



A COMPARATIVE STUDY TO EVALUATE EFFICACY OF GOKSHURA AND GUDUCHI AGAINST *KLEBSIELLA PNEUMONIAE* BY URINE CULTURE AND SENSITIVITY IN PITTAJA MUTRAKRICHRA (URINARY TRACT INFECTION)

Anand S¹, Ajantha², Shashirekha K S³, Anuradha B¹

¹P.G Scholar, Department of Roga Nidana Avum Vikriti Vigyana, Sri Dharmasthala Manjunatheshwara College of Ayurveda And Hospital, Hassan, India

²Professor, Department of Roga Nidana Avum Vikriti Vigyana, Sri Dharmasthala Manjunatheshwara College of Ayurveda And Hospital, Hassan, India

³Microbiologist, Department of Roga Nidana Avum Vikriti Vigyana, Sri Dharmasthala Manjunatheshwara College of Ayurveda And Hospital, Hassan, India

*Corresponding Author Email: dranandpala@gmail.com

DOI: 10.7897/2277-4572.085148

Received on: 11/06/19 Revised on: 13/07/19 Accepted on: 18/07/19

ABSTRACT

Urinary tract infections are associated with high morbidity and long-term complications. The major causative organisms of UTI are *Escherichia coli*, *Klebsiella pneumoniae*, *Proteus mirabilis* etc. *Klebsiella pneumoniae* is the foremost micro-organism causing nosocomial infections including UTI. In Ayurveda micro-organisms are identified under Krimi and many drugs with Krimighna action is mentioned. But knowledge of specific drug against specific bacteria re-established by modern methods of culture and sensitivity is very less. Hence, if evidence can be developed for these Ayurveda drugs action scientifically against specific causative bacteria, then these observations would pave way for administration in patient. This is achieved through tools like culture and sensitivity in vitro. Gokshura and Guduchi is indicated in mutrakrichra and said to possess krimighna property. The present study aims to compare antibacterial activity of Gokshura and Guduchi against *Klebsiella pneumoniae* by urine culture and sensitivity method in Pittaja mutrakrichra (Urinary tract infection). Patients of Pittaja mutrakrichra were subjected for urine culture and those with positive results for *Klebsiella pneumoniae* were further used. Alcoholic extract of Gokshura and Guduchi was prepared by Soxhlet method. Further sensitivity test was performed by Agar well diffusion method and zone of inhibition was measured. Alcoholic extract of Guduchi has showed better antibacterial effect against *Klebsiella pneumoniae* in comparison to alcoholic extract of Gokshura in same concentration as the mean value of zone of inhibition is higher for Guduchi. Phytochemical components present in alcoholic extracts aid in antibacterial action against cell membrane of micro-organism.

Keywords: Pittaja mutrakrichra, Urinary tract infection, *Klebsiella pneumoniae*, Urine culture and sensitivity, Antibacterial action of Gokshura and Guduchi

INTRODUCTION

Mutrakrichra patient suffers from difficulty in urination. In pittaja type of mutrakrichra, the symptoms include yellow coloured urine, urine mixed with blood, painful micturition, burning sensation during micturition, difficulty in urination and voids small quantity of urine frequently. Based on these signs and symptoms, pittaja mutrakrichra is co-related with Urinary Tract Infection.^{1,2} Urinary Tract Infection is defined as bacteriuria, that is the multiplication of bacteria in urine within the urinary tract and present in the mid-stream sample of urine. A person suffering from Urinary Tract Infection will show following signs and symptoms: Frequency/urgency, Suprapubic pain, Burning sensation, Painful Micturition, Yellowish Urine and Haematuria. *Klebsiella*, is a gram negative, non-motile, bacteria grouped under family Enterobacteriaceae. Among them, *Klebsiella pneumoniae* and *Klebsiella oxytoca* are medically important species and pathogenic to human beings. It is the second most pathogen causing UTI after *Escherichia coli*.

Gokshura (*Tribulus terrestris* Linn.) is mentioned in the context of pittaja mutrakrichra chikitsa and possess krimighna property. It is also included under krimighna dashemani.^{3,4} Guduchi (*Tinospora cordifolia* Miers.) is useful in mutrakrichra and possesses krimighna property. According to Kaiyyadeva Nighantu, Guduchi is mentioned as krimihara and useful in mutrakrichra.^{5,6} Testing for antimicrobial sensitivity determines appropriate drug to be used for a particular bacterial strain isolated from clinical specimen. So, culture and sensitivity techniques are helpful to confirm antibacterial effects of herbal drugs against pathogen. Hence, Present work is planned to evaluate sensitivity of Gokshura and Guduchi against *Klebsiella pneumoniae* isolated from patients suffering from Pittaja mutrakrichra with special reference to Urinary tract infection.

