ANTIMICROBIAL ACTIVITY OF CHITOSAN BASED TRIDAX PROCUMBENS STEM GEL- AN INVITRO STUDY TYPE OF STUDY- ORIGINAL RESEARCH

Samyuktha.P.S[1], M. Jeevitha[2]*, S. Rajeshkumar[3]

Article History: Received: 12.12.2022 **Revised:** 29.01.2023 **Accepted:** 15.03.2023

Abstract

Introduction: A wound is caused by skin injury from surgery, ulceration, cancer, wear and tear, and/or loss of the connective tissue beneath the skin. Green synthesis is a systematic method for producing a product without creating harmful byproducts. It is a long-term, effective, and replicable synthesis method. Tridax procumbens is a perennial weed taxonomically classified as a member of the Asteraceae family. Among other active components, T. procumbens's phytoconstituents contain sitosterol, carotenoids, luteolin, and linolenic acid. Beta-sitosterol, a plant steroid, aids in epithelialization and wound healing. The current study is focused on evaluating the antimicrobial effect of chitosan based T. procumbens stem gel.
Materials and method: Chitosan based T. procumbens stem gel was prepared and assessed for antibacterial activity at various concentrations against pathogenic microorganisms, Staphylococcus aureus, Pseudomonas and Escherichia coli and compared with that of the commercial wound healing gel. The results were tabulated and graphically presented.
Results: The Chitosan based T. procumbens stem gel exhibited higher antimicrobial activity than commercially available wound healing gel. The activity was dose dependent and was highest at 100 µL concentration.
Conclusion: This study concluded that the antimicrobial effect of chitosan based T. procumbens stem gel is better than the commercially available wound healing gel and thus can be used as an alternative in pathogenic oral diseases.

Keywords: antimicrobial, chitosan, innovative, sustainable, Tridax procumbens,

[1]Saveetha dental college and hospitals, Saveetha Institute of Medical and Technical Sciences (SIMATS), Chennai-77, India
[2*]Department of Periodontics, Saveetha dental college and hospitals, Saveetha Institute of Medical and Technical Sciences (SIMATS), Chennai-77, India
[3]Nanobiomedicine lab, Department of Pharmacology, Saveetha Dental College, Saveetha Institute of Medical and Technical Sciences (SIMATS), Chennai-77, India

DOI: 10.31838/ecb/2023.12.s2.069

1. Introduction

The biological process of wound healing is linked to the more general phenomena of growth and tissue regeneration. A variety of cellular and matrix components collaborate to repair damaged tissue integrity and replace lost tissue over the course of wound healing, which takes place in a series of interrelated and overlapping stages (Egbuna et al. 2022). A wound is caused by skin injury from surgery, ulceration, cancer, wear and tear, and/or loss of the connective tissue beneath the skin. It also results in a loss of epithelial continuity. Inflammation, proliferation, and remodeling are the three sequential and overlapping phases that make up the complex process of wound healing("Website," n.d.).

Chitosan is a **β**- 1,4-linked polymer of glucosamine (2-amino-2-deoxy—D-glucose) and trace quantities of N-acetylglucosamine("Website," n.d.). The use of chitosan as a wound-healing accelerator has been extensively studied, and there is compelling evidence that chitosan may benefit each stage of wound healing("Website," n.d.). By increasing the functions of inflammatory cells such polymorphonuclear leukocytes (PMN), macrophages, fibroblasts, and osteoblasts, chitosan and its derivatives may speed up the healing of wounds(Burdușel et al. 2018)(Jeevitha et al. 2022).

