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Development and HPLC Characterization of a Water-Soluble Supramolecular Complex of Propolis and Glycyrrhizic Acid Monoammonium Salt: Composition, Toxicity, and Pharmaceutical Potential

Khurshida Saidova[1]*, Ibrokhim Askarov[2], Akmal Islomov[1], Jamshid Ashurov[1], Dilnoza Abdugafurova[1], Lazizbek Mahmudov[1], Arofat Inkhonova[3] and Uchkun Ishimov[1]

[1]Department of Pharmacol A.Sadykov Institute of Bioorganic Chemistry of the Academy Sciences of Uzbekistan, Tashkent.
[2]Department of Chemistry, Andijan State University of Uzbekistan, Andijan, Uzbekistan.
[3]Department Pharmacy and Chemistry, Alfraganus University, Tashkent, Uzbekistan, Tashkent.
*Corresponding Author E-mail:xurshidasaidova335@gmail.com

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This study synthesized and evaluated a supramolecular complex of propolis with glycyrrhizic acid monoammonium salt (GMAS) in a 1:3 ratio, aiming to enhance its solubility, bioavailability, and therapeutic potential. The complex was characterized as a white to pale-yellow amorphous powder with high water solubility. Vibrational frequency analysis confirmed the presence of functional groups essential for bioactivity, while HPLC analysis identified significant levels of vitamins B2, B6, and C, suggesting antioxidant potential. In vivo evaluation using a carrageenan-induced paw edema model demonstrated dose-dependent anti-inflammatory activity, with a maximum inhibition of edema (60.7%) observed at 100 mg/kg. The complex significantly reduced swelling within 5 hours post-induction, comparable to conventional anti-inflammatory drugs. Acute toxicity studies classified the complex as non-toxic (LD50 > 2000 mg/kg), with no adverse effects observed at therapeutic doses. The potent anti-inflammatory activity is attributed to bioactive compounds, including flavonoids and phenolic acids, whose efficacy is enhanced by improved solubility through supramolecular complexation. These findings underscore the potential of the GMAS-propolis complex as a safe, natural alternative to conventional therapies for managing inflammation and oxidative stress. Future studies should focus on clinical trials and formulation optimization to establish its therapeutic applicability.

Keywords: Anti-inflammatory activity; (GAMS) Glycyrrhizic acid monoammonium salt; Pharmacological activity; Propolis; synergism.

Bees create propolis from plant resins, which has anti-inflammatory, anti-cancer, antimicrobial, analgesic, and antioxidant qualities that make it useful for treating a variety of illnesses.[1] Propolis, sometimes referred to as "bee glue," is a naturally occurring substance that resembles resin that bees gather from plant buds and exudates and combine with beeswax. By applying propolis to the inner walls and frames of their hives, bees can sterilize them and stop the growth of microbial illnesses. Additionally, propolis helps regulate humidity and temperature within the

hive throughout the year.[2] Propolis has been used since ancient times as a natural compound with antipyretic (fever-reducing) and wound-healing properties. Over the last 30 years, its medicinal properties have also been increasingly applied in hygiene, cosmetics, and the food industry. Scientific research has revealed propolis's antioxidant, antibacterial, antiviral, anti-inflammatory, and immune-supporting characteristics. Consequently, its use is expanding not only in traditional medicine but also in modern pharmaceutical and cosmetic products.[3] Bees gather propolis, a popular bee product, from plant resins. It contains a variety of bioactive compounds, such as lignans, coumarins, terpenes, stilbenes, phenylpropanoids, flavonoids, and their prenylated derivatives. These substances are the primary bioactive ingredients that give propolis its ability to effectively treat a wide range of illnesses.t Propolis is useful for treating a number of ailments because of its anti-inflammatory, antibacterial, antioxidant, anticancer, and wound-healing qualities. Its chemical composition, particularly flavonoids, terpenes, and phenols, enhances its effectiveness.u Propolis is widely used as an immunostimulant, aiding in the prevention of colds due to its antibacterial and antiviral effects. It is also a natural remedy for skin issues, minor wounds, oral ulcers, and irritation relief. Additionally, propolis supports urinary tract health and aids in restoring gastric mucosal balance.v Propolis exhibits antibacterial, antiviral, and antiprotozoal activity, showing potential for treating cardiovascular diseases, diabetes, immune modulation, and cancer. Its biological activity and chemical composition make it highly valuable.w The current research is of interest to the pharmaceutical industry, enabling the development of standardized propolis preparations such as mouthwashes, toothpaste, sprays, creams, ointments, suppositories, tablets, and capsules. Propolis can also serve as a source for isolating bioactive compounds. Studies on standardizing propolis aim to solve its variability issues and expand its applications in the pharmaceutical industry. Furthermore, propolis can be useful in the food industry for developing balanced food products.x Bee products, including honey, bee venom, wax, and propolis, are notable for their rich composition. Honey contains unsaturated fatty acids, vitamins (A, C, E, B group), flavonoids, and hydroxy acids, which play an essential role in nourishing the skin, acting as antioxidants, and reducing inflammation. Bee venom exhibits antibacterial, antifungal, anti-inflammatory, and antiviral properties. Beeswax acts as an emulsifier in cosmetics, combining liquids and oils to improve skin nutrition. The unique chemical composition of each product, when combined, contributes to enhanced skin health.y The medical application of propolis is linked to its polyphenolic compounds, particularly flavonoids and phenolic acids, which possess antioxidant, antibacterial, antifungal, and anti-inflammatory properties.[1]p Traditional medicine has made extensive use of propolis because of its distinct chemical makeup, potent pharmacological effects, and low toxicity.[11] The antibacterial activity of propolis is associated with its high concentration of flavonoids and phenolic acids. For instance, *P. nigra* L. (black poplar) propolis enhances protective properties against microorganisms.[12] Propolis acts as an antibacterial, antifungal, and antiparasitic agent against bacteria, fungi, and protozoa, making it an effective natural antiseptic and immunostimulant for treating infections and inflammation.[13] Rich in flavonoids, phenolic acids, and terpenes, Iranian propolis exhibits antibacterial, antifungal, and antiparasitic properties against pathogens like *Staphylococcus aureus, Escherichia coli*, and *Candida albicans*. This synergistic effect enhances the efficacy of antibiotics and helps prevent microbial resistance. Propolis has high redox potential, phenolic compounds, and radical-scavenging activity, confirming its antioxidant and anti-inflammatory properties. Compounds like phenolic acids and flavonoids contribute to its bioactive characteristics, making it effective in combating microorganisms.

