Research Article

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ANTIMICROBIAL ACTIVITY OF INGREDIENTS OF *HARITAKYADI YOGA* IN URINARY TRACT INFECTION

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ABSTRACT

Haritakyadi Yoga is used to treat Urinary tract infections, as mentioned in the Ayurvedic literature. In the present study, *Haritakyadi Yoga* formulation has been screened for antibacterial activity against selected bacterial species. The minimum inhibitory concentration of the extracts was performed by the broth dilution method. The Kirby-Bauer agar disc diffusion method studied the zone of inhibition at 2 and 4 mg/ml concentrations in DMSO solution. Nitrofurantoin drugs (5 µg/ml) were used as a reference control for the antibacterial study. The zone of inhibition study revealed concentration dependant nature of the extract with better effectiveness against gram-positive bacteria than gram-negative bacteria. The *Haritakyadi Yoga* formulation and individual components exhibited antibacterial activity against different bacterial strains responsible for Urinary tract infection.

Keywords: *Haritakyadi Yoga,* Nitrofurantoin, Urinary tract infection, Antibacterial activity, Zone of inhibition.

INTRODUCTION

Urinary tract infection is one of the most common and potentially severe bacterial infections. Bacteria *E. coli* remains the predominant uro-pathogen (80%) isolated in community-acquired uncomplicated infections.[1] *Haritakyadi Yoga* is described in Ayurvedic text *Bhaisajya Ratnavali Mutrakricchra chikitsa.* In the Ayurvedic System of Medicine, this formulation is prescribed against burning micturition, painful micturition, urine retention and Non-specific UTI.[2]

Antibiotic resistance has been rising globally due to antibiotics being prescribed unnecessarily or inappropriately.[3] Hence, *In - vitro antimicrobial activity* was taken as the subject of intervention with the drug *Haritakyadi Yoga* against selected uro-pathogens.

Several *In-vitro* antimicrobial studies revealed that aqueous and methanolic extracts of drugs of *Haritakyadi Yoga* had been well documented for their antimicrobial activity. The study result is as follows:

Terminalia chebula (Haritaki)

The aqueous and methanolic extract of *Terminalia chebula* was analyzed to testify its antibacterial activities against bacteria causing enteric disorder viz. *E. coli.* Methanol and aqueous extract of *T. chebula* has 14 mm and 13 mm zone of inhibition against *E. coli* which showed potential bactericidal activity.[4]

Tribulus terrestris (Gokshura)

The aqueous extract of *Tribulus terrestris* against *S. aureus, E. coli, P. aeruginosa*. Out of these three bacteria, *S. aureus* shows growth Inhibition Zone of 15 mm *E. coli* & *P. aeruginosa* did not show inhibition zone, i.e., antibacterial property.[5]

Bergenia lingulata (Pashanbheda)

The methanolic extract of *Bergenia lingulata* has a 06 mm mean diameter inhibition zone against *E. coli* & the minimum inhibitory concentration (MIC) for *E. coli* is 2500 (ug/ml).[6]

Fagonia cretica* (*Dhanvayasa)

The methanolic and aqueous extract of *Fagonia cretica* against different bacteria. Aqueous and methanolic extract of *F. cretica* has 16 mm & 14 mm zone of inhibition against *S. aureus*. Aqueous, methanolic extract of *F. cretica* has 15.4 mm & 15 mm zone of inhibition against *E. coli*. Aqueous, methanolic extract of *F. cretica* has 16.2 mm & 15 mm zone of inhibition against *P. aeruginosa*. MIC for *S. aureus* is 0.06 mg/ml, *P. aeruginosa* is 0.25 mg/ml.[7]

Cassia fistula (Aragvadha)

Cassia fistula's methanolic and aqueous extract against different gram-positive and gram-negative bacteria. Aqueous and methanolic extract of *C. fistula* has 11.5 mm & 16.5mm zone of inhibition against *S. aureus*. Aqueous and methanolic extract of *C. fistula* has 16.5 mm & 16 mm zone of inhibition against *E. coli*. Aqueous and methanolic extract of *C. fistula* has 10.5 mm & 14 mm zone of inhibition against *P. aeruginosa*. Aqueous and methanolic extract of *C. fistula* has 10 mm & 11.5 mm zone of inhibition against *Proteus spp*.[8]

Aim & Objective of the antimicrobial study

To evaluate the antimicrobial activity of methanolic extract of ingredients of *Haritakyadi Yoga* against UTI causing bacteria.

MATERIALS AND METHODS

Collection of Plant material

The ingredients of *Haritakyadi Yoga are Haritaki (Terminalia chebula)* fruits*, Gokshura (Tribulus terrestris)* fruits, *Pashanbheda (Bergenia lingulata)* root, *Dhanvayasa (Fagonia cretica)* whole plant*, Aragvadha (Cassia fistula)* fruit pulp; these medicinal plants were collected from the Dina Gola Nath, Varanasi, India.

