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ANTIBACTERIAL ACTIVITY OF VIBHĪTAKĪ KṢĀRA [TERMINALIA BELLIRICA (GAERTN.) ROXB.] ON CHRONIC WOUND MICROBIOTA AGAINST MUPIROCIN- A COMPARATIVE IN VITRO STUDY.

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ABSTRACT

The nonhealing ulcer is a major health problem and the presence of microbes on the wound surface is one of the main factors that delay wound healing. The common bacteria present on the wound surface are *Staphylococcus aureus, Pseudomonas aeruginosa, and Enterococcus faecalis*. In Ayurveda, nonhealing ulcers can be correlated to *duṣtavr̥aṇa*. *Kṣāra karma* (alkaline cauterisation) is one among *ṣaṣṭi upakrama* and is indicated in ulcers that are difficult to cure and that persist for a long time. *Kṣāra* also has the property *kr̥imighna. Vibhītakī* and *apāmārga* are drugs mentioned by *Ācārya Suśruta* for the preparation of *pr̥atisāranīya kṣāra.* This study aimed to explore the mode of action of the drug through antimicrobial properties. The study design was a comparative antibacterial in vitro study which consist of an antibacterial activity assay. Antibacterial assay was done using the good diffusion method. The result was compared with a standard antibiotic. On evaluation, *vibhītakī kṣāra* showed significant antibacterial activity when compared with the standard drug Mupirocin.

Keywords: Nonhealing ulcer, *Duṣtavr̥aṇa*, *Vibhītakī kṣāra* (*Terminalia bellirica*), Mupirocin, Antibacterial activity

INTRODUCTION

A nonhealing ulcer is a major health problem, and it affects nearly 6 million populations worldwide in India it is reported as 4.5 per 1000 population.[1] The incidence of chronic ulcers is expected to increase as the population age increase. During the course of the disease, the patient can experience pain, emotional and physical distress, reduced mobility, and social isolation.[2] There are several factors that affect wound healing which include infection, ischemia, metabolic diseases, and immunosuppression. The common bacteria present on the wound surface are *Staphylococcus aureus, Pseudomonas aeruginosa,* and *Enterococcus faecalis*.[3] Bacteria in an infected wound cause cell death which leads to an increase in local inflammation. The presence of necrotic tissue will prevent the growth of new tissue and it serves as a culture medium for bacterial proliferation.[4] The conventional management of non-healing ulcers includes debridement of necrotic tissue, infection management using topical antimicrobial agents, and management of co-morbidities.[5]

In *Ayurveda*, nonhealing ulcers can be correlated to *duṣtavr̥aṇa*.[6] *Āchārya Suśruta* has mentioned *ṣaṣṭi upakrama* (sixty treatments) for the management of *vr̥aṇa*.[7] *Kṣāra karma* (chemical cauterisation) is one among *ṣaṣṭi upakrama* and is indicated in ulcers that are difficult to cure and those persist for a long time.[8] *Kṣāra* is considered the best among *śastr̥a* and *anuśastr̥a* by *Suśruta* since it does the functions like *chedana* (excision), *bhedana* (cutting) *lekhana* (scraping), and *tr̥idoṣagna* (mitigates all the three *doṣa*). It is also having actions like *śodhana* (purification) and *kr̥imighna* (killing the worms & bacteria).[9] *Vibhītakī* is one among the drugs mentioned by *Āchārya Suśruta* for the preparation of *pr̥atisāranīya kṣāra* (externally applied caustic alkali).[10] It is having the pharmacological properties *Kaṣāya rasa, laghu, rūkṣa guṇa, uṣṇa vīrya, madhura vipāka, karma* like *kaphahara* and *kr̥imighna*. Mupirocin is a widely used topical antibiotic in the management of chronic ulcers. It shows a broad spectrum of antibacterial activity on Staphylococci, Streptococci, and several gram-negative bacteria. Conventionally antibiotics are being used for the management of chronic ulcers both internally and externally for a long time period. antibiotic-resistant bacteria are one of the biggest challenges that conventional medicine is facing,[11] studies regarding the antimicrobial activity of Ayurveda medicine are relevant in the current scenario. The study also aims to explore the mode of action of Ayurveda drugs through antimicrobial properties.

