JMBFS

Journal of Microbiology, Biotechnology and Food Sciences

International peer-reviewed scientific online journal

Published by Faculty of Biotechnology and Food Sciences

ANTIMCROBIAL ACTIVITY OF LICORICE (*Glycyrrhiza glabra*) EXTRACT AGAINEST SOME PATHOGENIC MICROORGANISMS AS A PRESERVATIVE AGENT IN MANGO NECTAR AS AN ALTERNATIVE TO SODIUM BENZOATE

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https://doi.org/10.55251/jmbfs.12298

ARTICLE INFO

Received 27. 1. 2025
Revised 2. 5. 2025
Accepted 9. 5. 2025
Published 1. 6. 2025

Regular article

OPEN ACCESS

ABSTRACT

Chemical preservatives cause great concern due to their unhealthy effects, and plant extract could be used as a natural antimicrobial alternative to chemical preservatives. The objective of the present research is to investigate the use of licorice extracted using 80% ethanol solution as a natural preservative in mango nectar and its impact on the physicochemical and sensory characteristics. The bioactive compounds in licorice extract and their antimicrobial activity were determined utilizing LC-MS/MS, HPLC, and disc diffusion techniques. The effect of licorice extract on mango nectar's physicochemical, sensory, and microbiological properties was examined. The results showed that the major antimicrobial compounds in licorice extract were ellagic acid, quercetin, rutin, and catechin. The extract exhibited substantial antibacterial action, effectively suppressing the development of all tested harmful bacteria, such as Bacillus *subtilis*, *Staphylococcus aureus*, and *Pseudomonas aeruginosa*, except *Escherichia coli*, where its effect was less pronounced. Additionally, licorice extract had comparable efficacy to mango nectar treated with sodium benzoate in reducing the overall bacterial count at 1% concentration. Furthermore, it fully suppressed fungal growth at all concentrations for seven days. The physicochemical and sensory properties of the mango nectar treated with licorice extract varied negligently compared to sodium benzoate treatments. Licorice extract is an effective natural preservative for mango nectar, showing comparable efficacy to sodium benzoate in controlling microbial growth while maintaining the physicochemical and sensory properties of the juice.

Keywords: Natural antimicrobial; chemical antimicrobial; licorice; HPLC; polyphenols; fruit juice

INTRODUCTION

A growing number of scientific researches indicate that artificial chemicals used as food additives might have harmful consequences on health, such as obesity **(Ul-Haq, Z et al 2012)**, cancer **(Pogoda, J.M., & Preston-Martin, S. 2001),** asthma **(Oo, K. M. 2019),** and attention deficit disorder-related hyperactivity **(Schaefer, A. 2019).** Certain chemicals used in food can disrupt hormone function and stunt growth and development. It is among the elements causing the high prevalence of obesity in children. Children are more prone than adults to eat specific types of dietary intakes **(Inetianbor, J.E et al 2015; Sharma S. 2015; Gupta R & Yadav R.K. 2021).** Sodium benzoate is used with caution in soft drinks since it has the propensity to undergo a chemical reaction and transform into benzene, which is a well-known cancer-causing substance **(Azuma, S. L et al 2020).**
Cellular damage is one of the health risks associated with sodium benzoate **(Sunitha J & Preethi R. 2000).** A high dietary sodium benzoate intake in children may be related to allergies, asthma, or attention deficit hyperactivity disorder. Benzoate has cognitive benefits by enhancing the activity of N-methyl-D-aspartate (NMDA) receptors in the brain. It achieves this by acting as a competitive inhibitor of the enzyme D-amino acid oxidase (DAAO), hence reducing the breakdown of D-serine catalyzed by DAAO. Consumption of high levels of benzoate can also lead to glycine deficiency, which is a common disorder of the brain's neurochemistry **(Magomya, A.M et al 2020; Piper, P. W. 2018).** In addition, benzoate may cause chromosome damage and the formation of micronuclei in lymphocytes, leading to mutagenic and toxic effects **(Pongsavee, M. 2015; Saatci, C et al 2016, Gaur et al. 2018)** strongly suggest that increasing concentrations of sodium benzoate cause harm to vertebrates **(Gaur, H et al 2018).** Furthermore, **(Zeghib & Boutlelis 2021** and **Khodaei et al 2019)** suggest that sodium benzoate may induce nephrotoxic substances **(Zeghib, K & Boutlelis, D. A. 2021; Khodaei, F et al 2019).**
Both conventional and elective pharmaceuticals have a long history of utilizing therapeutic plants. Later studies have upheld the bioactivities of natural compounds, including their antibacterial properties **(Gavarić, N et al 2015; Bouarab Chibane, L et al 2019).** Licorice, a widely available herb, has been used in traditional Chinese medicine for centuries. More than 20 triterpenoids and almost 300 flavonoids have been discovered in licorice. Recent studies have shown that these metabolites possess various pharmacological activities, such as antibacterial, antiviral, anti-inflammatory, anticancer, and other qualities **(Wang, L et al 2015; Dinteren, S et al 2022).** *Glycyrrhiza glabra* Linn has antioxidant and antibacterial properties **(Dong, Y et al 2014; Martins, N et al 2015).** Root and leaf extracts tested positive for gram-positive bacteria and illustrated antifungal activity against *Candida* in a dose-dependent way **(Irani, M et al 2010; Gupta, V. K et al 2008; Sultana, S et al 2010).** Regarding two gram-positive (*Staphylococcus aureus* and *Bacillus subtilis*) and two gram-negative (*Pseudomonas aeruginosa* and *Escherichia coli*) bacteria, licorice extracts showed substantial antibacterial activity **(Nitalikar, M.M et al 2010).** Licorice extract can be suggested as a natural antioxidant and antibacterial agent for use within the food sector and may have pharmacological promise in the control of breast cancer **(Hamad, G. M et al 2020).** So, the current work has focused on the use of a natural and safe agent (licorice extract) as an antimicrobial against some pathogenic bacteria and its application in mango nectar, which is more commonly used, especially by children.

