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Effect of gum Arabic and arginine on antioxidant activity and the growth of *Lactobacillus* in rats

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ABSTRACT

The aim of this study was to determine the antioxidant activities and their effect on the numbers of *Lactobacillus* of dietary gum arabic and arginine supplemented in female rat. In this study, the common test for measuring antioxidant activity of the plasma was evaluated using 2, 2-diphenyl-l-picrylhydrazyl assay (DPPH. The active compounds were detected using GC-mass and the *Lactobacillus* bacteria was evaluated by detecting them in the MRS culture medium, agar. The results showed that there were significant differences between the studied groups, where the highest (87.00 ± 2.00) rate of antioxidants was recorded in the group of gum Arabic and arginine, while the lowest (40.67 ± 2.51) percentage was recorded in the positive control group. The results of GC-mass also showed the presence of many active compounds in the extract of Arabic gum that act as antioxidants, microorganisms, allergens, and anti-cancer. The GA + AA group also recorded the highest average number of *Lactobacillus* bacteria (240.66) compared to the rest of the other groups. These findings support the notion that the gum arabic a good source of bioactive compounds. From the study it was observed that there was a significantly increased ($p \leq 0.05$) in plasma total antioxidant capacity and increase the growth of *Lactobacillus* in rat after the consumption of gum arabic and arginine.

Keywords: Antioxidant activity, Gum Arabic, Bioactive compounds, *Lactobacillus*

Introduction

Gum Arabic is known as a food fiber that is soluble in water and edible and is made of heterogeneous polysaccharides. It is formed in the form of dry mucilage secretions on the stems and branches of *Acacia Senegal* and *Acacia Sial* trees. It is also known as gum acacia, and it is rich in calcium, potassium, and magnesium(1). It has been used in the treatment of a variety of diseases, such as kidney failure, liver and heart disease, anemia, and diabetes. It has been used orally as a healthy anti-diarrheal and anti-inflammatory substance for the intestinal mucosa and skin infections and to improve the work of the digestive system. (2,3). Gum arabic is a probiotic that is widely used in human remedies and folk medicine and is used to treat

intestinal inflammation.(4). Stimulates the growth of normal gut bacteria (beneficial bacteria) and acts as a prebiotic(5). It has an antibacterial effect(6). The Scientific Board on Probiotics and Prebiotics defines beneficial bacteria that may also be called probiotics, Probiotics are defined as live, beneficial, and harmless microorganisms that give the host body various health benefits. They are necessary for digesting food and preventing harmful pathogenic bacteria from invading the body(7). They are also a vital stimulus for the immune system and its maintenance. It works to extract nutrients and manufacture some essential vitamins(8). The aim of the study is to detect the active compounds contained in the gum Arabic extract using the (GC-MS) technique, and to detect the activity of antioxidants.

Materials and methods

Experimental design

Experimental animals

Studies were carried out using thirty female rats each weighing between 150-200g. They were obtained from the animal house of the college of Science, University of Thi-Qar. The animals were acclimatized to laboratory condition for a week before commencement of the experiment. The rats were dosed daily with 10% of gum Arabic extract and arginine for four weeks

1- The first group ; Negative control
2- The second group; Positive control
3- The third group; Gum arabic at a concentration of 10% of body weight in an amount of 1 ml.
4- The fourth group; Arginine injected 35 mg/kg of body weight.
4- The fifth group ; Gam Arabic + Arginine
5-

Gas chromatography-mass spectrometry (GC-MS)

GC-MS analysis was performed in the methanol extract of gum Arabic extract by a GC-MS system (Shimadzu QP2010PLUS) equipped with a capillary column (30 m x 0.25 mm ID x 0.25 µm film thickness), Split-less injections were performed with a purification time of 0.1 min. The carrier gas was helium, with a flow rate of 1 ml per minute. The temperature of the column was maintained at 50°C for 3 minutes, then programmed at 5°C min-1 to 80°C, then at 10°C min-1 to 340°C. The inlet temperature was 250 °C, the detector temperature was 340 °C, and the solvent delay was 4 minutes. Peak identification was based on computer matching of mass spectra with the National Institute of Standards and Technology library (NIST 08 and NIST 08S) and by direct comparison with the data (9). .

