

## ANNOTATION OF MATERIALS

**B1. Stoyanova, M.; Gledacheva, V.; Nikolova, S.**

**Gut–Brain–Microbiota Axis in Irritable Bowel Syndrome: A Narrative Review of Pathophysiology and Current Approaches.**

*Appl. Sci.* **2025**, *15*, 6441. <https://doi.org/10.3390/app15126441>

Irritable bowel syndrome (IBS) is a widespread functional gastrointestinal disorder characterised by chronic abdominal discomfort and altered bowel habits. Despite its high impact on life quality and healthcare systems, the initial pathophysiology of IBS is not yet fully understood. The present narrative review aims to synthesise and integrate recent evidence regarding the multifactorial nature of IBS, focusing on the interplay between gut–brain interactions, microbiota, and immune responses, without proposing a novel model but rather reinforcing and updating existing conceptual frameworks. A comprehensive literature search of relevant studies published in English during the past two decades was conducted using PubMed, Scopus, and Google Scholar. The selected articles were thoroughly evaluated to provide a complete overview of IBS-related research. The review demonstrates that IBS is not only a multifactorial condition involving gut–brain axis dysregulation, altered gut motility, visceral hypersensitivity, and microbiome disturbances, but also a crucial psychosocial factor. Modern therapeutics targeting the microbiota and neurogastroenterology pathways show promising results but require further investigation. IBS represents a heterogeneous disorder with complex interrelated mechanisms. Improvements in understanding its multifaceted nature are of paramount importance in developing more effective diagnostic and therapeutic approaches. Continued research is essential to unravel the intricacies of IBS and improve patient outcomes.

**B2. Milusheva, M.; Stoyanova, M.; Gledacheva, V.; Stefanova, I.; Todorova, M.; Pencheva, M.; Stojnova, K.; Tsoneva, S.; Nedialkov, P.; Nikolova, S.**

**2-Amino-N-Phenethylbenzamides for Irritable Bowel Syndrome Treatment.**

*Molecules* **2024**, *29*, 3375. <https://doi.org/10.3390/molecules29143375>

Irritable bowel syndrome (IBS) is a common gastrointestinal (GI) disorder characterized by abdominal pain or discomfort. Mebeverine is an antispasmodic that has been widely used in

clinical practice to relieve the symptoms of IBS. However, its systemic use usually leads to side effects. Therefore, the current paper aimed to synthesize more effective medicines for IBS treatment. We used ring opening of isatoic anhydride for the synthesis in reaction with 2-phenylethylamine. In silico simulation predicted spasmolytic activity for 2-amino-N-phenethylbenzamides. The newly synthesized compounds demonstrated a relaxation effect similar to mebeverine but did not affect the serotonin or  $\text{Ca}^{2+}$ -dependent signaling pathway of contractile activity (CA) in contrast. Having in mind the anti-inflammatory potential of antispasmodics, the synthesized molecules were tested in vitro and ex vivo for their anti-inflammatory effects. Four of the newly synthesized compounds demonstrated very good activity by preventing albumin denaturation compared to anti-inflammatory drugs/agents well-established in medicinal practice. The newly synthesized compounds also inhibited the expression of interleukin- $1\beta$  and stimulated the expression of neuronal nitric oxide synthase (nNOS), and, consequently, nitric oxide (NO) synthesis by neurons of the myenteric plexus. This characterizes the newly synthesized compounds as biologically active relaxants, offering a cleaner and more precise application in pharmacological practice, thereby enhancing their potential therapeutic value.

**B3. Milusheva, M.; Todorova, M.; Gledacheva, V.; Stefanova, I.; Feizi-Dehneyebi, M.; Pencheva, M.; Nedialkov, P.; Tumbarski, Y.; Yanakieva, V.; Tsoneva, S.; Nikolova, S. Novel Anthranilic Acid Hybrids—An Alternative Weapon against Inflammatory Diseases.**

*Pharmaceuticals* 2023, 16, 1660. <https://doi.org/10.3390/ph16121660>

Anti-inflammatory drugs are used to relieve pain, fever, and inflammation while protecting the cardiovascular system. However, the side effects of currently available medications have limited their usage. Due to these adverse effects, there is a significant need for new drugs. The current trend of research has shifted towards the synthesis of novel anthranilic acid hybrids as anti-inflammatory agents. Phenyl- or benzyl-substituted hybrids exerted very good anti-inflammatory effects in preventing albumin denaturation. To confirm their anti-inflammatory effects, additional ex vivo tests were conducted. These immunohistochemical studies explicated the same compounds with better anti-inflammatory potential. To determine the binding affinity and interaction mode, as well as to explain the anti-inflammatory activities, the molecular

docking simulation of the compounds was investigated against human serum albumin. The biological evaluation of the compounds was completed, assessing their antimicrobial activity and spasmolytic effect. Based on the experimental data, we can conclude that a collection of novel hybrids was successfully synthesized, and they can be considered anti-inflammatory drug candidates—alternatives to current therapeutics.

**B4. Milusheva, M.; Gledacheva, V.; Stefanova, I.; Feizi-Dehmayebi, M.; Mihaylova, R.; Nedialkov, P.; Cherneva, E.; Tumbarski, Y.; Tsoneva, S.; Todorova, M.; Nikolova, S. Synthesis, Molecular Docking, and Biological Evaluation of Novel Anthranilic Acid Hybrid and Its Diamides as Antispasmodics.**

*Int. J. Mol. Sci.* 2023, 24, 13855. <https://doi.org/10.3390/ijms241813855>

The present article focuses on the synthesis and biological evaluation of a novel anthranilic acid hybrid and its diamides as antispasmodics. Methods: Due to the predicted *in silico* methods spasmolytic activity, we synthesized a hybrid molecule of anthranilic acid and 2-(3-chlorophenyl)ethylamine. The obtained hybrid was then applied in acylation with different acyl chlorides. Using *in silico* analysis, pharmacodynamic profiles of the compounds were predicted. A thorough biological evaluation of the compounds was conducted assessing their *in vitro* antimicrobial, cytotoxic, anti-inflammatory activity, and *ex vivo* spasmolytic activity. Density functional theory (DFT) calculation, including geometry optimization, molecular electrostatic potential (MEP) surface, and HOMO-LUMO analysis for the synthesized compounds was conducted using the B3LYP/6-311G(d,p) method to explore the electronic behavior, reactive regions, and stability and chemical reactivity of the compounds. Furthermore, molecular docking simulation along with viscosity measurement indicated that the newly synthesized compounds interact with DNA via groove binding mode. The obtained results from all the experiments demonstrate that the hybrid molecule and its diamides inherit spasmolytic, antimicrobial, and anti-inflammatory capabilities, making them excellent candidates for future medications.