MATERIAL AND METHODS

Materials

Licorice (*Glycyrrhiza glabra*), fully ripe mango fruits "Sukaria" (*Mangifera indica*), and sugar were obtained from supermarkets in Cairo, Egypt.

Pathogenic strains

Pathogen bacteria, including two Gram-negative (*P. aeruginosa* (ATCC27853) and *E. coli* (ATCC25922)) and two gram-positive (*B. subtilis* ATCC6633 and *S. aureus* (MRSA) ATCC43300).

Chemicals and reagents

All chemicals and reagents utilized in this study were of analytical grade. The chemicals citric acid, sodium benzoate, and bacteriology media were acquired from El-Gamhouria Trading for Chemicals and Drugs Company, located in Cairo, Egypt.

Preparation of licorice extract

A quantity of 100 grams of licorice was introduced into an excessive amount of distilled water containing ethanol in a ratio of 8 parts ethanol to 2 parts water by volume (80%). The mixture was then left at room temperature for the night. Subsequently, the mixture was filtered. The aqueous extract was concentrated using a rotary evaporator 60 ºC at reduced pressure, and the residual compounds were dissolved in 50 mL of distilled water.

LC-ESI-MS/MS analysis of licorice extract profile

Instrument

The sample analysis was performed using liquid chromatography-electrospray ionization–tandem mass spectrometry (LC–ESI–MS/MS), using a 3200 QTRAP mass spectrometer (AB Sciex, Framingham, MA, USA) linked to a column oven and a binary gradient solvent pump, an autosampler, and a degasser, an Agilent 1200 Series HPLC system (Agilent Technologies, Santa Clara, CA, USA) was used **(El-Ssayad, M. F et al 2023).**

Positive ionization mode:

Separation was conducted using an Ascentis® Express 90 Å C_{18} Column (2.1 × 150 mm, 2.7 µm). The mobile phases included two eluents: (A) 5 mM ammonium formate, pH 3, and (B) acetonitrile (LC grade). Using a recently devised, straightforward, and quick approach that combined tandem mass spectrometry, electrospray ionization, and liquid chromatography, the concentrations of free flavonoid aglycones were ascertained. On a SB-C_{18} column, the compounds were separated at 25 °C. There was a 5 µL injection volume. Acetonitrile (B) and water with 0.1% HCOOH (A) were used for gradient elution. The 200 µL/min flow rate was used **(El-Ssayad, M. F et al 2023).**

HPLC analysis of licorice extract

HPLC analysis was performed utilizing an Agilent 1260 series. The separation was conducted utilizing Eclipse C18 column (4.6 mm x 250 mm i.d., 5 µm) as described by **(Abdel-Aziz *et al*., 2021).**

Antimicrobial activity and minimal inhibitory concentration

Following the guidelines of the disk diffusion assay protocol, 1 mL of each strain culture was spread on the top surface of the media. Each agar plate was adorned with six aseptic paper disks (6mm), meticulously placed on its surface. Subsequently, these disks were impregnated with 15 µL of licorice extract at various doses. The plates were incubated at a temperature of 37 °C for duration of 24 hours. The antibacterial activity of the licorice extract was evaluated by measuring the minimum inhibitory concentration (MIC). The lowest concentration of the diluted extract that resulted in the formation of an inhibitory zone around a disc during a 24-hour incubation period was determined by calculation. The negative controls consisted of discs that were immersed in sterile distilled water.