Identification of Drug

The crude drugs were identified and authenticated from the Department of Botany, Institute of Science, BHU, Varanasi, with voucher specimens as:

- *Terminalia chebula* Retz. *Combreta*. 2019/1
- *Tribulus terrestris* Linn. *Zygophylla*. 2019/1
- *Bergenia lingulata* (Wall.) Engl. *Saxifraga.* 2019/1
- *Fagonia cretica* Linn. *Zygophylla.* 2019/1
- *Cassia fistula* Linn. *Caesalpinia*. 2019/1

Test Organisms

The bacterial cultures were procured from the Department of Microbiology, Institute of Medical Sciences, Banaras Hindu University, Varanasi, India. *Escherichia. Coli*, *Klebsiella pneumoniae, Pseudomonas aeruginosa, Proteus spp., Citrobacter koseri, Citrobacter freundii, Staphylococcus aureus* are 7 test organisms.

Preparation of extract

Methanolic extract of each drug was prepared in the Department of Medicinal Chemistry laboratory, IMS BHU, Varanasi, India. The fruits, rhizome, pulp of the drugs were taken, and the foreign matter was removed, air-dried at room temperature under shadow and then ground into small pieces using mortar and pestle.

The methanolic extract was prepared by taking 100 gm of the coarsely powdered sample of each drug separately and packed tightly in the Soxhlet apparatus; 250 ml of methanol was used as a solvent for extraction. The solvent was removed under reduced pressure for 24 hours, which gave a greenish-black coloured sticky residue. The dried residues were suitably diluted with dimethyl sulfoxide (DMSO) to get the final concentration of each extract 2mg/ ml and 4mg/ml. It was collected in separate sterile vials and preserved at 4˚C temperature.

Determination of Anti-microbial activity

Determination of Minimum Inhibitory Concentration (MIC)

Minimum inhibitory concentration (MIC) of methanolic extracts of all drugs was performed by broth dilution method at concentrations of the different extracts ranging from 25 µg/ml to 500 µg/ml in DMSO against all the test microorganisms.

Determination of zone of inhibition

The zone of inhibition of the extracts was performed by the agar disc diffusion method at concentrations of 2 and 4 mg/ml of the extract in DMSO. Nitrofurantoin (5 µg/ml) were used as reference controls for the antibacterial study.

PROCEDURE

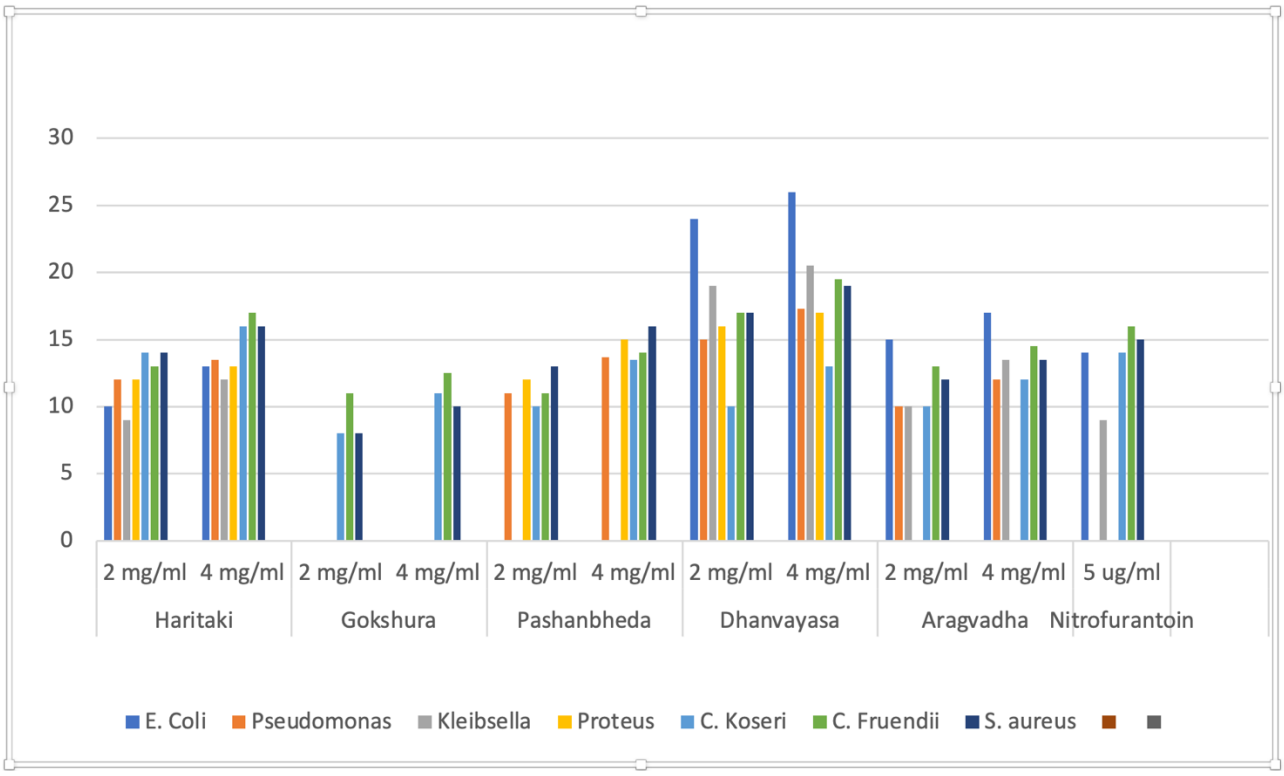
The modified Kirby-Bauer disk diffusion method in the routine laboratory is one of the most widely practiced antimicrobial susceptibility tests (AST). It was suggested by NCCLS (National Committee for Clinical Laboratory Services), USA, 2000.