AIMS & OBJECTIVES

To compare antibacterial activity of two drugs viz. Gokshura and Guduchi against *Klebsiella pneumoniae* by urine culture and sensitivity method in Pittaja mutrakrichra (Urinary tract infection)

MATERIALS & METHODS

Plant collection and Authentication was carried out. Dried fruits of Gokshura (*Tribulus terrestris* Linn.) and stems of Guduchi (*Tinospora cordifolia* Miers.) was collected from a shop. The

authentication of the raw drugs was done. It was cleaned, dried and a coarse powder was prepared. Alcoholic extract of Gokshura and Guduchi by Hot Extraction was prepared using Soxhlet method.⁷

Table 1: Preparation of Alcoholic extracts of Gokshura and Guduchi

Sl. No.	Drug	Day	Content	Time taken for total Extraction	Temperature	Quantity obtained
1.	Gokshura	22-06-2018 Friday	Coarse powder of dried Gokshura fruits – 50 grams Ethanol – 500 ml	11:45 am – 04:00 pm	60°C - 90°C	2 grams
2.	Guduchi	23-06-2018 Saturday	Coarse powder of dried Guduchi stems – 50 grams Ethanol – 500 ml	09:55 am – 04:00 pm	60°C - 90°C	2 grams

25 patients fulfilling diagnostic and inclusion criteria were included for study from OPD and IPD of Primary / Tertiary Ayurveda Hospital, Hassan and regional diagnostic labs.

Diagnostic criteria

Patients complaining of Krichramutrata (dysuria) associated with one or more following symptoms of Pittaja mutrakrichra (Urinary Tract Infection) and having urine culture positive for *Klebsiella pneumoniae* bacteria.

Table 2: Pittaja mutrakrichra and Urinary tract infection

Pittaja mutrakrichra	Urinary tract infection
Muhurmuhu mutra pravruithi	Frequency / urgency
Basthi shoola	Supra pubic pain
Mutra daha	Burning sensation
Saruja mutrata	Painful micturition
Peeta mutrata	Yellowish urine
Sarakta mutra	Hematuria

Inclusion criteria

Patients between the age of 18 – 70 years of either gender fulfilling the diagnostic criteria were included in the study.

Exclusion criteria

Patient with chronic kidney failure or any other disease, that may interfere in course of study. Institutional Ethical clearance number SDM/IEC/52/2017-2018.

Methodology

Culturing, isolation and identification of *Klebsiella pneumoniae* bacteria was conducted. The mid-stream sample of urine from the patients of urinary tract infection was collected and microscopical examination was carried out for microscopic characterization of the bacteria. The inoculum was transferred to Macconkey agar plate and culturing was done by Streak culture method under incubation for 24 hours under culture condition. The cultured organism was subjected to microscopic examination, Stig test and Gram's staining for the identification of *Klebsiella*

pneumoniae bacteria followed with sub-culturing and biochemical-serological tests for confirmation.

Evaluation of Sensitivity test was performed by Agar well diffusion method. Work place was cleaned in laminar air flow using 70% of ethyl alcohol and UV was switched on for 20 minutes. One loop full of *Klebsiella pneumoniae* from 24 hours culture was transformed into the Mueller-hinton agar media with a sterile non-toxic cotton swab and swabbing was done over the media (lawn culture). 6 equidistant wells were made on two separate plates with sterile cork borer. And the wells in the dishes were filled with different concentrations (20, 10, 5, 2.5, 1.25 and 0.625 µg/ml) of alcoholic extracts of Gokshura and Guduchi. Plates were kept for incubation at 37°C for 24 hours. After the incubation period, the zone of inhibition was measured and tabulated.

Assessment was done after incubation. Zone of inhibition was identified with presence of "Halo" around the wells. These "Halo" was measured with ruler and the results were tabulated as sensitive zone, moderately sensitive / intermediate sensitive zone and resistant zone.