Methodology

The study design was a comparative in-vitro trial. The methodology includes the preparation of *vibhītakī kṣāra* and antibacterial assay. The raw drug *vibhītakī phala* for the *kṣāra* was purchased from a GMP-certified company and *kṣāra* was prepared as per *Śusruta samhita.* The drugs were dried and burnt to ashes. The whole ashes were collected on the next day and mixed with 6 times of water and were allowed to settle down. A supernatant portion of *kṣārajala* was collected the next day and filtered using double-layered cloth. The obtained *kṣārajala* was taken in a big vessel and kept for boiling on mild fire and continuously stirred well. After the complete evaporation of *kṣārajala*, powdered *vibhītakī kṣāra* was obtained. The obtained *kṣāra* were collected and stored in an airtight glass container. On pH analysis, *vibhītakī kṣāra* had a pH of 11.3 ± 0.2 at 32º±0.2 Celsius.

Agar well diffusion method was used to evaluate the antimicrobial activity of *vibhītakī kṣāra.* Muller Hinton agar was used to prepare the sterile agar petriplates. For the preparation of inoculum, test organisms (*Staphylococcus aureus, Pseudomonas aeruginosa,* and *Enterococcus faecalis*) were inoculated into the broth medium and incubated at 37 ºc until turbidity of the culture matches with recommended turbidity standard (0.5 McFarland opacity standard tube).10 µl of inoculum was inoculated into the agar plate using a micropipette. Then uniformly spread with the help of a sterile cell spreader on the surface of the agar plate and it was allowed to dry for 3-5 minutes. A hole with a diameter of 3 mm has been punched aseptically with a sterile corn borer.6 well were prepared in this manner .20 µl of 6 concentra-

tions of extract solution (480 mg/ml, 240 mg/ml, 120 mg/ml, 60 mg/ml, 30 mg/ml and 15 mg/ml) were introduced in to the well.Agar plates were incubated at 37 ºc for 24 hours. Standard drug – Mupirocin 200µg disc was placed over a separate agar plate. The diameter of the zone of inhibition around each well was measured using Verniercalliper. The inhibition zone of different concentrations of *vibhītakī kṣāra* was compared with mupirocin.

Observations and analysis

Zone of inhibition (ZOI) of different concentrations of *vibhītakī kṣāra* on three bacterial strains w*as* compared with the standard drug Mupirocin 200µg. Hence it was multiple comparisons between groups Dunnett's multiple comparison test was used for the statistical evaluation.

Table 01: Dunnett's multiple comparison tests in the mean zone of inhibition of *vibhītakī kṣāra* with Mupirocin on *Staphylococcus aureus*

(I)group	ZOI(I) in mm	(J)group	ZOI(J) in mm	Mean difference (i-j)	Standard error	Sig.
480 mg	24.33	Mupirocin 200 µg	33.66	-9.33	0.84	0.00
240 mg	18.33			-15.33	0.84	0.00
120 mg	10.33			-23.33	0.84	0.00
60 mg	3.00			-30.66	0.84	0.00
30 mg	3.00			-30.66	0.84	0.00
15 mg	3.00			-30.66	0.84	0.00

Dunnett's multiple comparison tests were used to identify the pairs with significant differences. As per table, no 1, the mean zone of inhibition of mupirocin showed the highest value than *vibhītakī kṣāra*'s 6 concentrations against *Staphylococcus aureus* and all the pair showed a significant
mean difference. All the concentrations showed statistically significant but inferior results in comparison with Mupirocin at p<0.001 level of significance.

Regarding the antibacterial activity towards *Pseudomonas aeruginosa*, as per table no.2, the mean zone of inhibition of *vibhītakī kṣāra* at a concentration of 480 mg showed the highest zone of inhibition when compared with other 5 concentrations of *vibhītakī kṣāra* and mupirocin. 480 mg showed statistically insignificant and equal results at P>0.05. All the other concentrations showed significant and inferior results at p<0.001.

Table 02: Dunnett's multiple comparison tests in the mean zone of inhibition of *vibhītakī kṣāra* with Mupirocin on *Pseudomonas aeruginosa*.