**B5. Stoyanova, M.; Milusheva, M.; Gledacheva, V.; Stefanova, I.; Todorova, M.; Kircheva, N.; Angelova, S.; Pencheva, M.; Stojnova, K.; Tsoneva, S.; Nikolova, S. Spasmolytic Activity and Anti-Inflammatory Effect of Novel Mebeverine Derivatives.**

**Biomedicines 2024, 12, 2321. <https://doi.org/10.3390/biomedicines12102321>**

**Background:** Irritable bowel syndrome (IBS) has a major negative influence on quality of life, causing cramps, stomach pain, bloating, constipation, etc. Antispasmodics have varying degrees of efficacy. Mebeverine, for example, works by controlling bowel movements and relaxing the muscles of the intestines but has side effects. Therefore, more efficient medication is required. **Methods:** In the current study, we investigated the synthesis of novel mebeverine analogs and determined *ex vivo* their spasmolytic and *in vitro* and *ex vivo* anti-inflammatory properties. The ability to influence both contractility and inflammation provides a dual-action approach, offering a comprehensive solution for the prevention and treatment of both conditions. **Results:** The results showed that all the compounds have better spasmolytic activity than mebeverine and good anti-inflammatory potential. Among the tested compounds, **3**, **4a**, and **4b** have been pointed out as the most active in all the studies conducted. To understand their mechanism of activity, molecular docking simulation was investigated. The docking analysis explained the biological activities with their calculated Gibbs energies and possibilities for binding both centers of albumin. Moreover, the calculations showed that molecules can bind also the two muscarinic receptors and interleukin- $\beta$ , hence these structures would exert a positive therapeutic effect owed to interaction with these specific receptors/cytokine. **Conclusions:** Three of the tested compounds have emerged as the most active and effective in all the studies conducted. Future *in vivo* and preclinical experiments will contribute to the establishment of these novel mebeverine derivatives as potential drug candidates against inflammatory diseases in the gastrointestinal tract.

**B6. Stoyanova, M.; Milusheva, M.; Georgieva, M.; Ivanov, P.; Miloshev, G.; Krasteva, N.; Hristova-Panusheva, K.; Feizi-Dehnyayebi, M.; Mohammadi Ziarani, G.; Stojnova, K.; Tsoneva, S.; Todorova, M.; Nikolova, S.**

**Synthesis, Cytotoxic and Genotoxic Evaluation of Drug-Loaded Silver Nanoparticles with Mebeverine and Its Analog.**

**Pharmaceuticals 2025, 18, 397. <https://doi.org/10.3390/ph18030397>**

**Background:** Irritable bowel syndrome (IBS) is a prevalent gastrointestinal disorder with a complex pathogenesis that necessitates innovative therapeutic approaches for effective management. Among the commonly used treatments, mebeverine (MBH), an antispasmodic, is widely prescribed to alleviate IBS symptoms. However, challenges in delivering the drug precisely to the colonic region often hinder its therapeutic effectiveness. To address this limitation, silver nanoparticles (AgNPs) have emerged as promising drug delivery systems, offering unique physicochemical properties that can enhance the precision and efficacy of IBS treatments. **Objectives:** This study aimed to synthesize AgNPs as drug delivery vehicles for MBH and a previously reported analog. The research focused on evaluating the cytotoxic and genotoxic effects of the AgNPs and forecasting their possibly harmful effects on future sustainable development. **Methods:** AgNPs were synthesized using a rapid method and functionalized with MBH and its analog. The nanoparticles were characterized using different techniques. Cytotoxicity and genotoxicity were evaluated in vitro. Additionally, in silico docking analyses were performed to explore their safety profile further. **Results:** In vitro assays revealed concentration-dependent cytotoxic effects and a lack of genotoxic effects with MBH-loaded AgNPs. A molecular docking simulation was performed to confirm this effect. **Conclusions:** The study underscores the potential of AgNPs as advanced drug delivery systems for safe and significant therapeutic implications for IBS. Future in vivo and preclinical investigations are essential to validate the safe range of exposure doses and evaluation standards for assessing AgNPs' safety in targeted and personalized medicine.

**B7. Stoyanova, M.; Milusheva, M.; Gledacheva, V.; Todorova, M.; Kircheva, N.; Angelova, S.; Stefanova, I.; Pencheva, M.; Vasileva, B.; Hristova-Panusheva, K.; Krasteva, N.; Miloshev, G.; Tumbarski, Y.; Georgieva, M.; Nikolova, S.**

**Silver Nanoparticles with Mebeverine in IBS Treatment: DFT Analysis, Spasmolytic, and Anti-Inflammatory Effects.**

*Pharmaceutics* 2025, 17, 561. <https://doi.org/10.3390/pharmaceutics17050561>

**Background/Objectives:** Mebeverine hydrochloride (MBH) is an antispasmodic agent used to regulate bowel movements and relax intestinal smooth muscle, but its application is limited by specific side effects; therefore, this study investigates the effects of previously synthesized MBH-loaded silver nanoparticles (AgNPs) on smooth muscle contractile activity and their anti-

inflammatory potential as an alternative delivery system. **Methods:** The interactions of AgNPs with cholinergic inhibitors, selective antagonists, Ca<sup>2+</sup> blockers, and key neurotransmitters were analyzed. In vitro, albumin denaturation suppression and ex vivo assays evaluated the anti-inflammatory effects of AgNPs-MBH, validated using a DFT in silico approach. To comprehensively assess the systemic impact and IBS treatment potential of AgNPs-MBH, we also examined in vitro their antimicrobial activity and hepatic cell responses, as the liver is a key organ in evaluating the overall safety and efficacy of nanoparticles. Additionally, the drug-release capabilities of Ag NPs were established. **Results:** Our findings indicate that AgNPs with MBH do not affect blocked cholinergic receptors, but their effects are more pronounced and distinct in amplitude and character than MBH. MBH-loaded AgNPs showed a lower anti-inflammatory effect than MBH but were still better than diclofenac. They also affected hepatic cell morphology and proliferation, suggesting potential for enhanced therapeutic efficacy. Drug-loaded AgNPs are considered not bactericidal. **Conclusions:** Based on our results, drug-loaded AgNPs might be a promising medication delivery system for MBH and a useful treatment option for IBS. Future in vivo and preclinical experiments will contribute to the establishment of drug-loaded AgNPs in IBS treatment.

