International Journal of Advanced Biochemistry Research

ISSN Print: 2617-4693
ISSN Online: 2617-4707
IJABR 2024; 8(5): 322-326
www.biochemjournal.com
Received: 05-02-2024
Accepted: 17-04-2024

Sonali Mahore
Department of Veterinary Medicine, College of Veterinary Science and A. H., Ndvsu, Jabalpur, Madhya Pradesh, India

Shashi Pradhan
Department of Veterinary Medicine, College of Veterinary Science and A. H., Ndvsu, Jabalpur, Madhya Pradesh, India

Ranbir Jatav
Department of Veterinary Medicine, College of Veterinary Science and A. H., Ndvsu, Jabalpur, Madhya Pradesh, India

Devendra Gupta
Department of Veterinary Medicine, College of Veterinary Science and A. H., Ndvsu, Jabalpur, Madhya Pradesh, India

Anju Nayak
Department of Veterinary Microbiology College of Veterinary Science and A. H., Ndvsu, Jabalpur, Madhya Pradesh, India

Sachin Jain
Department of Veterinary Pharmacology and Toxicology College of Veterinary Science and A. H., Ndvsu, Jabalpur, Madhya Pradesh, India

Amita Tiwari
Department of Veterinary Medicine, College of Veterinary Science and A. H., Ndvsu, Jabalpur, Madhya Pradesh, India

Corresponding Author:
Sonali Mahore
Department of Veterinary Medicine, College of Veterinary Science and A. H., Ndvsu, Jabalpur, Madhya Pradesh, India

Evaluation of minimum inhibitory concentration of *Glycyrrhiza glabra* and *Zingiber officinale* against different bacteria

Sonali Mahore, Shashi Pradhan, Ranbir Jatav, Devendra Gupta, Anju Nayak, Sachin Jain and Amita Tiwari

DOI: https://doi.org/10.33545/26174693.2024.v8.i5d.1091

Abstract
The present study was conducted to study the antibacterial activity of fresh rhizome aqueous extract of *Glycyrrhiza glabra* and *Zingiber officinale* against *Escherichia coli, Klebsiella, and Staphylococcus* spp. *In vitro* minimum inhibitory concentration of extracts against test organisms were determined. The research was conducted at Department of Veterinary Microbiology, College of Veterinary Science and Animal Husbandry, NDVSU, Jabalpur Madhya Pradesh (M.P.). The aqueous extract of *Glycyrrhiza glabra* and *Zingiber officinale* was prepared and were tested on isolates of *Escherichia coli, Klebsiella, and Staphylococcus* spp. with the help of standard tube dilution method. Antibacterial activity of the aqueous extract of *Zingiber officinale* rhizomes showed minimum inhibitory concentration of 12.5 mg/ml, 12.5 mg/ml and 50 mg/ml against bacteria *E. coli*, *Klebsiella* spp. and *Staphylococcus* spp. respectively. The aqueous extract of combination of *Glycyrrhiza glabra* and *Zingiber officinale* rhizomes showed minimum inhibitory concentration of 25 mg/ml, 25 mg/ml and no inhibition against bacteria *E. coli*, *Klebsiella* spp. and *Staphylococcus* spp. respectively. The study indicated that *Zingiber officinale* plant can act as a potential source of antibacterial agent.

Keywords: *Zingiber officinale, Glycyrrhiza glabra, E. coli*, *Klebsiella* spp., *Staphylococcus* spp., minimum inhibitory concentration

Introduction
One of the main body parts is the respiratory system, which consists of the upper airways (larynx, pharynx, and trachea) and the lower airways (bronchi, bronchioles, and alveoli). The respiratory system is extremely susceptible to necrobiotic degeneration in the eco-morphological environment after repeated exposure to infectious pathogens that can enter through both haematogenous and aerogenous routes. Disorders affecting the nasal cavity and paranasal sinuses are frequently accompanied by symptoms such as nasal discharge, sneezing, facial deformity, stretor, lethargy, and intolerance. Numerous bacteria have been implicated as the most frequent aetiological agents in canine respiratory infections, including Bordetella brochiseptica, *Escherichia coli, Klebsiella, Streptococcus, and Staphylococcus* spp. Treatment of the upper respiratory tract infections typically involves the use of synthetic antimicrobial agents. However, the emergence of drug resistance to β-lactams has created increasing world-wide interest in alternative antimicrobial agents. Herbal medicines are being rediscovered because of growing awareness of the adverse side effects of the allopathic medicines (Dwarakan and Alagesaboopathi, 1999) [2].