Glycyrrhizic Acid Monoammonium Salt and Its Synergistic Effects with Propolis

Glycyrrhizic acid monoammonium salt, derived from licorice (*Glycyrrhiza glabra*), is a bioactive compound known for its antiviral, anti-inflammatory, and hepatoprotective effects. It is widely used in pharmaceutical and cosmetic applications due to its ability to enhance immune responses and modulate inflammation. Studies suggest that glycyrrhizic acid interacts with cellular pathways involved in oxidative stress, making it beneficial for treating liver disorders,

viral infections, and inflammatory diseases.[14] The combination of glycyrrhizic acid monoammonium salt with propolis may enhance its therapeutic effects, particularly in immune modulation and antimicrobial activity. Research indicates that glycyrrhizic acid can improve the bioavailability of polyphenolic compounds found in propolis, increasing their stability and absorption. This synergistic interaction may be valuable in developing novel formulations for wound healing, oral health, and antiviral treatments. Additionally, glycyrrhizic acid has been found to inhibit viral replication in conditions such as hepatitis and respiratory infections, supporting its potential as a complementary agent in propolis-based pharmaceutical products.[15] Given its diverse pharmacological properties, glycyrrhizic acid monoammonium salt is increasingly being integrated into formulations containing propolis to enhance efficacy in antimicrobial, anti-inflammatory, and immune-supporting treatments. Further studies are needed to explore the full potential of this combination in pharmaceutical and nutraceutical applications.

MATERIALS AND METHODS

Chemicals

GMAS (Glycyrrhizic Acid Monoammonium Salt), propolis, balance in analysis, Flasks for measuring, 40% ethanol, Boiling apparatus, or reactor Water-Soluble Standards for Vitamins: B2, B3 (PP), B6, B9, and C vitamins Analytical grade (1 mg/mL stock solutions made with Sigma-Aldrich 40% ethanol) In HPLC analysis, acetonitrile (HPLC grade) is utilized as the mobile phase (Merck). For HPLC separation, acetate buffer (pH 5.0) is made with sodium acetate and acetic acid. The Agilent-1200 HPLC system, Eclipse XDB C18 column (5 µm, 4.6 × 250 mm), and Diode Array Detector (DAD) are used to measure the reference medication, Indometacin (Sofarma), at a dosage of 25 mg/kg.[16]

Laboratory animals: Albino mice weighing 20 ± 2.0 g were used for testing Digital caliper (Insize, Model 7140): Paw diameter (PV…) is measured 1, 2, 3, 4, 5, and 24 hours following carrageenan injection (PVt).[17]

Experimental groups

Animals were divided into 14 groups, with 6 rats in each group, to evaluate the anti-inflammatory activity of the samples Specialized probe and other equipment Preparation of GMAS from Glycyrrhiza glabra (Licorice) Root: GMAS was extracted from the roots of *Glycyrrhiza glabra* following a known method reported in the literature.[18] The extraction process of glycyrrhizic acid (GA) and its monoammonium salt is illustrated (Figure 1).

Preparation of GMAS from Glycyrrhiza glabra (Licorice) Root

Licorice Root Dark Extract

The starting material is the dark extract derived from licorice root.[19]

Conversion to Technical Glycyrrhizic Acid

The licorice root dark extract is treated with concentrated sulfuric acid (H, $SO_{,,}$), resulting in the formation of technical glycyrrhizic acid.

Formation of Glycyrrhizic Acid 3-Ammonium Salt

The technical glycyrrhizic acid is further processed using acetone and 25% concentrated ammonium hydroxide ($NH_{,,}$ OH) to produce glycyrrhizic acid 3-ammonium salt.

Production of Glycyrrhizic Acid Monoammonium Salt

The licorice root dark extract is treated with 80% ethanol, yielding glycyrrhizic acid monoammonium salt with a purity of 80–82%.

Sample Preparation

A 300 ml round-bottom flask was filled with 5–10 g of the supramolecular complex after it had been weighed. A 40% ethanol solution (50 ml) was added. For one hour, the mixture was heated while being vigorously stirred with a magnetic stirrer and reflux condenser. Following heating, the mixture was cooled, filtered, and agitated for a further two hours at room temperature. Twenty-five milliliters of 40% ethanol were used twice to remove the residue.

In a volumetric flask containing 40% ethanol (5–10%), the filtrates were mixed and diluted to 100 ml. The supernatant was used for analysis after the solution was centrifuged for ten minutes at 7000 rpm.