Green synthesis is a systematic method for producing a product without creating harmful byproducts (Prathap et al. 2021; Santhakumar et al. 2021; Begum, Jeevitha, and Preetha 2020)(Rajeshkumar and Jeevitha 2021; Santhakumar et al. 2021).It is a long-term, effective, and replicable synthesis method. Green synthesis methods also seem to involve rapid and non-toxic substitutes (Rajeshkumar and Jeevitha 2021). Since ancient times, medicinal agents derived from plants have been used in alternative medicine. T. procumbens is a perennial weed that is native to tropical America and is found across the tropical and subtropical climates. It is taxonomically classified as a member of the Asteraceae family. A hispid, procumbent plant known as "Common button" or "Coat button" is used by traditional healers and tribal groups as a remedy for a number of illnesses and skin conditions. Among other active components, T. procumbens's phytoconstituents contain sitosterol, carotenoids, luteolin, and linolenic acid. Beta-sitosterol, a plant steroid, aids in epithelialization and wound healing. Analgesic, anti-inflammatory, antipyretic, antidiabetic, antioxidant, insecticidal, immunomodulatory, and antibacterial properties of T. procumbens have been demonstrated. It also has antiviral, antifungal, antimalarial, anticandidal, anti-cancerous, and analgesic properties (Andriana et al. 2019). Our team has extensive knowledge and research experience that has translated into high quality publications (Krishnan, Pandian, and Kumar S 2015; Ramesh Kumar et al. 2011; Felicita 2017b; Kumar 2017; Felicita 2017a; Sivamurthy and Sundari 2016; Sekar et al. 2019; Johnson et al. 2020; Jain, Kumar, and Manjula 2014; Keerthana and Thenmozhi 2016)

Considering all the prior mentioned information, the current study is focused on evaluating the antimicrobial effect of T. procumbens stem based chitosan gel against more common oral pathogens.

2. Materials And Method:

Preparation of 50 mL chitosan gel:

In 49 mL of distilled water and 1mL of glacial acetic acid, 0.5 mL of chitosan is dissolved. The solution is then placed on a magnetic stirrer for 24 hours. T. procumbens stem was taken and was crushed and made into powdered form. 1g of this powder was dissolved in 100 mL of distilled water. The solution was heated using a heating mantle. Then, the solution was filtered using a filter paper. The filtered solution was again heated, i.e. condensed, till the solution was in thick form. Now, 1 mL of the stem extract was dissolved in 9 mL of chitosan and was placed in a magnetic stirrer for 24 h to obtain the chitosan based T. procumbens stem gel.

Antibacterial activity:

Antibacterial activity of the chitosan based T. procumbens stem gel was determined against the strains Staphylococcus aureus, Pseudomonas and Escherichia coli. Mueller Hinton Agar was utilized for this activity to determine the zone of inhibition. Mueller Hinton agar culture plates were prepared and sterilized for 15 minutes at 121 °C. The media was poured into the sterilized plates and left undisturbed for solidification. The wells were cut using 9 mm sterile polystyrene tips and the test organisms were swabbed. The gels were diluted to different concentrations (25 µL, 50 µL and 100 µL) and were loaded in the wells. A similar set up was performed for commercial wound healing gel. The plates were incubated for 24 hours at 37°C. After incubation, the zones of inhibition were measured.

3. Results

After incubation of the culture plates for 24 hours, the zones of inhibition produced by various concentrations of the stem gel and commercially available gel were tabulated (Table 1). The observed zones were graphically represented for ease of understanding and clear assessment (Figure 1). The zone of inhibition was maximum at 100 µL concentration against all the studied pathogens (20 mm for E. coli, 18 mm for S. aureus and 20 mm for

Pseudomonas) and shown to be higher than that of the commercial wound healing gel (10 mm for E. coli, 9 mm for both S. aureus and Pseudomonas) (Figure 2).

Table 1: Table depicting the zones of inhibition (in mm) observed after 24 hours in various concentrations of chitosan based T. procumbens stem gel comparison to commercially available wound healing gel.

