

## IN VITRO STUDY OF GOKSHURA AND VARUNA AGAINST *Escherichia coli* BY URINE CULTURE AND SENSITIVITY WSR TO PITTAJA MUTRAKRICHRA (URINARY TRACT INFECTION)

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### ABSTRACT

Pittaja mutrakrichra is correlated to Urinary tract infection usually caused by *Escherichia coli* bacteria. UTI amounts from 1 to 3% of consultations and up to 50% of women suffer from Urinary tract infection. In Ayurveda various drugs are mentioned for mutrakrichra which is also ascribed with krimighna action, but indication of specific drug in specific causative micro-organism and stage of disease is missing and very few studies have been accomplished. Therefore it is the need of the hour to use diagnostic tools like culture and sensitivity to identify causative micro-organism, its characteristics and other attributes. Hence, present study is undertaken to evaluate and compare sensitivity of Gokshura and Varuna against *Escherichia coli* by urine culture and sensitivity method from urine samples of patients suffering from Pittaja Mutrakrichra wsr to Urinary Tract Infection.

**Key words:** UTI, Culture and sensitivity, Pittaja mutrakrichra

### INTRODUCTION

Mutrakrichra is mentioned in Ayurveda classics as krichrata or difficulty during mutra pravruithi affecting Basthi one among the trimarma shows the importance and gravity of the condition. Pittaja Mutrakrichra is correlated with Urinary tract infection and is the most common painful and annoying cause of health, usually caused by *Escherichia coli* bacteria. In general medical practice Urinary tract infection amounts of 1 to 3% of the consultations and up to 50% of women suffer from Urinary tract infection<sup>1</sup>.

Modern medical management of UTI includes chiefly antibiotics and presently chance of resistance is high. Considering the above it is the need of the hour to search for alternative drugs from natural sources, which are effective, cheap and easily available with no adverse effects so that they can bestow Upashaya. In Ayurveda various drugs are mentioned for mutrakrichra which is also ascribed with krimighna action, but indication of specific drug in specific causative micro-organism and stage of disease is

missing and very few studies have been accomplished. Therefore it is the need of the hour to use diagnostic tools like culture and sensitivity and identify causative micro-organism, its characteristics and other attributes by culturing these organisms in vitro. Before the drug is used clinically on patients, its activity needs to be checked on causative microorganisms in vitro and confirmed whether the drug is efficacious, so that preliminary evidence for Upashaya in vivo can be generated scientifically by in vitro study. Gokshura<sup>2</sup> and Varuna<sup>3</sup> are indicated in mutrakrichra and possess krimighna property hence, selected for the study. Adoption of new approaches like Culture and sensitivity methods would strengthen existing Ayurvedic knowledge and help in achieving improved diagnostic and curative abilities. Therefore, present study is undertaken to study various attributes of the micro-organism *E coli*, its laboratory diagnosis, its culture and evaluate Upashaya capability in vitro by sensitivity with south Indian grown Gokshura and Varuna.

#### **AIMS & OBJECTIVE**

- To evaluate and compare sensitivity of Gokshura and Varuna against *Escherichia coli* by urine culture and sensitivity method with urine samples of patients suffering from Pittaja Mutrakrichra (Urinary Tract Infection).

#### **MATERIALS AND METHODS**

**Source of Data:** A minimum of 30 patients fulfilling diagnostic and inclusion criteria was included for study from OPD & IPD of Sri Dharmasthala Manjunatheshwara College of Ayurveda and hospital, Hassan.

#### **DIAGNOSTIC CRITERIA**

Patients complaining of Krichramutrata (dysuria) associated with one or more symp-

toms of Pittaja Mutrakrichra (Urinary Tract Infection) such as Muhurmuhu mutra pravruithi (Frequency/ urgency), Basthi shoola (Supra pubic pain), Mutra daha (burning sensation), Saruja mutrata (painful micturation), Peeta mutrata (yellowish urine) and Sarakta mutram (haematuria).<sup>4,5</sup>

#### **INCLUSION CRITERIA**

- Patients between the age of 18 – 70 yrs of either sex fulfilling the diagnostic criteria.