Standard Preparation

1 mg/ml stock solutions of water-soluble vitamins (B2, B3, B6, B9, C) in 40% ethanol were prepared by dissolving 50 mg of each standard in a 50 ml volumetric flask.

Chromatographic Conditions

Agilent-1200 HPLC system with auto-sampler. Column: Eclipse XDB C18, 4.6 × 250mm 20, 5 µm, reverse phase. Diode Array Detector (DAD) at 250 nm is the detector. Acetonitrile and acetate buffer make up the mobile phase. Gradient: 96:4 for 0–5 minutes, 90:10 for 6–8 minutes, 80:20 for 9–15 minutes, and 0.8 ml/min for 15–17 minutes. 25°C is the thermostat temperature, while 5 µl is the injection volume.

Animal Experiments

The European Directive 2010/63/EU on the protection of animals used in scientific research (European Union, 2010) was followed in all animal procedures. The Institute of Bioorganic Chemistry, AS RUz Animal Ethics Committee approved the study protocol (Protocol Number: 133/1a/h, dated August 4, 2014).

Statistics

A paired t-test for combined data and an unpaired t-test for independent comparisons were used to assess the statistical significance between the control and experimental groups. To indicate statistically significant differences, a significance level of $P<0.001$ and $P<0.05$ were applied.

RESULTS

After recrystallization, the yield of GMAS salt relative to technical glycyrrhizic acid (TGA) was 28–30%. The purity of GMAS was determined to be 80–82% using the YuSSX method.[21] For the preparation of supramolecular complexes, GMAS with a purity level of 80–82% was primarily used (Table 1).

The OH and NH groups' valence vibration frequencies in the GMAS molecule create wide "bands" in the 3203.76 cm{ ¹ range. The CH*f* and CH, groups' valence vibration frequencies are found to be between 2927 and 2870 cm{ ¹. The carbonyl part of the carboxyl groups in the GMAS molecule is represented by the valence vibration frequencies that are recorded at 1705 cm{ ¹. The carbonyl group at position C11 in the aglycone portion of the GMAS molecule exhibits a high intensity valence vibration frequency at 1651 cm{ ¹. The carboxyl groups' (COO{) valence vibration frequencies are detected at 1589 cm{ ¹ with a medium degree of intensity. The CH*f* and CH, groups' deformation vibration frequencies are found to be between 1454 and 1417 cm{ ¹. The NH,, z ion's deformation vibrations in the GMAS molecule are visible at 1386.82 cm{ ¹ with medium intensity.[22] The valence vibration frequencies of the molecule's C-O-C and C-OH bonds are strongly visible in the area of 1035.77 cm{ ¹. The (=CH) group's deformation vibration frequencies are found to be 979.84 cm{ ¹ (Figure 2).

In this study, the primary objective was to utilize the unique physicochemical properties

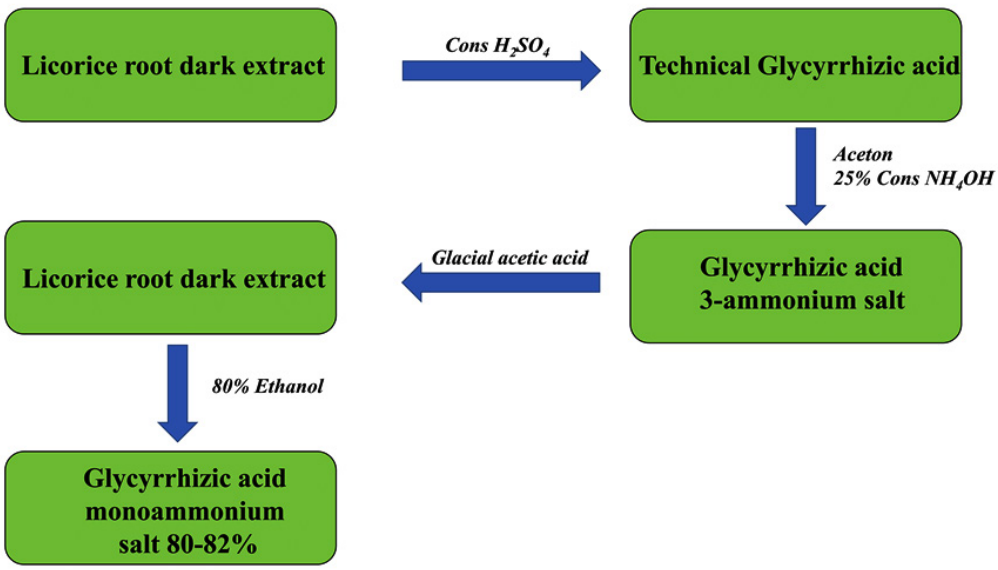

Fig. 1. Scheme for Obtaining GMAS from the Thick Extract of Glycyrrhiza glabra (Licorice) Root

of GMAS to synthesize novel, effective, water-soluble supramolecular complexes and identify their properties. One gram of glycyrrhizinic acid monoammonium salt (GMAS) was dissolved in five milliliters of water, and three grams of propolis were dissolved in ten milliliters of ethanol to create a supramolecular complex in a 1:3 ratio. A magnetic stirrer was used to mix the resultant solutions and agitate them constantly for 24 hours at room temperature. Ethanol was then evaporated, and the aqueous phase was dried using a lyophilization method. The supramolecular complexes of propolis and GMAS, obtained in a 1:3 ratio, appeared as white to pale yellow amorphous powders. These complexes formed gels when prepared as 0.1% aqueous solutions.

