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GCMS Analysis of Aqueous Extract of *Tamala Patra* (Cinnamomum tamala) and its Efficacy Against Escherichia Coli from *Pittaja Mutrakrichra* (UTI)

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ABSTRACT

Urinary tract infection is the second most common infectious presentation in community medical practice and are associated with high morbidity and long-term complications. *Lakshanas* of *pittaja mutrakrichra* and symptoms of urinary tract infection are analogues. In majority, causative organisms of urinary tract infections are gram negative bacteria viz. *Escherichia coli* followed by *klebsiella pneumoniae, Proteus mirabilis* etc. Acharyas explains drug possessing *krimigna* action but indication of specific drug on specific micro-organism in a disease has not been explored. Hence in present work, culture and sensitivity is taken as a tool to evaluate the concept of *upashaya* and *anupashaya* in vitro to revalidate the activity of *Tamala patra* against *E-coli* by culture and sensitivity in *Pittaja mutrakrichra* with special reference to UTI. Patients of *pittaja mutrakrichra* were subjected for urine culture and those with positive results for *E-coli* were further used. Aqueous extract of *Tamala patra* was prepared by cold maceration method.

Further sensitivity test was performed by Agar well diffusion method and zone of inhibition was measured. aqueous extract of *Tamala patra* has shown is having antimicrobial action against *E. coli* from *pittaja mutrakrichra* (UTI).

Key Words *Urine culture and sensitivity, E-coli, Antibacterial action of aqueous extract*

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INTRODUCTION

Mutrakrichra is defined as *krichrata* of *Mutrapravruthi* or difficulty during micturition affecting the *Basthi*, one among the *trimarma. Pittaja mutrakrichra* is characterised by *krichra mutratha, muhur muhur mutra pravruthi, Sarakta mutratha, sadaha mutra, saruja* and *peetha mutratha*[1]. These symptoms are analogues with the manifestation of urinary tract infection. *Escherichia coli* is one of the foremost causative organisms responsible for urinary tract infection. The pathology runs behind the UTI caused by *E. coli* may affect any part of the renal system[2]. In Ayurveda, there are many drugs possessing

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krimigna action, but an indication of a specific drug on a particular microorganism is missing. Therefore, it is the time to use diagnostic tools like culture and sensitivity and identify the causative microorganism. Before the drug is used clinically in patients, its activity needs to be checked to confirm whether the drug shows sensitivity. Therefore, preliminary evidence in-vitro studies can be generated scientifically so that the drug can later be used as *upashaya*. So, to find out the antibacterial activity of a drug possessing *krimigna* property[3] against a specific microorganism and to define the anti-bacterial property of that particular drug for known concentrations. Hence the implementation of new approaches like culture and sensitivity methods would strengthen the existing Ayurveda knowledge and help in accomplishing improved diagnostic and curative abilities. So, in the present study, revalidation of ayurvedic knowledge with the help of modern diagnostic tools like culture and sensitivity in-vitro was carried out.

AIMS AND OBJECTIVES

To study the sensitivity of *Tamala patra (Cinnamomum tamala*) against *Escherichia coli* by urine culture and sensitivity method in *Pittaja mutrakrichra* (Urinary tract infection).

MATERIALS AND METHODS

Tamala patra was collected from a nearby shop, and authentification was initially conducted. The authentification of drug *Tamala patra* was done at Sri Dharmasthala Manjunatheswara college of Ayurveda and hospital, Hassan (No. SDMCAH-DG/2019/22). It was cleaned, dried, and coarse powder was prepared.