(I)group	ZOI(I) in mm	(J)group	ZOI(J) in mm	Mean difference (i-j)	Standard error	Sig.
480 mg	24.33	Mupirocin 200 µg	23.33	1.00	0.92	0.76
240 mg	11.33			-12.00	0.92	0.00
120 mg	3.00			-20.33	0.92	0.00
60 mg	3.00			-20.33	0.92	0.00
30 mg	3.00			-20.33	0.92	0.00
15 mg	3.00			-20.33	0.92	0.00

Table 03: Dunnett's multiple comparison tests in the mean zone of inhibition of *vibhītakī kṣāra* with Mupirocin on *Enterococcus faecalis.*

(I)group	ZOI(I) in mm	(J)group	ZOI(J) in mm	Mean difference (i-j)	Standard error	Sig.
480 mg	24.66	Mupirocin 200 µg	23.33	2.66	0.87	0.038
240 mg	13.66			-8.33	0.87	0.00
120 mg	13.33			-8.67	0.87	0.00
60 mg	3.00			-19.00	0.87	0.00
30 mg	3.00			-19.00	0.87	0.00
15 mg	3.00			-19.00	0.87	0.00

On comparing the antibacterial activity of *Vibhītakī kṣāra* with Mupirocin on *Enterococcus faecalis* as per table no.3, the mean zone of inhibition of *vibhītakī kṣāra* at a concentration of 480 mg showed the highest zone of inhibition when compared with other 5 concentrations of *vibhītakī kṣāra* and mupirocin. All the pairs showed a statistically significant mean difference. Among this, 480 mg of *vibhītakī kṣāra* showed significant and better results at P<0.05. 240 mg, 120 mg, 60 mg, and 30 mg showed significant but inferior results in comparison with Mupirocin with 0.001 level significance.

DISCUSSION

Even though the drugs used for the preparation of *kṣāra* contain a lot of phytochemicals like tannin, saponin, etc., the prepared *kṣāra* only possess the thermostable inorganic metals and minerals like Zn, Cu, Ca, Fe, etc. which contribute the different actions.[12] In kṣāra, these minerals are present in their oxide form. Since the method of preparation of *kṣāra* is combustion, the final products will be in form of the nanoparticle. An article published by Sarala et. al explained the mineral composition of *Terminalia bellerica*, in which the presence of a higher concentration of minerals like zinc, copper, iron, and manganese was observed.[13] *Ācārya Suśruta* explained different *guna* and *karma* of *kṣāra* such as *kṣāraṇa*, *chēdana*, *lēkhana*, *vilayana*, *śodhana*, *kṛimigna etc*[11] by these properties, *kṣāra* acts in *duṣtavr̥ana* to make a healthy environment and augment the wound healing. By using the term *kṣāra*, *Āchārya* tries to explain the corrosive and alkaline nature of the drug. The alkaline nature of *kṣāra* has a positive impact on wound healing action. New researchers found that a slightly alkaline pH (8-8.5) is better for promoting the growth of fibroblasts and keratinocytes.[14] This alkaline nature also has a role in antimicrobial activity. It reduces the growth of bacteria over the wound. Debridement is recognized as a major component of wound management to prepare the wound bed for reepithelialisation. *kṣāraṇa*, *chēdana*, *and lēkhana* can be considered as chemical debridement which prepares a healthy environment for wound healing. The term '*vilayana*' has several meanings like removing, liquefying or destroying, etc. The membrane leaking action, protein coagulation, cell lysis, etc. action of *kṣāra* can be considered as *vilayana*. The wound healing action of *kṣāra* can be considered as *ropaṇa*. The inorganic metal ions present in the *kṣāra* may be the factor that contributes to this action. Zinc oxide has a positive impact on wound healing. The ultimate effect of zinc oxide seems to be the acceleration of re-epithelialization within the wound.[15]Copper is a trace mineral essential for many wound healing-related processes. It is associated with vascular endothelial growth factor expression, causing angiogenesis and remodelling of the extracellular matrix.[16]Calcium ions present in *kṣāra* have been shown to modulate the proliferation, differentiation, and maturation of keratinocytes and fibroblasts.[17] These ions also regulate angiogenesis. *kṛimighna* is a special property of *kṣāra* explained in our textbooks. The antimicrobial activity can be correlated as *kṛimighna*. The Zinc oxide showed the greatest antimicrobial activity against both Gram-positive and Gram-negative bacteria. The reactive oxygen species produced by the zinc oxides help to reduce bacterial cell

