Revista ARACÊ
ISSN: 2358-2472

ANTIMICROBIAL ACTIVITY OF GLYCYRRHIZA GLABRA (LICORICE) EXTRACT ON CLINICAL ESCHERICHIA COLI ISOLATES

https://doi.org/10.56238/arev7n2-159

Submitted on: 12/01/2025 **Publication date:** 12/02/2025

Waldemar de Paula-Júnior[1], Rebecca Alves Marinho[2], Yves André Rodrigues Gomes[3], Guilherme Henrique Azevedo dos Reis[4], Maria Tereza Carvalho Almeida[5], Viviane de Oliveira Vasconcelos[6], Thaisa de Almeida Pinheiro[7], Thales de Almeida Pinheiro[8], Valéria Farias Andrade[9], Luís Paulo Ribeiro Ruas[10], Eurislene Moreira Antunes Damasceno[11], Thiago Santos Monção[12], Janniny Fernanda Lopes Mendes Figueiredo Lacerda[13],

[1] Doctor in Pharmaceutical SciencesFederal University of Ouro Preto – Ufop
E-mail: waldemar.junior@unimontes.br
[2] Medical Student
State University of Montes Claros - Unimontes
E-mail: rebeccaalvesmarinho@gmail.com
[3] Graduating in Medicine
State University of Montes Claros - Unimontes
E-mail: yvesandrerg@outlook.com
[4] Graduating in Medicine
State University of Montes Claros - Unimontes
E-mail: ghadrmoc@gmail.com
[5] PhD in Health Sciences
State University of Montes Claros - Unimontes
E-mail: maria.almeida@unimontes.br
[6] PhD in Parasitology
State University of Montes Claros (Unimontes)
E-mail: viviane.vasconcelos@unimontes.br
[7] Master of Science in Health Sciences
State University of Montes Claros – Unimontes
E-mail: thaisa.pinheiro@unifipmoc.edu.br
[8] Master of Science in Health Sciences
State University of Montes Claros – Unimontes
Email: thales.pinheiro@unifipmoc.edu.br
[9] Master's Degree in Plant Production
Federal University of Minas Gerais – UFMG
Email: valeria.andrade@unifipmoc.edu.br
[10] Master's Degree in Health, Society and Environment from
Federal University of the Jequitinhonha and Mucuri Valleys – UFVJM
E-mail: luis.ruas@unifipmoc.edu.br
[11] Master of Science in Primary Health Care
United Colleges of Northern Minas (Funorte), State University of Montes Claros (Unimontes)
E-mail: lene.euris@yahoo.com.br
[12] Doctor student in Biotechnology
State University of Montes Claros (Unimontes), University Center FIPMoc (Unifipmoc Afya)
E-mail: professorthiagomoncao@gmail.com
[13] Medical residency in family and community health
State University of Montes Claros - Unimontes
E-mail: nynafigueiredo@gmail.com

Revista ARACÊ
ISSN: 2358-2472

Maria Cristina da Costa[14] and Flávio Júnior Barbosa Figueiredo[15]

ABSTRACT
Bacterial resistance to antimicrobials is a genetic phenomenon developed by microorganisms that leads to the ineffectiveness of most of the drugs used for the treatment of infectious diseases. Escherichia coli is a gram-negative bacterium associated with clinical conditions of complex control. This scenario points to the search for new treatment alternatives for this bacterium, especially based on medicinal plants, rich in pharmacologically active chemical compounds. The objective of this work is to evaluate the antimicrobial activity of the hydroalcoholic extract of Glycyrrhiza glabra (licorice) on clinical Escherichia coli isolates. The antimicrobial activity assays were carried out in independent triplicates using the minimum inhibitory concentration (MIC) investigation, in 96-well microplates, using the resazurin dye. The present study may contribute to the understanding of the antimicrobial activity of the extract in question and its possible limitations, in addition to directing research towards the development of active phytopharmaceuticals on clinical isolates of Escherichia coli.