#### **EXCLUSION CRITERIA**

- Patient with chronic kidney failure which may interfere in course of study.

**RESEARCH DESIGN:** An observational experimental study.

#### **METHODOLOGY**

- The mid-stream sample of urine from the patients with urinary tract infection was subjected to Microscopical examination for microscopic characterization of pus cells, epithelial cells and bacteria.<sup>6</sup> Then transfer one loop full of inoculum to Macconkey agar plate and culturing was done by Streak culture method. Incubate the petridishes at 37°C for 24 hours. After incubation the culture is subjected to colony morphology and differential staining for the identification of *Escherichia coli*. Then sensitivity test is performed by Agar well diffusion method.<sup>7</sup> The results showing positive cultures for *Escherichia coli* was subjected to sensitivity with different concentrations (0.25 µl, 0.125 µl, 0.0625 µl, 0.0313 µl and 0.015 µl ) of alcoholic extracts of Gokshura and Varuna. Incubate the petridishes at 37°C for 24 hours. After the incubation period, the zone of inhibition<sup>8</sup> is measured with a ruler.

#### **ASSESSMENT CRITERIA**

If the drug is sensitive a clear circular “ halo” ( technically known as Zone of Inhibition) will appear around the well, indicating an absence of bacteria, which shows that the particular drug is effective against the *Escherichia coli*. Based on in-vitro study susceptibility of *E.coli* against Gokshura and Varuna is fairly evident between 18-16mm zone of inhibition hence it is considered sensitive, 14- 12 is intermediate hence moderately sensitive, below 12 is resistant.

## RESULT

In vitro antibacterial activity of alcoholic extract of Gokshura and Varuna was evaluated by agar well diffusion method and zone of inhibition is measured as follows.

### Alcoholic extract of Gokshura

**Table 1: Mean values of zone of inhibition at Different concentrations of alcoholic extract of Gokshura**

Different concentrations of alcoholic extract of Gokshura	0.25µl	0.125 µl	0.0625 µl	0.0313 µl	0.015 µl
N (Total samples)	30	30	30	30	30
Mean value of zone of inhibition ( in mm)	16.00	14.30	12.67	11.27	9.33

Here 16mm is sensitive, 14.30mm and 12.67mm are moderately sensitive, 11.27mm and 9.33 are considered as resistant to *E. coli* in spite of possessing weak antibacterial action.

### Alcoholic extract of Varuna

**Table 2: Mean values of Different concentrations of alcoholic extract of Varuna**

Different concentrations of alcoholic extract of Varuna	0.25µl	0.125 µl	0.0625 µl	0.0313 µl	0.015 µl
N (Total samples)	30	30	30	30	30
Mean value of zone of inhibition ( in mm)	14.90	13.20	11.63	9.85	8.13

Here 14.90mm and 13.20 mm are moderately sensitive, 11.63mm, 9.85mm and 8.13mm are considered as resistant to *E. coli* in spite of possessing weak antibacterial action.

**Table 3: Comparison of antibacterial action of alcoholic extracts of Gokshura and Varuna**

Different concentrations of alcoholic extract	0.25µl	0.125 µl	0.0625 µl	0.0313 µl	0.015 µl
N ( total samples)	30	30	30	30	30
Mean of zone of inhibition of different conc. alcoholic extract of Gokshura (mm)	16.00	14.30	12.67	11.27	9.33
Mean of zone of inhibition of different conc. alcoholic extract of Varuna (mm)	14.90	13.20	11.63	9.85	8.13
Difference of mean in mm	1.1	1.1	1.04	1.42	1.2

Statistical analysis of the data was performed using SPSS 20.0 (IBM corp). The

means were compared using unpaired- t test. Furthermore, Tukey’s test was applied.