RESEARCH DESIGN

An observational Experimental study

METHODOLOGY

Plant collection and authentification were carried out. Aqueous extracts of *Tamala patra* were prepared by using the standard procedure of cold maceration[4]. Because it is easy to perform, cost-effective, and simple without using any complex instruments but yields highly potent extracts with many bioactive principles. Fresh and cleaned *Tamala* leaves of about 50gm were weighed by using a weighing balance. The leaves were then grinded in a clean mortar and pestle coarsely without adding water. Grinded *Tamala* leaves were added to 250 ml distilled water in a 1000ml capacity conical flask. Then plugged tightly with cotton and was sealed with tape. The conical flasks were shaken manually for 10-15min in every 3 hours during daytime.(Table No.1). The procedure was repeated for 7 days. On the 7th day, 150ml of filtrate was obtained by filtering the content of conical flask. Further 7.5gm of aqueous extract of *Tamala patra* was obtained by keeping the filtrate over water bath in a petri dish at 60^{0}C.

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Table 1 Frequency and duration of shaking in cold maceration process

	Time	Duration (min)
12/11/2020	12 pm	10 min
	2 pm	15min
	4 pm	15min
13/11/2020	10 am	15min
	11 am	15min
	1 pm	15min
	3 pm	10min
14/11/2020	10 am	10min
	12 pm	15min
	2 pm	10min
	4 pm	15 min
15/11/2020	10am	10min
	12pm	15min
	2pm	10min
	4pm	15min
	5pm	10min
16/11/2020	10am	15min
	12pm	10 min
	2pm	15min
17/11/2020	9.30am	10min
	11am	15min
	2pm	15min
	4.30pm	15min

Urine sample was collected from *pittaja mutrakrichra* patients and was subjected to wet mounting method under the microscope to examine for presence of bacteria[5]. Culturing was done by transferring the inoculum to MacConkey agar plate by Streak culture method. Incubation was done on 24 hours with culture condition. Further isolation of bacteria and subjected to gram staining for the identification of gram-negative bacteria. After the identification colony morphology, the biochemical test was conducted to confirm *Escherichia coli*[6].

Different concentrations of aqueous extract of *Tamala patra* were prepared by dissolving 5gm of aqueous extract in 15ml of distilled water that gave a stock solution carrying 5000µg/ml of drug concentration. From that main concentration, different concentration like 4000 µg/ml,3000 µg/ml,2000 µg/ml,1000 µg/ml and 900 µg/ml of the aqueous extracts were prepared. Sensitivity test was performed by agar well diffusion method.one loop full of *Escherichia coli* from 24 hours culture was transformed into the Muller Hinton agar media with a sterile non-toxic cotton swab and swabbed over the media (lawn culture). Six equidistant wells were made on the plate with sterile cork baurer.100 µl of each of different concentration of aqueous extract were poured into labelled wells on different plates. All these plates were kept for incubation at 37^{o}C for 24 hours. After the incubation period, zone of inhibition was measured in mm by using a ruler.

ASSESMENT CRITERIA

If *Tamala patra* is sensitive, a clear circular Halo (Zone of inhibition) that appears around the well, indicating the absence of bacteria. If that zone appears, it shows that *Tamala patra* has antibacterial action *against E. coli*. In current study it reveals that, the susceptibility of *E. coli* against aqueous extract of *Tamala patra* is quite marked between 20 to 18mm zone of inhibition. Hence it is considered as sensitive,16 to 14mm is considered as moderately sensitive and 12 to 10 mm is considered as resistant.

RESULTS

Gas chromatography and mass spectrometric analysis of *Tamala patra*

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The presence of the following compounds was observed (Table No.2) and Peaks observed in Gas chromatography and mass spectrometry of drug extract had shown in (Table No.3).

Table 2 Compounds observed on GCMS analysis of *Tamala patra*

COMPOUND	AREA %
1.Acetic acid	1.99%
2.Propane	3.84%
3.Propanoic acid	3.85%
4.2,3 – Butanediol	20.98%
5.Benzoic acid	1.80%
6.Silane	0.68%
7.Vitamin E	0.61%
8.Eugenol	2.12%
9.Benzene	0.38%
10.Thiophene	0.15%
11.Pyridine	0.87%
12.Dodecanoic acid	0.25%
13.Hexadecenoic acid	4.20%
14.Benzenamine	0.63%
15.Methyl ester	0.22%
16.Vitamin A aldehyde	0.16%
17.Phthalic acid	0.92%
18.Stigma stan	0.38%
19.Oxime,methoxy-phenyl-2-amino-6-methyl benzoic acid	4.35%
20.Cyclotrisiloxane,Hexamethyl-Benzo(h)quinoline	2.50%
21.Pentadecanoic acid	4.20%
22.14-methyl-ester	4.20%
23.9-Octadecenoic acid	1.91%

