PLANT SCIENCE TODAY
Vol x(x): xx–xx
https://doi.org/10.14719/pst.6928

eISSN 2348-1900

RESEARCH ARTICLE

Evaluation of the antimicrobial potential of *Dioscorea alata* and *Embelia ribes* extracts and their formulation against urinary tract pathogens

Bhakkia Rani R, Rajeshkumar Shanmugam* & Sulochana Govindharaj

Nanobiomedicine Lab, Department of Anatomy, Saveetha Medical College and Hospital, Saveetha Institute of Medical and Technical Sciences, Saveetha University, Chennai 602 105, Tamil Nadu, India

*Correspondence email - rajeshkumars.sdc@saveetha.com

Received: 27 December 2024; Accepted: 08 September 2025; Available online: Version 1.0: 10 November 2025

Cite this article: Bhakkiarani R, Rajeshkumar S, Sulochana G. Evaluation of the antimicrobial potential of *Dioscorea alata* and *Embelia ribes* extracts and their formulation against urinary tract pathogens. Plant Science Today (Early Access). https://doi.org/10.14719/pst.6928

Abstract

Women are susceptible to urinary tract infections (UTIs), especially during pregnancy and in the perimenopausal and postmenopausal stages. Significant disabilities and fatalities are linked to UTIs and they have a direct effect on the patients' quality of life. The aim of the current study was to prepare the aqueous extract of *Dioscorea alata*, *Embelia ribes* and their formulation extract and analyze the efficacy of antimicrobial activity against UTI pathogens and its cytotoxic effect. In the research work, the extract was prepared and the antimicrobial efficacy of *D. alata, E. ribes* and their formulation was analyzed using the agar well diffusion technique (50 µL) and the time-kill curve assay. The extracts and their formulation were used to determine toxicity and cytotoxic effects using brine shrimp lethality analysis. *D. alata, E. ribes* and their formulation was prepared and color of the extract was confirmed. In antimicrobial activity against UTI pathogens, especially *Pseudomonas* spp., a higher inhibitory zone was observed in *D. alata, E. ribes* and their formulation extract at the concentration (50 µL) and the cytotoxic effect showed lower toxicity in all extract at the higher concentration (80 µL). The formulation extract showed significant extract antimicrobial activity with lower toxicity, suggesting its potential use as an antimicrobial agent for the treatment of UTIs.

Keywords: agar well diffusion technique; antimicrobial activity; cytotoxic effect; formulation; UTI pathogens

Introduction

The infection of kidney, urethra and bladder was termed as urinary tract infection (UTI). The complication of the UTI, which refers to increase the risk of patient including the immunocompromised patient, diabetics, urinary track abnormality, stent (1). The common UTI pathogens are response to the 75 % of uncomplicated and 25 % of complicated, the pathogens are *Klebsiella* spp., *Pseudomonas* spp., *Proteus* spp., *Streptococcus* spp., *Staphylococcus* spp., *Enterococcus* spp. and *Candida albicans* (2). The microbial pathogens were bind and the biofilm formation on the catheter surface and it increased the death rate and the infections progress the urosepsis, pyelonephritis, etc. (3).

The natural therapy of herbal based treatments for UTI infections includes leaf/herb extracts, seed/root/resin derived products and phytochemical cluster/berry derived products (4). *E. ribes* is a medicinal plant that has been used to reduce microbial growth. The biomedical applications of *E. ribes* extract include antimicrobial, anti-diuretic, antifertility, hypoglycemia, anthelmintic, anti-inflammatory and antimalarial activities (5). Its efficacy in antimicrobial activity has been tested against *Pseudomonas* spp., *Klebsiella* spp. and *S. aureus* (6). It has been used to manage many kinds of medical conditions, including muscle weakness, autoimmune diseases, intestinal problems, neurological mental illnesses, healing from injuries and illnesses such as the common cold and influenza (7).

D. alata is a potent pharmaceutical and dietary plant that includes multiple bioactive ingredients. The aerial tuber has 68.51 % moisture, 5.61 % glucose and 1.39 % protein, while the underground tuber contains more calories, vitamin C and minerals such as iron and potassium (8). This biologically rich ingredient is an essential component of the traditional Odia meal Dalma. Along from nutrients, *D. alata* also contains further metabolites such as phenolic acid, flavonoids, coumarins, quinines, alkaloids, amines, terpenoids, phytosterols, tannin, diosgenin and saponins. *D. alata* has been demonstrated to possess anti-inflammatory, antidiabetic, disinfectant and antimicrobial properties (9-11). The ethanol extract showed a high phenolic content and had antibacterial activity against *E. coli, S. aureus* and *B. subtilis* (12). The aim of the current study was to prepare the aqueous extract of *E. ribes, D. alata* and their formulation extract, to analyze the efficacy of antimicrobial activity against UTI pathogens using agar well diffusion technique and time kill curve analysis and to determine toxicity using the cytotoxic effect using brine shrimp lethality analysis.