**B8. Stoyanova, M.; Gledacheva, V.; Milusheva, M.; Todorova, M.; Kircheva, N.; Angelova, S.; Stefanova, I.; Pencheva, M.; Tumbarski, Y.; Vasileva, B.; Hristova-Panusheva, K.; Gospodinova, Z.; Krasteva, N.; Miloshev, G.; Georgieva, M.; Nikolova, S. Functionalized Silver Nanoparticles as Multifunctional Agents Against Gut Microbiota Imbalance and Inflammation.**

*Nanomaterials* 2025, 15, 815. <https://doi.org/10.3390/nano15110815>

Human pathogenic fungi are the source of various illnesses, including invasive, cutaneous, and mucosal infections. One promising solution is using nanoparticles (NPs) as an antifungal agent. The current study aims to assess the antimicrobial and antifungal effects of drug-loaded silver nanoparticles (AgNPs) with previously reported mebeverine analogue (MA) as a potential drug candidate targeting gut microbiota and inflammation in the gastrointestinal tract. Density Functional Theory (DFT) calculations were conducted to identify possible mechanisms by which AgNPs could prevent microorganisms from growing. In vitro and ex vivo anti-inflammatory, in vitro antimicrobial, ex vivo spasmolytic activities, and in vitro hepatic cell

morphology and proliferation of drug-loaded AgNPs were assessed. The drug-loaded AgNPs were considered to have promising antifungal activity against all tested fungal strains, *Aspergillus niger*, *Penicillium chrysogenum*, and *Fusarium moniliforme*, and yeasts, *Candida albicans*, *Saccharomyces cerevisiae*, and good antimicrobial activity against Gram-positive and Gram-negative bacterial strains. The results of in vitro and ex vivo determination of anti-inflammatory activity indicated that the drug-loaded AgNPs preserved MA's anti-inflammatory activity and decreased inflammation. A similar effect was observed in spasmolytic activity measurements. Drug-loaded AgNPs also influenced the morphology and proliferation of hepatic cells, indicating a potential for improved gut and liver therapeutic efficacy. Each test was performed in triplicate, and the results were reported as mean values. Based on the results, drug-loaded AgNPs might be a promising antimicrobial agent, maintaining the MA's potential as a spasmolytic and anti-inflammatory agent. Future in vivo and preclinical experiments will contribute to establishing the in vivo properties of drug-loaded AgNPs.

**Г1. Lyudmilova, M.; Stoychev, L.; Yancheva, D.; Nikolova, S.; Todorova, M.; Xenodochidis, C.; Hristova-Panusheva, K.; Krasteva, N.; Genova, J.**

**Influence of Silver Nanoparticles on Liposomal Membrane Properties Relevant in Photothermal Therapy.**

*Appl. Sci.* 2026, 16, 220. <https://doi.org/10.3390/app16010220>

Silver nanoparticles (AgNPs) are promising agents for nanomedicine but their interactions with lipid membranes, which are a key interfaces for drug delivery, require a deeper understanding. This study investigates the influence of fructose-capped AgNPs on the physicochemical properties of SOPC-based liposomal bilayers, with potential implications for drug delivery and photothermal therapy. We employed a multitechnique approach, including infrared (IR) spectroscopy, differential scanning calorimetry (DSC), thermally induced shape fluctuation analysis, and laser irradiation at 343, 515, and 1030 nm. Our results show that AgNPs incorporated into the bilayer cause measurable perturbations: DSC reveals a decrease in the main phase transition enthalpy (from 0.280 to 0.234 J/g) and temperature (from 2.80 to 3.41 °C), while shape fluctuation analysis indicates a reduction in bending modulus (from  $1.18 \times 10^{-19}$  J to  $0.93 \times 10^{-19}$  J), confirming increased membrane fluidity. FTIR confirms interactions of fructose-capped nanoparticles and the lipid's carbonyl and phosphate groups.

Furthermore, the AgNPs-liposomes exhibit a strong, wavelength-dependent photothermal response with a temperature increase of  $\approx 22$  °C under 515 nm laser irradiation, compared to only 3–5 °C at 1030 nm. We conclude that fructose-capped AgNPs moderately fluidify lipid bilayers while enabling efficient, controllable photothermal capability, making them excellent candidates for the eventual design of advanced liposomal systems for combined therapy and diagnostics.

**Г2. Todorova, M.; Milusheva, M.; Kaynarova, L.; Georgieva, D.; Delchev, V.; Simeonova, S.; Pilicheva, B.; Nikolova, S.**

**Drug-Loaded Silver Nanoparticles—A Tool for Delivery of a Mebeverine Precursor in Inflammatory Bowel Diseases Treatment.**

*Biomedicines* 2023, *11*, 1593. <https://doi.org/10.3390/biomedicines11061593>

Chronic, multifactorial illnesses of the gastrointestinal tract include inflammatory bowel diseases. One of the greatest methods for regulated medicine administration in a particular region of inflammation is the nanoparticle system. Silver nanoparticles (Ag NPs) have been utilized as drug delivery systems in the pharmaceutical industry. The goal of the current study is to synthesize drug-loaded Ag NPs using a previously described 3-methyl-1-phenylbutan-2-amine, as a mebeverine precursor (MP). Methods: A green, galactose-assisted method for the rapid synthesis and stabilization of Ag NPs as a drug-delivery system is presented. Galactose was used as a reducing and capping agent forming a thin layer encasing the nanoparticles. Results: The structure, size distribution, zeta potential, surface charge, and the role of the capping agent of drug-loaded Ag NPs were discussed. The drug release of the MP-loaded Ag NPs was also investigated. The Ag NPs indicated a very good drug release between 80 and 85%. Based on the preliminary results, Ag NPs might be a promising medication delivery system for MP and a useful treatment option for inflammatory bowel disease. Therefore, future research into the potential medical applications of the produced Ag NPs is necessary.

