SHORT COMMUNICATION

Antimicrobial Activity of Piper betle, Ocimum sanctum and Trachyspermum ammi Leaves Against Enteropathogens: A Comparative Study

Deepali Shukla1, Vivek Kumar Shrivastav2, Piyush Shukla3, Jayandra K. Johri4* DOI: 10.18811/ijpen.v7i02.9

ABSTRACT

Food-borne infections are common in humans by several factors. Microorganisms such as bacteria, viruses, and parasites are involved in common food-borne infections. *Escherichia coli* and *Staphylococcus* are notably associated bacteria with food-borne illness or gastrointestinal tract-related disease. Prevention or treatment of bacterial-associated food poisoning is achieved by antibiotic therapy, which has many negative impacts on human health. Such concern makes it necessary to find out healthy, safer, and natural alternatives to combat the disease. In Indian culture, Ayurveda always suggested chewing some medicinal herbal leaves for the betterment of gastrointestinal disease. In this study, herbal extract of three Indian traditional plants viz., *Piper Betle, Ocimum sanctum Linn*, and *Trachyspermum ammi* were evaluated for the antimicrobial property against four enteropathogenic bacteria, i.e., *E. coli, Salmonella sp.*, *Staphylococcus sp.*, and *Bacillus cereus*, by disc diffusion technique. Ethanolic extract of herbs showed variable activities against four bacteria, and all extracts can potentially inhibit the growth of enteropathogens. Results prove that these herbal extracts can be used as a natural alternative against pathogens and harmless antimicrobials for humans.

Keywords: Antimicrobial activity, Entero-pathogens, Herbal Extract.

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INTRODUCTION

Bacterial originated food-borne disease is common worldwide, mostly found in developing countries due to poor hygiene that causes common illness and death (Newell et al., 2010). Food poisoning is mainly associated with numerous gram-negative bacteria like *E. coli, Pseudomonas* sp., *Salmonella typhi*, and gram-positive bacteria like *S. aureus*, and *B. cereus* (Mith et al., 2014). Antibiotic is a common treatment of bacterial-associated food-borne illness. Consumption of antibiotics also leads to health disorders like kidney and liver dysfunction. Many factors also lead to developing drug resistance against the antibiotic (Coates et al., 2002).

Indian Ayurveda and other natural medicine related literature documents, that several herbs used for chewing have enormous medicinal properties and are important for the human gastrointestinal tract (Sarkar et al., 2015). Most traditional Indian plants prove that they have novel antimicrobial properties against several pathogens and these are also safe for human health (Bag et al., 2012). Keeping this in mind, this study was undertaken to evaluate the antibacterial activity of leaves of three herbs, viz., Ajwain, Paan, and Basil, which are routinely used in our culture for chewing purposes against food-borne pathogens (Nazar et al., 2019).

Ajwain, scientifically known as *Carum coticum* is an aromatic herb that belongs to the family *Apiaceae*. It is grown in India and other Asian countries (Piri et al., 2020). Ajwain has been commonly used as a spice worldwide. Seeds of carom are traditionally used as herbal medicine from ancient times, and it is said that carom seed is good for the digestive system and liver disorder (Dhiman et al., 2014). Leaf of Ajwain contains many phenolic compounds like thyme and carvacrol, which exhibit many medicinal properties (Asif et al., 2020).

P. betle also termed as Paan belongs to the family Piperaceae. Leaves of *P. betle* gain an auspicious place in Indian rituals or traditions (Das et al., 2016). Many ancient Indian literatures like Charak, Shushrut Sahinta, and Ayurveda reported the various medicinal properties of the *P. betle* (Johri et al., 2018). Scientifically betel leaf is rich in various bioactive compounds like Eugenol, Chavicol, and Hydroxy Chavicol that have many medicinal properties for treating digestive disorders (Shukla et al., 2018).

