A preliminary study on the antibacterial effects of ethanolic extract of *Psidium guajava* leaves on bacteria isolated from urinary tract infection with special reference to *Escherichia coli* and *Staphylococcus aureus*

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Abstract
Increasing bacterial resistance against antibiotics, used in urinary tract infection (UTI) is an emerging problem for human health. Therefore, this study was aimed at using ethanolic extract of leaves of *Psidium guajava* (guava) and assessing their effect in vitro on bacterial agents causing urinary tract infections, and also compared the effect of these extracts with common antibiotics being used in the treatment of urinary tract infection.

In an experimental design, the *Escherichia coli* (*E.coli*) and *Staphylococcus aureus* (*S. aureus*) were isolated from the urine of UTI patients. Antibacterial effects of the leaves extract against these bacteria were evaluated by well diffusion method and resistance of antibiotics against isolates was evaluated by using hexa UTI disc 4 (Himedia pvt limited). Ampicillin, gentamycin, nitrofurantoin, ciprofloxacin, nalidixic acid, co-trimoxazole were the antibiotics present in hexa UTI disc-4, tested against *E. coli* and *S. aureus*. Among these ciprofloxacin showed greater zone of inhibition compared to ethanolic extract of guava leaves.
Today, detection of antibiotic resistance against isolates is important in prevention and control of infection. In this study, the extracts of *Psidium guajava* were found to be the second higher antibacterial agent against isolates.

**Keywords:** *Psidium guajava*; Urinary Tract Infection; Antibacterial agents; Antibiotic resistance.

### INTRODUCTION

Different parts of plants are good source of potent and powerful drugs and are used in medicine in different countries. Their extracts are used as raw medicine and believed to be the important source of new chemical substances with potential therapeutic properties. Phytochemicals produced by plants could be used directly for the production of new drugs. The phytomedicines are believed to be more acceptable by the human body, as compared to modern synthetic drugs (Chandra, 2013).

Extraction of complete phytoactive compounds from plant material is mostly dependent on the type of solvents being used in the extraction procedure. Mostly used organic solvents are ethanol, acetone, and methanol to extract phytocompounds from plants (Wendakoon et al., 2012).

*Psidium guajava* or guava is a plant, belongs to family Myrtaceae, native to tropical America and now cultivated in many tropical and subtropical countries as their its edible fruit (Perez et al., 2008). Guava leaves contain many chemical constituents such as comarins, essential oils, flavonoids, triterpenes etc., which are known to have antimicrobial properties. The leaves of *Psidium guajava* tree have a long history of medicinal use against microbes and they are still begin employed today inspite of with the lack of proper knowledge and literature (Wei et al., 2000).

Today the most common infection in women is Urinary tract infection (UTI), affecting throughout their lifespan (Sharma et al., 2009). It could even affect the kidneys, ureters, bladder or urethra and considered as one of the most common infections in humans especially in women (Geetha et al., 2011).

The present work was undertaken to study antibacterial properties of ethanolic extract of leaves of *Psidium guajava* against *Escherichia coli* and *Staphylococcus aureus* isolated from urine of UTI patients and compared with that of available drugs.

### MATERIAL AND METHOD

**Collection of urine sample:** Urine samples were collected from clinical laboratories of Gwalior (M.P.) for isolation of the affecting agents, from 50 subjects who were clinically suspected to suffer from urinary tract infection.
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**Isolation and identification of bacteria:** First, the microorganisms present in urine samples of UTI infected patients were cultured on nutrient agar medium and the morphology and colony characteristics of organisms were studied for the shape, size, odour, margin and surface characteristics.

Gram staining procedure was adopted to differentiate between gram positive and gram negative organisms. Selective agar medium like MacConkey and EMB were used broadly for further identification of *E. coli*.

After phenotypical identification of bacteria, further confirmation was done on the basis of their biochemical characterization *viz* amylase production, hydrolysis of gelatin, IMViC test, catalase test, skim milk test, carbohydrate fermentation test.

**Collection of plant material:** Plant material was collected from the garden area of Vijayaraje Institute of Science and Management, Gwalior (M.P.)

**Ethanolic Extraction of Medicinal Plants:** The plant part *viz* leaves were washed with distilled water, dried in the air and pulverized. Twenty grams of pulverized material was used for extraction with 99.9% ethanol using Soxhlet's apparatus. The extract was kept overnight in oven at 50°C to evaporate excess amount of ethanol. Hundred milligram of residue of ethanolic extract was dissolved in 10 ml of ethanol to get final concentration of 1%.

**Testing of antimicrobial activities:** Antimicrobial activity was studied by well agar plate diffusion method according to Pandey *et al.*, (2011). Antimicrobial activity test of ethanolic extract of *Psidium guajava* leaves was carried out against *Escherichia coli* and *Staphylococcus aureus* along with chosen antibiotics for their comparative study.

**RESULTS**

Results of the present study showed that the ethnolic extracts of *Psidium guajava* showed notable antibacterial activity against *Escherichia coli* and *Staphylococcus aureus*. When tested by the disc diffusion method, the ethanolic extracts of the leaves of *Psidium guajava* showed significant inhibitory activity against both the above microorganisms when compared to antibiotics present in Hexa UTI- 4 disc, which is mostly being used against UTI infections.