T. procumbens stem gel				Commercial gel		
Organisms	25 µL	50 µL	100 µL	25 µL	50 µL	100 µL
Eschersia coli.	15 mm	18 mm	20 mm	9 mm	9 mm	10 mm
S. aureus	12 mm	14 mm	18 mm	9 mm	9 mm	9 mm
Pseudomonas	15 mm	11 mm	20 mm	9 mm	9 mm	9 mm

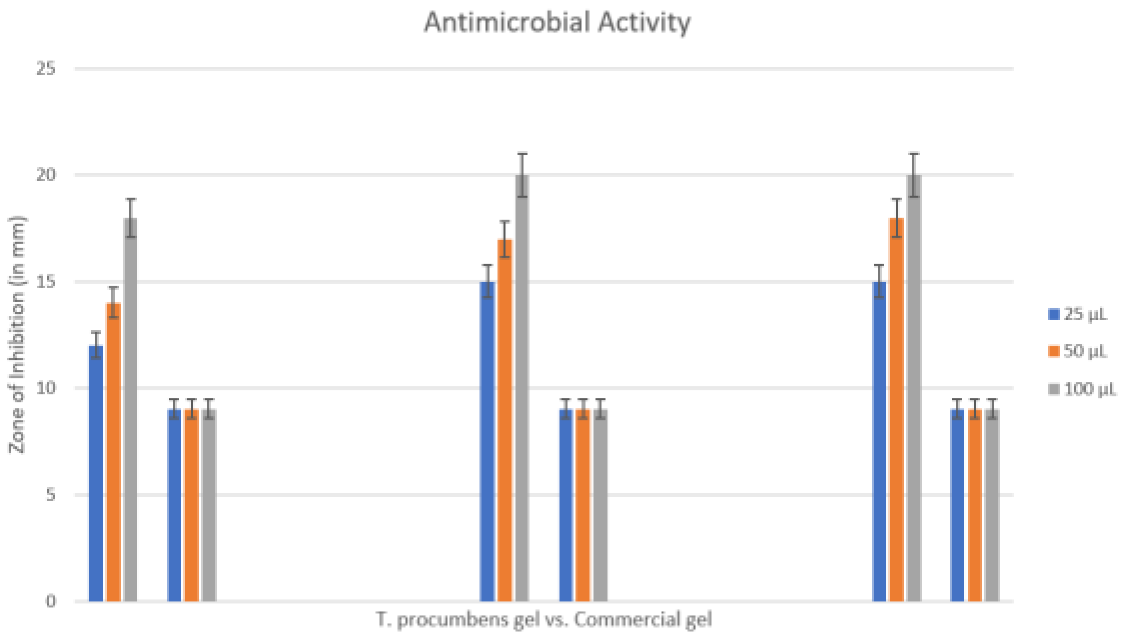

Figure 1: Bar chart depicting the antimicrobial activity exhibited by chitosan based T. procumbens stem gel in comparison with commercially available gel at various concentrations against S. aureus, Pseudomonas and E. coli.