OBSERVATIONS & RESULTS

Table 3: Observation on Antibacterial activity shown at different concentrations of alcoholic extracts of Gokshura against *Klebsiella pneumoniae*

Extract	*ZOI in mm Against <i>Klebsiella pneumoniae</i>	20 µg/ml N=25		10 µg/ml N=25		5 µg/ml N=25		2.5 µg/ml N=25		1.25 µg/ml N=25		0.625 µg/ml N=25	
		F	%	F	%	F	%	F	%	F	%	F	%
Alcoholic extract of Gokshura	0	18	72	19	76	12	48	3	12	2	8	2	8
	10	2	8	2	8	1	4	1	4	-	-	1	4
	12	1	4	-	-	-	-	-	-	-	-	-	-
	14	-	-	1	4	-	-	-	-	-	-	-	-
	18	1	4	-	-	-	-	1	4	-	-	1	4
	20	2	8	1	4	5	20	9	36	7	28	7	28
	22	1	4	1	4	4	16	3	12	6	24	3	12
	24	-	-	1	4	3	12	6	24	6	24	4	16
	26	-	-	-	-	-	-	-	-	2	8	1	4
	28	-	-	-	-	-	-	1	4	1	4	4	16
	30	-	-	-	-	-	-	-	-	-	-	1	4
	32	-	-	-	-	-	-	-	-	-	-	1	4
	34	-	-	-	-	-	-	1	4	1	4	-	-
Total		25	100	25	100	25	100	25	100	25	100	25	100

* ZOI = Zone Of Inhibition * F = Frequency of samples showing sensitivity against alcoholic extract of Gokshura
* N = Total no. of samples

Table 4: Observation on Antibacterial activity shown at different concentrations of alcoholic extracts of Guduchi against *Klebsiella pneumoniae*

Extract	*ZOI in mm against <i>Klebsiella pneumoniae</i>	20 µg/ml N=25		10 µg/ml N=25		5 µg/ml N=25		2.5 µg/ml N=25		1.25 µg/ml N=25		0.625 µg/ml N=25	
		F	%	F	%	F	%	F	%	F	%	F	%
Alcoholic extract of Guduchi	0	11	44	10	40	7	28	2	8	2	8	1	4
	10	3	12	1	4	1	4	1	4	-	-	-	-
	14	-	-	1	4	1	4	-	-	-	-	-	-
	16	-	-	1	4	-	-	1	4	-	-	-	-
	18	1	4	1	4	1	4	-	-	-	-	-	-
	20	7	28	5	20	5	20	5	20	2	8	-	-
	22	1	4	4	16	4	16	2	8	6	24	4	16
	24	1	4	1	4	3	12	9	36	6	24	6	24
	26	-	-	-	-	2	8	2	8	6	24	2	8
	28	1	4	1	4	1	4	2	8	1	4	5	20
	30	-	-	-	-	-	-	-	-	1	4	2	8
	32	-	-	-	-	-	-	1	4	-	-	3	12
	34	-	-	-	-	-	-	-	-	-	-	1	4
36	-	-	-	-	-	-	-	-	1	4	1	4	
Total		25	100	25	100	25	100	25	100	25	100	25	100

* ZOI = Zone of Inhibition * F = Frequency of samples showing sensitivity against alcoholic extract of Guduchi
* N = Total no. of samples

Table 5: Mean values of zone of inhibition in millimeter at different concentrations of alcoholic extract of Gokshura against *Klebsiella pneumoniae*

Different concentrations of alcoholic extract of Gokshura	20µg/ml	10µg/ml	5µg/ml	2.5µg/ml	1.25µg/ml	0.625µg/ml
N	25	25	25	25	25	25
Mean of zone of inhibition in mm	4.48	4	10.8	19.2	21.2	21.2

Table 6: Mean values of zone of inhibition in millimeter at different concentrations of alcoholic extract of Guduchi against *Klebsiella pneumoniae*

Different concentrations of alcoholic extract of Guduchi	20µg/ml	10µg/ml	5µg/ml	2.5µg/ml	1.25µg/ml	0.625µg/ml
N	25	25	25	25	25	25
Mean of zone of inhibition in mm	10.48	11.9	15.2	21.04	22.6	26

Table 7: Comparing the zone of inhibition of alcoholic extracts of Gokshura and Guduchi and ascertaining sensitivity

Concentrations	20 µg/ml			10 µg/ml			5 µg/ml			2.5 µg/ml			1.25 µg/ml			0.625 µg/ml		
	S	M	R	S	M	R	S	M	R	S	M	R	S	M	R	S	M	R
No. of samples (Gokshura)	3	1	21	3	0	22	13	0	12	20	1	4	23	0	2	21	1	3
No. of samples (Guduchi)	3	8	14	5	7	13	11	5	9	17	5	3	21	2	2	24	0	1

S=Sensitive zone (22-20 mm), M= Moderately sensitive zone (18-16 mm), R=Resistant zone (14-12 mm)

Table 8: Comparison between efficacy of Gokshura and Guduchi against *Klebsiella pneumoniae* by urine culture and sensitivity in Pittaja mutrakrichra (Urinary tract infection)