Basil plant, scientifically known as *O. sanctum*, is considered a holy plant in India belongs to the family Lamiaceae (Joseph and Nair, 2013). Leaves of basil contain valuable essential oils and flavonoids. The medicinal properties of basil are exploiting worldwide. In Ayurveda, it is also...
Antimicrobial Activity of herbal extracts Against Entero-pathogens

Table 1: Employed data of botanical species

<table>
<thead>
<tr>
<th>Plant species</th>
<th>Family</th>
<th>Local name</th>
<th>Common name</th>
<th>Plant part used</th>
<th>Extract PH</th>
<th>Extract yield (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Betel Vine</td>
<td>Piperaceae</td>
<td>Paan</td>
<td>Betel</td>
<td>Leaf</td>
<td>5.6</td>
<td>2.5</td>
</tr>
<tr>
<td>O. sanctum</td>
<td>Lamiaceae</td>
<td>Tulsi</td>
<td>Basil</td>
<td>Leaf</td>
<td>5.2</td>
<td>3.7</td>
</tr>
<tr>
<td>T. ammi</td>
<td>Apiaceae</td>
<td>Ajwain</td>
<td>Carom</td>
<td>Leaf</td>
<td>6.0</td>
<td>4.3</td>
</tr>
</tbody>
</table>

Fig. 1: Picture of all three employed plants

considered as good for the gastrointestinal tract (Nahak et al., 2011).

Material and Methods

Plant Material Collection and Extract Preparation
Leaves of the selected plant were collected from the medicinal garden of IPS Academy, Indore. Young leaves of all plants were collected to get high metabolite content (Ghasemzadeh, Ali et al., 2016). After that, leaves were washed and rinsed with distilled water and allow to dry in shade conditions, Fig. 1 shows the picture of leaves. Then after drying leaf material of all plants was crushed separately and make fine powder and passed the powder through a sieve.

For the preparation of ethanolic extract, 25 g of each leaf powder were soaked in 100 mL ethanol for 48 hours with continuous stirring, filtered with the muslin cloth, and centrifuge the filtrate at 10000 rpm for 10 minutes filter it again to attain the clear filtrate. The filtrate was placed in glass material and allow to drying by evaporation at 40°C by using a rotary evaporator. The net yield Weight of leaf extract was calculated according to Mostafa et al., 2018 (Table 1 shows net yield of herbal extracts) and stored in small glass bottles at 4°C.

Inoculum Preparation
Clinical strains of E. coli, Salmonella sp., Staphylococcus sp., and Bacillus cereus were isolated from the department of microbiology, IPS Academy Indore. Stock cultures were maintained at 4°C on nutrient agar slant. For active culture preparation, each bacterium was sub-cultured for 24 hours at 37°C in an MHA slant. To obtain the desirable cell count of bacteria, cells of each culture were suspended in freshly prepared nutrient broth at 37°C for 6–8 hours, and optical density should reach to 0.6.

Antibacterial Activity of Leaf Extract
For the Evaluation of in vitro antibacterial activity of all three ethanolic plant leaf extract against pathogenic bacteria associated with gastrointestinal disease, i.e., E. coli, Salmonella sp., S. aureus, and B. cereus disc diffusion method or also known as the Kirby Bauer method, were used (Monte et al., 2014).

Results and Discussion

In the present study, the antimicrobial efficacy of three herbal plant leaf extracts was evaluated against enteropathogens responsible for several food-borne infections (Mostafa et al., 2018). Results of the study show the agreement that herbs having good antimicrobial properties against test pathogens. The antibacterial activity of ethanolic extract of P. betle, Ocimum sp., and Ajwain leaf was evaluated against pathogenic bacteria. 25 ug/mL of Tetracycline was taken as a standard and 100 percent ethanol as the negative control.