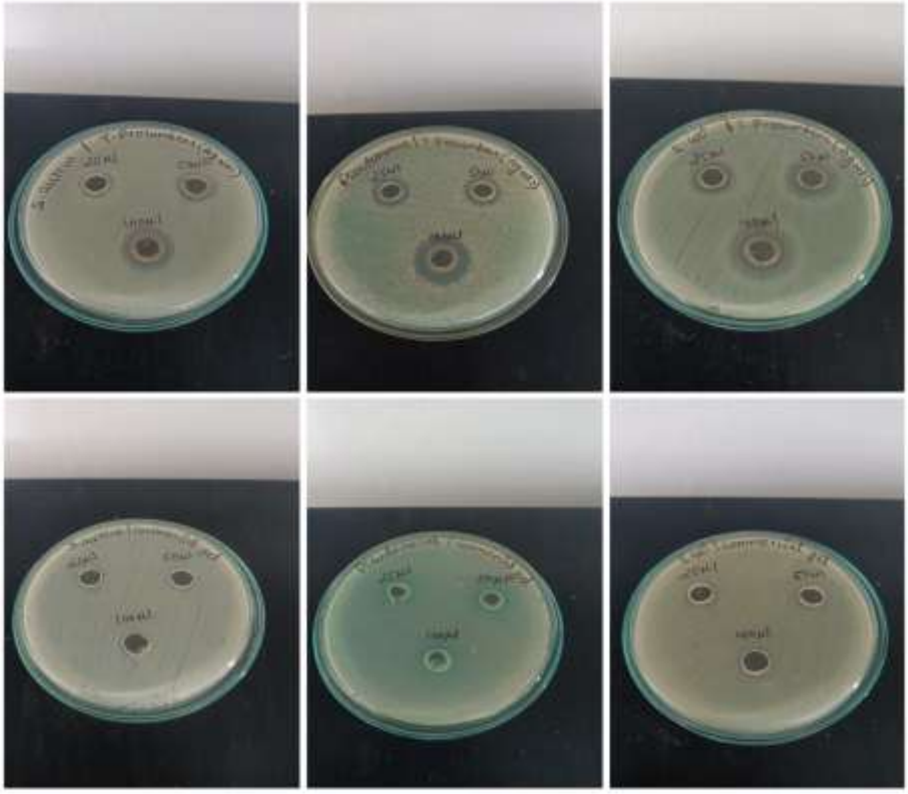

Figure 2: Evaluation of antimicrobial activity of chitosan based T. procumbens stem gel vs commercially available wound gel at various concentrations.

4. Discussion

Asepticity of the wounded area is vital in the process of wound healing. The presence of bacteria and other pathogens can affect the process, can delay the wound healing and can even cause complications such as inflammation and swelling of the area(Ravikumar, Shivashangari, and Devaki 2005). Hence, it is vital for any healing promoting agents to exhibit antimicrobial activity to ensure that the healing takes place.(Bhat et al. 2019). Previous studies conducted by Erick et al have studied antimicrobial activity of biogenic silver nanoparticles synthesized using T. procumbens L. This study is in correlation with the current study as the T. procumbens stem based gel had shown excellent antimicrobial activity against E. coli (Shukla and Iravani 2018).

The current study is a novel study, as it is the first of its kind to create a wound healing gel that is made from the stem extract of T. procumbens. Previous studies have formulated nanoparticles incorporating various plants, yet no formulation has been done. Previous studies conducted by Fatima et al have concluded that both gram-negative and gram-positive bacterial strains were resistant to the antibacterial effects of silver nanoparticles produced via green synthesis from T. procumbens. The biologically synthesized T. procumbens stem extract loaded with chitosan gel showed therapeutic efficacy and was effective against both gram-positive and gram-negative common oral pathogens as a topical antibacterial preparation. The new gel application could speed up wound healing by getting rid of the infection since the wound would host microbial strains (Fatima et al. 2021).

The current study observes a dose dependent antimicrobial activity. It is safe to say that as the concentration of the chitosan based T. procumbens stem gel increased, the antimicrobial activity increased. The antimicrobial activity was exhibited the most at 100 µL. The current study is limited by its in-vitro nature. The clinical efficacy is yet to be determined. The study can be further enhanced by performing animal model experiments, cytotoxic studies, and further clinical trials.

5. Conclusion

From the present study, it is revealed that the antimicrobial effect of chitosan based T. procumbens stem gel was higher than the commercially available wound healing gel against more common oral pathogens. Considering the fact that the gel is derived from most traditionally used medicine, economical and immediate effect, it can be applied as an antimicrobial wound healing gel as an alternative to commercial synthetic wound healing gel.

ACKNOWLEDGEMENT:
We would like to thank Saveetha Dental College and Hospitals, Saveetha Institute of Medical and Technical Sciences, Saveetha University for providing us support to carry out the study.

CONFLICT OF INTEREST:
The authors declare that there were no conflicts of interest in the present study.