Table 3 Peaks observed in gas chromatography and mass spectrometry of drug extract

Peak No.	RT min	First scan	Max scan	Last scan	Peak height	Corr.% Max	% Of Total
1.	**5.371**	**3**	**33**	**48**	**684211**	**9.46%**	**1.986%**
2.	**6.019**	**108**	**110**	**125**	**1355659**	**18.31**	**3.84**
3.	**6.170**	**125**	**128**	**186**	**1174972**	**18.36**	**3.85**
4.	**8.055**	**243**	**349**	**354**	**2204621**	**100.00**	**20.982**
5.	**9.472**	**493**	**516**	**540**	**1098418**	**8.60**	**1.80**
6.	**38.953**	**3967**	**3986**	**4002**	**401721**	**3.25**	**0.68**
7.	**73.495**	**8040**	**8052**	**80.69**	**335031**	**2.92**	**0.614**
8.	**29.771**	**2894**	**2906**	**2970**	**852042**	**10.10**	**2.12**
9.	**70.537**	**7695**	**7704**	**7714**	**318348**	**1.80**	**0.378**
10.	**35.292**	**3547**	**3556**	**3565**	**114969**	**0.72**	**0.150**
11.	**34.828**	**3480**	**3501**	**3517**	**292919**	**4.14**	**0.868**
12.	**35.436**	**3565**	**3572**	**3589**	**190341**	**1.21**	**0.253**
13.	**52.462**	**5562**	**5577**	**5604**	**3092839**	**20.04**	**4.204**
14.	**56.852**	**6081**	**6093**	**6109**	**238995**	**3.01**	**0.632**
15.	**69.685**	**7585**	**7604**	**7618**	**107026**	**1.03**	**0.215**
16.	**60.531**	**6513**	**6526**	**6532**	**95789**	**0.77**	**0.161**
17.	**64.477**	**6983**	**6991**	**7005**	**801754**	**4.39**	**0.921**
18.	**62.302**	**6723**	**6735**	**6748**	**282864**	**1.80**	**0.378**
19.	**10.123**	**560**	**593**	**646**	**929888**	**20.74**	**4.351**
20	**14.334**	**1056**	**1089**	**1093**	**376919**	**2.45**	**0.514**
21.	**52.462**	**5562**	**5577**	**5604**	**3092839**	**20.04**	**4.204**
22.	**52.462**	**5562**	**5577**	**5604**	**3092839**	**20.04**	**4.204**
23.	**56.659**	**6058**	**6071**	**6081**	**1071076**	**9.09**	**1.908**

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Test method adopted: CKL/ANL/GC-003