Preparation of mango nectar

Mango fruits were cleaned carefully by washing with potable water and then peels and seeds were removed. Fruits were cut into small pieces and meshed in an electric mixer to obtain mango pulp. Mango nectar was prepared by mixing mango pulp (40%) with white sugar (10 %), 0.5% citric acid and 0.5% CMC. The mixture was agitated incessantly until it reached a state of complete uniformity. The formula-2 contained 1% of licorice extract, while the formula-3 had 0.1% of Sodium benzoate. Next, the mango nectar was transferred into glass bottles that had been sterilized, and then it underwent pasteurization at a temperature of 80 ±2°C for duration of 5 minutes **(Lima-Filho et al 2014).**

Physicochemical analysis

The pH was determined using a digital pH meter (model 3505-JENWAY-UK) that was calibrated using pH 4.0 and 7.0 buffers. The filtered samples were analyzed for total soluble solids (TSS) using an MA871 digital refractometer (Milwaukee 0 to 85% Brix – Romania) at a temperature of 25°C. Prior to measuring the samples, the apparatus was calibrated using distilled water. The specimen was placed onto a prism of the refractometer and the total soluble solids (T.S.S) were measured directly. The AOAC 2012 method was used to determine the total titratable acidity (TTA) of mango nectar. Initially, 10 milliliters of the juice was measured and then mixed with 90 mL of distilled water **(Horwitz, W. 1975).** The mixture was then titrated with 0.1 mol/L NaOH, using phenolphthalein as an indicator **(Da Silva, L et al 2014).** The data are reported as a percentage of citric acid, with a molecular weight of 64. The provided equation was utilized for the calculation of titratable acidity: $\%\ acidity = \frac{N \times V1 \times Eq.wt.}{V2 \times 1000} \times 100$

Where, N = normality of NaOH (mEq/mL); V_1 = volume of titrant (mL); Eq. wt. = equivalent weight of acid (; V_2 = volume of sample (mL); 1,000 = factor relating milligrams to grams (mg/g) (1/10 = 100/1000).

Microbial assay

The Herrera 2001 method was employed to determine the total microbial count. A 10ml sample of mango nectar was obtained and subsequently combined with 90 ml of water peptone solution at a ratio of 1:10 **(Herrera, A.G. 2001).** Following the homogenization process, 1 mL of the substance was combined with 10 mL of total plate count agar medium in a sterilised Petri plate. Afterwards, the mixture was carefully mixed at a temperature of 45°C. In addition, the samples underwent incubation at a temperature of 37°C to assess and quantify the number of colonies that developed on each Petri dish. The recorded data consisted of the count of colonies detected inside a 1 mL sample, expressed as the logarithm of colony-forming units per millilitre (log cfu/mL). The identification of yeasts and moulds was conducted using identical experimental methods as the overall microbiological count, with the only distinction being the substitution of total plate count agar with a potato dextrose agar medium **(Herrera, A.G. 2001).**

Sensory evaluation

The sensory analysis was performed using a composite score scale in accordance with A panel of ten individuals, who underwent limited training from the department of Food Science and Technology at Al-Azhar University in Egypt, assessed the sensory attributes of mango nectar. An evaluation was conducted to assess the taste (30), odour (30), colour (20), texture (20), and acceptability (score 100) of three different treatments **(Chan, J. & Cavaletto, C. 1982).**
Every assessor in the sensory panel provided written consent to participate in the research project. Prior to the commencement of the study, participants were provided with prior notice on the objective and methodologies of the research. Participants were guaranteed that their data would be maintained in strict confidence. Enrollment in the research was optional, and participants had the freedom to discontinue their participation at any point. The participants exhibited remarkable physical well-being and had no documented sensitivities to the constituents. Obtaining institutional approval for the research is not required or essential because of the inherent characteristics of the food and drink being studied.

Statistical analysis

The experiments were replicated a minimum of three times, and the results are shown as the average ± standard deviation (SD). An analysis of variance (One Way ANOVA), was conducted to examine the disparities among the samples.

RESULTS AND DISCUSSION

In the current study, the licorice extract was subjected to an LC-MS/MS analytical scan to examine the active components shown in **Table 1 and Figures 1, 2**.

