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ANTIMICROBIAL ACTIVITY OF *BRIHATYADI KWATHA* AND *BHAVITA BRIHATYADI CHURNA* W.S.R. TO UTI

Chaithra G. T.[1*], Radhika Ranjan Geethesh P.[2], Ashok Kumar B. N.[3] and Ravindra Angadi[4]

[1]PG Scholar, [2,3]Associate Professor, [4]Professor

Department of PG and PhD Studies in Rasashastra and Bhaishajya Kalpana, Sri Dharmasthala Manjunatheshwara College of Ayurveda and Hospital, Kuthpady, Udupi, Karnataka, India.

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***Corresponding Author**
Dr. Chaithra G. T.
PG Scholar, Department of PG and PhD Studies in Rasashastra and Bhaishajya Kalpana, Sri Dharmasthala Manjunatheshwara College of Ayurveda and Hospital, Kuthpady, Udupi, Karnataka, India-574118.

ABSTRACT

Urinary tract infection is one of the common urological problems seen worldwide. In our classics, similar description is found in the disease *Mutrakrichra*. *Brihatyadi Kwatha* is indicated in this condition which is explained in *Astanga Hridaya*. *Bhavita Brihatyadi Churna* is also opted for the study keeping in mind the need for formulations with less dosage, more shelf life and therapeutic efficacy. The main etiology in causing UTI are microbial in nature. Thus the present study to evaluate the in-vitro Antimicrobial action of *Brihatyadi Kwatha* and *Bhavita Brihatyadi Churna* on specific pathogens was undertaken. There was no antimicrobial action of *Brihatyadi Kwatha* on the selected pathogens. The alcohol extract of *Bhavita Brihatyadi Churna* showed action against 3 pathogens- *Eshcherichia coli*, *Klebsiella pneumoniae* and *Pseudomonas aeruginosa.*

KEYWORDS: Urinary tract infection, *Brihatyadi Kwatha*, *Bhavita Brihatyadi Churna*, Antimicrobial study.

INTRODUCTION

Urinary tract infections is one of the commonly occurring infections. Usually women of childbearing age and older individuals are more vulnerable to this. It is also one of the hospital acquired infections due to frequent use of bladder catheters. The most common

symptoms are burning micturition with significant pain and having the urge to urinate frequently. Recurrent infection is common as found in recent research. Bacteria are most commonly responsible, although yeast, fungi and viruses may produce urinary infection. Bacteria which cause UTI are-*Eshcherichia coli, Klebsiella pneumoniae, Pseudomonas aeruginosa, Enterococcus faecalis* and fungi is *Candida albicans.*[1] Our classics describe *Basti* as one of the three vital organs of the body, the other two being *Hridaya* and *Shiras*. According to Ayurveda, *Vegavarodha* is one of the important causes for diseases of urinary tract. One such disorder which has been explained with respect to urinary tract is *Mutrakrichra*, where there is difficulty in micturition. *Brihatyadi Kwatha*[2] is a formulation described in *Astanga Hridaya* which has been indicated in all *Mutravikaras*. It comprises of drugs belonging to *Laghu panchamula* among which *Gokshura* is the main ingredient. Since *Kashaya* preparations have less shelf life another dosage form called *Bhavita Brihatyadi Churna* was selected where 21 *bhavanas* of *Brihatyadi Kwatha* was given to the *churna* of the same drugs-*Brihati, Kantakari, Shaalaparni, Prishniparni, Gokshura.* Both of these were assessed for anti-microbial activity in selected organisms causing UTI.

OBJECTIVES

To evaluate and compare the '*in-vitro'* anti-microbial action of *Brihatyadi Kwatha* and *Bhavita Brihatyadi Churna* by agar well diffusion method.

MATERIAL AND METHODS

In-vitro Antimicrobial action of *Brihatyadi Kwatha* and *Bhavita Brihatyadi Churna* was evaluated by Agar well diffusion method on.

- *Eshcherichia coli,*
- *Klebsiella pneumoniae,*
- *Pseudomonas aeruginosa,*
- *Enterococcus faecalis*
- *Candida albicans*

Preparation of sample in *Bhavita Brihatyadi Churna*

Preparation of Alcohol extract -2gm of *Bhavita Brihatyadi Churna* was taken and added with 6 ml of alcohol in a test tube and rotation was done in Rotospin test tube rotator for 1 day. Then the alcohol was changed and rotated again for 1 day. Then the filtrate was kept in

the evaporator until the alcohol evaporated. To this extract 600µl Dimethyl sulfoxide solution was added and dilution done which was stored in freezer and used as alcohol extract.