Table 1. Selected Physicochemical Properties of GMAS

Substances	$T_{.liquid}$ ^0C	$R_f^{*(system)}$	$[\alpha]_D$ 0,5% EtOH (50%)	IS (ν, cm^{-1})	UB nm
GMAS•$3H_2O$ (m=894)	225-227	0,32 (III)	+40	1042, (COC); 1655, (CO) 2948, (OH); 3239, (OH)	253

*I. Butanol, water, and acetic acid 3:1:1,

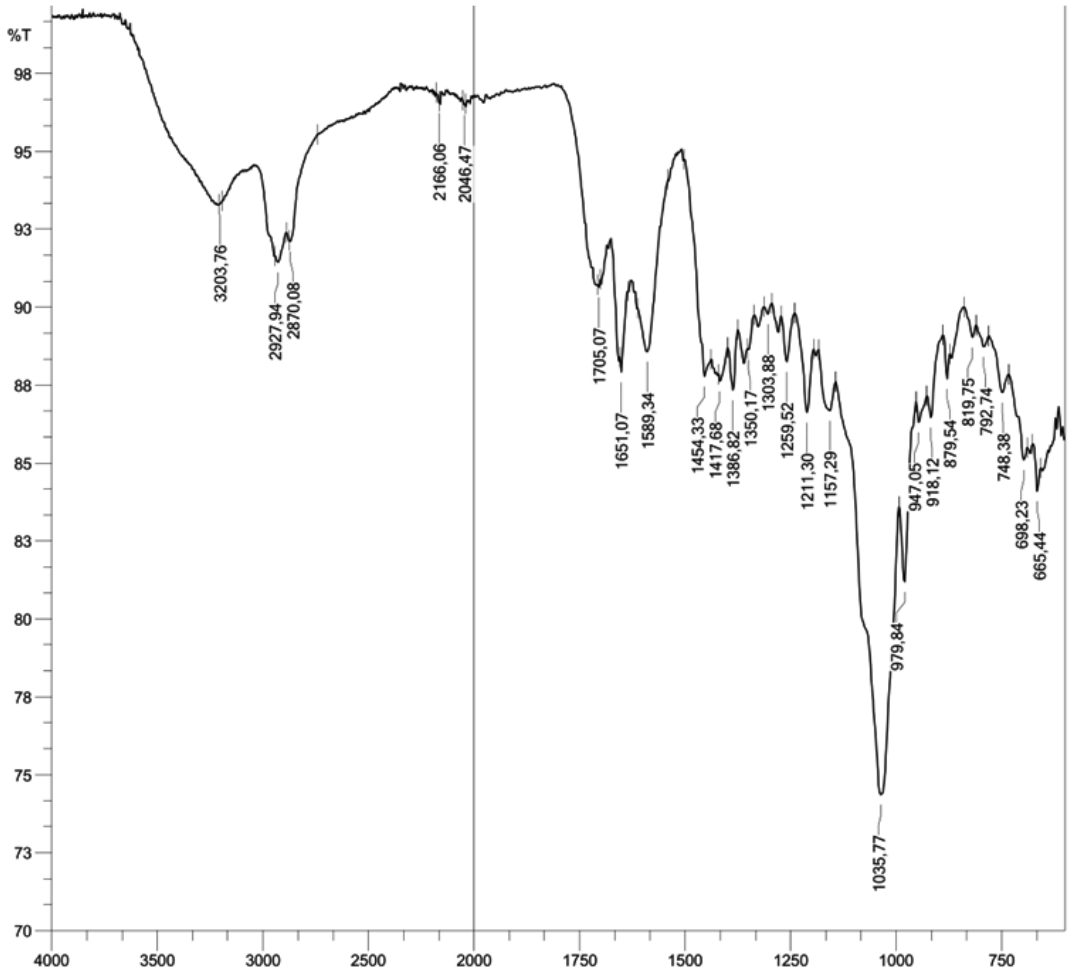

Fig. 2. IR spectrum of mono ammonium salt of glycyrrhizic acid (GMAS)

This study marks the first successful synthesis of a supramolecular complex of propolis with GMAS in a 1:3 ratio. Vitamin Analysis Using HPLC.[23]

High-performance liquid chromatography (HPLC) was used to identify the water-soluble vitamins in the propolis and GMAS (glycyrrhizinic acid monoammonium salt) supramolecular complex in a 1:3 ratio. The vitamin quantities were B1 = 0.00 mg, B2 = 0.63 mg, B6 = 1.14 mg, B9 = 0.00 mg, and Vitamin C = 1.25 mg, according to the data (Figure 3). Vitamins B2, B6, and C were verified to be present in the Propolis: GMAS supramolecular complex.[24] The free amino acids in the propolis and GMAS (glycyrrhizinic acid monoammonium salt) supramolecular complex in a 1:3 ratio was determined using high-performance