Table 1 Active components of licorice extract have been analyzed using LC-MS/MS

Compounds	Area	Chemical formula
Malic acid	9.07×10^8	$C_4H_6O_5$
Caffeic acid	6.69×10^8	$C_9H_8O_4$
Glycyrrhizate	1.63×10^9	$C_{42}H_{62}O_{16}$
Naringenin	6.08×10^8	$C_{15}H_{12}O_5$
Daphnetin	4.87×10^8	$C_9H_6O_4$
Thymidine 5' monophosphate	6.17×10^8	$C_{10}H_{13}N_2O_8P$
Riboflavin 5' monophosphate sodium Salt	5.16×10^8	$C_{17}H_{21}N_4O_9PNa$
Sinapoylmalate	8.8×10^8	$C_{17}H_{20}O_9$
Citramalate	7.49×10^8	$C_6H_{10}O_5$
Trans-cinnamate	4.47×10^8	$C_9H_8O_2$
L-Iditol	4.56×10^8	$C_6H_{14}O_6$
Apigenin	9.78×10^8	$C_{15}H_{10}O_5$
Cytidine 5' diphosphate	7.85×10^7	$C_9H_{15}N_3O_{11}P_2$
Maltitol	2.87×10^7	$C_{12}H_{24}O_{11}$
Methyl Xanthine	1.67×10^9	$C_7H_8N_4O_2$

The results showed that licorice extract has a high concentration of positive and negative charged chemicals, including caffeine, caffeic acid, glycyrrhizate, naringenin, daphnetin, thymidine 5' monophosphate, riboflavin 5' monophosphate sodium salt, sinapoylmalate, citramalate, trans-cinnamate, L-iditol, apigenin, cytidine 5' diphosphate, maltitol and methyl xanthine. These bioactive compounds

found in the licorice extract of *Glycyrrhiza glabra* contribute to its antimicrobial activity **(Quintana, S.E et al 2020).** In addition to its antimicrobial properties, licorice has antiviral and anticancer properties. Various phytochemicals, such as saponins, terpenoids, flavonoids, polyamines, and polysaccharides, are display in this species. Within the Glycyrrhiza class, there are over thirty species, most of which have not received much attention regarding their phytochemical or pharmacological properties. The major peaks of the NMR and MS spectra of glycyrrhizin, 4-hydroxyphenyl acetic acid, and glycosidic conjugates of liquiritigenin/isoliquiritigenin were identified as those that contributed to the species discrimination, according to multivariate analysis data from Glycyrrhiza uralensis, *Glycyrrhiza glabra*, Glycyrrhiza inflate, and Glycyrrhiza ivantata. When key metabolites were analysed using LC-MS/MS, cadeverine an amino acid display was discovered inside the roots of G. inflata **(Farag, M.A et al 2012).**
The licorice extract has been subjected to HPLC analysis to discover the bioactive substances that are responsible for its antimicrobial properties. The bioactive compounds of licorice that have been analyzed by HPLC **Table 2 and Figures 3, 4** have previously reported for their antimicrobial properties against varieties of pathogenic bacteria and fungi. The results have showed that the licorice contained Ellagic acid compound at the highest level 410.84 µg/ml followed by Catechin with the concentration of 91.47µg/ml. The licorice has also showed high concentrations of Rutin, Querectin, Chlorogenic acid, Naringenin, and Daidzein compounds which record 51.15, 21.22, 17.17, 15.87, 14.05 µg/ml, respectively. Gallic acid, Methyl gallate, Coumaric acid, Pyro catechol, Syringic acid, Coffeic acid, Apigenin, Vanillin, Hesperetin, Kaempferol, Ferulic acid and Cinnamic acid were found at gradually descending concentration respectively, the other antioxidant compound concentration was in the range 9.41-0.26 µg/ml. According to Pastorino *et al*. (2018), licoriceis one of the species that is widely used in feed and food products **(Pastorino, G et al 2018).** Licorice contains around 300 different types of flavonoids and over 20 different forms of triterpenoids, which are responsible for a wide range of antioxidant, anti-inflammatory, antibacterial, and antiviral properties **(Wang, L et al 2015, De et al. 2018)** demonstrated that ellagic acid has antibacterial and therapeutic effect against H. pylori infection. Bacterial cell membranes' structural disarray was the cause of the antibacterial properties of querectin, naringenin, and catechin **(Veiko, A.G et al 2023).**

Table 2 The bioactive compounds of licorice extract according to HPLC analysis.