**Г3. Nikolova, S.; Milusheva, M.; Gledacheva, V.; Feizi-Dehnayebi, M.; Kaynarova, L.; Georgieva, D.; Delchev, V.; Stefanova, L.; Tumbarski, Y.; Mihaylova, R.; et al.**

**Drug-Delivery Silver Nanoparticles: A New Perspective for Phenindione as an Anticoagulant.**

*Biomedicines* 2023, *11*, 2201. <https://doi.org/10.3390/biomedicines11082201>

Anticoagulants prevent the blood from developing the coagulation process, which is the primary cause of death in thromboembolic illnesses. Phenindione (PID) is a well-known anticoagulant that is rarely employed because it totally prevents coagulation, which can be a life-threatening complication. The goal of the current study is to synthesize drug-loaded Ag

NPs to slow down the coagulation process. Methods: A rapid synthesis and stabilization of silver nanoparticles as drug-delivery systems for phenindione (PID) were applied for the first time. Results: Several methods are used to determine the size of the resulting Ag NPs. Additionally, the drug-release capabilities of Ag NPs were established. Density functional theory (DFT) calculations were performed for the first time to indicate the nature of the interaction between PID and nanostructures. DFT findings supported that galactose-loaded nanostructure could be a proper delivery system for phenindione. The drug-loaded Ag NPs were characterized *in vitro* for their antimicrobial, cytotoxic, and anticoagulant activities, and *ex vivo* for spasmolytic activity. The obtained data confirmed the drug-release experiments. Drug-loaded Ag NPs showed that prothrombin time (PT, sec) and activated partial thromboplastin time (APTT, sec) are approximately 1.5 times longer than the normal values, while PID itself stopped coagulation at all. This can make the PID-loaded Ag NPs better therapeutic anticoagulants. PID was compared to PID-loaded Ag NPs in antimicrobial, spasmolytic activity, and cytotoxicity. All the experiments confirmed the drug-release results.

Ivanova, A.; Todorova, M.; Petrov, D.; Petkova, Z.; Teneva, O.; Antova, G.; Angelova-Romova, M.; Yanakieva, V.; Tsoneva, S.; Gledacheva, V.; Nikolova, K.; Karashanova, D.; Nikolova, S.

From *Spirulina platensis* to Nanomaterials: A Comparative Study of AgNPs Obtained from Two Extracts.

*Nanomaterials* 2025, 15, 1392. <https://doi.org/10.3390/nano15181392>

This study presents the synthesis and characterization of silver nanoparticles (AgNPs) using two *Spirulina platensis* extracts: one of them cultivated in a bioreactor in Bulgaria (near Varvara village), and the other one from the local market in Bulgaria (Dragon Superfoods). To assess their properties and stability, ATR-FTIR, TEM (Transmission Electron Microscopy) images, and zeta potential were used. Chemical content of the extracts and AgNPs obtained were assessed, as well as their antimicrobial and anti-inflammatory activities. We found that the extracts' origin significantly influenced nanoparticle morphology, surface charge, and bioactivity. AgNPs were spherical and different in size from Bioreactor 4–8 nm, while Dragon obtained larger particles, about 20 nm. We found that synthesis altered the chemical content of the extracts, particularly in lipid, protein, and tocopherol content, suggesting active involvement of *Spirulina*-derived biomolecules in nanoparticle formation. Antimicrobial assays showed

slightly higher activity for Dragon AgNPs against *P. aeruginosa* (21 mm) and *S. enteritidis* (23 mm), with similar effects against *L. monocytogenes* and *S. aureus*. At 2.5 mg/mL, both samples protected human albumin from thermal denaturation more effectively (23.36% and 20.07%) than prednisolone (16.99%). Based on the obtained results, AgNPs from *Spirulina platensis* can be attributed as multifunctional agents with anti-inflammatory and antimicrobial activity.

**G5. Andonova, V.; Nikolova, K.; Iliev, I.; Georgieva, S.; Petkova, N.; Feizi-Dehnyayebi, M.; Nikolova, S.; Gerasimova, A.**

**Spectral Characteristics, In Silico Perspectives, Density Functional Theory (DFT), and Therapeutic Potential of Green-Extracted Phycocyanin from *Spirulina*.**

*Int. J. Mol. Sci.* 2024, 25, 9170. <https://doi.org/10.3390/ijms25179170>

Phycocyanin (PC) is a naturally occurring green pigment in *Spirulina*. It was extracted by ultrasonic extraction using green technology, and its structure was studied using IR- and NMR-spectroscopy. Spectral data confirmed the PC structure. This study also involves an in silico assessment of the diverse applications of green pigment PC. Utilizing QSAR, PreADME/T, SwissADME, and Pro-Tox, this study explores the safety profile, pharmacokinetics, and potential targets of PC. QSAR analysis reveals a favorable safety profile, with the parent structure and most metabolites showing no binding to DNA or proteins. PreADME/T indicates low skin permeability, excellent intestinal absorption, and medium permeability, supporting oral administration. Distribution analysis suggests moderate plasma protein binding and cautious blood–brain barrier permeability, guiding formulation strategies. Metabolism assessments highlight interactions with key cytochrome P450 enzymes, influencing drug interactions. Target prediction analysis unveils potential targets, suggesting diverse therapeutic effects, including cardiovascular benefits, anti-inflammatory activities, neuroprotection, and immune modulation. Based on the in silico analysis, PC holds promise for various applications due to its safety, bioavailability, and potential therapeutic benefits. Experimental validation is crucial to elucidate precise molecular mechanisms, ensuring safe and effective utilization in therapeutic and dietary contexts. DFT calculations, including geometry optimization, MEP analysis, HOMO-LUMO energy surface, and quantum reactivity parameters of the PC compound, were obtained using the B3LYP/6–311G(d,p) level. This integrated approach contributes to a comprehensive understanding of PC's pharmacological profile and informs future research directions.

**Г6. Milusheva, M.; Gledacheva, V.; Batmazyan, M.; Nikolova, S.; Stefanova, I.; Dimitrova, D.; Saracheva, K.; Tomov, D.; Chaova-Gizdakova, V.**  
**Ex Vivo and In Vivo Study of Some Isoquinoline Precursors.**  
*Sci. Pharm.* 2022, 90, 37. <https://doi.org/10.3390/scipharm90020037>

This article concerns the synthesis and biological activities of some N-(1-(3,4-dimethoxyphenyl)propan-2-yl) amides as isoquinoline precursors and compounds with smooth muscle (SM) relaxant activity. Aim: find the biological activity of N-(1-(3,4-dimethoxyphenyl)propan-2-yl) amides and compare it with papaverine, an isoquinoline alkaloid that has been known as a brain and coronary vasodilator and SM relaxant. Materials and methods: In silico simulation with the PASS online program predicts SM relaxant activity for the compounds. The amides were tested on the isolated gastric SM preparations (SMPs) from rats to determine their effects on spontaneous contractile activity (CA) compared with papaverine. The in vivo effect on the learning and memory processes of rats was also assessed. Results: the data from the isometric measurements showed that one of the compounds caused ex vivo relaxation in circular SM tissues isolated from the stomach (corpus) of male Wistar rats. Conclusion: We found that the compound's SM relaxation uses the papaverine pathway. It also has an improving effect on the cognitive functions of learning and memory processes in rats.