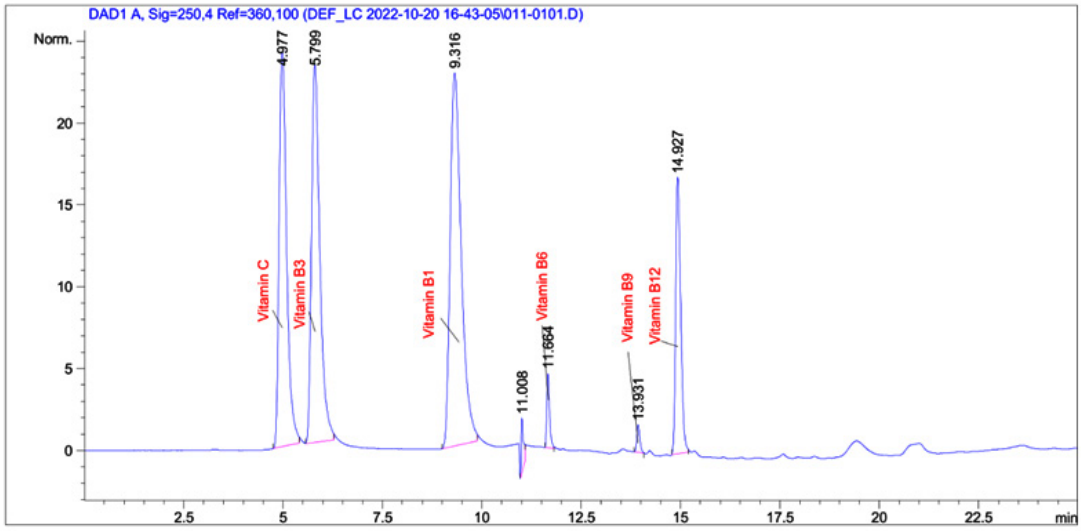

Fig. 3. Chromatography of the standard solution used to measure propolis's vitamin content

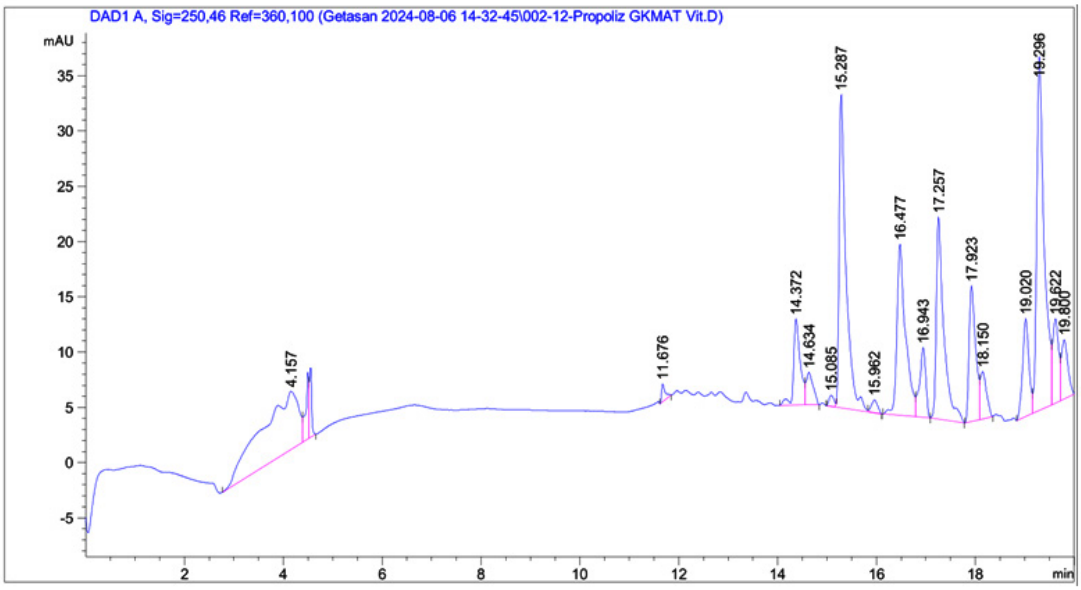

Fig. 4. Chromatography of the prepared solution for determining the vitamin content in the propolis and GMAS (glycyrrhizinic acid monoammonium salt) supramolecular complex in a 1:3 ratio

liquid chromatography (HPLC) (Figure 4). The preparation of the supramolecular complex from the aqueous extract of Propolis and GMAS involved the precipitation of proteins and peptides in centrifugal glasses. To do this, 1 ml of the test sample was added to 1 ml of 20% trichloroacetic acid (TCA). After 10 minutes, the precipitate was separated by centrifugation at 8000 rpm for 15 minutes. After removing 0.1 ml of the supernatant, it was frozen to dry. The hydrolysate was evaporated, and the dry residue was dissolved in a mixture of triethylamine-acetonitrile-water (1:7:1) and dried. This acid neutralization operation was repeated twice.[25] By reacting with phenylthioisocyanate, phenylthiocarbamyl derivatives (PTC) of amino acids were obtained using the method by Stephen A. and Koen Daviel. The determination of amino acid derivatives was carried out by HPLC. Chromatography conditions: Diode Array Detector (DAD), Agilent Technologies 1200 chromatograph, 75x4.6 mm Discovery HS C18 column. Solution B: CH3CN; Solution A: 0.14 M CH3COONa + 0.05% TEA, pH 6.4. Absorption at 269 nm, flow rate: 1.2 ml/min. Gradient percentage B/min: 0-2.5 min; 6-30%; 1-6% 30–60%; 2.5–40 minutes 40.1-45 minutes; 60-60% 60-0%; 45.1-50 min 50.1 to 55 minutes. The working standard solution was first made, followed by the propolis-prepared solution (Figure 5). The quantity of free amino acids was then ascertained by comparing the outcomes (Figure 6).