Bioactive compound	Area	Conc. (µg/ml)
Gallic acid	108.97	9.41
Chlorogenic acid	125.36	17.17
Catechin	369.35	91.47
Methyl gallate	127.43	6.96
Coffeic acid	27.95	2.15
Syringic acid	33.08	2.24
Pyro catechol	27.82	4.00
Rutin	440.46	51.15
Ellagic acid	2216.27	410.84
Coumaric acid	182.79	5.77
Vanillin	15.67	0.69
Ferulic acid	5.05	0.35
Naringenin	131.62	15.87
Daidzein	227.47	14.05
Querectin	154.09	21.22
Cinnamic acid	14.24	0.26
Apigenin	15.87	1.21
Kaempferol	4.95	0.38
Hesperetin	8.74	0.51

Antimicrobial activity of licorice extract

The antibacterial efficacy of licorice extract was evaluated using the agar disk diffusion method. The table displays the diameter (in millimeters) of the inhibition zone caused by licorice extract against various pathogenic organisms. The results demonstrate that the licorice extract displays potent inhibitory effects against *P. aeruginosa* (26 mm), *S. aureus* (18.5 mm), and *B. subtilis* (16 mm), although it's inhibitory activity against E. coli is modest (9 mm). The study investigated the minimum inhibitory concentration (MIC) of licorice extract against *S. aureus, B. subtilis, P. aeruginosa, and E. coli*. The concentrations tested ranged from 100% to 1.56%. The findings are displayed in Table 3 and Figure 5. The minimum inhibitory concentration (MIC) of licorice extract against S. aureus, *B. subtilis, and P. aeruginosa* was determined to be 1.56%. The excellent antibacterial activity of licorice extract was demonstrated by the observed inhibitory zones measuring 11 mm, 8.5 mm, and 16 mm, respectively. In contrast, *Glycyrrhiza glabra* extract exhibited a 25% minimum inhibitory concentration (MIC) against *E. coli*, resulting in an inhibition zone measuring 7.5 mm. The strong antimicrobial properties of *Glycyrrhiza glabra* extract can be attributed to its polyphenolic compounds as shown in **table 3**.