P=0.01- 0.001 is considered as statistically highly significant, P=0.01-0.05 is considered as statistically significant and P> 0.05 is considered as non significant.

**Table 47: Showing the statistical values of unpaired t-test**

PARAMETER ( zone of inhibition in mm)	Mean	Differ-ence	N	Mean			t- value	p val-ue	Interpretation
				GG (mm)	VG (mm)	SE (mm)			
0.25 µl	1.100		30	16.00	14.90	0.333	3.299	0.002	HS
0.125 µl	1.100		30	14.30	13.20	0.391	2.812	0.007	HS
0.0625 µl	1.033		30	12.67	11.63	0.398	2.598	0.012	S
0.0313 µl	1.417		30	11.27	9.85	0.412	3.441	0.001	HS
0.015 µl	1.200		30	9.33	8.13	0.339	3.537	0.001	HS

Here GG- Gokshura group, VG- Varuna group HS S

On comparing the same concentrations of alcoholic extracts of Gokshura and Varuna by unpaired- t test it can be concluded that Gokshura is having better antibacterial action than Varuna against *Escherichia coli*.

### DISCUSSION

In the current study out of 46 Urinary tract infection patients screened 30 (65.2%) were affected with *Escherichia coli*, 8 were affected with *Klebshiella*, 2 were affected with *Proteus* and 6 were affected with *Staphylococcus*. This shows the higher prevalence of *Escherichia coli* bacteria in causation of UTI. Gokshura was selected because, Acharya Charaka has included Gokshura in krimighna dashaimani and also included in the context of treatment of mutrakrichra. Varuna is a common drug used in urinary disorders, having krimighna action and also mentioned in mutrakrichra vikaras. Preclinical and clinical studies have shown that the stem bark of *C.nurvala* promote a healthy urinary system thus Varuna was selected for the present study. Alcoholic extract of Gokshura fruit and Varuna bark

was obtained by sox halation process in Soxhlet apparatus

consideration of mean value of zone of inhibition observed in alcoholic extract of Gokshura and Varuna against *E.coli* shows that, Mean value of zone of inhibitions at 0.25 µl concentration of Gokshura (mean=16.00mm) is greater than 0.25 µl concentration of Varuna (mean=14.90mm) with a mean difference of 1.1 mm. Mean value of zone of inhibitions at 0.125 µl concentration of Gokshura (mean=14.30mm) is greater than 0.125 µl concentration of Varuna (mean=13.20mm) with a mean difference of 1.1 mm. Mean value of zone of inhibitions at 0.0625 µl concentration of Gokshura (mean=12.67 mm) is greater than 0.0625 µl concentration of Varuna (mean=11.63 mm) with a mean difference of 1.04 mm. Mean value of zone of inhibitions at 0.0313µl concentration of Gokshura (mean=11.27 mm) is greater than 0.0313 µl concentration of Varuna (mean=9.85 mm) with a mean difference of 1.42 mm. Mean value of zone of inhibitions at 0.015µl concentration of Gokshura (mean=9.33 mm) is greater than 0.015 µl concentration of Varuna

(mean=8.13 mm) with a mean difference of 1.2 mm.

### CONCLUSION

Alcoholic extract of Gokshura and Alcoholic extract of Varuna independently has antimicrobial (Krimighna) action against *Escherichia coli* derived from urine samples of patient suffering from Pittaja mutrakrichra (Urinary tract infection). On comparing the mean values of zone of inhibition observed against different concentrations of alcoholic extracts of Gokshura and Varuna, the mean value of zone of inhibition of Gokshura (in mm) is higher than that of Varuna. Therefore it is concluded that Gokshura has slightly better anti microbial action than Varuna against *Escherichia coli* derived from urine samples of patient suffering from Pittaja mutrakrichra (Urinary tract infection). Further it is evident that as the concentration of alcoholic extract of Gokshura and Varuna increases progressively, zone of inhibition also increases respectively. Hence, concluded that as the concentration increases the antimicrobial activity also increases.

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