Materials and Methods

Preparation of herbal extract and their formulation

The fresh leaves were collected from the Nanoherbal garden in Saveetha Dental College, Chennai. *E. ribes* seeds were collected from the herbal shops in Poondhamali. Both the leaves and seeds were dried in sunshade 40 °C and dried leaves and seeds were ground into a fine powder. A total of 2 g of leaves and seeds were weighed in a separate flask, mixed with 100 mL distilled water. The solution was placed in heating mantle at 50 °C for 20 min. The boiled extract was filtered using muslin cloth and filtrated was concentrated up to 5 mL. The concentrated each extract was used for the formulation and their research purpose. A total of 2.5 mL of each extract was mixed under the sonication for 15 min (Fig. 1). The formulation extract (*E. ribes* + *D. alata*) was used for the further research purpose.

Antimicrobial activity

To determine the efficacy of antimicrobial activity of *D. alata*, *E. ribes* and their formulation extract using agar well diffusion technique against the UTI pathogens (13, 14). The sterilized Muller Hinton agar plates was swabbed the fresh microbial culture (UTI pathogens - *E. coli, Pseudomonas* spp., *Klebsiella* spp. and *S. aureus*) evenly. The wells were created using the polystyrene at 9 mm in diameter. The wells were filled with 100 µg/mL of *D. alata*, *E. ribes* and their formulation extract and the loaded plates were incubated for 24 hr at room temperature. The completion of incubation period, the plates were observed the inhibitory zone in millimetres (Fig. 2).

Time-kill curve assay

To analysis the potential of microbial broth analysis or time kill curve analysis of *D. alata*, *E. ribes* and their formulation extract against UTI pathogens (*E. coli, Pseudomonas* spp., *Klebsiella* spp. and *S. aureus*). 1 mL of microbial broth culture was mixed with 100 µL of extract and the tubes were incubated at room temperature for 0, 1, 2, 3 and 4 hr. The optical density of the time kill curve assay tubes were measured colorimetrically at 600 nm. The standard consisted of microbial broth culture with 20 µL of antibiotics (amoxyrite for bacteria), while the control contained only the microbial culture.

Cytotoxic effect

Brine shrimp lethality method

A 2 % of saltwater solution was prepared and used for the *Artemisia salina* (nauplii) eggs. *A. salina* eggs were added in the saltwater tank and aerated for 24 hr. under dark conditions. After completion of incubation, the newly hatched nauplii were collected in a separate container. The cytotoxic effect was determines using the brine shrimp lethality assay with the newly hatched *A. aalina*. In 6-well ELISA plates, the samples were added at different concentrations (5, 10, 20, 40, 80 and control) and 10-12 mL of saltwater was filled into each well. Ten newly hatched nauplii were added slowly to the wells. The plates were incubated for 24 hr at optimum temperature. After incubation, the number of nauplii in each well was recorded for all concentrations and the control (15, 16).

