Int J Ayu Pharm Chem
ISSN 2350-0204
www.ijapc.com

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Efficacy of Alcoholic Extract of *Tamala Patra* (Cinnamomum tamala) against Escherichia coli from *Pittaja Mutrakrichra* (Urinary Tract Infection)

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ABSTRACT

Pittaja mutrakruchra is a commonly reported disease resulting due to pathology of *Mutravaha Srotas*manifesting with the main complaint of difficulty in micturition. These symptoms manifested in *pittaja mutrakrichra* are analogous with the manifestation of UTI. Even though many drugs are attributed with *krimigna* action in Ayurveda, only a few worksare doneto establish the effectiveness of specific drug activity on specific microorganisms. Here in the present study, it is proven that the ethanolic extract of *Tamala patra*, which is enriched with active phytochemical constituents, can exert antimicrobial activity. So, the present study is intended to prove the antibacterial action of alcoholic extract of *Tamala patra* against *Escherichia coli* by urine culture and sensitivity.

Key Words *Pittaja mutrakrichra, Urinary tract infection, Tamala patra, Alcoholic extract, Urine culture and sensitivity*

Received 27th January 22 Accepted 03rd March 22 Published 10th March 2022

INTRODUCTION

Mutrakrichra is the disease of *Mutravaha srotas*[1] characterized by difficulty in micturition[2] the symptoms of *pittaja mutrakrichra* have similarities in signs and symptoms of urinary tract infection. *Pittaja mutrakrichra* are presenting with the symptoms like *peetha mootratha* (yellowish urine), *Saraktamutratha* (haematuria), *sadaha mutratha* (burning micturition), *saruja mutratha* (dysuria), and *muhurmuhur mutratha*(increased frequency of micturition)[3]. So based on the signs and symptoms, *pittaja mutrakrichra* is correlated with urinary tract infection. UTI is defined as bacteriuria i.e., the Multiplication of bacteria in the urinary tract is usually associated with the presence of neutrophils and > 10^5 organisms/ml in a mid-stream sample of urine (MSU). A person suffering from UTI may present with the following signs and symptoms frequency/urgency, suprapubic pain, burning

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sensation, painful micturition, yellowish urine, and haematuria. *E. coli* is one of the foremost causative organisms which is responsible for the causation of UTI. Even though many drugs are ascribed with *krimighna*[4] action in Ayurveda there are only a small number of works done on establishing the efficacy of specific drug activity on specific microorganisms. Hence in the present work, culture and sensitivity is taken as a tool to evaluate the concept of *upashaya* and *anupashaya* invitro to revalidate the activity of alcoholic extract of *Tamala patra* against *E. coli* by culture and sensitivity in *pittaja mutrakrichra* with special reference to UTI.

AIMS AND OBJECTIVES

To evaluate the sensitivity of alcoholic extract of *Tamala* (*Cinnamomum tamala*) *Patra* against *E. coli* from the urine sample of *pittaja mutrakrichra* (Urinary tract infection) patients by culture and sensitivity in vitro.

MATERIALS AND METHODS

The present study includes 30 subjects between 18-60 years of age, of either gender irrespective of religion and caste, presenting with urinary tract infection with following lakshanas like; *Muhurmuhu mutra pravruthi***,** *Basthi soola, Mutra daha, Saruja mutratha, Peeta mutratha, Sarakta mutram*from outpatient and inpatients department of the tertiary ayurvedic hospital, Hassan was included in the study. Subjects with the following were excluded from the study, like chronic kidney failure,HIV, Tuberculosis, CA prostate, STDs.

RESEARCH DESIGN

An observational experimental study

METHODOLOGY

Plant collection and authentication was carried out. The cold maceration method[5] was used for the preparation of alcoholic extract of *Tamala patra* as it is very easy to perform,cheap and simple without using any complex instruments but yields highly potent extract with many bioactive principles. Fresh and clean *Tamala* leaves of about 50gm were weighed using a weighing balance. The leaves were then crushed by using mortar and pestle without adding water to facilitate the easy release of active principles on the addition of extracting solvent ethanol. Crushed *Tamala Patra* was added to 250 ml Ethanol in a 1000 ml capacity of the conical flask. Later it was plugged tightly with cotton and was sealed with tape. The conical flask was shaken manually for 10-15 min at an interval of every 3 hours. The procedure was continued for 7 days during the daytime. On the 7thday, the content of the conical flask was filtered and obtained 210 ml of filtrate. The filtrate was then kept over a water bath in a China dish at 70 ºC. 7 gm of *Tamala Patra* extract was obtained from this process.