Glycyrrhiza glabra, family Leguminosa ('Mulethi' in Hindi), a genus of perennial herbs and shrubs, is widely distributed in the subtropical regions globally. The fibrous taproot and rhizomes are believed to be endowed with high medicinal potency. In folklore medicine, the root extracts are previously owned in the treatment of respiratory tract disorders. *Glycyrrhiza glabra* contains a wide range of phytochemicals: triterpenes, saponins, flavonoids (liquirtoside, isoliquiritoside) which are to contribute significantly to the bioactivity (Bakhane *et al*., 2014) [1].

Zingiber officinale, family Zingiberacae (popularly named Ginger in English) is a perennial herb with an aromatic pungent taste. The methanol, hydro-ethanol and aqueous extracts possess antibacterial, anti-inflammatory and antioxidant activity (Mahboubi, 2019) [5].

In perspective the present study was undertaken to evaluate the minimum inhibitory concentration of *Glycyrrhiza glabra* and *Zingiber officinale* against different bacteria found in upper respiratory tract infection.

Materials and Methods

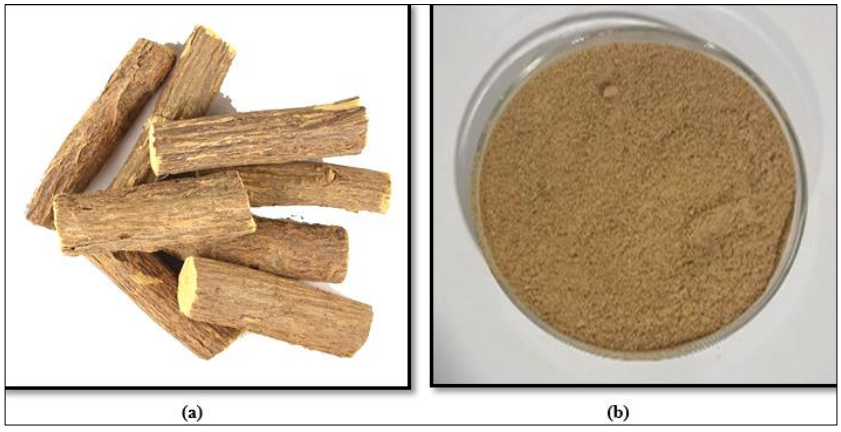
1. Location and place of work

The proposed study was carried out for six months i.e., from August, 2023 to January, 2024 at Department of Veterinary Medicine, College of Veterinary Science and Animal Husbandry, Nanaji Deshmukh Veterinary Science University (N.D.V.S.U), Jabalpur. The fresh rhizomes of the plant *Glycyrrhiza glabra* and *Zingiber officinale* was procured from the Department of Plant Physiology, Agriculture College, Jawaharlal Nehru Krishi Vishwa Vidyalaya, Jabalpur, Madhya Pradesh (M.P.). Further the isolation and identification bacterial culture of nasal sample was performed at Department of Veterinary Microbiology, College of Veterinary Science and Animal Husbandry, Nanaji Deshmukh Veterinary Science University (N.D.V.S.U), Jabalpur Madhya Pradesh (M.P.).

2. Preparation of aqueous extract

Aqueous extracts were prepared by soaking 50 g of crude powder of each plant material in 500 ml triple glass distilled water in an Erlenmeyer flask, stirring at hourly intervals initially 2-3 times, followed by 12 hours storage at room temperature. The soaked crude powder was filtered through Whatman filter paper No. 1 fitted in a separating funnel. The filtrate was dried in a water bath to obtain aqueous extract (Panicker, 2012) [6]. Two aqueous extracts of each plant and one combination aqueous extracts was prepared. As shown in figure 1, 2 and 3.