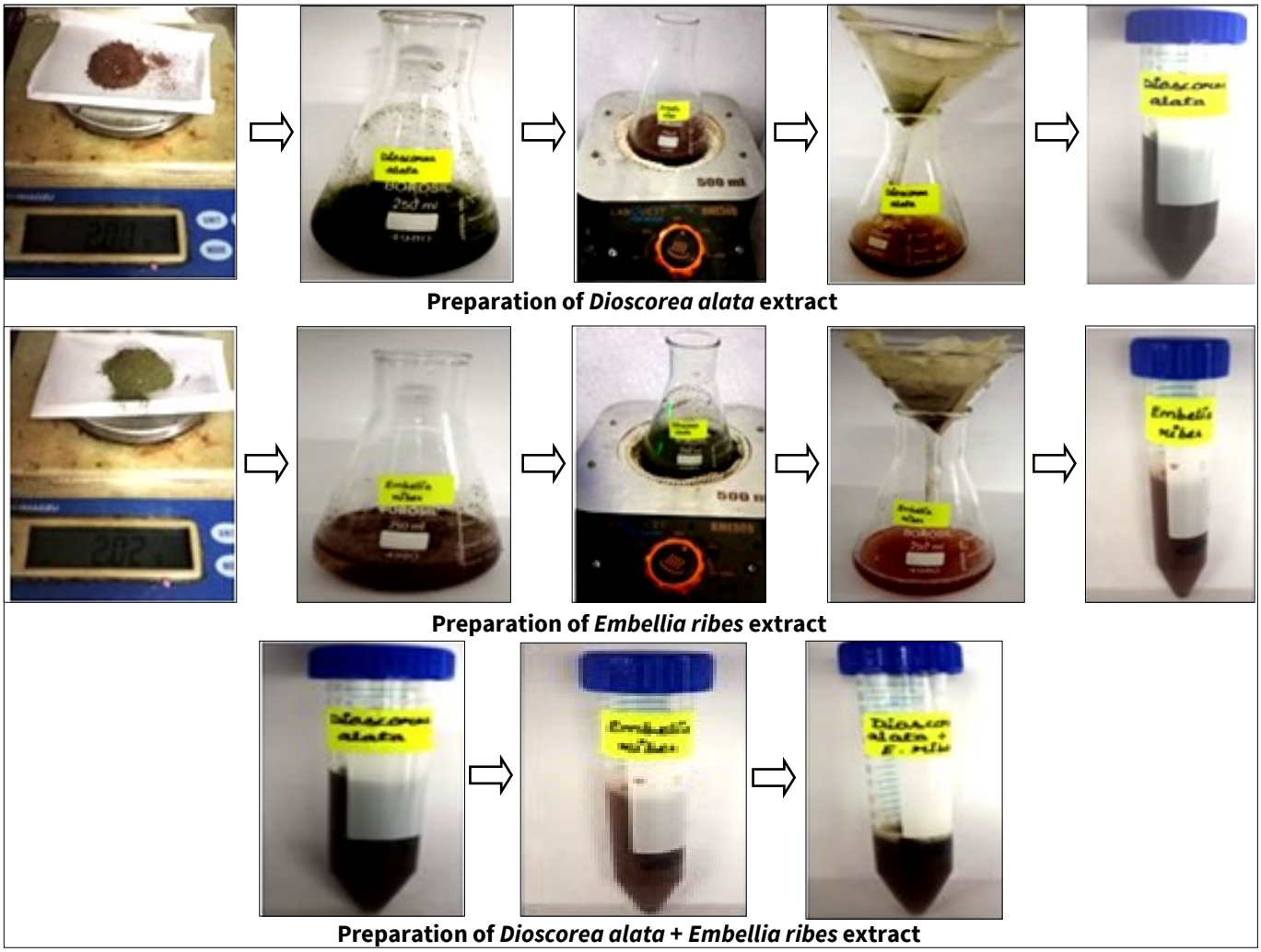

Fig. 1. The preparation of *D. alata, E. ribes* and their formulation extract.