Male albino laboratory mice weighing an average of 22±2.0g were used to test the toxicological (LD50) characteristics of the propolis and glycyrrhizinic acid monoammonium salt (GMAS) supramolecular complex in a 1:3

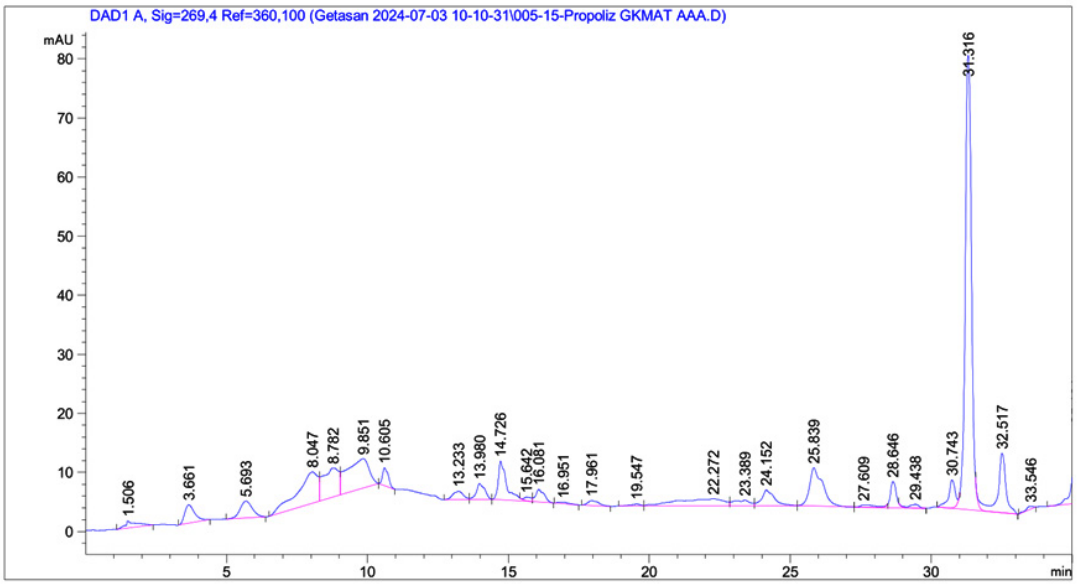

Fig. 5. Chromatography of the prepared solution to determine the amount of amino acids in the Propolis and GMAS (glycyrrhizinic acid monoammonium salt) supramolecular complex in a 1:3 ratio

Table 2. Evaluation indicators of acute toxicity of supramolecular complex substances of propolis and monoammonium salt of glycyrrhizic acid (MASGA) in a 1:3 ratio in mice (M±m, n=5)

Groups	Dose, mg/kg, ml	Number of animals /dead animals in the group	Average animal mass, gd Day 1	Day 7	Day 14	LD_{50}, mg/kg
Control	0,5 dis water	5/0	21,5 ± 0,3	23,6 ± 0,4	24,5± 0,5	-
Propolis: GAMS	2000	5/0	22,0± 0,3	23,9± 0,4	24,4 ± 0,6	>2000

ratio. Five mice were chosen for the experiment. Healthy, sexually mature mice that had been quarantined for 10–14 days participated in the pharmaceutical studies. Laboratory tests were conducted to evaluate the biological activity, effects, and acute toxicity features of the Propolis and GMAS supramolecular complex in a 1:2 ratio.[26,27] The complex's therapeutic effectiveness, toxicological safety, and possible safety limitations were investigated. There were two phases to the experiment: Two mice from the group were given 2000 mg/kg of each drug (0.4 ml volume) into their stomachs using a specialized probe in the first stage. They were monitored for two to three days without experiencing any deaths. The same dosage was given to the other three mice in the second stage. The control group was given the same amount of distilled water at the same time. On the first day of

Table 3. The effect of the supramolecular complex of propolis and monoammonium salt of glycyrrhizic acid (MASGA) in a 1:3 ratio at doses of 200, 150, and 100 mg/kg on carrageenan-induced paw swelling (expressed as a percentage relative to baseline, M±M; N=5).

Groups	Inhibition of Inflammation, %				
	One hour	Two hour	Three hour	Four hour	Five hour
Control	47,2±4,35	59,0 ± 5,4	94,4 ± 8,9	70,8 ± 6,8	41,0 ± 3,7
Propolis GAMS 150 mg/kg	28,8±2,6	40,0 ± 3,9	51,3 ± 4,8	28,8 ± 2,6	6,3 ± 0,56
Propolis GAMS 100 mg/kg	21,1±1,1	31,7 ± 2,9	42,2 ± 4,1	31,7 ± 3,0	2,2 ± 0,2

With the exception of carrageenan in the propolis extract form: GAMS at dosages of 200, 150, and 100 mg/kg, which showed an effect of 60.7%, all of the compounds under study have anti-exudative effects that fall within the range of 7.2%, according to the findings shown in Table 4.