**Г7. Todorova, M.; Bakalska, R.; Feizi-Dehnayebi, M.; Ziarani, G.M.; Pencheva, M.; Stojnova, K.; Milusheva, M.; Nedialkov, P.; Cherneva, E.; Kolev, T.; Nikolova, S.**  
**Synthesis, Anti-Inflammatory Activity, and Docking Simulation of a Novel Styryl Quinolinium Derivative.**  
*Appl. Sci.* 2025, 15, 284. <https://doi.org/10.3390/app15010284>

Stilbenes, like resveratrol, are natural small molecules with several applications in the treatment of chronic illnesses, diabetes, and neurological and cardiovascular conditions. Considering the molecular structure of stilbenes and the biological activities of resveratrol, we investigated the synthesis and biological activity of a novel styryl quinolinium (SQ) derivative. The SQ was synthesized using the alkylation of lepidine and methyl 4-methylbenzenesulfonate and further Knoevenagel condensation with 2-hydroxy naphthaldehyde. Its structure was determined using NMR, IR, Raman, UV-Vis, and MS. The current trend of research has shifted toward the

synthesis of novel SQ as resveratrol's analog. Therefore, its anti-inflammatory and antioxidant activities were evaluated. The compound exerted very good anti-inflammatory effects in preventing albumin denaturation, which were confirmed by ex vivo immunohistochemical studies. Density functional theory (DFT) analyses were conducted on the SQ structure to gain detailed insights into its active sites, energy gap, quantum properties, and electronic behavior. The biological evaluation of the compound was completed, assessing its antioxidant potential using DPPH radical scavenging assay. In addition, molecular docking simulations were utilized to assess the SQ compound's potential inhibitory effect on human serum albumin (HSA).

**I8. Tsoneva, S.; Milusheva, M.; Burdzhiev, N.; Marinova, P.; Varbanova, E.; Tumbarski, Y.; Mihaylova, R.; Cherneva, E.; Nikolova, S.**

**Antimicrobial Activity of Ethyl (2-(Methylcarbamoyl)phenyl)carbamate and Its Mixed Ligand Ni(II) and Co(II) Complexes.**

*Inorganics* 2025, 13, 267. <https://doi.org/10.3390/inorganics13080267>

The aim of this paper is to obtain ethyl (2-(methylcarbamoyl)phenyl)carbamate and its metal complexes as promising antimicrobial agents. The title compound was synthesized using the ring-opening of isatoic anhydride with methylamine and further acylation with ethyl chloroformate. All metal complexes were successfully obtained after mixing the ligand dissolved in DMSO and water solutions of the corresponding metal salts and sodium hydroxide, in a metal-to-ligand-to base ratio 1:2:2. As a result, mixed ligand complexes of ethyl 2-(methylcarbamoyl)phenylcarbamate and 3-methylquinazoline-2,4(1H,3H)-dione were obtained. The obtained complexes were characterized by their melting points, FTIR, NMR spectroscopy, and MP-AES. Then, the antimicrobial effect of the compounds against both Gram-negative and Gram-positive bacteria, yeasts, and fungi was studied. Only the Co(II) complex showed antimicrobial activity against almost all Gram-positive and Gram-negative bacteria. The cobalt complex exhibited promising antimicrobial activity against Gram-positive *Micrococcus luteus* with inhibition zones of 20 mm, *Listeria monocytogenes* (15 mm), *Staphylococcus aureus* (13 mm), as well as Gram-negative *Klebsiella pneumoniae* (13 mm) and *Proteus vulgaris* (13 mm). Given the potential of metal complexes as antimicrobial agents, understanding their cytotoxic effects is crucial for evaluating their therapeutic safety. To assess the in vitro biocompatibility of the experimental compounds, a range of cell viability assays was conducted using human malignant leukemic cell lines (LAMA-84, K-562) and

normal murine fibroblast cells (CCL-1). The Ni(II) complex shows  $IC_{50} = 105.1 \mu M$  against human malignant leukemic cell lines LAMA-84. Based on the reported results, it may be concluded that the mixed cobalt complex of 2-(methylcarbamoyl)phenyl)carbamate and 3-methylquinazoline-2,4(1*H*,3*H*)-dione can be attributed as a promising antimicrobial agent. Future in vivo tests will contribute to establishing the antimicrobial properties of this complex.

**Γ9. Mohammadi Ziarani, G.; Rezakhani, M.; Feizi-Dehneyebi, M.; Nikolova, S. Fumed-Si-Pr-Ald-Barb as a Fluorescent Chemosensor for the  $Hg^{2+}$  Detection and  $Cr_2O_7^{2-}$  Ions: A Combined Experimental and Computational Perspective. *Molecules* 2024, 29, 4825. <https://doi.org/10.3390/molecules29204825>**

The surface of fumed silica nanoparticles was modified by pyridine carbaldehyde and barbituric acid to provide fumed-Si-Pr-Ald-Barb. The structure was identified and investigated through diverse techniques, such as FT-IR, EDX, Mapping, BET, XRD, SEM, and TGA. This nanocomposite was used to detect different cations and anions in a mixture of  $H_2O:EtOH$ . The results showed that fumed-Si-Pr-Ald-Barb can selectively detect  $Hg^{2+}$  and  $Cr_2O_7^{2-}$  ions. The detection limits were calculated at about  $5.4 \times 10^{-3} M$  for  $Hg^{2+}$  and  $3.3 \times 10^{-3} M$  for  $Cr_2O_7^{2-}$  ions. A computational method (DFT) was applied to determine the active sites on the Pr-Ald-Barb for electrophilic and nucleophilic attacks. The HOMO-LUMO molecular orbital was calculated by B3LYP/6-311G(d,p)/LANL2DZ theoretical methods. The energy gap for the Pr-Ald-Barb and Pr-Ald-Barb+ion complexes was predicted by the  $E_{HOMO}$  and  $E_{LUMO}$  values. The DFT calculation confirms the suggested experimental mechanism for interacting the Pr-Ald-Barb with ions.