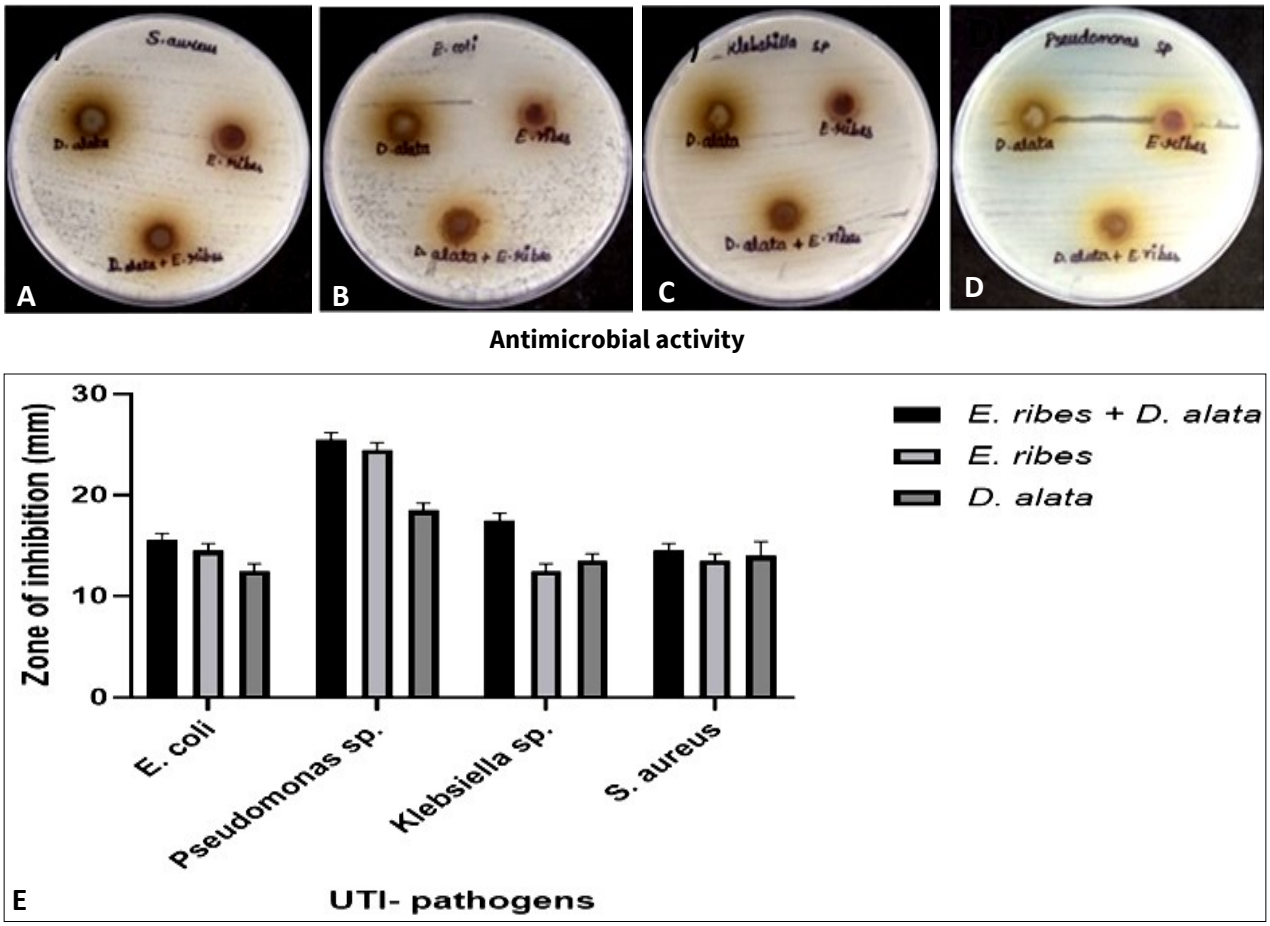

Fig. 2. The antimicrobial activity of *D. alata, E. ribes* and their formulation extract using the agar well diffusion technique against UTI pathogens. (**A**) *S. aureus,* (**B**) *E. coli*, (**C**) *Klebsiella* spp.**,** (**D**) *Pseudomonas* spp. and (**E**) represented graphical images of the extracts.

Results

Antimicrobial activity

The agar well diffusion technique was done by the antimicrobial activity of *E. ribes, D. alata* and their formulation extract against UTI pathogens (*Pseudomonas* spp.*, Klebsiella* spp.*, S. aureus* and *E. coli)* shown in Fig. 2. At 100 µg/mL, the *D. alata* extract produced inhibition zones of 18 mm against *Pseudomonas* spp.*,* 14 mm against *Klebsiella* spp.*,* 13 mm against *S. aureus* and 13 mm against *E. coli*. Similarly, at 100 µg/mL, the *E. ribes* extract produced inhibition zones of 24 mm against *Pseudomonas* spp.*,* 13 mm against *Klebsiella* spp.*,* 14 mm against *S. aureus* and 14 mm against *E. coli*. Their formulation extract was revealed inhibiton zone of 28 mm against *Pseudomonas* spp.*,* 17 mm against *Klebsiella* spp.*,* 15 mm against *S. aureus* and 16 mm against *E. coli*. The higher inhibitory was observed in their formulation extract, demonstrating excellent antimicrobial activity against UTI pathogens.

Time-kill curve kinetic analysis

The broth assay, or time-kill kinetic assay, showed that *D. alata, E. ribes* and their formulation extract exhibited the antimicrobial activity, as compared with standard (bacteria - amoxyrite 50 µL) and the control (only microbial culture). At 100 µL of extracts significantly reduced microbial growth, especially in *Pseudomonas* spp. and *Klebsiella* spp. (Fig. 3). Significantly, the formulation extract reduced bacterial growth through both bactericidal and bacteriostatic effects. Similarly, *E. ribes* and *D. alata* reduced bacterial cell counts in *Pseudomonas* spp. and *Klebsiella* spp. These results demonstrate the antimicrobial properties of the formulation extract against the tested UTI pathogens.

Cytotoxic effect

In Fig. 4, the cytotoxic effect of the *D. alata, E. ribes* and their formulation extract was analysed using the brine shrimp lethality assay. On day 1, 100 % of the nauplii remained alive across all concentrations and all three samples. On day 2, *D. alata* showed 100 % survival at the lowest concentration (5 µg/mL) and 70 % of survival was observed at the highest concentration (80 µg/mL). Similarly, to the *E. ribes,* 80 % of survival was observed at the highest concentration (80 µg/mL). For the formulation extract, the survival of nauplii at different concentration (5, 10, 20, 40 and 80 µg/mL) was 100 %, 90 %, 90 %, 80 % and 70 %, respectively. Overall, the herbal extracts and their formulation extract exhibited minimal toxicity even at higher concentrations.