Fig 1: Preparation of *Zingiber officinale* syrup a) Fresh Rhizome; b) *Zingiber officinale* Powder; c) Preparation of Aqueous Extract; d) Filteration of Crude Extract; e) *Zingiber officinale* Syrup



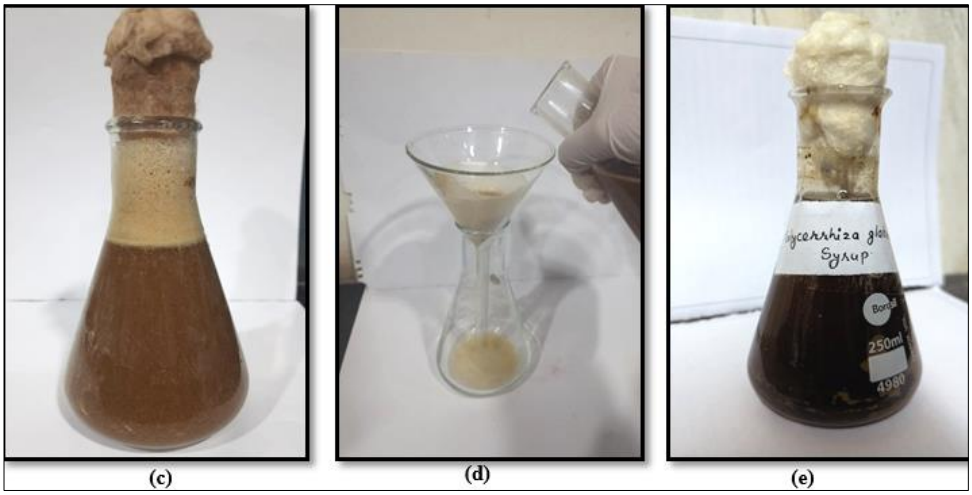


Fig 2: Preparation of *Glycyrrhiza glabra* syrup a) Fresh Rhizome; b) *Glycyrrhiza glabra* Powder; c) Preparation of Aqueous Extract; d) Filteration of Crude Extract; e) *Glycyrrhiza glabra* Syrup

Fig 3: Preparation of Combination syrup a) Powder; b) Preparation of Aqueous Extract; c) Filteration of Crude Extract; d) Syrup

3. Extracts preparation

The *Zingiber officinale* and *Glycyrrhiza glabra* rhizomes aqueous extracts were prepared at 100 percent concentration as the stock solution. The plant extracts were sterilized using the syringe filters (0.45 µm). These sterilized extracts were used for assessing minimum inhibitory concentrations of plants.

Procedure

A suspension of the test organisms was prepared by the direct colony suspension method. Morphologically similar colonies were touched with sterile loop and transferred into test tubes containing normal saline. After homogenization of the bacterial suspension, comparisons were made with the 0.5 McFarland turbidity standards, which were equal to approximately 1-5 × 10^6 CFU/ml (CLSI, 2006). To get various concentrations, the extracts were diluted two folds. Tubes numbered 1 to 6 were used for the separate serial dilutions of each extract. 1.9 mL of Brain Heart Infusion Broth, containing the extract stock solution was added to Tube 1. Tube 2 was filled with just 1 mL of the stock solution from tube 1, which was then diluted with 1 mL of Brain Heart Infusion Broth. The processes for the solutions in tubes two to six were repeated. Next, 1 mL of Brain Heart Infusion containing bacterial suspension was added to each tube. The resultant mixes were incubated for twenty-four hours at 37°C. Negative control was provided by the solvents. To determine the Minimum Bactericidal Concentration (MBC) values, 100 µL of the turbidity-free tubes' contents were cultured on the Nutrient agar medium and incubated at 37°C for 24 hours. Turbidity was taken as a sign of growth, and the lowest concentration that remained clear was recorded as the relative minimum inhibitory concentration.