**Γ10. Milusheva, M.; Gledacheva, V.; Stefanova, I.; Pencheva, M.; Mihaylova, R.; Tumbariski, Y.; Nedialkov, P.; Cherneva, E.; Todorova, M.; Nikolova, S. In Silico, In Vitro, and Ex Vivo Biological Activity of Some Novel Mebeverine Precursors. *Biomedicines* 2023, 11, 605. <https://doi.org/10.3390/biomedicines11020605>**

Irritable bowel syndrome (IBS) is a functional gastroenterological disorder with complex pathogenesis and multifaceted therapy approaches, aimed at alleviating clinical symptoms and improving the life quality of patients. Its treatment includes dietary changes and drugs from various pharmacological groups such as antidiarrheals, anticholinergics, serotonin receptor antagonists, targeting chloride ion channels, etc. The present article is focused on the synthesis and biological evaluation of some mebeverine precursors as potential antispasmodics. Methods:

In silico analysis aimed at predicting the pharmacodynamic profile of the compounds was performed. Based on these predictions, ex vivo bioelectrical activity (BEA) and immunohistochemical effects of the compounds were established. A thorough biological evaluation of the compounds was conducted assessing their in vitro antimicrobial and cytotoxic activity. Results: All the newly synthesized compounds exerted drug-like properties, whereby 3-methyl-1-phenylbutan-2-amine **3** showed a significant change in BEA due to Ca<sup>2+</sup> channel regulation, Ca<sup>2+</sup> influx modulation, and a subsequent change in smooth muscle cell response. The immunohistochemical studies showed a good correlation with the obtained data on the BEA, defining amine **3** as a leader structure. No cytotoxicity to human malignant leukemic cell lines (LAMA-84, K-562) was observed for all tested compounds. Conclusion: Based on the experimental results, we outlined 3-methyl-1-phenylbutan-2-amine **3** as a potential effective choice for orally active long-term therapy of IBS.

**Г11. Milusheva, M.; Stoyanova, M.; Gledacheva, V.; Stefanova, I.; Todorova, M.; Nikolova, S.**

**Spasmolytic Activity of 1,3-Disubstituted 3,4-Dihydroisoquinolines.**

*Biomedicines* **2024**, *12*, 1556. <https://doi.org/10.3390/biomedicines12071556>

This article concerns the spasmolytic activities of some novel 1,3-disubstituted 3,4-dihydroisoquinolines. These compounds can be evaluated as potential therapeutic candidates according to Lipinski's rule of five, showing high gastrointestinal absorption and the ability to cross the blood–brain barrier, which is a very important parameter in the drug discovery processes. In silico simulation predicted smooth muscle relaxant activity for all the compounds. Since smooth muscle contractile failure is a characteristic feature of many disorders, in the current paper, we concentrate on the parameters of the spontaneous contractile responses of smooth muscle (SM) cells compared to the well-known drug mebeverine. Two of the newly synthesized substances can be identified as essential modulating regulators and potentially used as therapeutic molecules. One of these molecules also showed significant DPPH antioxidant activity compared to rutin.

Г12. Slavchev, V.; Gledacheva, V.; Pencheva, M.; Milusheva, M.; Nikolova, S.; Stefanova, I.

Therapeutic Potential of 1-(2-Chlorophenyl)-6,7-dimethoxy-3-methyl-3,4-dihydroisoquinoline.

*Molecules* 2024, 29, 3804. <https://doi.org/10.3390/molecules29163804>

The synthesized compound 1-(2-chlorophenyl) 6-7-dimethoxy-3-methyl-3,4-dihydroisoquinoline (DIQ) was investigated as a biological agent. Its potential to affect muscle contractility was predicted through in silico PASS analysis. Based on the in silico analysis, its capabilities were experimentally investigated. The study aimed to investigate the effects of DIQ on the ex vivo spontaneous contractile activity (CA) of smooth muscle (SM) tissue. DIQ was observed to reduce the strength of Ca<sup>2+</sup>-dependent contractions in SM preparations (SMP), possibly by increasing cytosolic Ca<sup>2+</sup> levels through the activation of a voltage-gated L-type Ca<sup>2+</sup> channel. DIQ potently affected calcium currents by modulating the function of muscarinic acetylcholine receptors (mAChRs) and 5-hydroxytryptamine (5-HT) receptors at a concentration of 50 µM. Immunohistochemical tests showed a 47% reduction in 5-HT<sub>2A</sub> and 5-HT<sub>2B</sub> receptor activity in SM cells and neurons in the myenteric plexus (MP), further confirming the effects of DIQ. Furthermore, a significant inhibition of neuronal activity was observed when the compound was co-administered with 5-HT to SM tissues. The conducted experiments confirm the ability of the isoquinoline analog to act as a physiologically active molecule to control muscle contractility and related physiological processes.

Г13. Gledacheva, V.; Pencheva, M.; Nikolova, S.; Stefanova, I.

Ability of 2-Chloro-N-(1-(3,4-dimethoxyphenyl)propan-2-yl)-2-phenylacetamide to Stimulate Endogenous Nitric Oxide Synthesis.

*Appl. Sci.* 2022, 12, 4473. <https://doi.org/10.3390/app12094473>

Papaverine is one of the isoquinoline alkaloids derived from opium which is a vasodilator and smooth muscle relaxant. Using its chemical structure as a basic model, we synthesized 2-chloro-N-(1-(3,4-dimethoxyphenyl)propan-2-yl)-2-phenylacetamide as an isoquinoline precursor (IQP). Aim: Clarifying the nature of the relationship between IQP as a new biologically active molecule and the neurotransmitters acetylcholine (ACh) and serotonin (5-hydroxytryptamine, 5-HT), as well as with the nitric oxide (NO). Materials and methods: The IQP compound was tested on the isolated gastric smooth muscle preparations (SMPs) from rats to determine its

effects on spontaneous contractile activity. NO concentration in tissue homogenates was determined, and immunohistochemistry was used to visualize the expression of neuronal nitric oxide synthase (nNOS) and endothelial nitric oxide synthase (eNOS) in smooth muscle (SM) cells. Results: The data from the isometric measurements suggest that IQP has an additional specific action affecting the intracellular signaling pathways of 5-HT. Using immunohistochemistry, we found that the combination of 5-HT and IQP affected the density and intensity of nNOS-positive cells, which increase significantly in the myenteric plexus and SM cells. Conclusions: In conclusion, IQP is involved in the regulation of intestinal neurons expressing nNOS, affects the function of nNOS/NO, and, by this mechanism, probably regulates the spontaneous contractile activity of gastric SM.