viability. Copper oxide nanoparticles have the potential for external uses as antibacterial agents in surface coatings on various substrates to prevent microorganisms from attaching, colonizing, spreading, and forming biofilms.[18] Iron oxides show antimicrobial activity against Gram-negative bacteria than the Gram-positive variety. Calcium hydroxide has antimicrobial activity.[19] Calcium oxide has the ability to combine with other oxide forms like zinc, copper, or magnesium oxide and to increase its antibacterial properties. [20]The metal oxide nanoparticles present in *kṣāra* may be able to produce oxidative stress in bacterial cells thus resulting in an antibacterial action. Nanoparticles are able to cross the cellular membrane of bacteria, interfere with metabolic pathways, and induce changes in membrane shape and function. Inside the cells, these particles interact with the microbial cellular machinery, inhibit the enzymes present inside, deactivate the proteins, induce oxidative stress and electrolyte imbalance, and modify gene expression levels. Oxidative stress will also alter the bacterial membrane permeability which may lead to cell wall damage.[21]

On analysing the antimicrobial action of minerals in *kṣāra* it is understood that *kṣāra* may produce its antimicrobial action by different means at the same time. Hence, there may be less chance to develop antimicrobial drug resistance.

Here in this study, On *Staphylococcus aureus*, even though the zone of inhibition was high in mupirocin than *Vibhītakī kṣāra,* the ksara showed a good antibacterial activity towards the bacterial strain. In *Pseudomonas aeruginosa*, both *kṣāra* showed better results at higher concentrations. 480 mg of *vibhītakī kṣāra* showed equal antibacterial activity that of 200 µg of mupirocin. On *Enterococcus faecalis*, *Vibhītakī kṣāra* 480 mg concentration showed significant and better results than the standard drug.

CONCLUSION

In all three strains, *Vibhītakī kṣāra* showed significant antibacterial activity when compared with the standard drug.480 mg of *vibhītakī kṣāra* showed equal antibacterial activity to that of 200 µg of mupirocin in *Pseudomonas aeruginosa*.480 mg of *vibhītakī kṣāra* showed better results in antibacterial activity than 200 µg mupirocin in *Enterococcus faecalis*. Based on these data, the study concluded that *vibhītakī kṣāra* possesses significant antibacterial activity when compared with Mupirocin. The *kṣāra* can be used as a good alternative to modern antimicrobial agents in the management of wound infection.

REFERENCES

1. Shukla V K, Mumtaz A Ansari, Gupta S K. Wound healing research: A Perspective from India. Int J Low Extrem Wounds [Internet]. 2005 March;4(1):7-8. Available from: http://pubmed.ncbi.nlm.nih.gov/ doi: 10.1177/153473460427366.
2. Walshe C. Living with a venous leg ulcer: a descriptive study of patients' experiences. J Adv Nurs [Internet]. 1995;22(6):1092–100. Available from: http://pubmed.ncbi.nlm.nih.gov/ doi:10.1111/j.1365-2648. 1995.tb03110.x
3. Randall D Wolcott, John D hanson, Eric J Rees et al. Analysis of chronic wound microbiota of 2,963 patients by 16S rDNA pyrosequencing. Wound Repair Regen [Internet]. 2016;24(1):163-174. Available from: https://pubmed.ncbi.nlm.nih.gov/26463872/ doi:10.1111/wrr.12370.
4. Guo S, DiPietro L.A. Factors affecting wound healing. J Dent Res [Internet]. 2010 March;89(3):219-229. Available from: http://www.ncbi.nlm.nih.gov/pmc/article/ doi:10.1177/0022034509359125.
5. Manish Suthar, Saniya Gupta, Suhail Bukhari, et al. Treatment of chronic non-healing ulcers using autologous platelet-rich plasma: a case series. J Biomed Sci [Internet]. 2017; 24:16. Available from: http://www.ncbi.nlm.nih.gov/pmc/article/ doi:10.1186/s12929-017-0324-1.
6. Srikantha murthy K.R. Illustrated *Suśruta Samhita:* Vol 1. Varanasi: Chaukhambha orientalia; 2012. p.166.
7. Srikantha murthy K.R. Illustrated *Suśruta Samhita:* Vol 2. Varanasi: Chaukhambha orientalia; 2012. p.6.
8. Srikantha murthy K.R. Illustrated *Suśruta Samhita:* Vol 2. Varanasi: Chaukhambha orientalia; 2012. p.20.
9. Srikantha murthy K.R. Illustrated *Suśruta Samhita:* Vol 1. Varanasi: Chaukhambha orientalia; 2012. chapter 1. p.61-70.
10. Srikantha murthy K.R. Illustrated *Suśruta Samhita:* Vol 1. Varanasi: Chaukhambha orientalia; 2012. p.65.

