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An Experimental Study on Gum Arabic Preparations in the Treatment of Some Colon Problems

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ABSTRACT

The present study aimed to collect gum Arabic from local markets in Baghdad city and study its physical properties as well as its biological activity against pathogenic bacteria isolated from food that causes food poisoning. The results of our study indicated that the acidity of gum Arabic was 5.8 ,viscosity was 9.45 CPS, relative density was 1.0095 gram/cm^3 and specific weight was 1.0074, in term of antibacterial activity, the aqueous solution of Arabic gum showed inhibition activity against E.coli and S. aureus , with highest inhibition zone at a concentration of 500 (8.7 and 11.2 mm) .The alcoholic solution exhibited the highest inhibition activity at a concentration of 400 (10.1 and 12.3 mm).Additionally, a synergistic effect was observed when combining Rifampcin with the aqueous solution against E.coli and S. aureus at a concentration of 500 (9 and 12 mm) and with the alcoholic solution at a concentration of 400 (11 and 15 mm). These finding suggest the potential use of Arabic gum in various pharmaceutical industries due to its inhibitory effect on pathogenic microorganisms and fungi .

Keywords*: Arabic gum, Treatment of bacterial infection, colon problems.*

Introduction

Gum Arabic is found in a solid state, with no smell but a bitter taste, obtained from acacia trees found in the Arab world, it can be taken orally and used in the manufacture of many foods and drinks such as soft drinks [**1**]. It is an edible biopolymer obtained when the outer bark of branches or stems is removed or when insects attack, secreting it as a defense mechanism. The gum comes out in the form of a tear, dries when exposed to the outside air and becomes a ball. The secretions are a non-viscous liquid, including many substances insoluble in water, such as resins, latex and chicle **[2]**. The gum contains a complex mixture of large molecules of different sizes, consisting mainly of carbohydrates and proteins [**3**]. Gum arabic is rich in soluble fibers. When decomposed by chemical methods, it is found to contain the compound Arabin, which is the calcium salt of arabic acid, with traces of magnesium salts. It solidifies with a pale brown to orange-brown color. The pieces become lighter in color after being crushed or broken, displying a transparent, crystalline appearance [**4**]. Arabic gums are polysaccharides characterized by their ability to form very viscous solutions at low concentrations. These gums are commonly used in food applications as stabilizers or gel-forming agents [**5**]. The benefits of gum Arabic for the colon are numerous, as it contains elements beneficial for the body and fibers. The colon is organs that often requires quick and effective treatment to alleviate severe pain. To learn how to use gum Arabic in treating the colon, refer to sources [**6-7**]. Gum Arabic aids digestion by reducing stomach acid, increasing mucus production in the stomach, and relieving symptoms of irritable bowel syndrome. Its contains healthy

fibers that help food pass through the colon more easily, enhancing its functions. Additionally, it can help relieve stomach ulcers due to its antibacterial properties, which may combat stomach bacteria. Gum Arabic also reduces constipation by increasing stool volume and improving bowel movement. It can lower harmful cholesterol (LDL) by binding to it in the intestines, preventing absorption. Furthermore, it regulates blood sugar by slowing the absorption of glucose in the intestines and boosts immunity by reducing inflammation and increasing white blood cell production. [**8, 9**]

Materials and methods

Acacia Gum collection

Acacia Gum also known as Arabic gum, was collected from local markets in Baghdad City from January to April. The plants that produce gum Arabic gum were randomly collected from three different locations in the local markets. They were then cleaned of impurities, washed thoroughly, and dried at room temperature. After drying, the gums were ground into a powder using an electric grinder and stored in dry containers until ready for use

Physical tests of Arabic gum

Viscosity estimation of Arabic gum:

The relative viscosity was determined using an Ostwald viscometer size C to estimate the viscosity of the gum. This was done by calculating the time required for the liquid to flow at a temperature of 25°C following the method mentioned [**10**].

Gum Arabic Refractive Index Estimation

The refractive index of gum Arabic was calculated by preparing suspensions of gum Arabic at concentrations of 1% and 2% using a refract meter at a temperature of 20°C according the method in reference [**11**].

PH number:

The viscosity of gum arabic was measured at various pH values ranging from 1-9. The sample solution was prepared at a concentration of 1% in different buffer solutions : 0.1 M citrate buffer solution at pH 2, 4, 6, 8, phosphate buffer solution at pH 7 and Tris-HCL buffer solution at pH 8, 10. This methods followed according to [**12**].

Density and specific gravity determination

The density of gum Arabic at concentrations of 1%, 2%, 3%, 4% and 5% was estimated using a density bottle at a temperature of 25°C as mentioned in [**13**].

Preparation of aqueous extracts

The hot aqueous extract of oak plant was prepared using the method **[14]** . One gram of dry gum Arabic powder was added 200 ml of distilled water in a 500 ml glass flask. The flask was then placed on a magnetic thermal heater set at a temperature of 40°C. The mixture was left to mix well using a magnetic stirrer for 24 hours to allow for extraction of the active substance in the sample. The solution was then filtered using Whatman No. 1 filter papers and a Buechner funnel connected to an air vacuum device . the filtrate was transferred a centrifuge and spun at a speed of 3000 rpm for 15 minutes to precipitate any suspended plant parts and obtain a clear solution, then drying the filtrate using a rotary evaporator at a temperature of 40 °C for 24 hours. This process was reported several times to obtain sufficient quantities of dry extracts . The resulting powder was weighed and stored in the refrigerator at a temperature of 4 °C until use.