Discussions

Eupatorium adenophorum was collected form the Himalaya and its extract was analysed for antimicrobial activity against *E. coli* and *S. aureus*. At concentrations ranging from 32.6 to 500 µg/mL, inhibiton zones of 8-19 mm were observed against *S. aureus* and 8 mm at all concentrations against *E. coli* (17). Based on the previous study, aqueous extracts of *Sphagneticola calendulacea* (Chinese Wedelia) showed potential antibacterial activity against UTI pathogens. The tested organism, *E. coli,* exhibited an inhibition zone of 7 mm, *Proteus* spp., 7 mm, *Staphylococcus* spp., 10 mm, *Pseudomonas* spp., 8 mm and *Enterobacter* spp., 7 mm (18, 19). In a similar study, methanol extracts *of Andrographis paniculata* and *Rosa* were tested at concentrations of 25 µL, 50 µL and 100 µL against the UTI-causing microorganism, showing

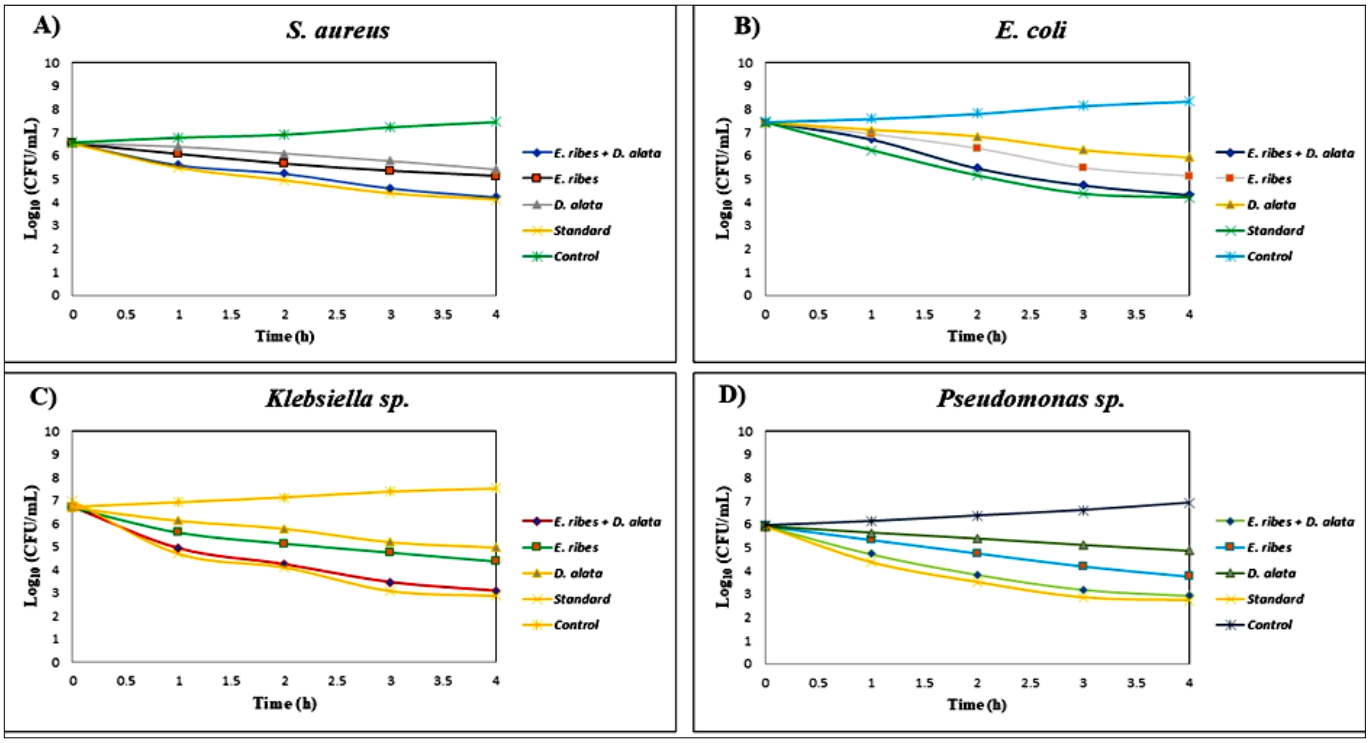

Fig. 3. The microbial growth reduction using the *D. alata, E. ribes* and their formulation extract against UTI pathogens.

