International Research Journal of Ayurveda & Yoga
Vol. 5 (10),27-32, Oct,2022
ISSN: 2581-785X;https://irjay.com/
DOI: **10.47223/IRJAY.2022.51004**

Antibacterial Activity of *Shwetavarshabuvadi* Formulation Extracts on Various Pathogens Causing Skin and Soft Tissue Infections – An In Vitro Study

Manita Ahlawat[1], Pawan Kumar Godatwar[2]
1. Assistant Professor, Dept. of Roga Nidana & Vikriti Vigyana, NIA Jaipur.
2. Technical officer, SEARO WHO, New Delhi.

Article Info

Article history:
Received on: 27-08-2022
Accepted on: 22-10-2022
Available online: 31-10-2022

Corresponding author-
Manita Ahlawat, Assistant Professor, Dept. of Roga Nidana & Vikriti Vigyana, NIA Jaipur,
Email: dr.ahlawatmanita16@gmail.com,

ABSTRACT:

Introduction: The present study was carried out with an objective to investigate the antimicrobial potentials of various extracts of *Shwetavarshabuvadi* formulation on common pathogens causing skin and soft tissue infections.

Methods: Aqueous and ethanolic extracts of study formulation with three concentrations 10%, 20%, 30% were prepared using Continuous extraction method by Soxhlet Apparatus. All extracts were tested against *Staphylococcus aureus* (ATCC 29213), *Escherichia coli* (ATCC 25922), and *Pseudomonas aeruginosa* (ATCC 27853). The susceptibility tests were performed using Agar ditch method. The antibacterial activity was assessed on the basis of Zone of inhibition and activity index.

Results :As compared with standard drug (doxycycline), the results revealed that all extracts possesses anti-bacterial activity in dose dependent manner but aqueous extract was found to have maximum activity against all pathogens taken in this study. Maximum antibacterial activity was reported against *S.aureus* followed by *P.aeruginosa* by all extracts. *E.Coli* was resistant at 10 % conc. of both extracts of the study formulation.

Conclusion: In vitro findings of this study confirmed antibacterial activity of *Shwetavarshabuvadi* formulation on common pathogens causing skin and soft tissue infections.

Keywords: *Shwetavarshabu*, *Varuna*, Antibacterial Activity, Zone Of Inhibition, Activity Index

INTRODUCTION

Over the past few decades, skin diseases have become more common, and they are putting a significant burden on health-care systems around the world. As per Global burden of disease study 2017, bacterial skin diseases (0.03 million, 95% uncertainty interval 0.02–0.04) reported with percentage increases of 50%, respectively from 1990 to 2017.[1] These bacterial skin and soft-tissue infections (SSTIs) range from simple cellulitis to rapidly progressive necrotizing fasciitis. Abscess is one of the skin and soft tissue infections most commonly caused by S.aureus in leading roles. It may be caused by other pathogens also. But the problematic situation is aroused by increasing incidence of MRSA from 30% to 70% of all S. aureus infections[2] The rapid spread of methicillin-resistant S.

aureus is presenting a significant health problem worldwide. The novel generation antibiotics although has contributed in reduction in mortality and morbidity due to infectious diseases, but their misuse and overuse has led to emergence of resistant microbial populations. Thus, researchers are in constant search of new herbal agents with immense antimicrobial potential. For the present study, one of the formulation *Shetavarshabuvadi*[3] i.e. *Shwetavarshabu* (Trianthema portulacastrum Linn.) & *Varuna* (Crataeva nurvala Buch-Ham.) indicated for the management of abscess in classical texts has been selected to explore its antimicrobial potential in skin infections.

MATERIALS AND METHODS

1. **Preparation of the extracts of study drug**: Aqueous and ethanolic extracts of study drug *Shwetavarshabu* (Trianthema portulacastrum Linn.) & *Varuna* (Crataeva nurvala Buch-Ham.) with three concentrations 10%, 20%, 30% were prepared using sterile water and ethanol as diluents. The method for extraction was Continuous extraction method by Soxhlet Apparatus.
2. **Bacterial isolates:** following bacterial strains were selected for the antimicrobial study:Table 1

   These are the most common microorganisms responsible for skin and soft tissue infections, thus selected for the present study.

   Culture media

   N- broth (Himedia) was used for preparing the inoculums.

   N broth Composition: Table 2

   Final pH (at 25º C) 7.4 +/- 0.2

   Mueller Hinton agar (Himedia) was used for the anti-microbial activity Table 3

   Final pH (at 25°C) 7.3±0.1

   Method

   Zone of inhibition by agar ditch method

   The pathogenic strains of above bacteria were produced and anti-bacterial study was done at Microbiology Unit, Central laboratory, NIA, Jaipur.

   Procedure

   A 24 hours old young culture was prepared of the organisms Staphylococcus aureus, Escherichia coli and Pseudomonas aeruginosa by inoculating isolated colony into 3 ml of sterile N- broth. The OD of each inoculated broth obtained was 0.6 when checked at 600 nm.