Preparation of Alcoholic extract

The same steps were followed to prepare the aqueous extract, except for using 70% ethyl alcohol instead of distilled water. [**14**]

Antibacterial activity of aqueous and alcoholic solution of Acacia gum against pathogenic bacteria

Four isolates, two isolates from *E.coli* and two isolates from *Staphylococcus aureus* were selected for their multidrug resistance to determine the antibacterial activity of aqueous and alcoholic extracts of Acacia gum. The Acacia gum solution was prepared according to methods [**15**], dissolving 50 grams of fresh gum Arabic in 100 ml of sterile distilled water to achieve a final concentration of 500 mg/ml. The solution was left covered for 24 hours to complete the dissolution process before being sterilized through filtration. Concentrations of **100,200,300,400,** and **500** mg/ml were prepared from this stock solution, and stored in sterile bottles in the refrigerator until needed for biological activity. The agar well diffusion method was employed to assess the antibacterial activity of the Acacia gum aqueous and alcoholic solutions [**16-17**]. Bacterial suspensions were prepared by diluting the bacteria colonies in normal saline to match the turbidity of a standard McFarland tube (0.5) ,equivalent to 1.5×10^8 CFU\ml .One hundred microliters of the bacterial suspension were spread on the surface of Muller- Hinton agar using a sterile cotton swab. The plates were allowed to sit for 15 minutes to absorb the bacterial suspension. Aseptic holes were then made in the agar using a cork borer, and 0.5 ml of each prepared dilution (100-500 mg/ ml) of Acacia aqueous and alcoholic extract was placed in each hole using a micropipette with added distilled water. Rifampcin disk (5 µg) was used as positive control, with only to one hole serving as negative control.

Statistical Analyses

The results were subjected to statistical analysis to determine any significant differences. The analysis utilized two-factor experiments and the two-way analysis of variance (ANOVA) test, with the least significant difference (LSD) applied at a significance level of 5% **. [18].**

Results and Discussion

Physical properties of Arabic gum

The results of the physical properties of the gum arabic under study, including; viscosity, specific weight, pH and relative density are shown in (Table. 1**)**.

Table 1 shows the results of the physical properties of gum Arabic. The pH of gum Arabic was 5.8 , the viscosity was 9.45 CPS, and the specific weight was 1.0074.The relative density of the gum was 1.0095 gram/cm^3.

Table 1: The physical properties of gum arabic

Arabic gum sample	Relative density	pH number	Viscosity	Specific weight
1	1.0095	5.8	9.45	1.0074

The results of pH in the current study were agreement with previous research [**19**], which demonstrate that Acacia gum is an acidic extract and is considered one of the multiple acidic gums due to its content of calacturonic and chloroquinic acids. The results agreement with finding from another study [**20**], which showed that gum Arabic has a pH value of 5.9.

The results of viscosity were agreement with [**21**], proving that the difference in viscosity values between gums may be due to the difference in molecular weights of the gums. Higher molecular weight leads to higher viscosity. The results were also agreement with [**22**], who emphasized that the decrease in the viscosity of gum arabic is due to increased branching of the hydroxyl groups that make up the gum structure. Gum arabic is characterized by high solubility and low viscosity. The low viscosity of raw gum can be attributed to the low resistance of the gum to flow resulting from impurities in the main structure of the polysaccharide chains and

the presence of various metal ions that have a negative effect on viscosity. However, the results were in agreement with [**23**]. A viscosity difference was observed in Hashab gum, a type of gum arabic, with a viscosity range of 18.35- 53.5.

The results of relative density and specific weight was agreement with previous study [**24**], in that study, highest values of density and specific gravity of acacia gum were found to be 1.0131 and 1.0133 grams/cm^3, respectively. It is commonly observed that the specific gravity values of gums extracted from raw materials are typically higher than those of high purity gum arabic. Relative density serves as a measure of the degree of compact packing of large molecules in the gums. Additionally, the density values of the gum solution generally increase with higher concentrations of the gum being used.