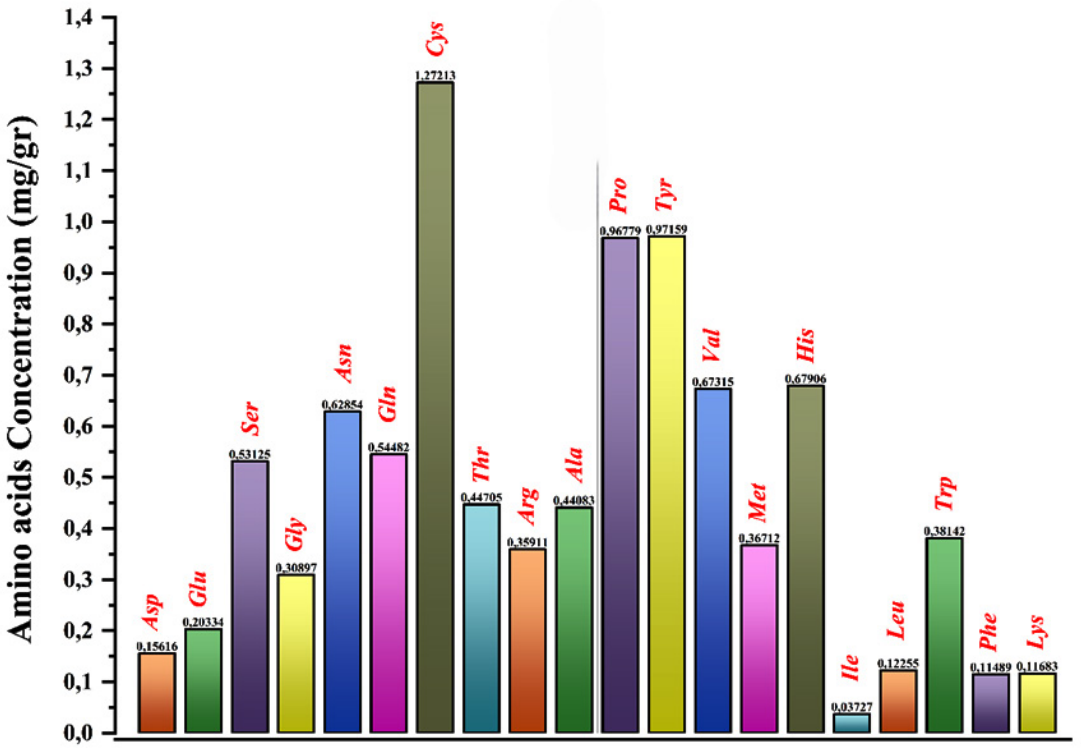

Fig. 6. Amount of amino acids identified in the Propolis and glycyrrhizinic acid monoammonium salt (GMAS) supramolecular complex in a 1:3 ratio

Table 4. Maximum increase in paw volume in rats, studied for the anti-exudative activity of the supramolecular complex of propolis and monoammonium salt of glycyrrhizic acid (GAMS) in a 1:3 ratio at doses of 200, 150, and 100 mg/kg in extract form, compared to the control group (M ± m; n=5)

Groups	Increase in leg size 3 hours after induction, %	Anti-exudative effect in percent
Control	94,4 ± 8,9	———
Propolis GMAS 200 mg/kg	42,2 ± 4,1	55,3
Propolis GMAS 150 mg/kg	51,3 ± 4,8	45,6
Propolis GMAS 100 mg/kg	42,2 ± 4,1	55,3

both phases of the experiment, the overall health of the research animals was checked hourly for any indications of tremors or death. For two weeks, the animals' general health, activity, skin and fur quality, breathing rate and depth, urine, body weight fluctuations, and other characteristics were checked every day. The animals had unrestricted access to food and water and were housed in standard feeding circumstances. The average lethal dose (LD50) of the propolis compound and its toxicity class were established at the conclusion of the study. The arithmetic mean (M) and standard error (m) were used to statistically process the data, with significance set at $p<0.05$. The findings showed that within 15 minutes of receiving a 2000 mg/kg dose of the Propolis and GMAS complex, the animals showed signs of tightness of the eyelids, fast breathing, and a concentration in one area.[28,29] After 20 to 25 minutes of these symptoms, the mice's condition reverted to normal. Over the course of the study (7 and 14 days), there was no discernible drop in body weight in the experimental mice when compared to the control group ($p>0.05$). It was discovered that the Propolis and GMAS supramolecular combination had an average fatal dose (LD50) of more than 2000 mg/kg (Table 2).

According to OECD guidelines, mice were given a single oral dose of 2000 mg/kg of the supramolecular complex of propolis and monoammonium salt of glycyrrhizic acid (GAMS) in a 1:3 ratio.[30,31] The results showed that these samples were classified as chemically non-toxic Class V substances (LD50 > 2000 mg/kg). The traditional "carrageenan model" was employed in the initial evaluation of the anti-inflammatory properties of the supramolecular complex of propolis and MASGA in a 1:3 ratio at different dosages. Eight rats of the same sex, weighing 190±20 g, participated in the investigation. Thirteen groups were created from the animals: four experimental groups and a control group, each consisting of four animals.[32,33] Rats' rear paws were injected with 0.1 ml of a 1% carrageenan solution to cause inflammation. One hour following the carrageenan injection, the preparations were given orally in extract form at varying doses (200, 150, and 100 mg/kg). Paw volume changes were used to measure the inflammatory response 1, 2, 3, 4, and 5 hours after injection. The following formula was used to determine the percentage decrease in swelling in comparison to the control group:

$$AEF = \Delta Vtaj - \Delta Vn / \Delta Vn \times 100\%$$

AEF - Anti-exudative activity, in percentage;
ΔVn - The control group's hind paw volume;
$\Delta Vtaj$ - Volume of the experimental group's hind paw.

In the control group, maximum swelling was observed at 3 hours, reaching 94.4 ± 8.9% compared to the baseline, and even after 5 hours, it remained 41.0 ± 3.7% higher. In the experimental groups, maximum swelling was also observed 3 hours after carrageenan administration, but the effect of the preparations brought the swelling nearly back to normal by 5 hours.[34,35] The percentage results for each hour (1, 2, 3, 4, and 5 hours) are provided (Table 3).