Antioxidant extraction

(15 µ) of PCA) was added to remember 300 µL of pre-transfer data 5 minutes before the centrifuge at a speed of 13,000 rpm. After that, 64 µL of potassium hydroxide KOH was added to the filtrate after dissolving it in 100 ml of water, then mixing by vortex, centrifuging at 13000 rpm for 15 minutes, and removing the filtrate as antioxidants.

DPPH radical scavenging activity

The determination of antioxidant activity through the 2,2-diphenyl-1-picrylhydrazyl (DPPH) scavenging system was carried out according to the methods (10). The stock solution was prepared by dissolving 40 mg DPPH in 100 ml methanol and keeping it at -20°C until used. About 350 mL stock solution was mixed with

350 ml methanol to obtain an absorbance of 0.70±0.01 unit at 516 nm wavelength by using a spectrophotometer (Epoch, Biotek, USA). About 100 µL sample with a 1 ml methanolic DPPH solution prepared were kept overnight for scavenging reactions in the dark. The percentage of DPPH scavenging activity was determined as follows: DPPH scavenging activity (%) = [(A blank -A sample) / A blank] × 100. Where A is the absorbance.

***Lactobacillus* bacteria**

Transfer approximately 1 g of the stool sample into a test tube containing 9 mL of liquid MRS medium sterilized and after growing them in the incubator at a temperature of 37 ° C for 24 h under anaerobic conditions. Transfer 1 ml from the growth of 9 ml of sterile liquid MRS medium to incubate again under the same conditions, it was repeated process three times. Then, serial dilutions were made with peptone water, and 1 ml of each dilution was cultured in prepared solid MRS medium and then incubated at 37 °C for 48 h (11).

Statistical analysis

The results were expressed as mean ± standard error (n = 6). The data were analyzed statistically by one way ANOVA and different group means were compared by Duncan's multiple range test. For all analyses, P values ≤ 0.05 were considered significant.

Results and Discussion

Phytochemicals identified by GC-MS of gum Arabic extract

Gas chromatography-mass spectrometry (GC-MS) analysis of an extract of gum Arabic revealed the presence of various groups of bioactive compounds (Figure 1). The bioactive compounds with their retention time (RT), molecular formula, area, molecular weight, and biological activity are exhibited in Table 1. 2,4-Dimethoxycinnamic acid(Cytotoxic activity), Acetylenedicarboxylic acid(Antibacterial potency), Butanedioic acid(Antimicrobial), 2,4,5-Trihydroxypyrimidine(Antioxidant, Anticancer, Antibacterial), 9-Octadecenal(Antibacterial), 2,4-Dimethoxycinnamic acid(Antioxidant, Anti-inflammatory), Lupulon(Antimicrobial), Catechol(Antimicrobial), β-Carotene(Antioxidant), Benzoic acid 2-methylpentyl ester(Antimicrobial), Astaxanthin(Antioxidant, Anticancer), 7-Methyl-Z-tetradecen-1-ol acetate(Anti-inflammatory , Antibacterial), Lycopene(Antioxidant), Betamethasone(Antimicrobial), Oleic Acid(Anticancer, Antimicrobial).

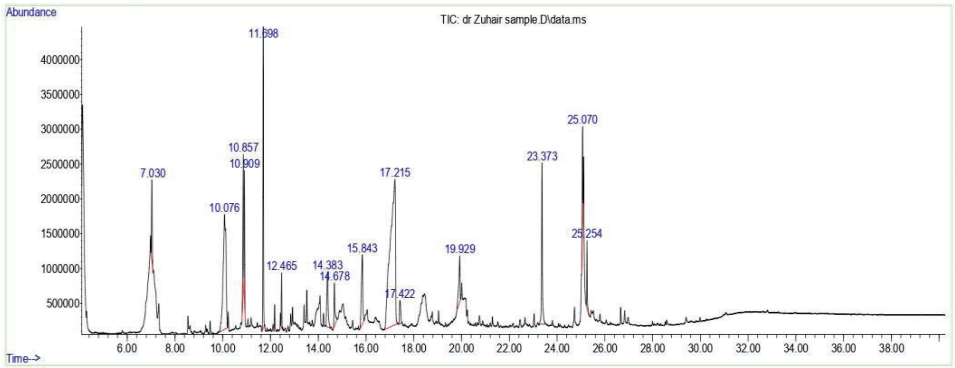

Figure 1: Gas chromatogram of methanolic Acacia sengal extract

Table 1: The active chemical compounds that were identified using GC- mass in the extract of gum arabic