Keywords: Antimicrobials. Escherichia coli. Glycyrrhiza Glabra. Bacterial Resistance.

[14] Graduated in Medicine
State University of Montes Claros - Unimontes
E-mail: tchayna.mc@gmail.com
[15] Doctor in Health Science (Fiocruz)
FIPMoc University Center - Afya and University Center of the North of Minas Gerais
Email: figueiredofjb@gmail.com

Revista ARACÊ
ISSN: 2358-2472

INTRODUCTION

Bacterial resistance is a genetic phenomenon that occurs due to mutations in bacteria and corresponds to a global health problem, influenced by the inappropriate use of antimicrobials by the population. Studies to identify new antimicrobials have been carried out to reverse this important problem (Loureiro et al., 2016). Some of these studies were done in hospitals and showed the resistance of the *Staphylococcus aureus* bacterium against methicillin. Health professionals who have direct contact with the infected patient have a high chance of developing resistance to the drugs used to fight diseases caused by microorganisms (Franco et al., 2015).

Markwart and colleagues (2020) reported that mortality in Intensive Care Units (ICUs) ranges from 9 to 38% and can reach 70% when they have a healthcare-associated infection (HAI), and mortality can reach 50% when related to infections by resistant microorganisms (Markwart *et al*., 2020). Among them, *Escherichia coli* stands out, a gram-negative bacterium, belonging to the *Enterobacteriacea* family, not sporulated, flagellated, with an excellent temperature for development of 37°C (Denamur *et al*., 2021). Its size varies from 1.1 to 1.5μm by 2 to 6μm, with facultative anaerobic metabolism, being part of the group of fecal coliforms, thus being a more specific indicator of fecal contamination (Fernandes et al., 2015). E. *coli* lives in the digestive tract and makes up the gut microbiota. Commonly, the bacterium is present in a way that does not cause diseases, while, in other cases, mutagenic strains can be acquired and cause an infectious process in the intestine, such as acute diarrhea, meningitis, urinary tract infection, among other diseases (Pokharel *et al*., 2023).

In addition to the studies that are carried out to combat the resistance of microorganisms in the attempt of new drugs with antimicrobial capacity or synergism, there is the use of medicinal plants in vogue (Oliveira et al., 2015). Used in folk medicine, they have activity against various microorganisms. Considered easily accessible and economically viable, plants represent an important source of new medicines (Flor, et al., 2015). For the World Health Organization, it is essential to carry out experimental investigations with plants for their use in alternative medicine. In 2006, the Ministry of Health approved and included the use of medicinal plants in the SUS so that the population can be treated not only with industrialized products but also with natural products (Boccolini & Boccolini, 2020).

Revista ARACÊ
ISSN: 2358-2472

One of the plants that are used for the treatment of diseases is licorice, which belongs to the *Leguminosae family* with the scientific name *Glycyrrhiza glabra*. Originally from Asia, the parts used are root, stem, and leaves, and the root is used by folk medicine against asthma, cough, bronchitis, and gastric inflammation (Wahab *et al*., 2021). A study proved the protection of this plant against gastritis, justified by its antacid and antispasmodic properties, which help the digestive system by improving bile functions, reducing stomach acidity, and increasing mucus secretion. Secondary metabolites that are related to these effects are saponins and flavonoids (Murugan *et al*., 2022).

In addition, a systematic review study of clinical trials conducted by Dorsareh *et al.* (2023) highlighted the effects of licorice root extract in reducing signs and symptoms of recurrent aphthous stomatitis, a common condition of the oral cavity which is identified by the presence of lesions in the oral mucosa. A study carried out with licorice extract at a concentration of 100 mg/mL, observed an average reduction in *Staphylococcus aureus* biofilm compared to the control group (Araujo *et al*., 2016). Therefore, the literature shows the potential of *Glycyrrhiza glabra* as a medicinal product, although there are still gaps to be filled regarding the quantification of its antibacterial activity against specific pathogens.