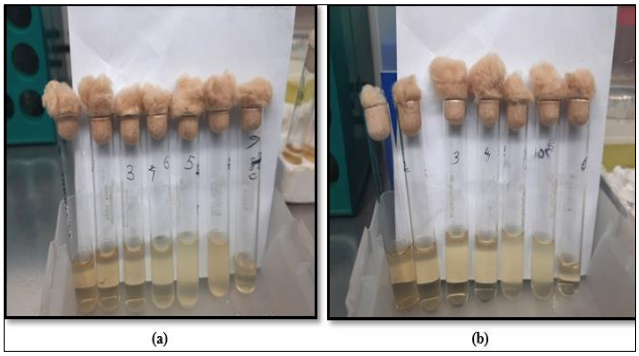
Results

1. *In vitro* minimum assessment of the inhibitory concentration (MIC)

In the present study *in vitro* minimum inhibitory concentration of plant extracts against bacterial isolates *E. coli*, *Klebsiella* spp. and *Staphylococcus* spp. was analysed. Antibacterial activity of the aqueous extract of *Zingiber officinale* rhizomes showed minimum inhibitory concentration of 12.5 mg/ml, 12.5 mg/ml and 50 mg/ml against bacteria *E. coli*, *Klebsiella* spp. and *Staphylococcus* spp. respectively. (as shown in table 1 and plate 4). The aqueous extract of combination of *Glycyrrhiza glabra* and *Zingiber officinale* rhizomes showed minimum inhibitory concentration of 25 mg/ml, 25 mg/ml and no inhibition against bacteria *E. coli*, *Klebsiella* spp. and *Staphylococcus* spp. respectively.

Table 1: *In-vitro* minimum inhibitory concentration for different bacteria

Bacteria	Extract	MIC Dose
E. coli	*Zingber offiinale*	12.5 mg/ml
Klebsiella spp.	*Zingber offiinale*	12.5 mg/ml
Staphylococcus spp.	*Zingber offiinale*	50 mg/ml
E. coli	*Glycyrrhiza glabra*	Turbidity in all tubes
Klebsiella spp.		
Staphylococcus spp.		
E. coli	Combination	25 mg/ml
Klebsiella spp.	Combination	25 mg/ml
Staphylococcus spp.	Combination	Turbidity in all tubes



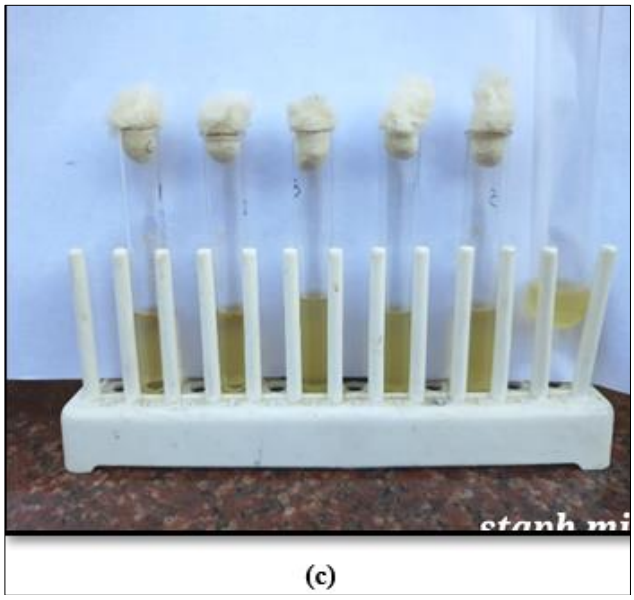


Fig 4: Minimum inhibitory concentration of *Zingiber officinale*
a) Against *Klebsiella* spp. at 12.5 mg/ml
b) Against *E. coli* at 12.5 mg/ml
c) Against *Staphylococcus* spp. at 50 mg/ml

Discussion

The results are in partial agreement to the findings of Gull *et al*. (2012) [3] who evaluated minimum inhibitory concentrations of aqueous extract of *Zingiber officinale* as 0.1 mg/ml, 0.2mg /ml and 0.2 mg/ml against bacteria *E. coli*, *Klebsiella* spp. and *Staphylococcus* spp. respectively. Whereas, Yusuf *et al*. (2018) [7] investigated minimum inhibitory concentrations of methanolic extract of *Zingiber officinale* as 0.025 mg/ml, 0.012 mg/ml and 0.025 mg/ml against bacteria *E. coli*, *Klebsiella* spp. and *Staphylococcus* spp. respectively.