o.	Compound name	Formula	RT	Area %	M. W	Biological Activity
1	2,4-Dimethoxycinnamic acid	$C_{11}H_{12}O_4$	7.03	0.256685	208.2	Cytotoxic activity
2	Acetylenedicarboxylic acid	C_4H2O_4	10.07	0.284901	114	Antibacterial potency
3	Butanedioic acid	$C_4H_6O_4$	10.85	0.207004		Antimicrobial
4	2,4,5-Trihydroxypyrimidine	$C_5H_5NO_3$	10.90	2.255241	170.1	Antioxidant, Anticancer, Antibacterial
5	9-Octadecenal	$C_{18}H_{34}$	11.69	0.212967	250.5	Antibacterial
6	2,4-Dimethoxycinnamic acid		12.46	0.192331		Antioxidant, Anti-inflammatory
7	Lupulon	$C_{26}H_{38}O_4$	14.38	1.504738	414.6	Antimicrobial
8	Catechol	$C_6H_6O_2$	14.67	0.557845		Antimicrobial
9	β-Carotene	$C_{40}H_{56}$	15.84	0.183511	536.9	Antioxidant
10	Benzoic acid 2-methylpentyl ester	$C_{13}H_{18}O_2$	17.25	0.814571	206	Antimicrobial
11	Astaxanthin	$C_{40}H_{52}O_4$	17.42	0.322663	596.8	Antioxidant, Anticancer
12	7-Methyl-Z-tetradecen-1-ol acetate		19.92	3.179591	268.4	Anti-inflammatory , Antibacterial
1	Lycopene	$C_{40}H_{56}$	23.3	3.4573	536.	Antioxidant

3			7	62	9	
14	Betamethasone	$C_{22}H_{29}FO_5$	25.07	1.620588	392.5	Antimicrobial
15	Oleic Acid	$C_{18}H_{34}O_2$	25.25	0.449054	282.4	Anticancer, Antimicrobial

Table2: Antioxidants activity of extract of Gum Arabic and arginine

Groups	DPPH %
G1	40.67 ± 2.51 [e]
G2	54.00 ± 3.00 [d]
G3	75.67 ± 1.52 [b]
G4	66.33 ± 3.05 [c]
G5	87.00 ± 2.00 [a]

a-e Mean with different letters within each column are significantly different (P < 0.05).

as the highest percentage was recorded for the gum arabic and amino acid treatment (87.00 ± 2.00), while the lowest percentage was in the control treatment (40.67 ± 2.51). It was also noted that the gum arabic treatment was superior to the amino acid treatment and the control treatment. The reason for this may be due to the fact that gum arabic contains effective compounds that act as antioxidants, such as (2,4,5-Trihydroxypyrimidine), (2,4-Dimethoxycinnamic acid), (β-Carotene), Astaxanthin), (Lycopene). These results agreed with the results of (11), where the highest percentage was recorded in the group treated with gum arabic and olive leaves, which amounted to (12.80 ± 1.41).

Table3: The number of *Lactobacillus*

Groups	Numbers of colonies
G1	27.67 ± 3.51 e
G2	48.67 ± 5.03 d
G3	116.67 ± 4.50 b
G4	81.67 ± 7.02 c
G5	240.67 ± 11.01 a

[a-e] Mean with different letters within each column are significantly different ($P < 0.05$).

