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Original Research Article

Anti-Bacterial Activity of *Shadanga Ghrita* & Its *Ghana Vati* on Diaarrhoea Causing Enteropathogens

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Abstract

Background: In Ayurvedic classics Diarrhoea is described with the name of *Atisaar*. In modern science it is closely correlated with watery Diarrhoea which is defined as having loose or watery stools at least three times per day or more frequently than normal for an individual. Diarrhoea caused by bacterial pathogens is a global health problem, especially in developing countries and enteric bacterial pathogens are the main cause of infectious Diarrhoea. Ayurvedic literature have lot of unexplored or least tested medicine, *Shadanga Ghrita* is one of those Ayurvedic formulation used in the management of *Tridoshaja Atisaar*. But it has some disadvantages such as bitterness in taste, unpalatability, feasibility and inconvenience in transportation. Considering these, here an attempt was made to transform it into a new dosage form i.e., *Ghana Vati*. ***Aim & Objectives:*** Aim & objectives of present study was to evaluate the anti-bacterial activity of *Shadanga Ghrita* and its new dosage form on Diarrhoea causing enteropathogens. ***Material and Methods:*** Samples of *Shadanga Ghrita* & its *Ghana Vati* were prepared as per Sharangdhar Samhita Madhyama Khanda. All the samples of finished products were analyzed on organoleptic and physico-chemical parameters. Anti-bacterial study was done by using Well Diffusion Method and Muller Hinton Agar Media was used to evaluate anti-bacterial activity against *S. aureus*, *E. coli*, *S. enterica*, *S. boydii*, *Y. enterolitica*, *A. species*, and *C. perfringens*. ***Result:*** Both samples showed significant anti-bacterial activity against Diarrhoea causing enteropathogens. ***Conclusion:*** *Shadanga Ghrita* is more effective Antibacterial agent, as it was formulated by *Sneha Paka Kalpana* method; *Gou- Ghrita* has potentiated the anti-bacterial effect of it.

Keywords: *Shadanga Ghrita*, *Atisaar*, Anti-baterial, *Gou-ghrita*.

INTRODUCTION

The term *Atisaar* is the combination of two words i.e., *Ati* (excessive) and *Saar* (passing of liquid matter through anus). That means excessive flow of watery stool through anus [1]. In modern science it is closely correlated with watery Diarrhoea, which is defined as having loose or watery stools at least three times per day or more frequently than normal for an individual. More than 1 billion cases and at least 4 million deaths per year are attributed to Diarrhoea worldwide [2]. Enteric bacterial pathogens are the main cause of infectious Diarrhoea & currently, *Escherichia coli*, *Shigella*, *Salmonella*, *Campylobacter*, *Clostridium difficile*, and *Aeromonas* [3] are mainly considered to be the most common Diarrhoea causing enteropathogens. *Shadanga Ghrita* is an important herbal formulation quoted by *Acharya* Chakradatta [4] for the management of *Atisaar*. Common problems with Ayurvedic oleaginous preparations are difficult to administer, transport and is generally unpalatable. Hence it is quite difficult to take it, which urged the need for conversion of *Shadanga Ghrita* into other dosage forms like *Ghana Vati* to meet the requirement of the patients such as ease administration, transportation and handling etc.

MATERIAL & METHODS

Shadanga Ghrita & *Shadanga Ghana Vati* were prepared by as per Sharangdhar Samhita Madhyama Khanada [5] while Murchana of Ghrita was done as per Bhaishajya Ratnavali [6].

Collection of Bacterial Strains

Bacterial strains were procured from Institute of Microbial Technology (IMTECH), Chandigarh (India). The organisms tested were *S. aureus, E. coli, S. enterica, S. boydii, Y. enterolitica, A. species, C. perfringens.* The procured samples were sub-cultured & maintained in Sabouraud Dextrose Agar slants at 40 ^{0}C.

In Vitro Assay

Culture Preparation

Freshly prepared slant of *S. aureus, E. coli, S. enterica, S. boydii, Y. enterolitica, A. species, C. perfringens* were used and the slant were washed by using 10 ml of sterile normal solution.

METHODOLOGY

Cylinder Plate Method.

Media Preparation

Muller Hinton Agar was used for determining the activity of *S. aureus, E. coli, S.enterica, S. boydii, Y. enterolitica, A. species, C. perfringens*. Media was prepared as per manufacturer's instruction. The media was then autoclaved at 121^{0}C temperature and 15lbs pressure for 20 minutes.

Sample Preparation for *Shadanga Ghrita* and *Shadanga Ghana Vati*

Approximately 1 gm and 2 gm of *Shadanga Ghana Vati*, 1 gm, 2gm, 5 gm and 10 gm of *Shadanga Ghrita* were weighed into different 150 ml conical flasks. 12 ml mixture of Methanol: DMSO (10:2) was added. Then the samples were sonicated for 10 minutes. The samples were refluxed for 1 hour at 80˚C on a water bath followed by filtration. After that, filtrate was evaporated up to 5 ml sample and remaining was used as a test sample for the in-vitro antibacterial efficacy study.