   Agar ditch method Procedure

   Mueller – Hinton (MH) agar plates were prepared by solidifying 20 ml of sterile MH agar into petri plates. In sterile conditions, 100 ul of test organisms were inoculated by spread plate method. With the help of sterile cup borer, wells were prepared of 8 mm in every plate. 200 ul of different concentrations of the samples were added into the wells. The plates were incubated then at 37º C for 24 hours. Next day the plates were observed for the zone of inhibition.

   Positive control

   - Doxycycline 30 µg

   Experiment was carried out in triplicate and the average diameter of zone of inhibition was measured in mm. with the help of a scale. Then the mean was calculated of the three readings taken and then activity index was calculated.

   - Determination of the activity index[4]

   The activity index of the test samples extract was calculated as

   Activity index (AI) = Zone of inhibition of the extract Zone of inhibition obtained for standard antibiotic drug

   Each experiment was done in triplets. The average diameter of zone of inhibition was measured in mm. with the help of a scale. Then the mean was calculated of the three readings taken and then activity index was calculated.

RESULTS

Table 4. Mean of ZOI (in mm) of aqueous extract at 10%, 20%, 30% concentration of study drug against S.Aureus, E.Coli and P.aeruginosa with negative and positive control.

Table 5. Mean of ZOI (in mm) of ethanolic extract at 10%, 20%, 30% concentration of study drug against S.Aureus, E.Coli and P.aeruginosa with negative and positive control.

DISCUSSION

Probable mode of action of drug:

i. ***Krimighan* (antibacterial) activity** --As far as the ayurvedic concept of *kṛmi* is concerned, *kṛmi* are said to be of *śleṣmala o*rigin. *Kapha and purisha* both are conducive to the growth of *kṛimi. So, the dravya* which are opposite to the properties of kapha perform the function of *prakṛti vighata* in context of *krimi.* Acharya caraka has advised the administration of kaṭu, tikta, *kaṣhaya rasa dravya and ushna virya, kṣhara* in mode of treatment of *kṛimi*. Table 6 On analysis of *rasa panchaka* of study drug we found that they possess the above-mentioned properties *laghu,* drugs act as *kṛmighna.*

ii. **Potential disruption in the membrane of microorganisms**: it was assumed that extracts might cause hyperpolarization of cell membrane resulting into changes in the membrane potential and thus disruption.[5] Suzuki et al. (2003) used DiBAC4 (3) (a fluorescent dye) in a study to document changes in membrane potential following treatment with plant extracts. Normally, this dye has higher permeability and accumulation in bacterial cells whereas in hyperpolarization, the uptake of this dye gets reduced leading to low fluorescence intensity. Sanchez et al., 2010 reported the same in research.

iii. **Changes in microbial cell cytoplasmic pH (pH_{int}):** On the basis of previous researches, it is assumed that the drug extracts lead to significant reduction in cytoplasmic pH_{int}. In a study, using the cFSE technique for measuring the pH_{int} of bacteria, changes were observed in pH_{in} which also indicate the cell membrane damage.[6]

iv. **Effect on cytoplasmic ATP concentration:** Researches have shown that plant extracts cause membrane damage and thus decrease the cytoplasmic ATP. As both Membrane integrity and the capacity of cells to maintain a pH gradient along with intracellular pool of ATP is very crucial in determining cellular viability. Hence the drug extracts possess antibacterial activity by cumulative result of these abovementioned factors.

CONCLUSION

In vitro findings of this study confirmed antibacterial activity of *Shetavarshabuvadi* formulation on various pathogens causing skin and soft tissue infections. As compared with standard drug, the results revealed that all extracts possesses anti-bacterial activity but aqueous extract was found to have maximum activity against all pathogens taken in this study.

Acknowledgements - Nil
Conflict of interest - None
Source of finance & support – Nil

ORCID

Manita Ahlawat, https://orcid.org/0000-0003-2588-5135

REFERENCES

1. Thakur JS, Narang T. The burden of skin diseases in India: Global burden of disease study 2017.
2. Mahakalkar C et al. Management of acute bacterial skin and skin structure infections in India: are we equipped to meet the challenges of the growing menace of methicillin-resistant Staphylococcus aureus? Int Surg J. 2020 Sep;7(9):3172-3179
3. *Dvivedy R, Cakradatta*, of Chakrapani, edited Chaukhamba Sanskrit Bhawan Varanasi, *Vidradhi Cikitsā,* 43/14 Reprint- 2014.pp.256.
4. Jayanthi P, Lalitha P. Antimicrobial activity of solvent extracts of Eichhornia crassipes (Mart.) Solms. Der Pharma Chemica. 2013;5(3):135-40.
5. Faraja D. Gonelimali et al, Antimicrobial Properties and Mechanism of Action of Some Plant Extracts Against Food Pathogens and Spoilage Microorganisms, Front Microbiol. 2018 Jul 24;9:1639. doi: 10.3389/fmicb.2018.01639. PMID: 30087662; PMCID: PMC6066648.
6. Sánchez et al, Extracts of Edible and Medicinal Plants Damage Membranes of Vibrio cholera, Appl Environ Microbiol. 2010 Oct; 76(20): 6888–6894.