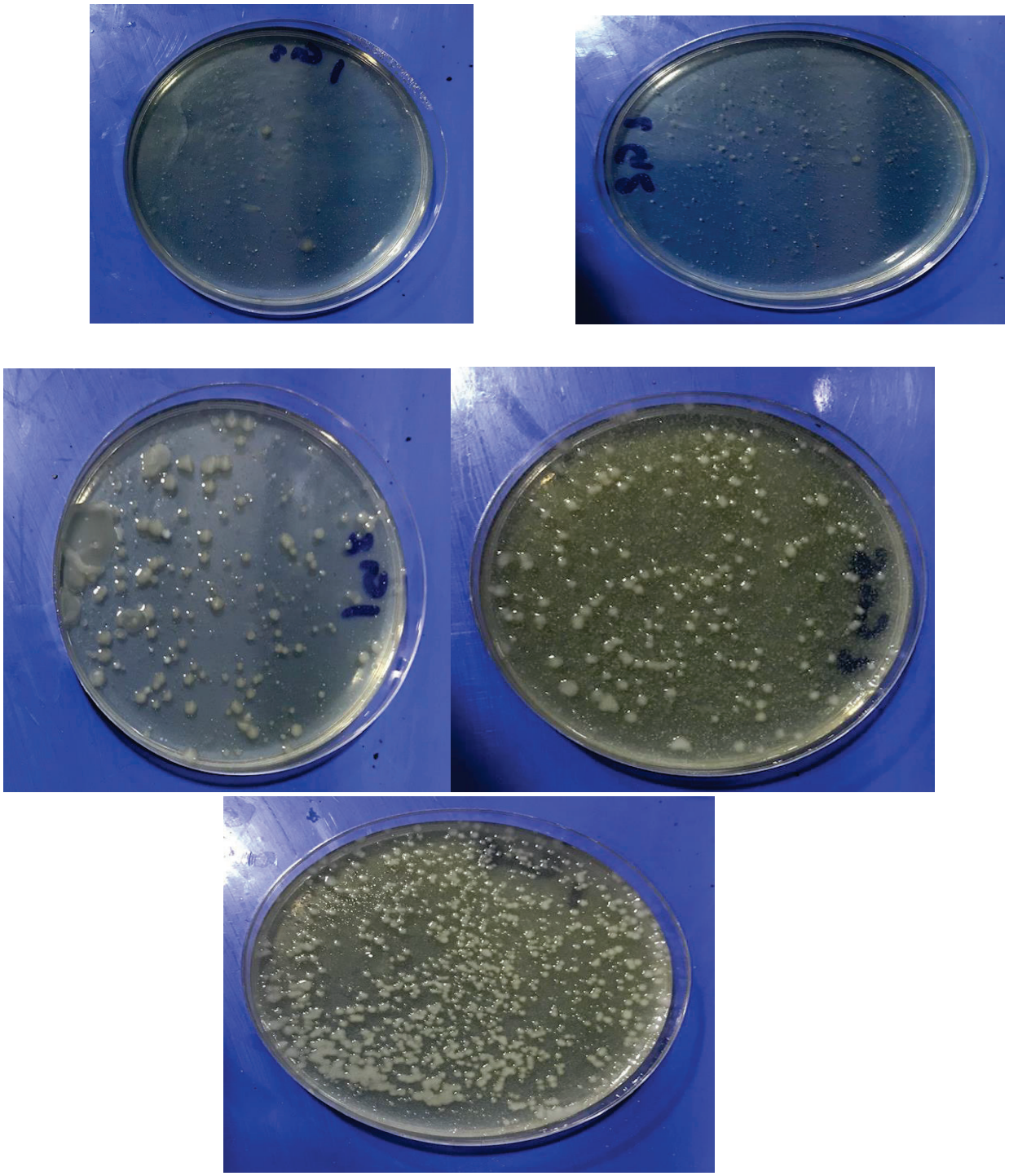

Figure1: The number of *Lactobacillus* colonies; A Group Positive control, B Group Negative control, C Group GA, D Group AA and E Group GA + AA

The results of the statistical analysis of the number of colonies of *Lactobacillus* bacteria in Table 3 showed that there were significant differences between the studied treatments, as the highest number of colonies of *Lactobacillus* bacteria was recorded in the treatment of gum arabic and amino acids (240.67±11.01), while the lowest percentage was in the control treatment (27.67±3.51). It was also noted that the gum arabic treatment was superior to the amino acid treatment and the control treatment. The reason for this may be due to the fact that gum arabic contains polysaccharide dietary fibers that are used as food for *Lactobacillus* bacteria, which enhances their activity and resistance to harmful bacteria inside the body, leading to an increase in the number of colonies. These results are consistent with the findings of (12), where it was shown that gum stimulates the growth of *Lactobacillus* bacteria and acts as a prebiotic.

Conclusion

In the current study that was conducted on the use of GA and amino acids to evaluate the effectiveness of antioxidants using the DPPH test and to promote *Lactobacillus* bacteria, the results of this study proved that gum arabic contains many biologically active compounds and their antioxidant activities, as well as the promotion of *Lactobacillus* bacteria and their resistance to harmful bacteria inside the body.

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