Given the above, the present article aimed to determine the minimum inhibitory concentration (MIC) of the hydroalcoholic extract of *Glycyrrhiza glabra* on clinical isolates of *Escherichia coli*.

METHODOLOGY

OBTAINING THE STATEMENT

The hydroalcoholic extract of the leaves of *Glycyrrhiza glabra* (licorice) obtained from the Harmonize Laboratory (Ipatinga, Minas Gerais, Brazil) was filtered, subjected to the evaporation route at 50 °C and lyophilized. The generated material was packed in an amber vial at 4°C until the moment of use, and then weighed and used to prepare a stock solution at 1mg/mL in 5% dimethylsulfoxide (DMSO - Synth, São Paulo, Brazil), and was immediately used in the biological tests.

PHYTOCHEMICAL SCREENING

The qualitative phytochemical tests were based on colorimetric methods and were conducted as follows: steroids and triterpenoids were investigated using the Lieberman-Burchard test, flavonoids were screened by Shinoda's test, tannins were identified by

Revista ARACÊ
ISSN: 2358-2472

observing the formation of blue precipitates after the addition of FeCl3, and saponins and alkaloids were investigated using the Drangendorff test.

OBTAINING AND CULTIVATING THE MICROORGANISMS USED

The microorganisms were obtained from the Microbiology Laboratory of the Vale do Rio Doce University (UNIVALE), and identified as E1 - E5 (uropathogenic Escherichia coli strains). They were submitted for identification with the Vitek 2.0 automated system (version R04.02, bioMérieux), following the manufacturer's instructions. All strains were kept frozen at -20 °C in BHI broth (Difco, Becton Dickinson, USA) with 10% glycerin. To activate the crops, the aliquots of the frozen crop were transferred to BHI broth, supplemented with 5% sucrose, and incubated at 35 ± 2 °C. After 24 hours, the cultures containing the bacteria were seeded in Petri dishes containing Nutrient agar (Difco, Becton Dickinson, USA) and placed in an incubator at 37°C for 48 hours. After visualization of the growth, colonies of the bacteria were selected and transferred to the BHI broth.

DETERMINATION OF THE MINIMUM INHIBITORY CONCENTRATION (MIC)

The MIC of the hydroalcoholic extract of *Glycyrrhiza glabra* (licorice) root was determined on polystyrene microplates from 96 sterile wells as previously described by our group (Pereira *et al*., 2015). Briefly, the bacterial cultures were prepared in BHI broth on the 0.5 McFarland scale, with 100µL dispensed in the wells. Subsequently, 100µL of the hydroalcoholic extract of the leaves of *Glycyrrhiza glabra* was added and diluted in series. The concentrations tested ranged from 1mg/mL to 7.8µg/mL. The plates were then incubated in a bacteriological incubator at 35 ± 2 °C for the entire night. After this time, 10µL of resazurin solution (Sigma-Aldrich Missouri, USA) previously diluted in sterile distilled water at a final concentration of 0.001mg/mL were added to each well, and the plates were preincubated under the previous conditions for 30 minutes. MIC was established as the lowest concentration at which the resazurin color remained unchanged (without changing from blue to pink). All experiments were carried out in independent triplicates.

RESULTS

The data obtained in the tests of antimicrobial activity of *Glycyrrhiza glabra* were organized in Table 1.

Revista ARACÊ
ISSN: 2358-2472

Table 1: Minimum Inhibitory Concentration (MIC) of hydroalcoholic extract of *Glycyrrhiza glabra* leaves on clinical isolates of *E. coli*.

STRAINS	MIC (ug/ml)
E1	> 1000
E2	> 1000
E3	> 1000
E4	> 1000
E5	> 1000

Image Source: Figueiredo *et al*. (2025)

DISCUSSION

Bacterial resistance has become a public health issue, thus being a growing threat in the treatment of infectious diseases by multidrug-resistant microorganisms (Costa & Júnior, 2017).

