EVALUATION OF THE ANTIBACTERIAL ACTIVITY OF BARK OF LITCHI CHINENSIS AGAINST ESCHERICHIA COLI, A UTI CAUSING ORGANISM

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Abstract: Main focus of present study was to screen the UTI patients, classification of patient on the basis of sex, age and antimicrobial activity of different ethanol, aqueous extracts of bark of Litchi chinensis L. against Escherichia coli. Agar well diffusion method was used to evaluate antibacterial activity against E. coli. Result suggested that Ethanol extract of Litchi chinensis shows more antibacterial activity as compared to aqueous extract, and norfloxacin against E. coli. On the basis of microbial count in urine sample, 30 out of 97 suspects were UTI positive. 70% females were UTI positive. Most infections were seen in age group of 16-30yr in both male (13.3%) as well as female (30%). Ethanol extract (30mg/ml) showed 31.86% more inhibition zone as compared to norfloxacin (30mg/ml). Aqueous extract (30mg/ml) also showed 23.56% more inhibition zone as compared to norfloxacin (30mg/ml)

Keywords: Litchi chinensis, Antibacterial, E. coli, UTI

INTRODUCTION

India enjoys the privilege of having time tested traditional system of medicines based on the natural products. Plants are being used as medicines by mankind since the ancient times and they are being taken as a good source of drugs (Deshwal and Siddiqui, 2011a). The word Ayurveda is derived from Ayus (r) meaning life, and Veda, meaning knowledge, thus Ayurveda literally means science of life and it is the ancient Indian system of healthcare and longevity (Sukh dev, 2006). Development of antibiotic resistant strains has forced scientists to search for new antimicrobial substances from various sources as novel antimicrobial chemotherapeutic agents (Singh et al., 2010). Urinary tract infections (UTIs) are serious problems (Jacobsen et al., 2008). Uropathogenic Escherichia coli (UPEC) is the most common etiologic agent, responsible for 80 to 85% of community- acquired UTIs (Ronald et al., 2001).

The lychee (Litchi chinensis), a member of the Sapindaceae family, has its origin in China and is now widely spread in the tropical and subtropical regions of the world (Menzel, 1983). The lychee is one of the best fruit trees growing in the subtropics. Bhoopat et al. (2011) explained the antioxidant properties of the Gimjeng and Chakapat lychees as evidenced by the vitamin C and phenolic compounds, anti-lipid peroxidation and anti-apoptosis could explain the hepatoprotective effects in CCl(4)-induced hepatotoxicity. Besra et al. (1996) conducted pharmacological studies on Litchi chinensis and observed that extract was found to possess antiinflammatory, analgesic and antipyretic activity. Deshwal and Vig (2011) reported that Aqueous, ethanol and chloroform extracts of Tribulus terrestris seeds showed more inhibition zone as compared to norfloxacin. So, aim of present study was to screen the UTI patients, classification of patient on the basis of sex and age and antimicrobial activity of different ethanol, aqueous extract of bark of Litchi chinensis L. against Escherichia coli.

MATERIAL AND METHOD

Isolation of microorganism: Urine sample was collected in a sterilized container from a patient suffering from urinary tract infection. The mid-stream urine was collected after carefully cleaning the genitalia and mid-stream urine was collected because the first portion of urine may contain most of contaminants. Pathogens were isolated and counted by standard plate method and MacConkey agar without crystal violet and Blood agar medium was used for isolation of various pathogens. The plates were incubated at 37°C for 24-48h.

Characterization of pathogens: Thirty suspects out of 97 samples were UTI positive and were analyzed for present study. Pathogens were characterized according to Bergey’s Manual of Determinative Bacteriology (Holt et al., 1994).

Preparation and selection of different extracts:
Two extracts such as aqueous extract, ethanol extract were selected for present study:
(a) Preparation of Aqueous extracts: 100g dried finely powdered bark of plant were infused in distilled water until completely exhausted. The extract was then filtered using Whatman No. 1 filter paper and the filtrate was evaporated and dried using rotary evaporator at 60°C. The final dried samples were stored at low temperature.
(b) Preparation of Ethanol extracts: Dried bark of plant was grounded and extracted in a percolator with 95% ethanol. About 10ml of ethanol per gram of sample was used. The ethanol extract was dried
under reduced pressure at 40°C. The dried extract was stored in sterile bottles for further use.

**Sterilization and preparation of different concentration of extract:** The dried extracts were exposed to ultra violet light (UV rays) for 24h to sterilize (Ekwenny and Elegalam, 2005). Liquid extracts were sterilized using a membrane filter (0.2µm sterile filter). Dry powder extracts were initially dissolved in 1ml of dimethyl sulfoxide (DMSO). Different dilutions of extract were prepared. Norfloxacin antibiotic worked as control drug.