A Midstream urine sample was collected from the patients fulfilling the diagnostic criteria[6]. After that culturing was done on Muller Hinton

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Agar (MHA) and McConkey agar plates by streaking method using one loop full of inoculum. The plates were then kept for 24 hours culture in an incubator at 37ºC.After 24-48 hours of incubation, the cultural characters like colony morphology were studied and microscopic examination was done by gram staining to confirm the organisms as gram-negative (Table No.1)[7]. Different concentrations of alcoholic extract of *Tamala Patra* were prepared by dissolving 5gm of alcoholic extract in 15ml of ethanol that gave a stock solution carrying 5000µl of drug concentration from the stock solution,different concentrations like 4000µl,3000µl,2000µl,1000µland 900µl of the alcoholic extract were prepared. MHA plates were uniformly swabbed with a sterile non-toxic cotton swab (lawn culture). Different drug concentrations were then subjected to antibacterial sensitivity by the agar well diffusion method. Six equidistant wells were made on the plates with the help of a sterile cork borer.100µl of alcoholic extract of different concentrations were poured into labelled wells on different plates. All the plates were incubated at 37^0C for 24-48 hours. Later, the zone of inhibition was measured with a ruler in mm.

Table 1 Colony morphology and identification

CULTURE CHARACTERS	GRAM STAINING	ORGANISM IDENTIFIED
Size (in mm) – 2-3mm **Shape - Round** **Surface - smooth** **Elevation-low convex** **Edge -circular** **Opacity- opaque** **Colour of the colony -Grey to white** **Consistency – buttery** **Hemolysis - Nil** **Other properties-lactose fermenting**	Gram-negative	*E. coli*

ASSESSMENT CRITERIA

Sensitivity of drug to a bacterium is assessed by a clear circular 'Halo' (Zone of inhibition) that appears around the well-representing absence of organism. That indicates that the drug is effective against that bacterium. This study reveals that susceptibility of *Escherichia coli* against the alcoholic extract of *Tamala patra* is quite marked between 20 to 22 mm zone of inhibition. Hence it is considered as sensitive, 18 to 16mm is considered as moderately sensitive, 14 to 12 mm is considered as resistant.

OBSERVATION AND RESULTS

In vitro, the antibacterial activity of alcoholic extract of *Tamala patra* was evaluated by agar well diffusion method and zone of inhibition was measured as shown in table no. 2.

Table2 Mean values of the zone of inhibition at different concentrations of aqueous extract of *Tamala patra* against *E. coli*.

Different concentrations of Alcoholic Extract of *Tamala patra*(µl)	**5000**	**4000**	**3000**	**2000**	**1000**	**900**
Patients (N)	**30**	**3030**	**30**	**30**	**30**	

Mean zone of inhibition (mm)	**18.57**	**17.97**	**16.97**	**12.80**	**12.33**	**8.00**

The present study shows the susceptibility of *E. coli* against the alcoholic extract of *Tamala patra* is fairly evident between 20-16hence, it is considered as sensitive.15 to 12mm is considered as moderately sensitive. Below 12mm is considered as resistant**.**Therefore, with the current study it is proven that the *E. coli* organism is sensitive to 5000 µl,4000 µl and 3000µl; moderately sensitive to 2000 µl,1000 µl whereas it is resistant to 900 µl of alcoholic extract of *Tamala patra***.**

DISCUSSION

In the present study, 50 subjects with *pittaja mutrakrichra* (Urinary tract infection) were screened. Among them, 30 subjects fulfilled the diagnostic inclusion criteria, and the remaining 20 subjects were excluded. Among them, 4 subjects presented with chronic kidney failure, and 16 samples had bacteria other than *E. coli* on culture.

Tamala patra the drug possesses *krimigna* and *basthikandutridoshagna* action mentioned in *Raja nigantuand Madanadi nighantu.* And it contains *Madhura* and *tikta rasa*; *teekshna*, *laghuguna,ushnavirya* and *Madhura vipaka*. It is *tridoshagna,basthi dosha hara*, *Krimigna*, and *Kandugna*[8,9].In the present study,the cold maceration method was selected as it is easy to perform. Alcoholic extract of *Tamala patra* has many phytochemical constituents that alter the surface tension of the extracellular medium of organism cell, complexing with soluble and extracellular proteins and obtruding with DNA of the organism that contributes to the sensitivity of the bacterium against the extract. Various strains of gram-negative bacteria have antimicrobial effects including inhibition of various cellular processes followed by an increase in plasma membrane permeability and finally, ion leakage from the cells[10]. Different concentrations of alcoholic extract of *Tamala patra* showed different zone of inhibition. It is because different components diffuse at different rates that produce varying zones of inhibition against *E. coli*bacteria. In a higher concentration of alcoholic extract, the drug content is more, hence showing a significant zone of inhibition.

CONCLUSION

In the current study, it is proven that the mean zone of inhibition of alcoholic extract of *Tamala patra* (*Cinnamomum tamala*) contains antimicrobial action against *E. coli* obtained from the urine sample of patients diagnosed with *pittaja mutrakrichra* (Urinary tract infection). It is also proven that as the concentration of alcoholic extract of *Tamala patra* (*Cinnamomum tamala*) increases the zone of inhibition of *E. coli* also increases.

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