Total 73 compounds were observed

Peak 21 was observed in the highest concentration

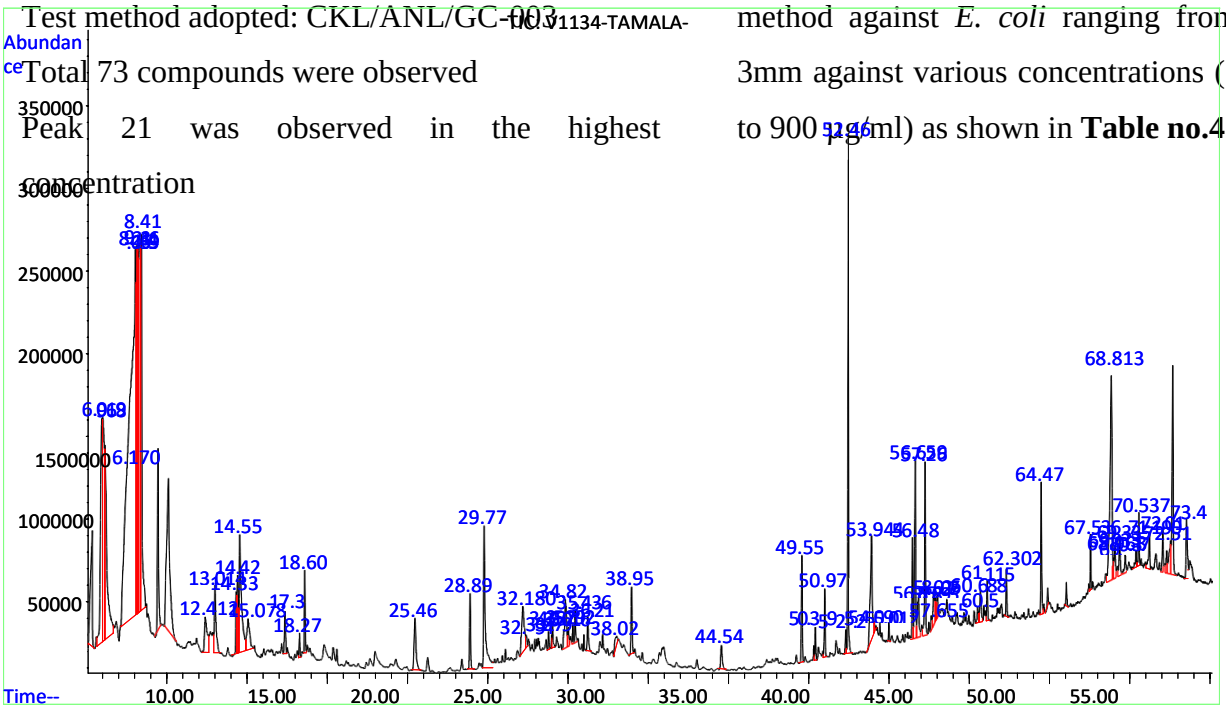

Figure 1 Chromatogram of Drug extract

Aqueous extract of *Tamala patra* had shown various zone of inhibition by agar well diffusion method against *E. coli* ranging from 24mm to 3mm against various concentrations (5000 µg/ml to 900 µg/ml) as shown in **Table no.4**

Maximum zone of inhibition (24mm) was recorded for aqueous extract of *Tamala patra* at 5000 µg/ml and minimum zone of inhibition (3mm) was recorded at 3000 µg/ml concentration.

Table 4 Observation on Antibacterial activity shown at different concentrations of Aqueous extract of *Tamala patra* against *Escherichia coli*

Extract	ZOI in mm	5000µg/ml N=30		4000 µg/ml N=30		3000 **µg/ml** N=30		2000 µg/ml N=30		1000 µg/ml N=30		900 µg/ml N=30	
		F	**%**	**F**	**%**	**F**	**%**	**F**	**%**	**F**	**%**	**F**	**%**
Aqueous Extract of Tamala patra	0	1	3.3	1	3.3	-	-	-	-	2	6.7	8	26.7
	3	-	-	-	-	1	3.3	-	-	-	-	-	-
	5	-	-	1	3.3	-	-	1	3.3	2	6.7	3	10.0
	6	-	-	-	-	1	3.3	-	-	2	6.7	-	-
	7	-		1	3.3	-	-	1	3.3	2	6.7	5	16.7
	8	-		-	-	-	-	2	6.7	-	-	-	-
	9	-		-	-	-	-	-	-	-	-	2	6.7
	10	1	3.3	-	-	2	6.7	2	6.7	3	10.0	2	6.7
	11	-	-	-	-	-	-	4	13.3	4	13.3	4	13.3
	12	1	3.3	1	3.3	1	3.3	1	3.3	1	3.3	-	-
	13	-	-	2	6.7	-	-	3	10	3	10	1	3.3

