm) was significantly higher(P<0.05) higher than Suwayda gum (2.46 \pm 0.19 ml oil/gm) and Arabic gum(2.23 \pm 0.09 ml oil/gm). According to the literature [22], the high OHC value of polysaccharides was due to the presence of non-polar side chains and hydrophobic fractions such as protein and fat, which can bind the hydrocarbon units of oil, resulting in increased oil

absorption capacity. The good OHC value suggests that cherry gums can improve the texture of food products.

3-3-Emulsifying capacity

Emulsifying capacity (EC) measures the ability of emulsion to retain its system after centrifugal force [23]. The gums were significantly different(p<0.05) in their EC values as shown in Table-2. EC of the Arabic gum (96.11%) was higher than Serghaya gum (66.43%) and Suwayda gum (5.6%).

The presence of protein and ferulic acid increases the emulsion capacity, while it was stated that the peach gum exudates showed a better emulsion capacity (95%) and stability than Arabic gum [24].

Gum	Emulsifying capacity (%)
Serghaya gum	66.43±2.44 ^b
Suwayda gum	$5.6 \pm 0.0.63^{\circ}$
Gum Arabic	96.11±2.36 ^a

a, b, c Values within a column followed by different letters are significantly different at P < 0.05 using Fisher's unprotected range test However, the mechanisms of water-soluble polysaccharides reducing the surface tension of oil-water system are still under studying, with a focus on the role of protein or polysaccharides-protein complexes on O/W surface. Some researchers suggested that the emulsification activity of polysaccharides is dependent on its protein impurities [25]. According to the literature [24] emulsifying properties of gum's polysaccharides are due to the high molecular weight and high branching. The 'wattle blossom model' shows the possible mechanism of Arabic gum adsorbing at the oil-water interface. The hydrophobic protein has an affinity for the oil phase which leaves the hydrophilic carbohydrates outside the oil droplet in the water phase, acting as an emulsifiers [26]as shown in Figure-4.

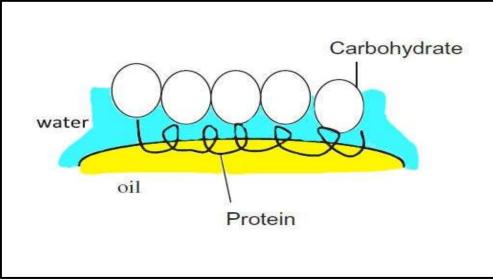


Figure 4-Wattle blossom model (after Dickinson, 2003).

3-4-Phenolic compounds

In the three gum types, phenolic compounds could not be detected, a result that is consistent with the study conducted by Abbasi and co-workers[27],who reported the precipitation of gums with alcoholic compounds in the removal of phenolic compounds.

3-5-DPPH-scavenging activity

The results of radical scavenging activities of gums are presented in Table-3. Arabic gum showed the highest(51.52%) whereas Suwayda gum showed the lowest (21.19%) DPPH scavenging activities. The variation in DPPH radical scavenging activity of treated gums may be due to the different sources of gums and a variety of chemical compositions, molecular weights and structures, resulting in the different molar quantities of active sites to eliminate free radicals [28]. Furthermore, the treatments of purification might affect the activities of polysaccharide fractions [29]. The radical scavenging ability was related to the number of hydroxyl or amino groups in polysaccharide molecules [30]. According

to the results, Arabic gum had more hydroxyl groups than cherry gums, which act as hydrogen donors to scavenge DPPH free radicals and, consequently, to neutralize the effects of oxidative stress. Also, Arabic gumcontains several types of amino acid residues such as lysine, tyrosine, and histidine, which are commonly considered as antioxidant biomolecules

Gums	free radical scavenging activities(%)
Serghaya gum	23.23±0.40
Suwayda gum	21.19 ±0.2
Arabic gum	51.52±1.34

Table 3-Radical	scavenging	activities	of gums
I ubic o Ituaicai	beu venging	ucu vitico	or guins

The lowest IC₅₀ value corresponds to the highest DPPH scavenging activity. The results demonstrated that the radical scavenging activity decreased in the following order: Ascorbic acid>Arabic gum>Serghayagum>Suwayda gum, with significant differences(p<0.05) as shown in Figure-5. The IC₅₀values of theSuwayda, Serghayaand Arabic gumswas found to be1078,1025 and 62.92 mg/L, respectively, as compared to the standard ascorbic acid which exhibited a value of 0.61 mg/L. Cherry gum showed the lowest effect on DPPH, possibly due to its special structure that should be studied further. Arabic gum showed stronger free radical scavenging activities, which may be due to higher uronic acid content than that in cherry gum [31].The IC₅₀ value of cherry gums was reported to be lower than that found in Almond gum(6.6mg/ml)[32] and higher than that found in sour cherrygum (*Prunuscerasoides*)(IC₅₀ = 0.98 mg/mL)[33].