11. Stephen T Odonkor, Kennedy K Addo. Bacteria resistance to antibiotics: recent trends and challenges. Int J Biol Med Res [Internet]. 2011;2(4):1204-1210. Availablefrom: https://www.biomedscidirect.com/353/bacteria_resistance_to_antibiotics_recent_trends_and_challenges/articlescategories.
12. Kumara J.P et al. Antibacterial activity of pratisaraniya Kṣāra (*Achyranthes aspera*) in the management of the fistula in ano. International journal of Ayurvedic and Herbal medicine [Internet]. 2016; 6(3):2220-2228. Available from: http://www.interscience.org.uk.
13. Sarala P, Krishnamurthy S.R. Distribution, Nutritive Value and Mineral Composition of a Few Medicinal Plants of Shimoga District, Karnataka India. Int. J. Pharm. Sci. Rev. Res [Internet]. 2021;69(2):150-162. Available from: http://dx.doi.org/10.47583/ijpsrr.2021.v69i02.023.
14. Nostro A, Cellini L, Di Giulio M, D'Arrigo M, Marino A, Blanco AR, Favaloro A, Cutroneo G, Bisignano G. Effect of alkaline pH on staphylococcal biofilm formation. APMIS [Internet]. 2012;120(9):733-42. Available from: DOI: 10.1111/j.1600-0463.2012.02900.x.
15. Lin PH, Sermersheim M, Li H, Lee PHU, Steinberg SM, Ma J. Zinc in Wound Healing Modulation. Nutrients [Internet]. 2017;10(1):16. Availabe from: DOI: 10.3390/nu10010016.
16. Melamed Eyal et al. Healing of Chronic Wounds by Copper Oxide-Impregnated Wound Dressings-Case Series. Medicina (Kaunas) [Internet]. 2021;57(3):296. Available from: DOI: 10.3390/medicina57030296.
17. Subramaniam T, Fauzi MB, Lokanathan Y, Law JX. The Role of Calcium in Wound Healing. Int J Mol Sci [Internet]. 2021;22(12):6486. Available network: doi: 10.3390/ijms22126486.
18. Ahamed M, Alhadlaq HA, Khan MA, Karuppiah P, Al-Dhabi NA. Synthesis, characterization, and antimicrobial activity of copper oxide nanoparticles. Journal of Nanomaterials [Internet]. 2014 Oct. Available from: https://dl.acm.org/doi/abs/ 10.1155/2014/637858.
19. Sergey V. Gudkov Dmitriy E. Burmistrov Dmitriy A. Serov Maksim B. Rebezov Anastasia A. Semenova Andrey B. Lisitsyn. Antibiotics. Basel [Internet]. 2021; 10(7):884. Available from: DOI: 10.3390/antibiotics10070884.
20. Yousefshahi H, Aminsobhani M, Shokri M, Shahbazi R. Anti-bacterial properties of calcium hydroxide in combination with silver, copper, zinc oxide or magnesium oxide. Eur J Transl Myol [Internet]. 2018;28(3):7545. Available from: DOI: 10.4081/ejtm.2018.7545.
21. Shaikh Sibhghatulla et al. Mechanistic Insights into the Antimicrobial Actions of Metallic Nanoparticles and Their Implications for Multidrug Resistance. International journal of molecular sciences [Internet]. 2019; 20(10):2468. Available from: DOI: 10.3390/ijms20102468.

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