Antibacterial activity of aqueous and alcoholic solution of Acacia gum against pathogenic bacteria

Table 2 shows the antibacterial activity of Arabic gum aqueous solution against *E.coli* and *S. aureus* determined using the well diffusion method. The highest inhibition activity of Arabic gum at a of concentration **500** was observed against the *E.coli* and *S. aureus* isolates with inhibition zones of (8.7 and 11.2 mm) ,respectively. Additionally, the synergistic effect of Rifampicin against *E.coli* and *S. aureus* was recorded at a concentration of 500 with inhibition zones of (9 and 12 mm) as shown in (**Table 2**)

Table 2: Antibacterial activity of Arabic gum aqueous solution against *E.coli* and *S. aureus*

Concentration mg/ml	***E.coli***	***E.coli***	**Rifampicin 5 µg**	***Con. average***	***S. aureus***	***S. aureus***	**Rifampicin 5 µg**	***Con. average***
100	**2.1**	**0**	**0**	**1.05**	**2.5**	**2.3**	**2**	**2.4**
200	**3.9**	**3.1**	**2**	**3.5**	**4.1**	**4.3**	**3**	**4.25**
300	**4.1**	**4.2**	**3**	**4.15**	**5.2**	**5.7**	**5**	**5.55**
400	**4.5**	**4.3**	**5**	**4.4**	**7.7**	**7.1**	**8**	**7.75**
500	**8.2**	**8.7**	**9**	**8.45**	**9.5**	**11.2**	**12**	**10.35**
Isolate average	**4.56**	**4.06**	**3.8**	**/**	**5.8**	**6.12**	**6**	**/**
p- value	**0.0001**			**0.0001**		**0.0001**		
LSD	**0.894 ****			**1.027 ****		**1.174 ****		
**** (P≤0.01).**								

Table 3 shows the antibacterial activity of Arabic gum alcoholic solution against *E.coli and S. aureus*. The highest inhibition activity of Arabic gum was observed at a concentration of 400 against the *E.coli* and *S. aureus* isolates with inhibition zones of (10.1 and 12.3 mm) , respectively. The synergistic effect of Rifampicin against *E.coli* and *S. aureus* was recorded at a concentration of 400 with inhibition zones of (11 and 15 mm) as shown in (**Table 3**)

Table 3: Antibacterial activity of Arabic gum alcoholic solution against pathogenic bacteria

Concentration	*E.coli*	*E.coli*	Rifampcin 5 µg	*Con. Average*	*S. aureus*	*S. aureus*	Rifampcin 5 µg	*Con. average*
100	**2.9**	**2**	**2**	**2.45**	**3.1**	**2.9**	**3**	**3**
200	**3.1**	**4.0**	**4**	**3.55**	**3.5**	**4.6**	**5**	**4.05**
300	**4.5**	**4.9**	**6**	**4.7**	**5.9**	**6.2**	**7**	**6.05**
400	**9.1**	**10.1**	**11**	**9.6**	**10.2**	**12.3**	**15**	**11.25**
500	**5.2**	**5.8**	**8**	**5.5**	**8.2**	**8.8**	**10**	**8.5**
Isolate average	**4.96**	**5.36**	**6.2**	**/**	**6.18**	**6.96**	**8**	**/**
p- value	**0.0001**			**0.0001**		**0.0001**		
LSD	**1.183 ****			**1.0775 ****		**1.207 ****		
**** (P≤0.01).**								

The results of the present study on gum arabic against *Escherichia coli* and Staphylococcus aureus showed an effective inhibitory effect. There was a difference in antimicrobial activity between the aqueous and alcoholic extracts (table 2 and 3). Agar tests revealed that the alcoholic extracts of gum arabic had the highest activity against gram-positive bacteria and also demonstrated excellent activity against gram-negative bacteria compared to the aqueous extract. These results agreement with previous studies[**25,26**]. It has been proven that gram-negative bacteria differ from gram-positive bacteria due to the morphological differences between these microscopic organisms. Gram-negative bacteria contain an outer membrane of phospholipids that carries the structural components of lipopolysaccharides, making the cell wall impermeable to fat-loving dissolved materials. This is unlike the structure of the wall of gram-positive bacteria, which is more exposed because it contains only an outer layer of peptidoglycan that does not form an effective permeability barrier. The results were agreement with [**27**], who found the diameter rates increase as the extract concentration increases, The average diameter of bacterial colony inhibition was between 10.33-15.1 mm in the alcoholic extract treatments, while in the aqueous extract treatments, the average diameter of bacterial colony inhibition was between 9.1-13 mm . The results show that the alcoholic extract of gum Arabic is superior to the aqueous extract of the same gum in inhibiting bacterial growth. These results are agreement with [**28**] who found that the antimicrobial properties of gum arabic are mainly due to secondary metabolites such as saponins, glycosides, saponin, hydrolyzable tannin, volatile oil, triterpenoids, phenols and alkaloids. Gum arabic also contains a variety of chemical compounds such as neutral sugars arabinose, galactosic acids like 4-methoxyglucuronic acid and glucuronic acid, magnesium, calcium, sodium, and potassium. Due to its high terpene content, gum arabic has antibacterial properties. Therefore using it with antibiotics for synergistic effect increases its effectiveness against microbes and fungi.

Conclusion

The potential use of Arabic gum in various pharmaceutical industries is due to its inhibitory effect against pathogenic microorganisms, fungi, bacteria found in soft drinks. Additionally, Arabic gum possesses important functional properties, such as pH of 5.8 and viscosity of 9.45 CPS. Studies have shown that both aqueous and alcoholic solutions of gum arabic exhibit antibacterial activity against variety of foodborne bacteria. The size of the inhibition zone created by the alcoholic extract varied depending on the concentration used. Furthermore, the combination of the antibiotic rifampicin with gum arabic was found to have synergistic and effective effect.

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