The choice of *E. coli* was because it is a common bacterium in infections, which is the main cause of urinary tract infections (Whelan *et al*., 2023). They also cause gastroenteritis and can be triggered by five different mechanisms, responsible for different clinical conditions: *enterotoxigenic* E. coli (ETEC), enteropathogenic E. *coli* (EPEC), enteroinvasive *E. coli* (EIEC), *enterohemorrhagic* E. coli (EHEC) and *enteroaggregative E. coli-EAEC (Braz et al*., 2020). Despite being a component of the normal microbiota of the gastrointestinal system of humans, specific tests are needed to identify possible diarrheagenic strains (Mims *et al*., 1999).

E. coli is a bacterium that is very resistant to several antibiotics, such as polymyxin B, which studies reveal that it would be one of the last resources in the treatment of infections caused by resistant bacteria and that no other drug has been able to fight them (Fernandes *et al*., 2016; Olsson *et al*., 2021; Meijden *et al*., 2023).

Only up to 1000ug/ml was tested, as this was the highest concentration of the extract used in the study, since the highest concentrations did not present antimicrobial activity, consequently, being difficult to use in the treatment of bacterial infections (Mendes *et al*., 2011).

In the study carried out by Sedighinia et al. (2012), the ethanolic extract of *G. glabra was tested*, which exhibited higher values of MIC against *E.coli*. So, its antibacterial activity suggests that *G. glabra* has greater activity against gram-positive bacteria.

In a study conducted by Figueiredo *et al*. (2013), extracts of natural plant products were associated with antimicrobials and were tested against isolates of *S. aureus* and *E. coli* from humans, and it was observed that these extracts may represent an alternative to natural products with antimicrobial action, which are responsible for the positive effect

Revista ARACÊ
ISSN: 2358-2472

observed in his study. This may explain the differences in results between the present study and the one carried out by Figueiredo *et al*. (2013), showing that associating antimicrobial adjuvants with natural plant products can be decisive for better results in future research.

Considering that the American Type Culture Collection (ATCC) strains have known responses, unlike the clinical isolates (Brumano *et al.*, 2011), the present work was developed with different strains of *E. coli* to obtain the minimum inhibitory concentration of each of them against the extract of *Glycyrrhiza glabra.*

CONCLUSION

The extract *Glycyrrhiza glabra* (licorice) at the concentrations studied did not show activity on clinical isolates of *Escherichia coli*. It is concluded that further studies should be carried out to verify the activity of this extract as an antimicrobial, and modifying the methodology, using adjuvants in an attempt to improve its effect, seems to be a promising strategy.

Revista ARACÊ
ISSN: 2358-2472

REFERENCES

1. Araujo, M. M. de, & Longo, P. L. (2016). Testing of the in vitro antibacterial action of commercial essential oil of Origanum vulgare (oregano) against Escherichia coli and Staphylococcus aureus strains. Arquivos do Instituto Biológico, 83, 1–7.

2. Boccolini, P. de M. M., & Boccolini, C. S. (2020). Prevalence of complementary and alternative medicine (CAM) use in Brazil. BMC Complementary Medicine and Therapies, 20(1), 51.

3. Braz, V. S., et al. (2020). Escherichia coli as a multifaceted pathogenic and versatile bacterium. Frontiers in Cellular and Infection Microbiology, 10, 548492.

4. Brumano, L. P., et al. (2011). Standard bacterial strains, ways to obtain donation, and their maintenance in teaching and research laboratories. Interdisciplinary Journal of Experimental Studies, 3, 21–26.

5. Costa, A. L. P. da, & Júnior, C. S. S. (2017). Bacterial resistance to antibiotics and public health: a brief literature review. Scientific Station (UNIFAP), 7(2), 45–57.

6. Denamur, E., et al. (2021). The population genetics of pathogenic Escherichia coli. Nature Reviews Microbiology, 19(1), 37–54.

7. Dorsareh, F., et al. (2023). Topical licorice for aphthous: A systematic review of clinical trials. Iranian Journal of Medical Sciences, 48(5), 437–447.