Testing Procedure

For Antibacterial activity

Sterile media was cooled down up to 55°C and 10µl of different bacterial cultures were added into MHA flasks and mixed slowly. The plates were labeled & then 25 ml of media was poured by a sterile measuring cylinder. The plate was solidified and required wells were prepared at a proper distance by sterile borer on plates. Test samples & blank were added in respectively labeled wells. When samples were diffused completely in respective wells, MHA plate was incubated into Bacteriological incubator at 35°C for 48 hours to observe the Zone of inhibition. By using the Zone of inhibition data, the Activity index was also calculated.

OBSERVATION & RESULTS

Table 1: Showing *In-vitro* anti-bacterial activity of *Shdanga Ghana Vati* and *Shdanga Ghrita*

Name of organism	Name of sample							
	Blank	Reference Standard (Cefixime)	*Shdanga Ghana Vati*		*Shdanga Ghrita*			
		200 mg	1 gm	2 gm	1 gm	2 gm	5 g	10 gm
S.aureus	NZI	18 mm	15 mm	15 mm	16 mm	16 mm	23 mm	25 mm
E.coli	NZI	22 mm	15 mm	16 mm	NZI	NZI	10 mm	13 mm
S. enterica	NZI	34 mm	10 mm	14 mm	NZI	NZI	NZI	NZI
S. boydii	NZI	32 mm	15 mm	17 mm	NZI	NZI	16 mm	18 mm
Y.enterolitica	NZI	27 mm	11 mm	12 mm	NZI	NZI	15 mm	18 mm
A. species	NZI	15 mm	15 mm	17 mm	16 mm	15 mm	18 mm	17 mm
C.perfringens	NZI	23 mm	13 mm	16 mm	10 mm	12 mm	18 mm	20 mm

Table 2: Showing Activity index of *In-vitro* anti-microbial study done on *Shadanga Ghana Vati* and *Shadanga Ghrita*

Name of organism	Name of Sample					
	Shadanga Ghana Vati		*Shadanga Ghrita*			
	1 gm	2 gm	1 gm	2 gm	5 gm	10 gm
S. aureus	0.83	0.83	0.89	0.89	1.28	1.39
E. coli	0.68	0.72	NIA	NIA	0.45	0.59
S. enterica	0.29	0.41	NIA	NIA	NIA	NIA
S. boydii	0.47	0.53	NIA	NIA	0.50	0.56
Y. enterolitica	0.41	0.44	NIA	NIA	0.56	0.67
A. species	1	1.13	1.07	1	1.2	1.13
C. perfringens	0.57	0.70	0.43	0.52	0.78	0.87

DISCUSSION

From these results, it was very clear that, Cefixime (200 mg) was highly effective against *S. enterotica* and least against *A. species.* Antibacterial activity of *Shadanga Ghrita* against *S. aureus* was found useful even below usual dose and zone of inhibition increases with increasing the dose of the *Ghrita*. No zone of inhibition seen in *S. enterica, E. coli, S. boydii and Y. enterolitica* below usual dose but at therapeutic dose (10 gram) its activity was half of the standard drug, but in case of *S. enterica* even at a therapeutic dose no zone of inhibition was seen. Antibacterial activity of *Shadanga Ghana Vati* against *S. boydii E.coli, C. perfringens* and *A. species* was found useful even below usual dose and zone of inhibition increases with the increasing the dose of the drug.

The activity index > 0.50 implies the significant activity of *Shadanga Ghrita* and *Shadanga Ghana Vati* toward the bacteria. It is obtained by dividing the inhibition zone of positive control by inhibition zone of pathogenic strain.

Order of Antibacterial Activity in 7 strains was as follows:

i. ***S. aureus,*:** *Shadanga Ghrita* > Cefixime > *Shadanga Ghana Vati*
ii. ***E. coli*:** *Cefixime* > *Shadanga Ghrita* > *Shadanga Ghana Vati*
iii. ***S. enterica***: Cefixime > *Shadanga Ghana Vati* > *Shadanga Ghrita*
iv. ***S. boydii***: Cefixime > *Shadanga Ghrita* > *Shadanga Ghana Vati*
v. ***Y. enterolitica***: Cefixime > *Shadanga Ghrita* > *Shadanga Ghana Vati*
vi. ***A. species***: *Shadanga Ghrita* > *Shadanga Ghana Vati* > Cefixime
vii. ***C. perfringens***: *Cefixime* > *Shadanga Ghrita* > *Shadanga Ghana Vati*

The activity index > 0.50 implies the significant activity of *Shadanga Ghrita* and *Shadanga Ghana Vati* toward the bacteria. It is obtained by dividing the inhibition zone of positive control by inhibition zone of pathogenic strain.

CONCLUSION

From Anti-bacterial Study, it can be concluded that *Shadanga Ghrita & Shadanga Ghana Vati* showed good antibacterial activity on *S. aureus, E. coli, S. enterica, S. boydii, Y. enterolitica, A. species, C. perfringens. Shadanga Ghrita at 5 gm & 10 gm* is highly effective against *S. aureus* & *A. species* & show the equivalent result to Cefixime. In the case of *E. coli, S. enterica, S. boydii, Y. enterolitica & C. perfringens* the activity of *Shadanga Ghrita* was 2/3rd as compared to Cefixime (200 mg). *Shadanga Ghrita* did not show any antibacterial activity on *S. enterica* at any dose & is more effective anti-bacterial agent as it was formulated by *Sneha-Paka Kalpana* method, *Gou-ghrita* has potentiated the antibacterial activity of it.

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