**Antibacterial activity of plant extract:** Antibacterial activity was performed according to Deshwal and Vig (2011a). The microorganism was activated by inoculating a loopful of the strain in nutrient broth (30ml) and incubated on a rotary shaker. Then 0.2ml of inoculum (inoculum size was 10⁸ cells/ml as per McFarland standard) was inoculated into the molten Muller Hinton agar media and after proper homogenization it was poured into the Petri plate. For agar well diffusion method, a well was made in the seeded plates with the help of a sterilized cup-borer. 20µl test compound was introduced into the well and the plates were incubated at 37°C for 24 h. Microbial growth was determined by measuring the diameter of zone of inhibition.

**RESULT AND DISCUSSION**

On the basis of microbial count in urine sample, 30 out of 97 suspects were UTI positive. Isolated strains were characterized on the basis of Biochemical test and founded that strains were of *Escherichia coli*. Similar observation has been mentioned in Bergey’s manual of determinative bacteriology (Holt et al., 1994).

UTI patients were divided into age and sex. Four group of age were formed and these were 1-15yr, 16-30yr, 31-45yr and grater then 45. In all case, 70% females were UTI positive. Most infection was seen in 16-30yr in both male (13.3%) as well as female (30%) (Table-1). Our results showed that isolated strains were *E. coli* and infection was more common in female as compared to male. It could be possible because in females, the urethra is short and the area around the urethral opening is densely colonized with potential pathogens that make the females susceptible to UTI. Other scientists also observed the same results (Williams et al., 2006; Hansson and Jodal, 2004).

Further, present study showed that bark of *Litchi chinensis* showed antibacterial activity. Antibacterial activity of water extract of *Litchi chinensis* was tested against *E. coli* culture at different concentration. The various concentrations of water extract of *Litchi chinensis* that were used were 15mg/ml, 20mg/ml, 25mg/ml and 30mg/ml. Ethanol extract showed more inhibition zone as compared to aqueous extract and norfloxacin antibiotics. Ethanol extract (30mg/ml) showed 31.86% more inhibition zone as compared to norfloxacin (30mg/ml). Aqueous extract (30mg/ml) also showed 23.56% more inhibition zone as compared to norfloxacin (30mg/ml) (Table 2). Similarly, Ekwenny and Elegalam (2005) reported that ethanolic extract of ginger (*Zingiber Officinale*) inhibited growth of *E. coli*. Further, Parekh et al. (2005) reported that methanolic extract of medicinal plant extract was effective against 5 medically important bacterial strains. Many researchers observed that extract of medicinal plants inhibited the growth of human pathogenic bacteria (Indul et al., 2006; Nair and Chanda, 2007; Al-Bayati and Al-Mola, 2008; Elekwa et al., 2009; Deshwal and Vig, 2011a). Getachew et al. (2011) observed antibacterial activity of volatile fractions from *Artemesia abyssinthium*, *Croton macrostachyus*, *Echinops kebericho* and *Satureja punctata* tested against selected Gram-positive and Gram-negative bacterial strains. Recently, Gillitzer et al. (2012) reported the antimicrobial activity of *Betula papyrifera* and *Rhus typhina* against various pathogens.

All above facts and figures suggested that water and ethanol extract of *Litchi chinensis* inhibited the growth of *E. coli*. Further, other researches suggested that medicinal plants are good substitute of chemical drugs.

<table>
<thead>
<tr>
<th>S.No.</th>
<th>Age (years)</th>
<th>Sex</th>
<th>Concentration</th>
<th>Inhibition zone*</th>
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<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>water extract</td>
<td><em>Litchi chinensis</em> Norfloxacin</td>
</tr>
<tr>
<td>1</td>
<td>1-15</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>2</td>
<td>16-30</td>
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<td>Total</td>
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**Table 1. Distribution of patients according to age and sex**

**Table 2. In vitro antibacterial activity of water, ethanol extract of *Litchi chinensis* on the growth of *E. coli***
REFERENCES


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<tr>
<td>1</td>
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<td>16.1±0.8 mm</td>
<td>17.5±0.5 mm</td>
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<tr>
<td>2</td>
<td>20 mg/ml</td>
<td>19.3±0.4 mm</td>
<td>20.8±0.6 mm</td>
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<td>3</td>
<td>25 mg/ml</td>
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<td>23.6±0.5 mm</td>
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<tr>
<td>4</td>
<td>30 mg/ml</td>
<td>25.2±0.4 mm</td>
<td>26.9±0.5 mm</td>
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*Values are mean of 3 replicate ± SD