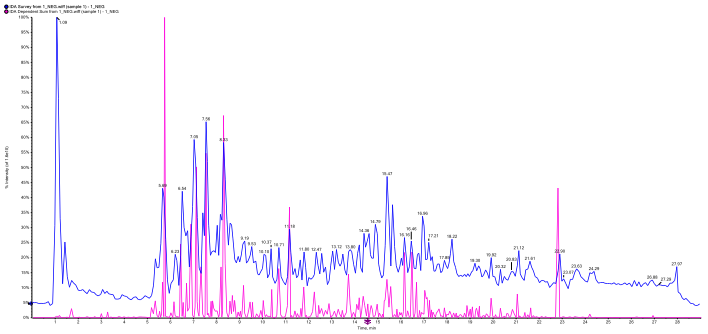

Figure 1 LC-MS/MS analytical scan of licorice extract- NEG

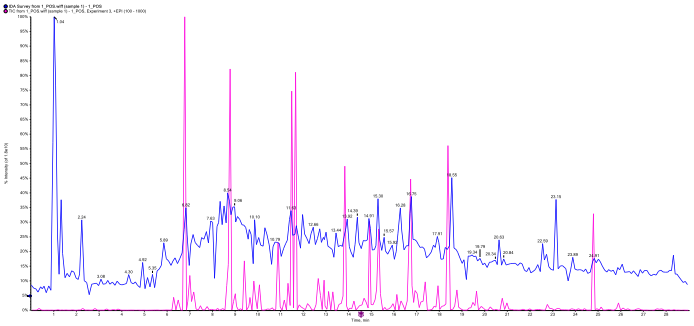

Figure 2 LC-MS/MS analytical scan of licorice extract POS

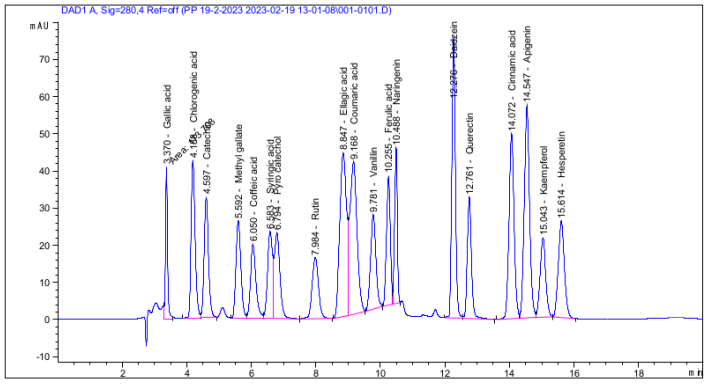

Figure 3 HPLC standard chromatogram profile

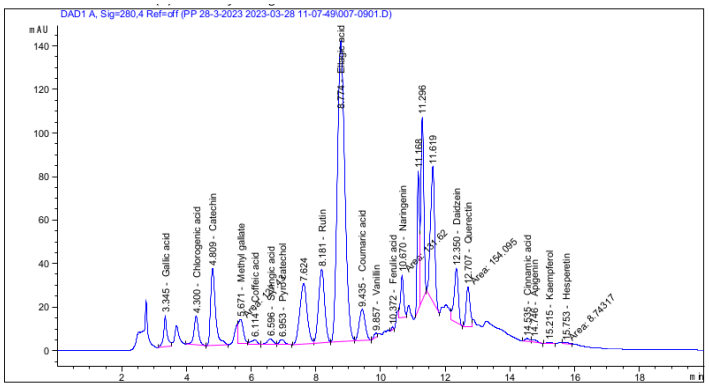

Figure 4 HPLC chromatogram of licorice *extract*

In the most recent investigation, researchers inspected the antibacterial properties of a methanolic extract of *Glycyrrhiza glabra*. They observed that MIC significantly inhibited the growth of *S. aureus* and *B. cereus* bacteria. Higher quantities of this extract prevent E. coli, while high concentrations of the *Glycyrrhiza glabra* extract had the least impact on *P. aeruginosa* bacteria and significantly different susceptibilities for G. glabra L. among the bacteria ($p < 0.05$) **(Jafari-Sales, A. & Bolouri, P. 2018).** The antibacterial activity of licorice was also examined by **(Gupta, V. K et al 2008),** and it emerged at a concentration of 500 µg/mL. The antibacterial assay of *Glycyrrhiza glabra* was also explored by **(Karahan *et al* in 2016, Karahan, F et al 2016),** and the findings showed that the plant root extracts were more efficient against Gram-positive bacteria than against Gram-negative ones. The extracts exhibited better antioxidant properties with a medium inhibitory concentration (IC_{50}) ranging from 588 ± 0.86 µg/mL to 2190 ±

1.73 µg/mL, the extracts also demonstrated better antioxidant properties and had a stronger antibacterial impact against *Candida* species than against bacteria.

Table 3 Effect of *Glycyrrhiza glabra* extract on pathogenic bacteria (gram positive and negative bacteria) and minimal inhibitory concentration (MIC)

Organisms	Inhibitory activity against the tested organism (zone of inhibition in mm)						
	Concentration						
	100%	50%	25%	12.5%	6.25%	3.13%	1.56%
Gram-positive bacteria							
Staphylococcus aureus **(MRSA) - ATCC43300**	18.5^{a} ±0.80	17^{a} ±0.50	15^{b} ±0.56	13^{c} ±0.70	12^{c} ±0.80	11^{c} ±0.15	11^{c} ±0.75
Bacillus subtilis **ATCC6633**	16^{a} ±0.25	15.5^{ab} ±0.71	14^{b} ±0.54	12^{c} ±0.57	11^{c} ±0.45	6^{e} ±0.64	8.5^{d} ±0.15
Gram-negative bacteria							
Escherichia coli) ATCC**25922(**	9.5^{a} ±0.25	8.5^{ab} ±0.50	7.5^{bc} ±0.34	NA	NA	NA	NA
Pseudomonas aeruginosa **(ATCC27853)**	26^{a} ±0.74	26^{a} ±0.55	25^{a} ±0.60	22^{b} ±0.91	19^{c} ±0.52	18^{c} ±0.35	16^{d} ±0.25

NA= No effect. The means within the same row with different superscripts significantly vary (P ≤0.05).

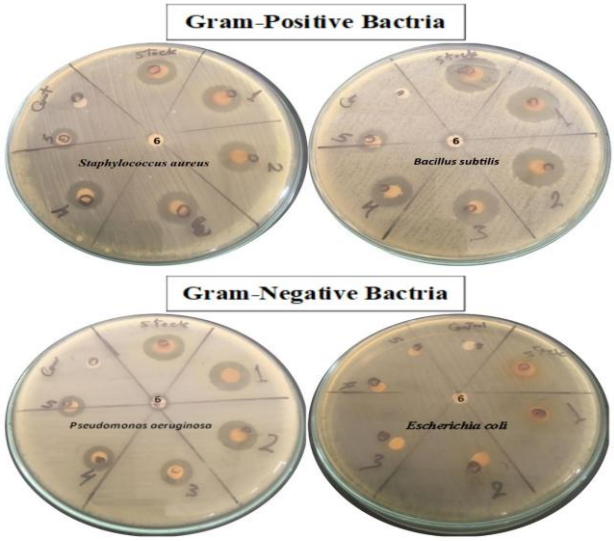

Figure 5 Pictures showing the antimicrobial effect of *Glycyrrhiza glabra* extract and it's MIC.