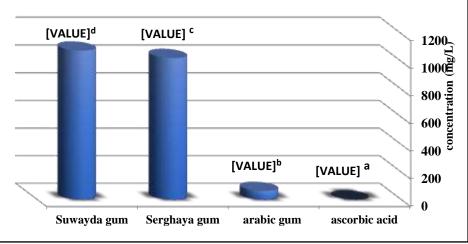


Figure 5-Concentration for scavenging 50% DPPH radical for different gums

3-6-Reducing power assay

Reducing power activity, measures the reductive ability, evaluated using the transformation of $[Fe(CN)_6]^{-3}$ to $[Fe(CN)_6]^{-4}$ in the presence of polysaccharides, by donating an electron. Hence, the formation of Fe⁺² can then be measured by the formation of Perl's Prussian Blue at 700 nm; the higher absorbance values indicate higher ferric iron-reducing power activity [34]. According to Table- 4, the reducing power activity of Serghaya and Suwayda gums were 312.93 and 83.43µg/100gm, respectively. On the other hand, Arabic gum did not exhibit any Fe⁺³reducing activity (data not shown). The differences in the antioxidant activities among gums were attributed to a combination of many factors such as their composition, molecular weight, water solubility, monosaccharide component, structure of chain conformation, polarity, and intramolecular hydrogen bonds [34]. Uronic acids are usually considered as a key factor in antioxidant activity. Based on the reported literature, polysaccharides with a higher content of uronic acids possessed stronger antioxidant activities [35]. Furthermore, some researchers have reported that polysaccharides with more mannose and rhamnose displayed higher antioxidant activity[36].

Gum	Reducing power(µg/100gm)
Serghaya gum	312.93±8.3
Suwayda gum	83.43±3.11
Arabic gum	$\mathrm{N.D}^{**}$

ND**: Non-Detected

The results of DPPH method were different from those of the FRAP method, possibly due to various kinds of antioxidants present in the samples which react differently with the radicals used and mechanism of reaction for DPPH assay as opposed to FRAP assay. We suppose that the high molecular weight polysaccharides had more repetitive structures and electron donors such as hydroxide groups, which could reduce Fe^{+3} to $Fe^{+2}[37]$.

3-7-FTIR analysis of gums

FTIR spectrum was used to analyze the functional groups of polysaccharides, as displayed in Figure-6, over the range of 4000-400 cm⁻¹. FTIR spectra of the cherry and Arabic gum showed a broad band of strong intensity at 3321 cm⁻¹ and 3428 cm⁻¹ which might be due to intra and inter O-H bond stretching (3500-3000cm⁻¹) of the sugar molecules present in the gum. The weak absorption peak of the C–H stretching vibration was found at 2925cm⁻¹ and 2926cm⁻¹,including CH, CH₂ and CH₃ stretching and bending vibrations[38].Moreover, the signals at 1618 cm⁻¹, 1617cm⁻¹, 1430 cm⁻¹ and 1427 cm⁻¹belonged to gums that had uronic acids that corresponded with the asymmetrical (C=O) and symmetrical (C–O) stretching vibrations[39].The peaks at 1039 cm⁻¹corresponded to cherry gums while at 1074 cm⁻¹ the peaks represented the Arabic gum, which were further attributed to glycosidic linkages[40].The peaks in the range of 330-960 cm⁻¹ were characteristic of xyloglucan and xylogalacturonans [41]. These data demonstrate that the cherry gum was mainly composed of polysaccharides.

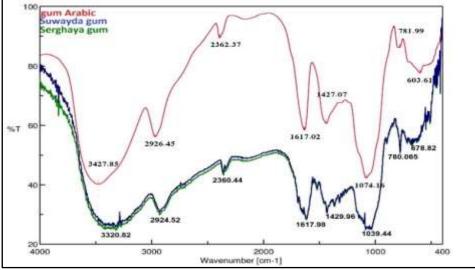


Figure 6-FT-IR spectroscopy of gums

4-Conclusion

The present study shows the great potential of cherry gums, which were obtained by alcohol extractions, for incorporation into human food products. The water solubility of cherry gums was lower, whereas they exhibited better water and oil holding capacity than the Arabic gum. Results from DPPH activity and FRAP indicated that the gums possess antioxidant properties and can be used, following further analysis, in various fields of medicine, pharmaceuticals and food industries. Determination of functional groups by FTIR spectroscopy may provide an important tool to confirm the identity of cherry gums.

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