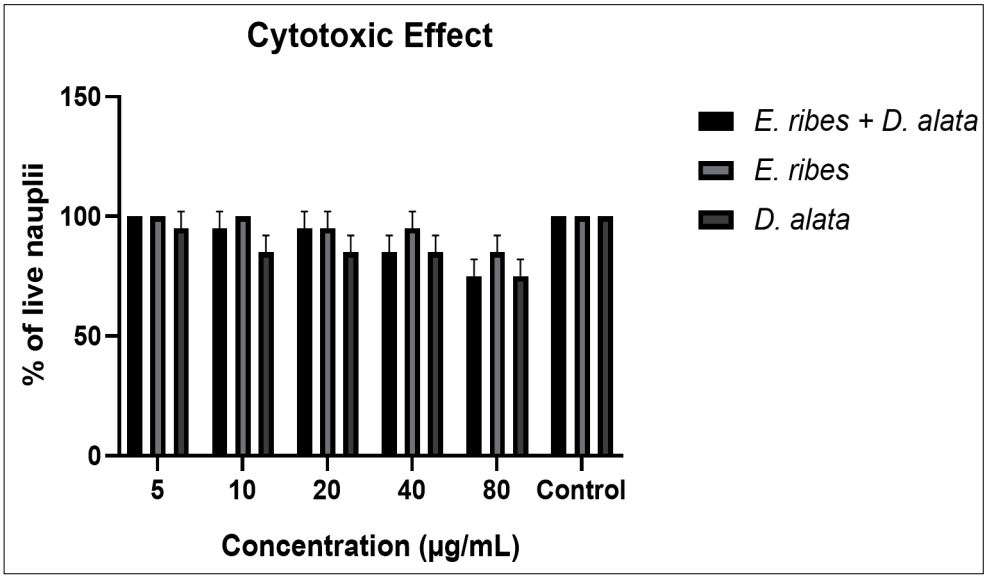

Fig. 4. Representation of cytotoxic effect using brine shrimp lethality assay.

higher zones of inhibition at higher concentrations: *E. coli* (10 mm), *Klebsiella* spp. (9 mm) and *Enterococcus faecalis* (15 mm) (20).

In previous research, *Acorus calamus* DMSO extract was analyzed using the time-kill curve assay at concentrations of 25, 50 and 100 µg/mL against *S. mutans* and *Pseudomonas aeruginosa.* At higher concentrations, bactericidal efficacy was observed against *Pseudomonas* spp. and *S. mutans* (21, 22). Similarly, *Croton bonplandianum*-mediated ethanolic extract was tested with the time-kill curve assay at different duration against cariogenic pathogens. For *Lactobacillus* spp., the highest concentration (100 µg/mL) reduced bacterial growth (23, 24).

Ficus platyphylla was used to synthesize an aqueous methanolic extract, which was analysed using the brine shrimp lethality assay at various concentration of 10, 100 and 1000 µg/mL. Thrity nauplii were added to each concentration and after 24 hr incubation, 29, 28 and 28 nauplii remained alive, respectively. The aqueous methanolic extract showed low toxicity even at higher concentrations (25). Similarly, methanolic extracts of *Justicia adhatoda* leaves were tested for cytotoxic effects at concentrations of 10, 100 and 1000 ppm Thirty nauplii were added a teach concentration and survival rates were 27, 19 and 7, respectively, indicating minimal toxicity at lower concentrations (26). *Theobroma cacao*-mediated acetone extract at 500, 100 and 10 ppm showed mortality rates of 36.67 %, 56.67 % and 66.70 %, respectively (27).

Conclusion

The studies conducted highlight the diverse pharmacological activities of *D. alata, E. ribes* and their formulation extract. The extract exhibited strong antibacterial and cytotoxic effects, with efficacy comparable to or greater than that of conventional agents under certain conditions. The results demonstrate the traditional use of *D. alata, E. ribes* and their formulation extract in

alternative medicine, while also providing a scientific basis for their therapeutic potential. Further research is required to isolate and identify the individual bioactive molecules responsible for these benefits. In addition, *in vivo* experiments should be conducted to validate and expand upon these promising findings. *D. alata, E. ribes* and their formulation extract represent potential natural sources of bioactive compounds that could be developed for application in nutraceutical and pharmaceutical production.

Acknowledgements

We would like to thank Saveetha Institute of Medical and Technical Sciences for support.

Authors' contributions

Study design and conception was created by RS. Data Collection was carried out by SG and BR. Analysis and interpretation of results was performed by RS, SG and BR. Draft manuscript preparation was executed RS and SG. All authors reviewed the results and approved the final version of the manuscript.

Compliance with ethical standards

Conflict of interest: Authors do not have any conflict of interest to declare.