How to cite this article: Ahlawat M, Godatwar P.K "Antibacterial Activity Of *Shetavarshabuvadi* Formulation Extracts On Various Pathogens Causing Skin And Soft Tissue Infections – An In Vitro Study"
IRJAY.[online]2022;5(10); 27-32.
Available from: https://irjay.com
DOI link- https://doi.org/10.47223/IRJAY.2022.51004

Table 1 Bacterial isolates: following bacterial strains were selected for the antimicrobial study:

S.No	Bacteria	ATCC no.	Supplier Company
1.	Staphylococcus aureus	ATCC 29213	Hi media Laboratories Pvt. Ltd. Mumbai- 400086, India
2.	Escherichia coli	ATCC 25922	
3.	Pseudomonas aeruginosa	ATCC 27853	

Table 2 Culture media

Ingredients	Grams/ litre
Peptic digest of animal tissue	5.0
Sodium chloride	5.0
Beef extract	1.5
Yeast extract	1.5

Table 3 Mueller Hinton agar (Himedia) was used for the anti-microbial activity

Ingredients	Grams/ litre
Beef infusion	300.000
Casein acid hydrolysate	17.500
Starch	1.500
Agar	17.000

Final pH (at 25°C) 7.3±0.1

Table 4. Mean of ZOI (in mm) of aqueous extract at 10%, 20%, 30% concentration of study drug against S.Aureus, E.Coli and P.aeruginosa with negative and positive control

Extract	**Bacterial strain**	**Parameter**	**10% conc.**	**20% conc.**	**30% conc.**	**Positive Control**	**Negative Control**
Aqueous	**S.Aureus**	Mean ZOI	11	16.33	23.67	52.67	0
		Activity index	0.21	0.31	0.45		-
	E.Coli	Mean ZOI	0	19.67	22.33	23	0
		Activity index	0	0.85	0.97		-
	P.aeruginosa	Mean ZOI	0	14.67	22.67	26.33	0
		Activity index	0	0.56	0.86		-

Table 5. Mean of ZOI (in mm) of ethanolic extract at 10%, 20%, 30% concentration of study drug against S.Aureus, E.Coli and P.aeruginosa with negative and positive control

Extract	Bacterial strain	Parameter	10% conc.	20% conc.	30% conc.	Positive Control	Negative Control
Ethanolic	**S.Aureus**	Mean ZOI	12	13.33	19.66	49.67	0
		Activity index	0.24	0.26	0.39		-
	E.Coli	Mean ZOI	0	16.33	21	21.67	0
		Activity index	0	0.75	0.97		-
	P.aeruginosa	Mean ZOI	0	11.67	16.33	23.67	0
		Activity index	0	0.49	0.69		-

Fig.1.a Aqueous extract of drug on Staph. aureus

Fig.1.b Ethanolic extract of drug on Staph. aureus

Fig.2.a Aqueous extract of drug on E.Coli

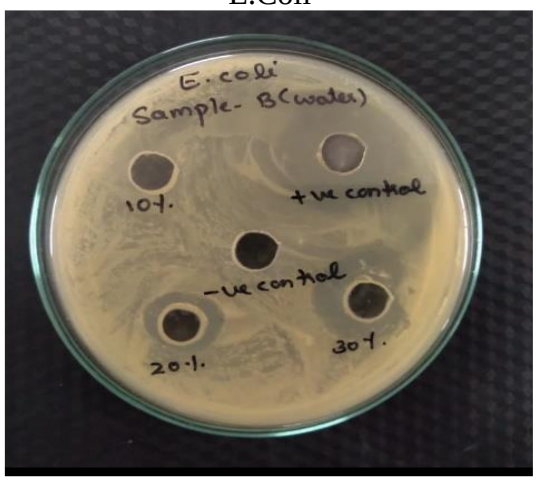

Fig.2.b Ethanolic extract of drug on E.Coli

Fig.3.a Aqueous extract of drug on P.Aeruginosa

Fig.3.b Ethanolic extract of drug on P.Aeruginosa

Table 6 Pharmacodynamics

Drug	***Rasa***	***Guna***	***Virya***	***Vipaka***	***Prabhava***
Shweta Varṣhabhumula	*Tikta, Kashaya, Kaṭu, Madhura*	*Laghu, Ruksha*	*Ushna*	*Kaṭu*	*Kaphavatahara*
Varuna	*Tikta, Kashaya*	*Laghu, Ruksha,*	*Ushna*	*Kaṭu*	*Kaphavatahara*