*Psidium guajava* showed maximum zone of the inhibition against *E.coli* with 10 mm diameter as compared to Ampicillin, gentamycin, nitrofurantoin, nalidixic acid and co-trimoxazole with 4mm, 7mm, 5mm, 5mm, 6 mm zone of inhibition respectively and is next to ciprofloxacin with 15 mm diameter. It was also observed that *Psidium guajava* leaves extract worked against *Staphylococcus aureus*. In case of *Staphylococcus aureus*, ampicillin, gentamycin, ciprofloxacin, , co-trimoxazole was
showed 7mm, 8mm, 8mm, 10mm respectively where as *Psidium guajava* showed 7mm zone of inhibition which is greater than nitrofurantoin and nalidixic acid which did not show any zone of inhibition at all.

### Table 1: Activity of several antibiotics and *Psidium guajava* leaves extract against *Escherichia Coli* and *Staphylococcus aureus*

<table>
<thead>
<tr>
<th>S.No.</th>
<th>Name of the antibiotics and plant material</th>
<th>Zone of Inhibition (mm)</th>
<th></th>
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<tbody>
<tr>
<td></td>
<td></td>
<td><em>Escherichia coli</em></td>
<td><em>Staphylococcus aureus</em></td>
</tr>
<tr>
<td>1.</td>
<td>Ampicilin (AMP)</td>
<td>4 mm</td>
<td>7 mm</td>
</tr>
<tr>
<td>2.</td>
<td>Gentamicin (GEN)</td>
<td>7 mm</td>
<td>8 mm</td>
</tr>
<tr>
<td>3.</td>
<td>Nitrofurantoin (NIT)</td>
<td>5 mm</td>
<td>0 mm</td>
</tr>
<tr>
<td>4.</td>
<td>Ciproflaxacin (CIP)</td>
<td>15 mm</td>
<td>8 mm</td>
</tr>
<tr>
<td>5.</td>
<td>Nalidixic acid (NA)</td>
<td>5 mm</td>
<td>0 mm</td>
</tr>
<tr>
<td>6.</td>
<td>Co-trimoxazole (COT)</td>
<td>6 mm</td>
<td>10 mm</td>
</tr>
<tr>
<td>7.</td>
<td><em>Psidium guajava</em> (Guava)</td>
<td>10 mm</td>
<td>7 mm</td>
</tr>
</tbody>
</table>

**Fig. 1. Comparison between antibiotics and ethanolic leaves extract of *Psidium guajava* against UTI infecting agents**

**AMP:** Ampicilin  
**GEN:** Gentamicin  
**NIT:** Nitrofurantoin  
**CIP:** Ciproflaxacin  
**NA:** Nalidixic acid  
**COT:** Co-trimoxazole  
**Guava:** *Psidium guajava*
DISCUSSION

Recently many scientists have showed their interest in the use of different plant parts as alternative agents to control the pathogenic and antibiotic resistant microorganisms (Aqil et. al., 2005, Nostro et. al., 2006). The increasing drug resistance of many pathogens is a serious problem in developing countries like India (Gopalakrishna Sarala et. al., 2010). Many reports are available on the antiviral, antibacterial, antifungal, anthelmintic, antimolluscal and anti inflammatory properties of plants and plants products (Mahesh and Satish, 2008).

Urinary tract infections (UTI) are common condition in the world and the agents of UTI showed day by day increasing resistance against available drugs. We conducted this work to isolate different agents of UTI from several patients and assessed their antibiotic susceptibilities. E. coli was the most predominant bacteria involved in UTI. There are earlier studies which are in agreement with the present findings. Escherichia coli is recognized as most important agent followed by Staphylococcus saprophyticus and Klebsiella pneumoniae. Several reports are also in argument with the findings of UTI agent detection (David and vrahas 2000; Ronald 2001; Gales et al., 2002).

The results obtained in the present study indicated that ethanolic extract of Psidium guajava leaves exhibited more inhibitory effect as compared to ampicillin, gentamycin, nitrofurantoin, nalidixic acid and co-trimoxazole and is second to ciprofloxacin. Because of the increase in the level of antibiotic resistance, some herbal medicinal plant extracts could be considered as new source of material for the treatment of patients suffering from the infection. It is also reported that plant extracts could eliminate chemotactic behavior of these agents with less possibility of causing side effects.

The plants extracts have no or minimum side effects so plant parts may be one of our choices because it contains hydrophobic liquid which can be easily extracted by the process of distillation. Plant products or oils contain volatile aroma and phytochemicals which show the antimicrobial activity. More over plants can be grown easily and proportion of their products is less sophisticated and expenditure of their product is bearable by common people than using antibiotics. Therefore more attempts may be made towards the development of effective natural, non-toxic drug for treatment of UTI.

CONCLUSION

The present study was undertaken to identify effective herbal medicines to control UTI caused by bacterial organisms. Results shown that the extract of guava plants origin has remarkable antibacterial activity as compared to antibiotics therefore can be...
used by all human beings as these are easily available in our environment, less expensive as well as safe.

It is also envisaged that further work should be done in this direction.

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REFERENCES
A preliminary study on the antibacterial effects of ethanolic extract of Psidium...