Ethical issues: None

References

1. Aslam S, Albo M, Brubaker L. Recurrent urinary tract infections in adult women. Jama. 2020;323(7):658-59. https://doi.org/10.1001/jama.2019.21377
2. Bunduki GK, Heinz E, Phiri VS, Noah P, Feasey N, Musaya J. Virulence factors and antimicrobial resistance of uropathogenic *Escherichia coli* (UPEC) isolated from urinary tract infections: a systematic review and meta-analysis. BMC Infectious Diseases. 2021;21(1):753. https://doi.org/10.1186/s12879-021-06435-7
3. Klein RD, Hultgren SJ. Urinary tract infections: microbial pathogenesis, host–pathogen interactions and new treatment strategies. Nat Rev Microbio. 2020;18(4):211-26. https://doi.org/10.1038/s41579-020-0324-0
4. Hsu YT, Wu HC, Tsai CC, Tsai YC, Kuo CY. Plant extracts and natural compounds for the treatment of urinary tract infections in women: Mechanisms, efficacy and therapeutic potential. Curr Issues Mole Biol. 2025;47(8):591. https://doi.org/10.3390/cimb47080591
5. Wankhade PR, Gupta RD, Das RJ, Awandekar NB, Umekar MJ. Review on pharmacological and phytochemistry of *Embelia ribes* plant. Int J Pharmacogn Life Sci. 2021;2:34-43. https://doi.org/10.33545/27072827.2021.v2.i1a.25
6. Afzal M, Gupta G, Kazmi I, Rahman M, Upadhyay G, Ahmad K, et al. Evaluation of anxiolytic activity of embelin isolated from *Embelia ribes*. Biomed Aging Pathol. 2012;2(2):45-47. https://doi.org/10.1016/j.biomag.2012.03.003
7. Singh R, Mewara DK. Therapeutic potential of novel tin metal complex of embelin isolated from *Embelia ribes* fruits. Indian J Pharmaco. 2025;57(3):166-72. https://doi.org/10.4103/ijp.ijp_678_23
8. Induar S, Dubey D, Rath S, Meher RK, Swain SK, Tripathy SK. Evaluation of the antioxidant and antimicrobial activity of the nutritionally rich plant, *Dioscorea alata* L. Biomed Pharmaco J. 2024;17(2):1265-78. https://doi.org/10.13005/bpj/2940
9. Amarasekara R, Wickramarachchi SR. Antioxidant activity of phenolic compounds in *Dioscorea alata* L. (Raja Ala) tuber cooking water. Acta Chem Iasi. 2021;29(2):183-200. https://doi.org/10.47743/achi-2021-2-0013
10. Abubakar AR, Haque M. Preparation of medicinal plants: Basic extraction and fractionation procedures for experimental purposes. J Pharm Bioalli Sci. 2020;12(1):1-10. https://doi.org/10.4103/jpbs.JPBS_175_19
11. Fauziah F, Mas' udah S, Hapsari L, Nurfadilah S. Biochemical composition and nutritional value of fresh tuber of water yam (*Dioscorea alata* L.) local accessions from East Java, Indonesia. AGRIVITA J Agri Sci. 2020;42(2):255-71. https://doi.org/10.17503/agrivita.v0i0.2552
12. Aung MT, Myint PP, Win C, Myint YY. Study on nutritional quality and antimicrobial, antioxidant and anti-proliferative activities of *Dioscorea alata* l. (Myauk U). J Myanmar Acad Arts Sci. 2020;18:21.
13. Sebastian S, Rajeshkumar, Shanmugam SN. Evaluation of anti-microbial activity of Aloe vera, Chamomile and Propolis formulation against oral pathogens. Cuestiones de Fisioterapia. 2025;54(3):276-85. https://doi.org/10.48047/4h58t174
14. Subash CS, Jaisankar V, Parameswari A, Leo Edward M. Development of novel palm resin-poly (Acrylamide) based hydrogel silver nanocomposite for antibacterial activity. Iran J Chem Chem Eng. 2024;43(2). https://www.ijcce.ac.ir/index.php/article_707545_815825e394de3a406a08f53fbc594421.pdf
15. Shyla RS, Shanmugam R, Rahman AK, Narayanan DR. Evaluating the cytotoxic impact of a methanolic extract of *Cassia auriculata* combined with alpha-tocopherol and povidone-iodine in an herbal-based wound dressing on a fibroblast cell line. Tpm-testing, Psychomet Meth Appl Psycho. 2025;32:277-85. https://tpmap.org/submission/index.php/tpm/article/view/224/110
16. Snehaa Baskaran BG, Kumar R. Cytotoxic effect of ginger and blue pea herbal formulation-based mouthwash-an *in vitro* study. Cuestiones de Fisioterapia. 2025;54(4):537-48. https://doi.org/10.48047/wh8ybk45
17. Panda J, Behera S, Ahmad R, Nayak D, Behera SK. Antimicrobial efficacy of microbial-derived nanoparticles: An alternative, ecofriendly and green approach against multidrug-resistant pathogens. In: Mohanta YK, Mishra B, Bhuyan T, editors. Microbial Nanotechnology for Sustainable Future. CRC Press; 2025. pp. 1-17 https://doi.org/10.1201/9781003487142-1
18. Rai S, Jas R. Antimicrobial activity of *Eupatorium adenophorum* (Crofton weed) extract on *Staphylococcus aureus* and *Escherichia coli* isolates from wound infection. J Vet Anim Sci. 2025;56(2):321-24. https://doi.org/10.51966/jvas.2025.56.2.321-324
19. Acharjee M, Zerin N, Ishma T, Mahmud MR. *In-vitro* anti-bacterial activity of medicinal plants against urinary tract infection (UTI) causing bacteria along with their synergistic effects with commercially available antibiotics. New Microbes New Infect. 2023;51:101076. https://doi.org/10.1016/j.nmni.2022.101076
20. Sivaperumal P, Ganapathy D, Kamala K. Evaluating the efficacy of doripenem against *Staphylococcus aureus* in vancomycin-resistant strains. Microbial Pathogenesis. 2025;202:107449. https://doi.org/10.1016/j.micpath.2025.107449
21. Thiraviarajan A, Shanmugam R, Munusamy T. Preparation of *Andrographis paniculata* and Rosa formulation and its antibacterial activity against Urinary Tract Infection causing pathogens. J Pharm Bioallied Sci. 2024;16(Suppl2):S1360-64. https://doi.org/10.4103/jpbs.jpbs_574_23
22. Haran P, Shanmugam R, Deenadayalan P. Free radical scavenging, anti-inflammatory and antibacterial activity of *Acorus calamus* leaves extract against *Pseudomonas aeruginosa* and *Staphylococcus aureus*. Cureus. 2024;16(3). https://doi.org/10.7759/cureus.55987
23. Shanmugam R, Deenadayalan P, Manigandan P. Anticariogenic, antidiabetic and toxicology evaluation of the ethanolic extract of *Croton bonplandianum*: An *in vitro* study. Cureus. 2024;16(7).