8. Fernandes, L. L., & Gois, R. V. (2015). Evaluation of the main methodologies applied to the microbiological analysis of water for human consumption aimed at the detection of total and thermotolerant coliforms. Scientific Journal of the Faculty of Education and Environment, 6(2), 49–64.

9. Fernandes, M. R., et al. (2016). Silent dissemination of colistin-resistance Escherichia coli in South America could contribute to the global spread of the mcr-1 gene. Eurosurveillance, 21(17).

10. Figueredo, F., et al. (2013). Modulation of the antibiotic activity by extracts from Amburana cearenses A. C. Smith and Anadenanthera macrocarpa (Benth.) Brenan. BioMed Research International, Article ID 640682.

11. Flor, A. S. S. de O., & Barbosa, W. L. R. (2015). Popular wisdom in the use of medicinal plants by the residents of the neighborhood of peace in the district of Marudá-PA. Revista Brasileira de Plantas Medicinais, 17(4), 757–768.

12. Franco, J. M. P. L., et al. (2015). The role of the pharmacist in the face of bacterial resistance caused by the irrational use of antimicrobials. Revista Científica Semana Acadêmica, 1(72), 1–17.

13. Loureiro, R. J., et al. (2016). The use of antibiotics and bacterial resistance: brief notes on their evolution. Revista Portuguesa de Saúde Pública, 34(1), 77–84.

Revista ARACÊ
ISSN: 2358-2472

14. Markwart, R., et al. (2020). Epidemiology and burden of sepsis acquired in hospitals and intensive care units: a systematic review and meta-analysis. Intensive Care Medicine, 46(8), 1536–1551.

15. Meijden, A. van der, et al. (2023). Pharmacokinetic and pharmacodynamic properties of polymyxin B in Escherichia coli and Klebsiella pneumoniae murine infection models. Journal of Antimicrobial Chemotherapy, 78(3), 832–839.

16. Mendes, L. P. M. (2011). Antimicrobial activity of ethanolic extracts of Peperomia pellucida and Portulaca pilosa. Revista Ciência Farmacêutica Básica e Aplicada, 32(1), 121–125.

17. Mims, C. (1999). Medical Microbiology (2nd ed.). Manole.

18. Murugan, S. K., et al. (2022). A flavonoid-rich standardized extract of Glycyrrhiza glabra protects intestinal epithelial barrier function and regulates the tight-junction proteins expression. BMC Complementary Medicine and Therapies, 22(1), 38.

19. Oliveira, D. R., et al. (2015). In vitro antimicrobial and modulatory activity of natural products silymarin and silibinin. Journal of Biomedical Research International.

20. Olsson, A., et al. (2021). Interactions of polymyxin B in combination with aztreonam, minocycline, meropenem, and rifampin against Escherichia coli producing NDM and OXA-48-group carbapenemases. Antimicrobial Agents and Chemotherapy, 65(12), e0106521.

21. Pereira, N., et al. (2015). Antimicrobial potential of Passiflora alata and Piper methysticum hydroalcoholic extracts, phytotherapics of anxiolytic-like activity. Journal of Applied Pharmaceutical Sciences, 2(2), 31–33.

22. Pokharel, P., Dhakal, S., & Dozois, C. (2023). The diversity of Escherichia coli pathotypes and vaccination strategies against this versatile bacterial pathogen. Microorganisms, 11(2), 344.

23. Sedighinia, F., et al. (2012). Antibacterial activity of Glycyrrhiza glabra against oral pathogens: an in vitro study. Avicenna Journal of Phytomedicine, 2(3), 118–124.

24. Wahab, S., et al. (2021). Glycyrrhiza glabra (licorice): A comprehensive review on its phytochemistry, biological activities, clinical evidence and toxicology. Plants, 10(12), 2751.

25. Whelan, S., Lucey, B., & Finn, K. (2023). Uropathogenic Escherichia coli (UPEC)-associated urinary tract infections: The molecular basis for challenges to effective treatment. Microorganisms, 11(9), 2169.