The licorice extract at an effective concentration of 1% against pathogenic bacteria was applied to mango nectar as a preservative agent alternative to sodium benzoate, and the bacterial quality was measured at zero time and during storage for 14 days, as tabulated in the **table 4.** The results showed that the licorice extract decreased the microbial load to 0.5 log cfu/mL after 1.7 log cfu/mL in control, while the juice containing sodium benzoate was free from microorganisms. After 7 days of storage, the microbial load slightly increased to 1.8 log cfu/mL for mango nectar samples containing licorice extract and sodium benzoate, while the control sample gave 3.4 log cfu/mL. The microbial load was increased after 14 days for both the samples containing *Glycyrrhiza glabra* extract and sodium benzoate and recorded almost equal microbial loads of 2.9 and 3 log cfu/mL, while the control sample recorded 4.8 log cfu/mL. It is worth mentioning that the *Glycyrrhiza glabra* extract and sodium benzoate completely inhibited mold and yeast counts at zero time and during storage for 7 days, but some colonies appeared after 14 days to record 3.1, 1.9, and 2.1 log cfu/mL for control, *Glycyrrhiza glabra* extract, and sodium benzoate juice samples, respectively.

Table 4 Effect of *Glycyrrhiza glabra* extract as preservative agent on the microbial quality of prepared mango nectar alternative to sodium benzoate.

Storage at 4 °C	Control	Licorice extract (1%)	Sodium benzoate (0.1%)
Total bacteria count by Log cfu/mL			
Zero	$1.7±0.08^{c}$	$0.5±0.01^{b}$	Nil^{a}
7	$3.4±0.11^{b}$	$1.8±0.05^{a}$	$1.8±0.09^{a}$
14	$4.8±0.10^{b}$	$2.9±0.09^{a}$	$3±0.11^{a}$
Mold and yeast count by Log cfu/mL			
Zero	Nil	Nil	Nil
7	Nil	Nil	Nil
14	$3.1±0.00^{b}$	$1.9±0.30^{a}$	$2.1±0.06^{a}$

The means within the same row with different superscripts significantly vary (P ≤0.05).

G. glabra's antibacterial activities were tested on *Candida albicans Staphylococcus aureus*, *Escherichia coli* and *Pseudomonas aeruginosa* using a variety of extracts. All the investigated isolates were extract-sensitive. Higher sensitivity was seen in *S. aureus* and *E. coli*, with the greatest effective inhibition for G. glabra aqueous extract against S. aureus and lowest inhibition against *P. aeruginosa*. Alcoholic extracts of G. glabra had the greatest impact on *P. aeruginosa* and the least impact on C. albicans, according to **(Al Mousawi*et al* in 2022).** Additionally, GE extracts significantly inhibited the growth of two gram-positive (*S. aureus and B. subtilis*) and two gram-negative (*E. coli and P. aeruginosa*) bacteria **(Hamad, G. M et al 2020).** The results of the antimicrobial tests on the plant root methanolic extracts showed that the extracts were more potent against Gram-positive bacteria than Gram-negative ones. In addition, **Karahan *et al.* 2016** found that the extracts had a stronger antibacterial activity against Candida species than against bacteria **(Farag, M.A et al 2012).**

Table 5 Effect of *Glycyrrhiza glabra* extract and sodium benzoate on total soluble solids (TSS), pH value, and acidity of prepared mango nectar.

Parameter	Control	Licorice extract	Sodium benzoate
T.S.S	$17.20±0.12^{a}$	$17.20±0.10^{a}$	$17.20±0.13^{a}$
pH value	$4.50±0.00^{a}$	$4.50±0.00^{a}$	$4.21±0.00^{a}$
Acidity (as citric acid)	$0.25\%±0.02^{a}$	$0.25\%±0.01^{a}$	$0.31\%±0.01^{a}$

The means within the same row with different superscripts significantly vary (P ≤0.05).

The effect of *Glycyrrhiza glabra* extract and sodium benzoate on total soluble solids (TSS), pH value, and acidity of prepared mango nectar were analyzed as shown in **Table 5**, and the results showed there are no differences or changes in the studied factors due to additional *Glycyrrhiza glabra* extract or sodium benzoate compared with the control sample. TSS was in the range of 17 for all analyzed samples of mango nectar incorporated with *Glycyrrhiza glabra* extract or sodium benzoate as well as the control juice sample, and the same trend and pattern for pH value that recorded 4.50 for control and the mango nectar incorporated with *Glycyrrhiza glabra* extract, but slightly decreased of mango nectar contained with sodium benzoate was noted and recorded 4.21. Also, the same trend for acidity; no difference was noted in all studied mango nectar samples, and the control and mango nectar incorporated with Glycyrrhizaglabra extract had the same acidity of 0.25%, but a slight increase in mango nectar contained with sodium benzoate was noted and recorded 0.31%.