**F14. Ivanova, S.; Ivanova, A.; Todorova, M.; Gledacheva, V.; Nikolova, S.**  
***Echinops* as a Source of Bioactive Compounds—A Systematic Review.**  
***Pharmaceuticals* 2025, 18, 1353. <https://doi.org/10.3390/ph18091353>**

**Background:** *Echinops* is a genus of spiny, herbaceous perennials in the *Asteraceae* family, known for its distinct morphology and broad pharmacological potential. Both traditional and modern medicinal systems have identified species in this genus as sources of bioactive compounds with anti-inflammatory, antimalarial, antidiabetic, anticancer, and neuroprotective effects. **Aims:** This study aimed to conduct a systematic literature review and update previous overviews of the recently reported phytochemicals and pharmacological properties of *Echinops*, systematically summarizing biological activities and their therapeutic applications. **Methods:** Major electronic medical databases—PubMed, Scopus, Science Direct, Web of Science, and Google Scholar—were systematically searched for publications from 1990 to 2025. **Results:** A total of 134 studies met our inclusion criteria. Thiophenes and terpenes emerged as characteristic metabolites of the genus, and along with flavonoids and alkaloids, contributed to a wide range of bioactivities. Experimental evidence supports the potential of these compounds as multifunctional agents, although clinical validation remains limited. **Conclusions:** *Echinops* is a promising source of structurally diverse metabolites with therapeutic relevance. Further pharmacological and toxicological studies are needed to establish their efficacy and ensure safe medical application.

**Г15. Todorova, M.; Petkova, N.; Ivanov, I.; Tumbarski, Y.; Yanakieva, V.; Vasileva, I.; Barakova, Y.; Cherneva, E.; Nikolova, S.**

**Chemical Characteristics and Biological Potential of *Prunus laurocerasus* Fruits.**

*Life* 2025, 15, 1847. <https://doi.org/10.3390/life15121847>

Fruits of the *Laurocerasus officinalis* Roem., known as cherry laurel, are found in the Black Sea region. This study examines the phytochemical characterization, antioxidant properties, and antimicrobial potential of cherry laurel's fruits, variety Novita, cultivated in Southern Bulgaria. The study is significant since it examines the phytochemical profile of this variety's fruits for the first time. The carbohydrate composition of the fruit was identified. The total polyphenols and flavonoids of five fruit extracts (96% ethanol, 70% ethanol, 50% ethanol, 80% methanol, and water) were determined. The antioxidant potential of these five extracts was evaluated by three methods: DPPH, ABTS, and FRAP. We found that the 96% ethanol extract had the highest content of polyphenols and flavonoids and the highest antioxidant activity values by all three methods. A correlation was established between the content of polyphenols, flavonoids, and antioxidant activity based on the calculated correlation coefficient. The antimicrobial potential of methanolic and aqueous extracts of the fruit of the laurel cherry was evaluated against twenty microorganisms. It was found that the methanolic extracts exhibited moderate to high sensitivity against both Gram-positive and Gram-negative strains, yeast *Saccharomyces cerevisiae*, and five fungi, while water extracts had moderately sensitive activity against *Micrococcus luteus* only. Based on the results, we can conclude that the fruits demonstrate good antioxidant and antimicrobial potential.

**Г16. Panova, N.; Gerasimova, A.; Todorova, M.; Pencheva, M.; Dincheva, I.; Batovska, D.; Gledacheva, V.; Slavchev, V.; Stefanova, I.; Nikolova, S.; et al.**

**Metabolite Signatures and Particle Size as Determinants of Anti-Inflammatory and Gastrointestinal Smooth Muscle Modulation by *Chlorella vulgaris*.**

*Foods* 2025, 14, 3319. <https://doi.org/10.3390/foods14193319>

*Chlorella vulgaris* is a nutrient-dense microalga with recognized antioxidant, anti-inflammatory, and metabolic regulatory properties, making it an attractive candidate for functional food applications. In such contexts, both chemical composition and particle size can influence dispersibility, bioactive release, and physiological effects. In this study, two commercial *C. vulgaris* powders from India (Sample 1) and the UK (Sample 2) were compared

with respect to particle size, metabolite composition, and biological activity. Sample 1 exhibited finer particles, while Sample 2 was coarser. GC–MS profiling revealed distinct compositional differences: Sample 1 displayed a higher relative abundance of saturated fatty acids,  $\beta$ -sitosterol,  $\beta$ -amyrin, and glucitol, whereas Sample 2 contained higher levels of unsaturated fatty acids, betulin, salicylic acid, and specific carbohydrates. In vitro assays showed stronger inhibition of albumin denaturation by Sample 1 compared with Sample 2 and prednisolone. Ex vivo tests indicated that both samples induced tonic contraction of gastric smooth muscle through muscarinic acetylcholine receptors (mAChRs) and L-type calcium channels, as evidenced by the marked reduction in responses after atropine and verapamil treatment, with Sample 1 producing a more pronounced effect. Immunohistochemistry further demonstrated broader IL-1 $\beta$  upregulation with Sample 1 and localized nNOS modulation with Sample 2. Overall, the results demonstrate that the interplay between composition and particle size shapes the bioactivity of *C. vulgaris*, supporting its targeted use in digestive, neuroimmune, and cardiometabolic health.

Г17. Panova, N.; Gerasimova, A.; Gentsheva, G.; Nikolova, S.; Makedonski, L.; Velikova, M.; Beraich, A.; Talhaoui, A.; Petkova, N.; Batovska, D.; et al. ***Moringa oleifera* Lam.: A Nutritional Powerhouse with Multifaceted Pharmacological and Functional Applications.** *Life* 2025, 15, 881. <https://doi.org/10.3390/life15060881>

*Moringa oleifera*, often referred to as the “miracle tree”, has gained widespread recognition for its exceptional nutritional profile and broad pharmacological potential. This review provides a comprehensive synthesis of the plant’s botanical characteristics, taxonomy, cultivation practices, and biochemical composition. Special emphasis is placed on its rich content of bioactive secondary metabolites—such as flavonoids, alkaloids, phenolic acids, saponins, isothiocyanates, and glucosinolates—which underlie its diverse therapeutic effects. The paper compiles and analyzes evidence from over 200 peer-reviewed studies, documenting antioxidant, anti-inflammatory, antimicrobial, antidiabetic, anticancer, hepatoprotective, neuroprotective, and anti-obesity effects, among others. For instance, leaf extracts have demonstrated potent antioxidant and antidiabetic effects in both animal models and clinical trials, while seed-derived isothiocyanates have shown significant antibacterial and anticancer activity. In addition, clinical and in vivo data support *M. oleifera*’s role in fertility regulation,

cardiovascular protection, and neurodegenerative disease mitigation. Beyond its medicinal applications, the review highlights its growing use in functional foods, dietary supplements, and cosmeceutical products, reflecting its commercial and industrial relevance. By consolidating findings across disciplines, this review underscores the multifaceted value of *M. oleifera* as a nutraceutical and therapeutic resource.