The following conclusions can be drawn from the study's findings about the anti-inflammatory properties of the supramolecular complex of propolis and monoammonium salt of glycyrrhizic acid (MASGA) in a 1:2 ratio at extract form doses of 200, 150, and 100 mg/kg: The dose

of 100 mg/kg exhibited the highest efficacy and the strongest anti-exudative action among the various dosages of these drugs.[36] The dose of 100 mg/kg was chosen for additional investigation based on the research findings (Table 4).

DISCUSSION

The present study successfully synthesized a supramolecular complex of propolis with glycyrrhizinic acid monoammonium salt (GMAS) in a 1:3 ratio, demonstrating promising results in terms of its physicochemical properties, vitamin composition, anti-inflammatory activity, and toxicity profile. The findings provide insights into the potential pharmaceutical applications of this complex, particularly in managing inflammation and associated disorders.

Physicochemical Properties and Composition

The GMAS-propolis complex was characterized as a white to pale-yellow amorphous powder with high water solubility, a critical feature for pharmaceutical formulations. The vibrational frequency analysis confirmed the presence of functional groups responsible for its bioactive properties, such as hydroxyl (OH), amine (NH), and carbonyl groups. Additionally, the presence of vitamins B2, B6, and C, as determined by HPLC, highlights the nutritional value and potential antioxidant benefits of the complex.

Anti-inflammatory Activity

The carrageenan-induced paw edema model in rats revealed significant anti-inflammatory effects of the GMAS-propolis complex at various doses (200, 150, and 100 mg/kg). The highest anti-exudative activity (60.7%) was observed at the 100 mg/kg dose, suggesting dose-dependent efficacy. Notably, the inflammatory response peaked at 3 hours post-carrageenan administration in both control and experimental groups. However, the GMAS-propolis complex significantly reduced swelling compared to the control, returning paw volume closer to baseline levels within 5 hours. This effect underscores the complex's potential as a potent anti-inflammatory agent, likely due to its bioactive compounds, such as flavonoids and phenolic acids, known for inhibiting inflammatory mediators.

Toxicological Profile

The acute toxicity studies demonstrated that the GMAS-propolis complex falls under Class V (non-toxic) substances, with an LD50 > 2000 mg/kg according to OECD guidelines. This finding confirms the safety of the complex at doses tested, as no significant adverse effects or mortality were observed. The absence of notable weight changes or behavioral abnormalities in treated animals further supports its low toxicity and suitability for therapeutic use.

Mechanistic Insights

The anti-inflammatory efficacy of the GMAS-propolis complex can be attributed to its rich composition of bioactive compounds, particularly flavonoids and phenolic acids, which possess well-documented anti-inflammatory, antioxidant, and immunomodulatory properties. The synergistic effect of these compounds, combined with the improved solubility afforded by the supramolecular complexation, likely enhances the bioavailability and therapeutic potential of the active ingredients.

Comparison with Existing Therapies

Compared to conventional anti-inflammatory agents, such as indomethacin (used as a reference drug in this study), the GMAS-propolis complex offers a natural, potentially safer alternative with comparable efficacy. Its multi-faceted properties—antioxidant, antibacterial, and anti-inflammatory—extend its therapeutic scope beyond inflammation to conditions involving oxidative stress and microbial infections.

Limitations and Future Directions

While this study provides compelling evidence for the anti-inflammatory and non-toxic nature of the GMAS-propolis complex, further research is warranted to elucidate its precise mechanisms of action, long-term safety, and efficacy in different disease models. Clinical trials are essential to validate its therapeutic potential in humans. Additionally, efforts to optimize the formulation and standardize the composition will enhance its applicability in pharmaceutical and nutraceutical industries.

CONCLUSION

1. The method for obtaining the supramolecular complex of propolis and monoammonium salt of glycyrrhizic acid (MASGA) in a 1:3 ratio was developed, and it was found that the complex

contains water-soluble vitamins B2, B6, and C.
2. The supramolecular complex of propolis and MASGA in a 1:3 ratio was found to contain the amino acids Aspartic acid, Glutamic acid, Serine, Glycine, Aspartine, Glutamine, Cysteine, Threonine, Arginine, Proline, Valine, Histidine, Isoleucine, Leucine, Tryptophan, Phenylalanine, and Lysine.
3. It was determined that the average lethal dose (LD50) of the supramolecular complex of propolis and MASGA in a 1:3 ratio, administered once to mice, is greater than 2000 mg/kg, classifying it as a non-toxic substance in Class V.
4. The pharmacological activity of the supramolecular complex of propolis and MASGA in a 1:3 ratio at doses of 100 mg/kg was found to have high anti-inflammatory activity.

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Conflict of interest

The authors do not have any conflict of interest.

Data Availability Statement

This statement does not apply to this article

Ethics statement

This research did not involve human participants, animal subjects, or any material that requires ethical approval.

Informed Consent Statement

This study did not involve human participants, and therefore, informed consent was not required.

Clinical Trial Registration

This research does not involve any clinical trials.

Permission to reproduce material from other sources

Not Applicable.

Author Contributions

Xurshida Saidova: Conceptualization, methodology, and preparation of the original manuscript draft. Ibrokhim Askarov: Supervision and guidance on the research framework and study design. Akmal Islomov: Supervision and critical revision of the manuscript. Jamshid Ashurov: HPLC analysis and interpretation of chromatographic data. Dilnoza Abdugafurova: Contribution to in vivo experimental procedures and data collection. Lazizbek Mahmudov: In vivo experimentation and data validation. Arofat Inkhonova: Support in research activities and manuscript review. Uchkun Ishimov: HPLC analysis and data verification.

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