SOURCE OF FUNDING:

The present project is supported by

- Saveetha Institute of Medical and Technical Sciences
- Saveetha Dental College and Hospitals, Saveetha University
- Sri Ramatheertham trust

6. References

Andriana, Yusuf, Tran Dang Xuan, Tran Ngoc Quy, Truong Ngoc Minh, Truong Mai Van, and Tran Duc Viet. 2019. "Antihyperuricemia, Antioxidant, and Antibacterial Activities of L." Foods (Basel, Switzerland) 8 (1). https://doi.org/10.3390/foods8010021.

Begum, Akifa, M. Jeevitha, and S. Preetha. 2020. "Knowledge and Awareness on Vitamin D Deficiency among IT Employees." Journal of Pharmaceutical Research International. https://doi.org/10.9734/jpri/2020/v32i1830683.

Bhat, Aamir Hussain, Imran Khan, Mohammad Jawaid, Fakhreldin O. Suliman, Haider Al-Lawati, and Salma Muhamed Al-Kindy. 2019. Nanomaterials for Healthcare, Energy and Environment. Springer.

Burdușel, Alexandra-Cristina, Oana Gherasim, Alexandru Mihai Grumezescu, Laurențiu Mogoantă, Anton Ficai, and Ecaterina Andronescu. 2018. "Biomedical Applications of Silver Nanoparticles: An Up-to-Date Overview." Nanomaterials (Basel, Switzerland) 8 (9). https://doi.org/10.3390/nano8090681.

Egbuna, Chukwuebuka, Jaison Jeevanandam, Kingsley C. Patrick-Iwuanyanwu, and Eugene N. Onyeike. 2022. Application of Nanotechnology in Food Science, Processing and Packaging. Springer Nature.

Fatima, Farhat, Mohammed F. Aldawsari, Mohammed Muqtader Ahmed, Md Khalid Anwer, Maimuna Naz, Mohammad Javed Ansari, Abubaker M. Hamad, Ameeduzzafar Zafar, and Mohammed Jafar. 2021. "Green Synthesized Silver Nanoparticles Using Tridax Procumbens for Topical Application: Excision Wound Model and Histopathological Studies." Pharmaceutics 13 (11). https://doi.org/10.3390/pharmaceutics13111754.

Felicita, A. Sumathi. 2017a. "Quantification of Intrusive/retraction Force and Moment Generated during En-Masse Retraction of Maxillary Anterior Teeth Using Mini-Implants: A Conceptual Approach." Dental Press Journal of Orthodontics 22 (5): 47–55.
2017b. "Orthodontic Management of a Dilacerated Central Incisor and Partially Impacted Canine with Unilateral Extraction - A Case Report." The Saudi Dental Journal 29 (4): 185–93.

Jain, Ravindra Kumar, Sridhar Prem Kumar, and W. S. Manjula. 2014. "Comparison of Intrusion Effects on Maxillary Incisors among Mini Implant Anchorage, J-Hook Headgear and Utility Arch." Journal of Clinical and Diagnostic Research: JCDR 8 (7): ZC21–24.

Jeevitha, M., Chandra Sekhara Prabhahar, M. Narendra Reddy, V. K. Vijay, M. Navarasu, and M. Umayal. 2022. "Clinical Evaluation of Lateral Pedicle Flap Stabilized with Cyanoacrylate Tissue Adhesive: A Randomized Controlled Clinical Trial." Contemporary Clinical Dentistry 13 (1): 24–29.

Johnson, Jayapriya, Ganesh Lakshmanan, Biruntha M, Vidhyavathi R M, Kohila Kalimuthu, and Durairaj Sekar. 2020. "Computational Identification of MiRNA-7110 from Pulmonary Arterial Hypertension (PAH) ESTs: A New microRNA That Links Diabetes and PAH." Hypertension Research: Official Journal of the Japanese Society of Hypertension 43 (4): 360–62.

Keerthana, B., and M. S. Thenmozhi. 2016. "Occurrence of Foramen of Huschke and Its Clinical Significance." Research Journal of Pharmacy and Technology 9 (11): 1835–36.