https://doi.org/10.7759/cureus.63813

24. Jabeen N, Prabhalakshmi K, Dhanraj G, Ramasubburayan R. Biosynthesis of titanium dioxide nanoparticles using *Sargassum tenerrimum* as reductant and deciphering its antibiofilm role against cariogenic *Candida albicans*. Microbial Pathogenesis. 2025;202:107452. https://doi.org/10.1016/j.micpath.2025.107452
25. Shina SI, Mohamed CI, Sammer Y, Abdulganiyyu IA, Abduljelil A, Balogun SU, et al. Preliminary anticancer activity, antimicrobial activity and brine shrimp lethality bioassay fractions of *Ficus platyphylla* del. Stem Bark J Chem B Nat Prod Med Chem. 2024;6:182-99. https://doi.org/10.48309/ajcb.2024.436738.1227
26. Nasir M, Ramash R, Fatima H, Ashraf S, Munir I, Asghar S, et al. Phytochemical characterization and assessment of crude extracts from *Justicia adhatoda* for phytotoxic and cytotoxic activity. Scientifica. 2024;2024(1):1374346. https://doi.org/10.1155/2024/1374346
27. Febrina RV, Yahya M, Windananti S. Toxicity of methanolic extract from cocoa pod husk (*Theobroma cacao* L) using brine shrimp lethality test (BSLT). In: IOP Conference Series: Earth and Environmental Science. IOP Publishing; 2024;1356(1):012110. https://doi.org/10.1088/1755-1315/1356/1/012110

Additional information

Peer review: Publisher thanks Sectional Editor and the other anonymous reviewers for their contribution to the peer review of this work.

Reprints & permissions information is available at https://horizonepublishing.com/journals/index.php/PST/open_access_policy

Publisher's Note: Horizon e-Publishing Group remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

Indexing: Plant Science Today, published by Horizon e-Publishing Group, is covered by Scopus, Web of Science, BIOSIS Previews, Clarivate Analytics, NAAS, UGC Care, etc
See https://horizonepublishing.com/journals/index.php/PST/indexing_abstracting

Publisher information: Plant Science Today is published by HORIZON e-Publishing Group with support from Empirion Publishers Private Limited, Thiruvananthapuram, India.