Concentrations	N	Mean of zone of inhibition in mm		Mean Difference	SE	f - Value	p value	Interpretation
		Gokshura	Guduchi					
20 µg/ml	25	4.48	10.48	6	1.344	5.437	0.024	S
10 µg/ml	25	4	11.9	7.9	1.408	9.234	0.004	S
5 µg/ml	25	10.8	15.2	4.4	1.523	2.217	0.143	NS
2.5 µg/ml	25	19.2	21.04	1.84	1.122	0.668	0.418	NS
1.25 µg/ml	25	21.2	22.6	1.4	1.035	0.479	0.492	NS
0.625 µg/ml	25	21.2	26	4.8	1.080	5.383	0.025	S

Statistical analysis: The means were compared using One-Way ANOVA test. p value between 0.01 - 0.001 is considered as statistically highly significant, p value between 0.01-0.05 is considered as statistically significant and p value > 0.05 is considered as non-significant.

Comparison between efficacy of alcoholic extract of Gokshura and Guduchi against *Klebsiella pneumoniae* by urine culture and sensitivity in Pittaja mutrakrichra (Urinary tract infection) in 25 patients showed that mean of zone of inhibition was higher against *Klebsiella pneumoniae* by Guduchi than Gokshura in all different concentrations except 2.5 µg/ml concentration. Further it may be noted that mean of zone of inhibition increased with decrease in concentration of drug in both the groups except for Gokshura at concentration of 10 µg/ml.

On comparing efficacy by mean of zone of inhibition at same concentrations of alcoholic extracts of Gokshura and Guduchi and applying One-Way ANOVA test, it is observed that at 20 µg/ml, 10 µg/ml and 0.625 µg/ml, it was found to be statistically significant with p<0.05. Mean difference suggest that Guduchi has better action as mean of zone of inhibition is higher compared to Gokshura and on statistical analysis it is statistically significant with p< 0.05.

At concentration of 5 µg/ml, 2.5 µg/ml and 1.25 µg/ml mean of zone of inhibition was demonstrated to be higher by Guduchi than Gokshura showing better efficacy, but it was statistically non-significant with p>0.05.

DISCUSSION

Research studies have been conducted in the past on antibacterial effect of Gokshura. Previous study conducted on antibacterial activity of Gokshura and Varuna against *E. coli* showed that Gokshura possess better sensitivity against *E. coli* than Varuna.⁸

In the present study on comparing the antibacterial action of alcoholic extracts of Gokshura and Guduchi, the mean difference between the zone of inhibition of alcoholic extracts of Guduchi and Gokshura at different concentrations (20 µg/ml, 10 µg/ml, 5 µg/ml, 2.5 µg/ml, 1.25 µg/ml, 0.625 µg/ml) are 6 mm, 7.9 mm, 4.4 mm, 1.84 mm, 1.4 mm and 4.8 mm respectively, with the

mean higher for Guduchi showing Guduchi has better action than Gokshura against *Klebsiella pneumoniae*.

It is reported that cytological components of *Klebsiella pneumoniae* comprises of group of virulent factors such as polysaccharide, lipopolysaccharide, fimbriae and outer membrane proteins. These factors play pivotal role in pathogenesis in an infected person. Depending on type of strain and species, virulence level may vary. Cell constituents aid in attachment of bacteria to host cell, invasion into host cell and prevention of phagocytosis from other immune-modulator helper cells.

Further it is also reported that, alcoholic extracts of Gokshura and Guduchi possess antibacterial constituents like alkaloids, tannins, flavonoids, saponins and terpenoid compounds possessing properties like alteration of surface tension of extra cellular medium of bacteria cell wall, ability to complex with extracellular soluble proteins, intruding and destruction of DNA of microbial cell etc.

In the present study, preliminary phyto-chemical screening of alcoholic extract of Gokshura and Guduchi has been conducted and revealed the presence of alkaloids, tannins, flavonoids, saponins and terpenoid.

Previous studies on Gokshura and Guduchi revealed that alcoholic extract shows more significant zone of inhibition than aqueous and chloroform extracts. Because it maximizes the bioavailability of the active principles from the plant. Ethanol contains, both the polar and non-polar ends to extract both groups of compounds of a drug. Thereby, phytochemical compounds are completely dissolved in solvent. While doing sensitivity, phytochemical constituents interact with enzymes and proteins of cell membrane of bacteria, causing its disruption to disperse a flux of protons towards cell exterior, that results in cell death or inhibit amino acid biosynthesis of microbial cell. It is noteworthy that hydrophobic characters of these extracts enable to react with protein of microbial cell membrane and mitochondria disturbing their cell structures and permeability. Likewise, for different strains of gram-negative bacteria it has been proposed that the mechanism of the antimicrobial effects involves the inhibition of various cellular processes followed by an increase in plasma membrane permeability and finally ion leakage from the cells.