Preparation of Chloroform extract - 2gm of *Bhavita Brihatyadi Churna* was taken and added with 6 ml of chloroform in a test tube and rotation was done in Rotospin test tube rotator for 1 day. Then the chloroform was changed and rotated again for 1 day. Then the filtrate was kept in the evaporator until the chloroform evaporated. To this extract 600µl Dimethyl sulfoxide solution was added and dilution done which was stored in freezer and used as chloroform extract.

Control sample taken in *Brihatyadi Kwatha* and *Bhavita Brihatyadi Churna*- In case of *Brihatyadi Kwatha*, distilled water was taken as control and Dimethyl sulfoxide solution was taken as control while testing extracts of *Bhavita Brihatyadi Churna*.

METHODOLOGY

The whole procedure was subdivided into following steps:

- Preparation of Nutrient Medium
- Preparation of Inoculum
- Well diffusion method

Preparation of Nutrient agar media[3] for *Escherichia coli, Klebsiella pneumonae, Pseudomonas aeruginosa*

Beef extract (1 g), yeast extract (2 g), peptone (5 g) and Sodium Chloride (5 g) were dissolved in 990 ml of distilled water. The pH was adjusted to 7.2 and the volume was made up to 1000 ml. Finally 15 g agar was added to the media and autoclaved at 121˚C for 20 minutes.

Preparation of Trypticase Soya Agar Medium (TSAM)[3] for *Enterococcus faecalis*

Glucose (5g), Casein peptone (15g), Soya peptone (5g), Sodium chloride (5g), Yeast extract (3g) were taken and dissolved in 990 ml distilled water and pH was adjusted to 7-7.2 and the volume was made to 1000 ml. Finally 15 g agar was added to the media and autoclaved at 121˚C for 20 minutes. Fresh blood was added at a concentration of 5% to prepare the plates.

Preparation of yeast extract dextrose agar media[3]for *Candida Albicans*

Yeast extract (3 g), peptone (10 g) and dextrose (20 g) were dissolved in 990 ml of distilled water. The pH was adjusted to 7.4 and the volume was made up to 1000 ml. Finally 15 g agar was added to the media and autoclaved at 121˚C for 20 minutes.

Preparation of the inoculum

Bacteria/Fungi were procured from culture collection centre, IMTECH, Chandigarh. Loopful of 48 h old culture from the slants was transferred to sterile saline and mixed well to prepare a homogenous inoculum.

Well diffusion method[4]

The media was cooled to around 45-55˚C, around 20 ml each was poured into sterile petriplates. One ml of the inoculum was immediately added to the plate, swirled for uniform distribution. Wells were bored using a sterile borer. The samples and the antibiotic were dispensed into the wells. Plates were incubated overnight at 37˚C and observed after 24h/ 48 h.

RESULTS

Table 1: *In vitro* antibacterial activity test for *Brihatyadi Kwatha* against *Escherichia coli.*

Brihatyadi Kwatha	Zone of inhibition (mm)
15 µl	0
30 µl	0
50 µl	0
Control (Distilled water) 30 µl	0
Standard (*Ampicilin*) 30 µg	08

Conclusion: Antibacterial effect was not seen at the concentration used against *Escherichia coli*.

Table 2: *In vitro* antibacterial activity test for *Bhavita Brihatyadi Churna* against *Escherichia coli.*

Bhavita Brihatyadi Churna		Zone of inhibition (mm)
Alcohol Extract	15 µl	0
	30 µl	10
	50 µl	13
Chloroform Extract	15 µl	0
	30 µl	0
	50 µl	0

Direct *Churna*	05 mg	0
	10 mg	0
Control (DMSO)	30 µl	0
Standard (*Ampicilin)*	30 µg	10

Conclusion: Antibacterial effect was seen at the concentration used against *Escherichia coli*.