Г18. Panova, N.; Gerasimova, A.; Tumbarski, Y.; Ivanov, I.; Todorova, M.; Dincheva, I.; Gentsheva, G.; Gledacheva, V.; Slavchev, V.; Stefanova, I.; Petkova, N.; Nikolova, S.; Nikolova, K.

**Metabolic Profile, Antioxidant, Antimicrobial, Contractile, and Anti-Inflammatory Potential of *Moringa oleifera* Leaves (India).**

*Life* 2025, 15, 583. <https://doi.org/10.3390/life15040583>

Background: *Moringa oleifera* is one of the most famous plants used for medicinal purposes. Its leaf extracts have antimicrobial activity and antioxidant activities, and reduce swelling in ulcers. Objectives: The present article aimed to determine the metabolic profile of *Moringa oleifera* leaf extracts from two samples originating from India and to evaluate in vitro and ex vivo their biological activities. Methods: The antioxidant properties of *Moringa oleifera* leaf extracts (methanol, 50% ethanol, and 70% ethanol) were studied in vitro for antimicrobial, anti-inflammatory, and antioxidant activities. The ex vivo contractile effects of the extracts were determined by assaying circularly isolated smooth muscle (SM) strips from a rat's stomach. Results: The obtained results indicated that one of the samples had amino acid and organic acid content approximately twice that of the second sample. In all the tests, the 50% ethanol extract of both samples showed better antioxidant activity (209 mM TE 100 g<sup>-1</sup> for the DPPH method). The 70% ethanolic extract of Sample 1 exhibited the strongest antimicrobial activity, inhibiting Gram-positive *Bacillus cereus*, *Bacillus subtilis*, and *Staphylococcus aureus*. The 50% ethanolic extract of both samples exhibited the highest anti-inflammatory activity, demonstrating a twice better effect than the reference drug diclofenac. Finally, the pre-incubation of SM preparations with both samples significantly enhanced the ACh-induced contractile response, increasing it by 134% and 111%, respectively. Conclusions: The findings suggested potential applications of *Moringa oleifera* as a suitable candidate for antimicrobial, antioxidant, anti-inflammatory, and antispasmodic supplementation for alternative pharmaceutical and nutraceutical products.

**Г19. Popstoyanova, D.; Gerasimova, A.; Gentsheva, G.; Nikolova, S.; Gavrilova, A.; Nikolova, K.**

***Ziziphus jujuba*: Applications in the Pharmacy and Food Industry.**

***Plants* 2024, 13, 2724. <https://doi.org/10.3390/plants13192724>**

*Ziziphus jujuba* has been used since ancient times in traditional Eastern medicine. It is widely cultivated in numerous countries between the tropical and temperate climatic zones due to its high ecological plasticity and resilience to adverse weather. The different classes of chemical compounds contained in the plant are the reason for its medicinal properties. Research shows that every part of *Ziziphus jujuba*, the leaves, fruits and seeds, demonstrate therapeutic properties. This review focuses on the chemical composition in order to establish the relationship between the plant and its clinical use. Various biological effects are summarized and discussed: anticancer, anti-inflammatory, immunostimulating, antioxidant, hepatoprotective, gastrointestinal, etc. Apart from medicinal uses, the fruits of *Ziziphus jujuba* are edible and used in fresh and dried form. This literature review reveals possible medical applications of *Ziziphus jujuba* and its great potential for improving the diet of people in areas where the plant is abundant.

**Г20. Gerasimova, A.; Nikolova, K.; Petkova, N.; Ivanov, I.; Dincheva, I.; Tumbarski, Y.; Yanakieva, V.; Todorova, M.; Gentsheva, G.; Gavrilova, A.; et al. Metabolic Profile of Leaves and Pulp of *Passiflora caerulea* L. (Bulgaria) and Their Biological Activities. *Plants* 2024, 13, 1731. <https://doi.org/10.3390/plants13131731>**

At present, there are no data in the scientific literature on studies aimed at characterizing *Passiflora caerulea* L. growing in Bulgaria. The present study aimed to investigate the metabolic profile and elemental composition of the leaves and pulp of this *Passiflora*, as well as to evaluate the antioxidant, antimicrobial and anti-inflammatory activities of its leaf and pulp extracts. The results showed that the pulp predominantly contained the essential amino acid histidine ( $7.81 \text{ mg g}^{-1}$ ), while it was absent in the leaves, with the highest concentration being tryptophan ( $8.30 \text{ mg g}^{-1}$ ). Of the fatty acids, palmitoleic acid predominated both in the pulp and in the leaves. A major sterol component was  $\beta$ -sitosterol. Fructose (7.50%) was the predominant sugar in the pulp, while for the leaves, it was glucose—1.51%. Seven elements were identified: sodium, potassium, iron, magnesium, manganese, copper and zinc. The highest concentrations of K and Mg were in the pulp ( $23,946 \text{ mg kg}^{-1}$  and

1890 mg kg<sup>-1</sup>) and leaves (36,179 mg kg<sup>-1</sup> and 5064 mg kg<sup>-1</sup>). According to the DPPH, FRAP and CUPRAC methods, the highest values for antioxidant activity were found in 70% ethanolic extracts of the leaves, while for the ABTS method, the highest value was found in 50% ethanolic extracts. In the pulp, for all four methods, the highest values were determined at 50% ethanolic extracts. Regarding the antibacterial activity, the 50% ethanolic leaf extracts were more effective against the Gram-positive bacteria. At the same time, the 70% ethanolic leaf extract was more effective against Gram-negative bacteria such as *Salmonella enteritidis* ATCC 13076. The leaf extracts exhibited higher anti-inflammatory activity than the extracts prepared from the pulp. The obtained results revealed that *P. caerulea* is a plant that can be successfully applied as an active ingredient in various nutritional supplements or cosmetic products.