Meantime for different concentrations of a same drug, it may exhibit different zone of inhibition.⁹ Because, the different components diffuse at different rates may have been responsible for the varying zone of inhibition against the bacteria. In lower concentrations, the molecular size of the active components will be too small via complete dissolution and thereby it can penetrate easily through cell membrane of bacteria. So it will show maximum zone of inhibition than other higher concentrations. The variation of susceptibility of the bacteria can be attributed with their intrinsic properties and permeability of cell surface to the extracts. Porosity of cell membrane varies cell to cell and the membrane inhibits cell structure perturbations by phytochemical components because of these unique characteristics.¹⁰

CONCLUSION

In the present study, it is evident that alcoholic extract of Guduchi has showed better antibacterial effect against *Klebsiella pneumoniae* in comparison to alcoholic extract of Gokshura in same concentration as the mean value of zone of inhibition is higher for Guduchi. As the concentration of the alcoholic extract of Guduchi and Gokshura decreased, progressively zone of inhibition increased respectively. So, it is concluded that as the concentration decreases, the antibacterial activity increases by Guduchi and Gokshura. Further, in-vitro evidence for indication of Guduchi and Gokshura in the treatment of Pittaja mutrakrichra (urinary tract infection) caused by *Klebsiella pneumoniae* is established.

REFERENCES

1. Ramkaran Sharma, Agnivesa's Caraka Samhitha chikitsa sthana, 26 th chapter. Chaukhambha Sanskrit series Varanasi; 2012, 26 th chapter p. 477-478.
2. Leo AS, Braun E, Neil KJ, Wilson JD, Martin JB, Kasper DL, et al. Harrison's principles of internal medicine. 17th ed. New York: Health Professions Division; 2008. P. 1620.
3. K.R Srikantha Murthy, Bhavaprakasha of Bhavamisra, Purva khanda, Chowkhamba Krishnadas Academy, 2004, Guduchyadi varga, p. 234
4. R.K. Sharma & Bhagwan Dash, Agnivesa's Caraka samhitha sutra sthana, Chaukhambha Sanskrit series Varanasi; 2009, 4th chapter, p. 91-92.
5. K.R Srikantha Murthy, Bhavaprakasha of Bhavamisra, Purva khanda, Guduchyadi varga, Chowkhamba Krishnadas Academy, 2004, p. 228.
6. Priyavrata Sharma & Guruprasada Sharma, Kaiyyadeva Nighantu, Aushadi varga, Chowkhamba Orientalia Varanasi; 1979, p.5.
7. K.R. Khandelwal and Vrunda K. Sethi, Practical Pharmacognosy- Techniques and Experiments, Nirali Prakashan,Pune, 2016.
8. Dr. Ajith. A Et Al: In Vitro Study Of Gokshura And Varuna Against Escherichia Coli By Urine Culture And Sensitivity W.S.R To Pittaja Mutrakrichra (Urinary Tract Infection) http://www.iamj.in/posts/images/upload/1130_1134.pdf
9. L. M. Kaur, N.K. Aggarwal and R. Dhiman, 2016. Antimicrobial Activity of Medicinal Plant: Parthenium hysterophorus L. Research Journal of Medicinal Plants, 10: 106-112. DOI: 10.3923/rjmp.2016.106.112.
10. Rosina Khan, Barira Islam, Mohd Akram, Shazi Shakil, Anis Ahmad, S. Manazir Ali, Mashiatullah Siddiqui, Asad U. Khan. 2009. Antimicrobial Activity of Five Herbal Extracts Against Multi Drug Resistant (MDR) Strains of Bacteria and Fungus of Clinical Origin. Journal of *Molecules*. 586-597. DOI:10.3390/molecules14020586.

How to cite this article:

Anand S et al. A comparative study to evaluate efficacy of gokshura and guduchi against *Klebsiella pneumoniae* by urine culture and sensitivity in pittaja mutrakrichra (urinary tract infection). J Pharm Sci Innov. 2019;8(5):165-169. <http://dx.doi.org/10.7897/2277-4572.085148>

Source of support: Nil, Conflict of interest: None Declared

Disclaimer: JPSI is solely owned by Moksha Publishing House - A non-profit publishing house, dedicated to publish quality research, while every effort has been taken to verify the accuracy of the content published in our Journal. JPSI cannot accept any responsibility or liability for the site content and articles published. The views expressed in articles by our contributing authors are not necessarily those of JPSI editor or editorial board members.