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	14	1	3.3	-	-	-	-	-	-	1	3.3	1	3.3
	15	-	-	1	3.3	1	3.3	1	3.3	1	3.3	-	-
	16	3	10	2	6.7	9	30	7	23.3	3	10	-	-
	17	4	13.3	4	13.3	9	30	3	10.0	2	6.7	2	6.7
	18	4	13.3	9	30	2	6.7	1	3.3	3	10	-	-
	19	4	13.3	4	13.3	3	10.0	3	10.0	1	3.3	-	-
	20	6	20	4	13.3	1	3.3	1	3.3	-	-	1	3.3
	21	3	10	-	-	-	-	-	-	-	-	-	-
	22	1	3.3	-	-	-	-	-	-	-	-	1	3.3
	24	1	3.3	-	-	-	-	-	-	-	-	-	-

***ZOI- Zone of inhibition**
F – Frequency of samples showing sensitivity against aqueous extract of *Tamala patra
***N – Total number of samples**

Table 5 Mean values of zone of inhibition at different concentrations of aqueous extract of *Tamala patra* against *Escherichia coli*

Different concentrations of alcoholic extract of *Tamala patra*	5000 **µg/ml**	4000 **µg/ml**	3000 **µg/ml**	2000 **µg/ml**	1000 **µg/ml**	900 **µg/ml**
N	**30**	**30**	**30**	**30**	**30**	**30**
Mean	**18.57**	**17.97**	**16.97**	**12.80**	**12.33**	**8.00**

Table 6 Sensitivity test for aqueous extract of different concentrations of *Tamala patra*

Concentrations-rations	5000 µg/ml			4000 µg/ml			3000 µg/ml			2000 µg/ml			1000 µg/ml			900 µg/ml		
	S	M	R	S	M	R	S	M	R	S	M	R	S	M	R	S	M	R
No. of samples	22	4	4	20	6	4	1		5	7	12	11	6	7	17	4	2	2

Here S-Sensitive, M-Moderately sensitive, R-Resistant

DISCUSSION

In this study 50 subjects with *pittaja mutrakrichra* (UTI) were screened and 30 subjects fulfilling the diagnostic and inclusion criteria were included. Remaining 20 subjects were excluded as 16 organisms are other than *E. coli*, 3 were with chronic kidney failure and 1 was with haematuria.

Tamala patra has been mentioned under *krimigna* and its therapeutic uses like *basthi-kandu tridoshagnam. Tamala Patra* possesses *Madhura* and *tikta rasa; teekshna, laghu guna, ushna* virya and *Madhura vipaka*. It is *tridoshagna, basthi dosha hara, Krimigna*, and *kandugna*[7,8]. Aqueous extract of Tamala patra have different phytochemical constituents that alter the surface tension of extra cellular medium of organism cell, complexing with extracellular and soluble proteins and obtruding with DNA of the organism that contributes to sensitivity of the bacterium against the extract[9].

Aqueous extract of *Tamala patra* had shown various zone of inhibition by agar well diffusion method against *E. coli* ranging from 24mm to 3mm against various concentrations (5000 µg/ml to 900 µg/ml) as shown in Table no.4. Gas chromatography, and mass spectrometry it is a modified technique useful to analyze and separate volatile compounds from organic or inorganic substances[10]. So, to know the concentration of different components and number of peaks present in the *Tamala patra* conveyed that there were 18 multiple volatile

compounds that covered the maximum area of the graph depicting the highest concentration in the *Tamala patra* sample. It is followed by the abundance of 2,3-butanediol, propanoic acid, propane, eugenol, and acetic acid. All these compounds have proven antimicrobial activities and can be assumed as the components that are involved in the antimicrobial action of the extract. Hence higher concentration shows maximum zone of inhibition than lower concentration because of more concentration of active molecules.

CONCLUSION

From the zone of inhibition found in this study it is clear that aqueous extract of Tamala patra is having antimicrobial action against *E. coli* from *pittaja mutrakrichra* (UTI). Further it is evident that better sensitivity is observed with higher concentrations of aqueous extract of *Tamala patra***.**

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