Similarly, minimum inhibitory concentration of aqueous extract of *Glycyrrhiza glabra* was evaluated. The aqueous extract of *Glycyrrhiza glabra* rhizomes showed no inhibition against *E. coli*, *Klebsiella* spp. and *Staphylococcus* spp. bacteria (as shown in table 1). The results are contrary to the findings of Irani *et al*. (2010) [4] who documented that aqueous extract of *Glycyrrhiza glabra* root extract exhibited minimum inhibitory concentrations of 5 mg/ml and 1.25 mg/ml against *Staphylococcus aureus* and *Candida albicans.*

The results of the present study promise potent antibacterial activity of *Zingiber officinale.*

Conclusion

The study showed that *Zingber offiinale* plant can act as a potential antimicrobial agent. However, the rhizome extract

of *Glycyrrhiza glabra* failed to exhibit antibacterial property. In accordance with the present study *Zingiber officinale* displayed greater effectiveness against gram negative organisms *i.e*., *E. coli* and *Klebsiella* spp. in contrast to gram positive organism *i.e*., *Staphylococcus* spp. Hence, it is recommended that *Zingiber officinale* and *Glycyrrhiza glabra* could be incorporated into herbal antibacterial formulations as these contains anti-inflammatory, immunostimulant and bactericidal properties.

Future Scope

Thus, the current research offers pertinent data minimum inhibitory concentration of *Glycyrrhiza glabra* and *Zingber offiinale*. Further comprehensive research mat be done for Characterization of bioactive constituents and determining the exact mechanism of action of *Glycyrrhiza glabra* and *Zingiber offcinale* must be investigated to access their antibacterial properties.

Acknowledgement

It is with great gratitude that the authors acknowledge the Dean, College of Veterinary Sciences and Animal Husbandry, NDVSU, Jabalpur for providing the necessary facilities for carrying out the study. No potential conflict of interest was reported by the author(s).

References

1. Bakhane Y, Yadav AS, Bajaj A, Sharma AK, Raghuwanshi DK. *Glycyrrhiza glabra* L.: a miracle medicinal herb. Indo American Journal of Pharmaceutical Research. 2014;4(12):5809-5816.
2. Dwarakan P, Alagesaboopathi C. Traditional crude drug resources used for human and livestock diseases in Salem district, Tamil Nadu. Journal of Economic and Taxonomic Botany. 1999;23(1):421-424.
3. Gull I, Saeed M, Shaukat H, Aslam SM, Samra ZQ, Athar AM. Inhibitory effect of *Allium sativum* and *Zingiber officinale* extracts on clinically important drug resistant pathogenic bacteria. Annals of Clinical Microbiology and Antimicrobials. 2012;11(1):1-6.
4. Irani M, Sarmadi M, Bernard F, Bazarnov HS. Leaves antimicrobial activity of *Glycyrrhiza glabra* L. Iranian Journal of Pharmaceutical Research: IJPR. 2010;9(4):425.
5. Mahboubi M. *Zingiber officinale* Rosc. Essential oil, a review on its composition and bioactivity. Clinical Phytoscience. 2019;5(1):1-12.
6. Panicker A. Antibacterial activity of cow urine, aloe vera and wheat grass in poultry. M.V.Sc. thesis (Veterinary Pharmacology and Toxicology), Nanaji Deshmukh Veterinary Science University, Jabalpur. 2012.
7. Yusuf AA, Lawal B, Abubakar AN, Berinyuy EB, Omonije YO, Umar SI, *et al*. *In-vitro* antioxidants, antimicrobial and toxicological evaluation of Nigerian *Zingiber officinale*. Clinical Phytoscience. 2018;4(1):1-8.