Paan is used in several Indian rituals, is a rich source of antioxidants like chavicol, hydroxychavicol, eugenol, leaf of betle is rich in several flavonoids and alkaloids (Johnri et al., 2018). The present study demonstrates that P. betle, L. leaf extract inhibit all four enteric pathogens growth at different concentrations. 100mg/ml concentration of P. betle leaf extract gives maximum inhibition against the pathogen E. coli, Salmonella sp., B. cereus, and S. aureus with the zone of inhibition of 14, 15, 15, and 15.5 mm, respectively (Table 2, Figure 2 & 3). work done by Sarma et al., 2018 and Lubis, 2020 against Staphylococcus and another...
bacterial pathogen also reveal that in the extract of P. betle leaf can inhibit the growth of pathogens as present work shows.

Tulsi, scientifically known as Ocimum, counted as a holy plant. A biochemical study reveals that tulsi leaves contain many active compounds with antimicrobial, antiparasitic, antiviral, and antioxidant properties (Pattanayak et al., 2010). Tulsi is frequently used as an edible agent in many rituals; keeping this in mind, the study was planned against enteropathogens. The present study reveals ethanolic extract of Ocimum leaf extract is also a good inhibitor of bacterial enteropathogens. 100 mg/mL of Ocimum leaf extract gives a zone of inhibition of 15, 16, 14, and 16.5 mm against all the test pathogens (Table 2, Figure 2 & 3). Shokrian, 2016 also shown that ajwain leaf having a good antibacterial property.

Results of all three herbal leaf extracts show a zone of inhibition against both gram-positive and gram-negative bacteria results are given in Table 2, and figure 2 demonstrate that all three plants extract to give a different zone of inhibition against pathogens, but all extracts are potentially effective to inhibit the growth of taken bacteria.

**Table 2:** Antimicrobial activity of A) P. betle B) Ocimum sp. and C) Ajwain leaf extracts

<table>
<thead>
<tr>
<th>Bacterial strain</th>
<th>Zone of inhibition in mm</th>
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<tbody>
<tr>
<td></td>
<td>10 mg/mL</td>
</tr>
<tr>
<td><strong>A. Piper betle leaf extract</strong></td>
<td></td>
</tr>
<tr>
<td>E. coli</td>
<td>6.33 ± 0.5</td>
</tr>
<tr>
<td>Salmonella sp.</td>
<td>9.33 ± 0.02</td>
</tr>
<tr>
<td>Bacillus cerus</td>
<td>10.6 ± 0.1</td>
</tr>
<tr>
<td>Staphylococcus</td>
<td>9.22 ± 0.03</td>
</tr>
<tr>
<td><strong>B. Ocimum leaf extract</strong></td>
<td></td>
</tr>
<tr>
<td>E. coli</td>
<td>-</td>
</tr>
<tr>
<td>Salmonella sp.</td>
<td>7.99 ± 0.02</td>
</tr>
<tr>
<td>Bacillus cerus</td>
<td>-</td>
</tr>
<tr>
<td>Staphylococcus</td>
<td>7.67 ± 0.03</td>
</tr>
<tr>
<td><strong>C. Carom leaf extract</strong></td>
<td></td>
</tr>
<tr>
<td>E. coli</td>
<td>9.66 ± 0.01</td>
</tr>
<tr>
<td>Salmonella sp.</td>
<td>8.5 ± 0.02</td>
</tr>
<tr>
<td>Bacillus cerus</td>
<td>-</td>
</tr>
<tr>
<td>Staphylococcus</td>
<td>7.67 ± 0.03</td>
</tr>
</tbody>
</table>

**Conclusion**

In recent years health research inclined to observe traditional therapies of herbal products because of their safe and eco-friendly health benefits. The present study evaluated some Indian traditional herbs against enteropathogens. Observation shows that all three herbs used as an edible agents in routine life having good antibacterial properties. This study is preliminary level work that needs to be finishing and purification of the active compound having medicinal value. Quantification and
identification of toxicity of active ingredient is also necessary. Result suggest that the use of herbal extract is more cost-effective and harmless to humans.

References