Mueller-Hinton agar media (MH) (pH – 7.2 to 7.4) was prepared and uniformly spread across the plate and allowed it to set in the laminar airflow. The nutrient broth was prepared, and the Colony of bacteria from the culture was inoculated and kept for incubation. From the primary culture MHA plate, tops of 3-4 colonies of similar appearance were touched and suspended in saline with loop help. Turbidity was matched with the standard tube by adding more growth or saline. After the sterile swab was dipped into the suspension, excess fluid was removed by pressing and rotating the swab against the side of the tube above the fluid level. The plate was turned at an angle of 60˚ after completing each surface. The MHA plate dried with the lid closed for a few minutes at room temperature. Then a hole diameter of 5-8 mm is punched aseptically with a sterile micro tip. 6 wells are made.

RESULT

Table 1: MIC (µg/ml) and zone of inhibition (mm) of methanolic extract of Ingredients of *Haritakyadi Yoga*

Bacterial strains	Category	MIC (µg/ml)	Zone of Inhibition (mm)										
			Haritaki mg/ml		*Gokshura* mg/ml		*Pashanbheda* mg/ml		*Dhanvayasa* mg/ml		*Aragvadha* mg/ml		Nitrofurantoin µg/ml
			2	4	2	4	2	4	2	4	2	4	5
Staphylococcus aureus	Gram (+)	50	14	16	08	10	13	16	17	19	12	13.5	15
Escherichia coli	Gram (-)	150	10	13	-	-	-	-	24	26	15	17	14
Pseudomonas aeruginosa	Gram (-)	300	12	13.5	-	-	11	13.7	15	17.3	10	12	R
Klebsiella pneumonia	Gram (-)	200	09	12	-	-	-		19	20.5	10	13.5	09
Proteus mirabilis	Gram (-)	400	12	13	-	-	12	15	16	17	-	-	R
Citrobacter koseri	Gram (-)	50	14	16	08	11	10	13.5	10	13	10	12	14
Citrobacter freundii	Gram (-)	75	13	17	11	12.5	11	14	17	19.5	13	14.5	16



Graph 1: Zone of Inhibition of Ingredients of Haritakyadi Yoga

Zone of Inhibition values are mean of three readings Standards: Antibacterial study - Nitrofurantoin- 5 µg/ml

The study used the previously prepared 2 and 4 mg/ml DMSO extract solution and 5µg/ml nitrofurantoin solution. A volume of each methanolic extract and control drug solution, i.e. 20 µl, is introduced into the well with the help of a micropipette. Further, the plates were keptfor incubation at 37 ^{0}C for 24 hours. At the end of incubation, the inhibition zone formed around the disc was measured with a transparent ruler in millimetres (mm). The lowest concentration of extract, which shows inhibition of the growth of bacteria, was determined. The observations are the mean values of three replicates.

DISCUSSION

Table 1 shows the methanolic extract's antimicrobial activity of *Haritakyadi Yoga's* ingredients. The results of the Minimum Inhibitory Concentration (MIC) study revealed the antimicrobial activity of the methanolic extracts against thetested strains of microorganisms between concentration ranges between 50 and 400 µg/ml. The zone of inhibition study results revealed that the extract possessed antimicrobial activity in a concentration-dependent manner against the testorganisms and was comparable with the standard drug Nitrofurantoin.

The gram-positive bacteria were observed to be more susceptible than gram-negative bacteria.These observations are more likely due toan outer membrane in gram-negative bacteria, which acts as a barrier to many environmental substances, including antibiotics.[11] Among the tested strains of bacteria, the extract was most effective against *S. aureus* and least against *Klebsiella pneumonia*.

Presently available antibiotics are rapidly developing resistance against bacterial strains. Conjoint use of naturally occurring antibacterial substances and formulations such as *Haritakyadi Yoga* proved useful.

CONCLUSION

The methanolic extracts of ingredients of *Haritakyadi Yoga* possess the definite in-vitro antimicrobial property with the zone of inhibition and MIC against *Escherichia. Coli*, *Klebsiella pneumoniae, Pseudomonas aeruginosa, Proteus spp., Citrobacter koseri, Citrobacter freundii, Staphylococcus aureus* but to a different extent. *In-vitro* antibacterial activity of *ingredients of Haritakyadi Yoga* extract was determined against different organisms associated with the UTI. *Haritakyadi Yoga* was effective against harmful bacterial strains causing Urinary Tract Infection, as mentioned in the Ayurvedic literature.

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