After 24 days of storage, **(Babarind*e et* al., 2019),** studied the quality characteristics and phytochemical activities of fresh juice made from different mango varieties. They found that the juice from the Kent type had the lowest titratable acidity (7.83 0.2 mg/100 g), pH (4.41 0.2 mg/100 g), and brix (11.83 0.2 mg/100 g), while the juice from the Lippen variety had the highest values. Another study looked at the physicochemical composition of tetra pack mango juices, including acidity, pH, brix, and total sugar. These samples' physicochemical analysis revealed that Brand A (Pepsico) had a pH of 3.73, an acidity of 0.27%, a brix of 12.9, and a total sugar content of 10.4%. brix 9.8, pH 4.20, acidity 0.13%, and total sugar 2.17% for Brand B (Enjoy). Acidity 0.19%, pH 4.03, 0brix 5.1, and total sugar 2.73% for Brand C (Tops).Acidity 0.23%, pH 3.97, 0brix 5.1, and total sugar 7.8% are all values for Brand D (Nestle). Acidity 0.17%, pH 4.02, 0brix 10.3, and total sugar 7.41% for Brand E (Kool). pH 4,05, acidity 0.18%, 0brix 6.5, and total sugar 5.37% for Brand F Tropico **(Jalal, A et al 2017).**

The effect of the studied preservatives on the sensory quality of prepared mango nectar was studied as shown in **Table 6.** The sensory quality of prepared mango nectar is a primary test in our study, and the main task at the same time was how to use the *Glycyrrhiza glabra* extract at the level that hasn't affected the sensory quality of produced mango nectar and is acceptable for panelists and after that consumer, so from the obtained results of sensory evaluation, it didn't affect the

addition level due to the use of *Glycyrrhiza glabra* extract as a preservative agent and the produced treated mango nectar was acceptable for panelists in test, , odour, colour, texture, and general acceptability scores were 97, 91and 94 % for the control, *Glycyrrhiza glabra* extract and sodium benzoate nectar samples respectively, The development of the mango nectar sensory profile is crucial, especially considering that sensory and color changes may exist during industrial processes **(Horwitz, W. 1975).**

Table 6 Effect of *Glycyrrhiza glabra* extract and sodium benzoate on sensory evaluation of prepared mango nectar.

Factor	Control	*Glycyrrhiza glabra extract*	Sodium benzoate
Taste (30)	29.6±0.8[a]	26.6±1.3[c]	28.0±0.7[b]
Odor (30)	28.1±1.6[a]	28.8±1.2[a]	28.0±1.4[a]
Color (20)	19.2±0.8[a]	18.6±0.8[a]	18.6±0.8[a]
Texture (20)	19.4±0.5[a]	18.0±0.6[b]	19.4±0.5[a]
Acceptability (score 100)	97.0±2.7[a]	91.0±1.6[c]	94.2±2.7[b]

The means within the same row having different superscripts are significantly varied (P ≤0.05).

CONCLUSION

Glycyrrhiza glabra extract has optimal capability as a valuable source of numerous bioactive compounds with significant antimicrobial activity. HPLC analysis has demonstrated several antioxidant compounds, with the domination of Ellagic acid. These compounds have several beneficial properties, such as antioxidant, anti-inflammatory, antibacterial, and antiviral effects. *Glycyrrhiza glabra* extract's antimicrobial activity was thoroughly investigated, revealing its potency against several pathogenic bacteria, including, *S. aureus, B. subtilis,P. aeruginosa* and *E. coli*. The reliable minimum inhibitory concentration (MIC) results in this study attributed *Glycyrrhiza glabra* antibacterial properties to its content of polyphenolic compounds. Examining *Glycyrrhiza glabra extract* as a suitable preservative agent in mango nectar demonstrated apromising result, in comparing totraditional preservatives such as sodium benzoate, due to its significant efficacy in reduction of the microbial load. Moreover, the addition of *Glycyrrhiza glabra* extract did not negatively affect the sensory quality of the mango nectar, making it a better natural alternative for consumers. Overall, this study highlighted the importance of *Glycyrrhiza glabra* as a natural source of antimicrobial compounds with a promising application in the food industry, which is leading to providing a safer and more natural way to increase the items' shelf life and improve their quality. Further research in this area could lead to the development of novel and healthier food preservation techniques.

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Declaration of Competing Interest: The authors declare that none of the work revealed in this study appears to have been impacted by any known competing financial interests or personal relationships.

Ethical Concern: The sensory tests were conducted according to established ethical guidelines, and informed consent obtained from the participants. Institutional approval for the research is not available and not required because edible drink.

Data Availability: This is a research article. All relevant information has been included in the manuscript itself. Thanks.

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