Krishnan, Sindhuja, Saravana Pandian, and Aravind Kumar S. 2015. "Effect of Bisphosphonates on Orthodontic Tooth Movement-an Update." Journal of Clinical and Diagnostic Research: JCDR 9 (4): ZE01–05.

Kumar, Santhosh. 2017. "The Emerging Role of Botulinum Toxin in the Treatment of Orofacial Disorders: Literature Update." Asian Journal of Pharmaceutical and Clinical Research 10 (9): 21.

Prathap, Lavanya, Selvaraj Jayaraman, Anitha Roy, Preetha Santhakumar, and M. Jeevitha. 2021. "Molecular Docking Analysis of Stachydrine and Sakuranetin with IL-6 and TNF-α in the Context of Inflammation." Bioinformation 17 (2): 363–68.

Rajeshkumar, S., and M. Jeevitha. 2021. "Plant-

Mediated Biosynthesis and Characterization of Zinc Oxide Nanoparticles.” Zinc-Based Nanostructures for Environmental and Agricultural Applications. https://doi.org/10.1016/b978-0-12-822836-4.00023-9.

Ramesh Kumar, K. R., K. K. Shanta Sundari, A. Venkatesan, and Shymalaa Chandrasekar. 2011. “Depth of Resin Penetration into Enamel with 3 Types of Enamel Conditioning Methods: A Confocal Microscopic Study.” American Journal of Orthodontics and Dentofacial Orthopedics: Official Publication of the American Association of Orthodontists, Its Constituent Societies, and the American Board of Orthodontics 140 (4): 479–85.

Ravikumar, Vilwanathan, Kanchi Subramanian Shivashangari, and Thiruvengadam Devaki. 2005. “Effect of Tridax Procumbens on Liver Antioxidant Defense System during Lipopolysaccharide-Induced Hepatitis in D-Galactosamine Sensitised Rats.” Molecular and Cellular Biochemistry 269 (1-2): 131–36.

Santhakumar, Preetha, Lavanya Prathap, Anitha Roy, Selvaraj Jayaraman, and M. Jeevitha. 2021. “Molecular Docking Analysis of Furfural and Isoginkgetin with Heme Oxygenase I and PPARγ.” Bioinformation 17 (2): 356–62.

Sekar, Durairaj, Ganesh Lakshmanan, Panagal Mani, and M. Biruntha. 2019. “Methylation-Dependent Circulating microRNA 510 in Preeclampsia Patients.” Hypertension Research: Official Journal of the Japanese Society of Hypertension 42 (10): 1647–48.

Shukla, Ashutosh Kumar, and Siavash Iravani. 2018. Green Synthesis, Characterization and Applications of Nanoparticles. Elsevier.

Sivamurthy, Gautham, and Shantha Sundari. 2016. “Stress Distribution Patterns at Mini-Implant Site during Retraction and Intrusion—a Three-Dimensional Finite Element Study.” Progress in Orthodontics 17 (1): 1–11.

Cañedo-Dorantes L, Cañedo-Ayala M. Skin Acute Wound Healing: A Comprehensive Review [Internet]. Vol. 2019, International Journal of Inflammation. 2019. p. 1–15. Available from: http://dx.doi.org/10.1155/2019/3706315.

Konop M, Damps T, Misicka A, Rudnicka L. Certain Aspects of Silver and Silver Nanoparticles in Wound Care: A Minireview [Internet]. Vol. 2016, Journal of Nanomaterials. 2016. p. 1–10. Available from: http://dx.doi.org/10.1155/2016/7614753.

Ueno H, Mori T, Fujinaga T. Topical formulations and wound healing applications of chitosan [Internet]. Vol. 52, Advanced Drug Delivery Reviews. 2001. p. 105–15. Available from: http://dx.doi.org/10.1016/s0169-409x(01)00189-2.