Fig 1: Action of BK on *E.coli.*

Fig 2: Action of BBC on *E.coli.*

Table 3: *In vitro* antibacterial activity test for *Brihatyadi Kwatha* against *Klebsiella pneumonae.*

Brihatyadi Kwatha	**Zone of inhibition (mm)**
15 µl	0
30 µl	0
50 µl	0
Control (Distilled water) 50 µl	0
Standard (*Ampicilin*) 50 µg	07

Conclusion: Antibacterial effect was not seen at the concentration used against *Klebsiella pneumonae.*

Table 4: *In vitro* antibacterial activity test for *Bhavita Brihatyadi Churna* against *Klebsiella pneumonae.*

Bhavita Brihatyadi Churna		**Zone of inhibition (mm)**
Alcohol Extract	15 µl	0
	30 µl	6
	50 µl	7
Chloroform Extract	15 µl	0
	30 µl	0
	50 µl	0
Direct *Churna*	05 mg	0
	10 mg	0
Control (DMSO)	30 µl	0
Standard (*Ampicilin*)	50 µg	8

Conclusion: Antibacterial effect was seen at the concentration used against *Klebsiella pneumonae.*

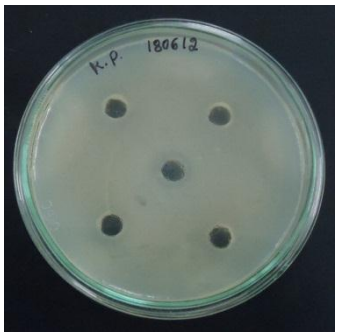

Fig.3: Action of BK on *K.pneumonae.*

Fig.4: Action of BBC on *K.pneumonae.*

Table 5: *In vitro* antibacterial activity test for *Brihatyadi Kwatha* against *Pseudomonas aeruginosa*.

Brihatyadi Kwatha	**Zone of inhibition (mm)**
15 µl	0
30 µl	0
50 µl	0
Control (Distilled water) 30 µl	0
Standard (*Gentamicin)* 240µg	16

Conclusion: Antibacterial effect was not seen at the concentration used against *Pseudomonas aeruginosa.*

Table 6: *In vitro* antibacterial activity test for *Bhavita Brihatyadi Churna* against *Pseudomonas aeruginosa*.

Bhavita Brihatyadi Churna		**Zone of inhibition (mm)**
Alcohol Extract	15 µl	0
	30 µl	7
	50 µl	8
Chloroform Extract	15 µl	0
	30 µl	0
	50 µl	0
Direct *Churna*	05 mg	0
	10 mg	0
Control (DMSO)	30 µl	0
Standard (*Gentamicin)*	240µg	16

Conclusion: Antibacterial effect was seen at the concentration used against *Pseudomonas aeruginosa.*

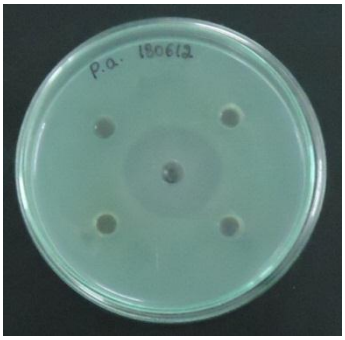

Fig.5: Action of BK on *P.aeruginosa.*

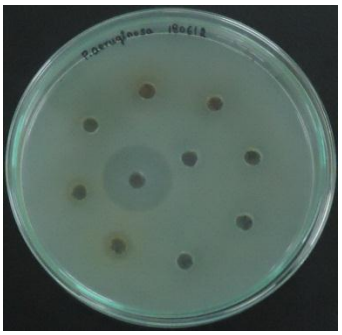

Fig.6: Action of BBC on *P.aeruginosa.*

Table 7: *In vitro* antibacterial activity test for *Brihatyadi Kwatha* against *Enterococcus faecalis*.

Brihatyadi Kwatha	**Zone of inhibition (mm)**
15 µl	0
30 µl	0
50 µl	0
Control (Distilled water) 30 µl	0
Standard (*Ampicilin)* 30µg	17

Conclusion: Antibacterial effect was not seen at the concentration used against *Enterococcus faecalis.*

Table 8: *In vitro* antibacterial activity test for *Bhavita Brihatyadi Churna* against *Enterococcus faecalis*.

Bhavita Brihatyadi Churna		**Zone of inhibition (mm)**
Alcohol Extract	15 µl	0
	30 µl	0
	50 µl	0
Chloroform Extract	15 µl	0
	30 µl	0
	50 µl	0
Direct *Churna*	05 mg	0
	10 mg	0
Control (DMSO)	30 µl	0
Standard (*Ampicilin*)	30 µg	17

Conclusion: Antibacterial effect was not seen at the concentration used against *Enterococcus faecalis.*

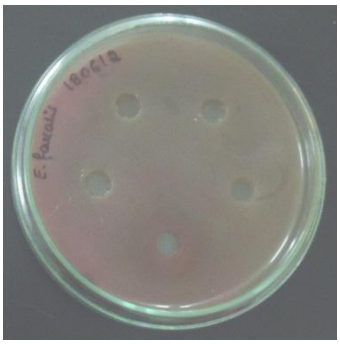

Fig. 7: Action of BK on *E.faecalis.*

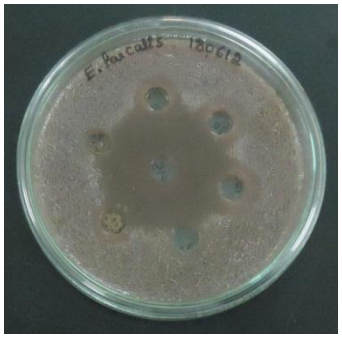

Fig. 8: Action of BBC on *E.faecalis.*

Table 9: *In vitro* antifungal activity test for *Brihatyadi Kwatha* against *Candida albicans.*

Brihatyadi Kwatha	**Zone of inhibition (mm)**
15 µl	0
30 µl	0
50 µl	0
Control (Distilled water) 30 µl	0
Standard (*Clotrimazole*) 10 µl	13

Conclusion: Antifungal effect was not seen at the concentration used against *Candida albicans*.

Table 10: *In vitro* antifungal activity test for *Bhavita Brihatyadi Churna* against *Candida albicans.*

Bhavita Brihatyadi Churna		**Zone of inhibition (mm)**
Alcohol Extract	15 µl	0
	30 µl	0
	50 µl	0
Chloroform Extract	15 µl	0
	30 µl	0
	50 µl	0
Direct *Churna*	05 mg	0
	10 mg	0
Control (DMSO)	30 µl	0
Standard (*Clotrimazole*)	10 µl	14

Conclusion: Antifungal effect was not seen at the concentration used against *Candida albicans*.

Fig. 9: Action of BK on *C. albicans.*

Fig. 10: Action of BBC on *C. albicans.*

(BK- Brihatyadi Kwatha, BBC- Bhavita Brihatyadi Churna)

DISCUSSION

Table 1 & 2: BK showed no anti-bacterial activity on *Escherichia coli*. Alcohol extract of BBC showed anti-bacterial activity at 2 concentrations 30 µl and 50 µl where Zone of inhibition was more at higher concentration indicating that it has antimicrobial effect at higher dosage. No anti-bacterial effect was seen in chloroform extract or when the *churna* was put directly.

Table 3 & 4: There was no antibacterial effect of BK on *Klebsiella pneumonae*. In alcohol extract of BBC, anti-bacterial effect was seen at 2 concentrations- 30 µl and 50 µl where zone of inhibition was more at 50 µl indicating that the drug has more action at higher concentration. No activity was seen in chloroform extract or in *churna* put directly.

Table 5 & 6: Alcohol extract of BBC showed antibacterial activity against *Pseudomonas aeruginosa* which was more at concentration of 50 µl than 30 µl. This may indicate that the drug acts better when its concentration is more. BK, Chloroform extract of BBC and the *churna* loaded directly showed no activity on *Pseudomonas aeruginosa.*

Table 7 & 8: BK had no antibacterial effect on *Enterococcus faecalis.* Alcohol and Chloroform extract of BBC, Direct *churna* also had no effect on *Enterococcus faecalis.*

Table 9 & 10: Anti-fungal effect on *Candida albicans* was not seen in BK, Alcohol and chloroform extract of BBC or in *churna* put directly.

Although *Brihatyadi Kwatha* failed to produce the anticipated activity, extract of *Bhavita Brihatyadi Churna* displayed remarkable action against *Escherichia coli* which is supposedly the main perpetrator in causing UTI. Significant effect was also seen against *Klebsiella*

pneumonae and *Pseudomonas aeruginosa* which are the other two common bacterial strains found in a person afflicted with UTI. The absence of desired action on the specific microbes in the case of *Brihatyadi Kwatha* may be attributed to various other factors involved in the manifestation of this disease.

CONCLUSION

Thus the study depicts that *Bhavita Brihatyadi Churna* is more effective than *Brihatyadi Kwatha* against the specific microbes causing UTI. This indicates that the mode of action of the two dosage forms is different from each other.

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