SHORT COMMUNICATION

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

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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 cerus,* 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.

Kewwords: 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 copticum* 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).

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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

Table 1: Employed data of botanical species

Plant species	*Family*	*Local name*	*Common name*	*Plant part used*	*Extract PH*	*Extract yield (%)*
Betel Vine	Piperaceae	Paan	Betel	Leaf	5.6	2.5
O. sanctum	Lamiaceae	Tulsi	Basil	Leaf	5.2	3.7
T. ammi	Apiaceae	Ajwain	Carom	Leaf	6.0	4.3

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 shady 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).

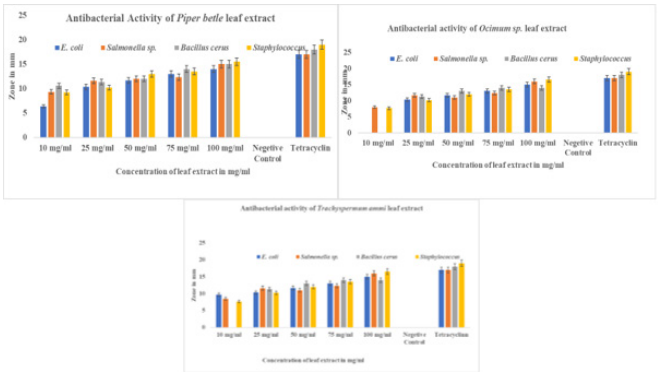

Fig. 2: Graph shows zone of inhibition of the antimicrobial activity of plant leaf extracts against pathogens

Muller Hinton agar plates were prepared by pouring 15 mL of molten agar on a sterile Petri dish, and then after, plates were allowed to solidify. 100 microlitres of inoculum suspension were swabbed uniformly in each plate and stand for 15 minutes. Different dilutions of each extract from the initial concentration to the final were loaded in 8 mm sterile filter paper disc. Ethanol disk was taken as a negative control. The loaded disc was placed on the surface of the medium. The compound was allowed to diffuse in an agar plate for 5 minutes and the plate was incubated at 37°C for 24 hours. After incubation inhibition zone was measured with the ruler, all studies were performed in triplicate.

Statistical Analysis

All the experiments were performed in triplicate data represented in the form of mean values and calculated using MS excel 2010 software.

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 (Johri *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. cerus,* 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

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

	Zone of inhibition in mm						
Bacterial strain	*10 mg/mL*	*25 mg/mL*	*50 mg/mL*	*75 mg/mL*	*100 mg/mL*	*Negetive control*	*Antibiotic*
A. Piper betle leaf extract							
E. coli	6.33 ± 0.5	10.33 ± 0.01	11.67 ± 0.5	13 ± 0.25	14 ± 0.5	0	17
Salmonella sp.	9.33 ± 0.02	11.6 ± 0.04	12 ± 0.6	12.33 ± 0.15	15 ± 0.25	0	17
Bacillus cerus	10.6 ± 0.1	11.33 ± 0.1	12 ± 0.1	14 ± 0.1	15 ± 0.15	0	18
Staphylococcus	9.22 ± 0.03	10.22 ± 0.9	13 ± 0.12	13.5 ± 0.2	15.5 ± 0.1	0	19
B. Ocimum leaf extract							
E. coli	-	10.33 ± 0.01	11.67 ± 0.5	13 ± 0.25	15 ± 0.2	0	17
Salmonella sp.	7.99 ± 0.02	11.6 ± 0.04	11 ± 0.6	12.33 ± 0.15	16 ± 0.25	0	17
Bacillus cerus	-	11.33 ± 0.1	13 ± 0.1	14 ± 0.1	14 ± 0.15	0	18
Staphylococcus	7.67 ± 0.03	10.22 ± 0.9	12 ± 0.12	13.5 ± 0.02	16.5 ± 0.01	0	19
C. Carom leaf extract							
E. coli	9.66 ± 0.01	10.33 ± 0.01	11.67 ± 0.5	13 ± 0.25	15 ± 0.2	0	17
Salmonella sp.	8.5 ± 0.02	11.6 ± 0.04	11 ± 0.6	12.33 ± 0.15	16 ± 0.225	0	17
Bacillus cerus	-	11.33 ± 0.1	13 ± 0.1	14 ± 0.1	14 ± 0.15	0	18
Staphylococcus	7.67 ± 0.03	10.22 ± 0.9	12 ± 0.12	13.5 ± 0.2	16.5 ± 0.1	0	19

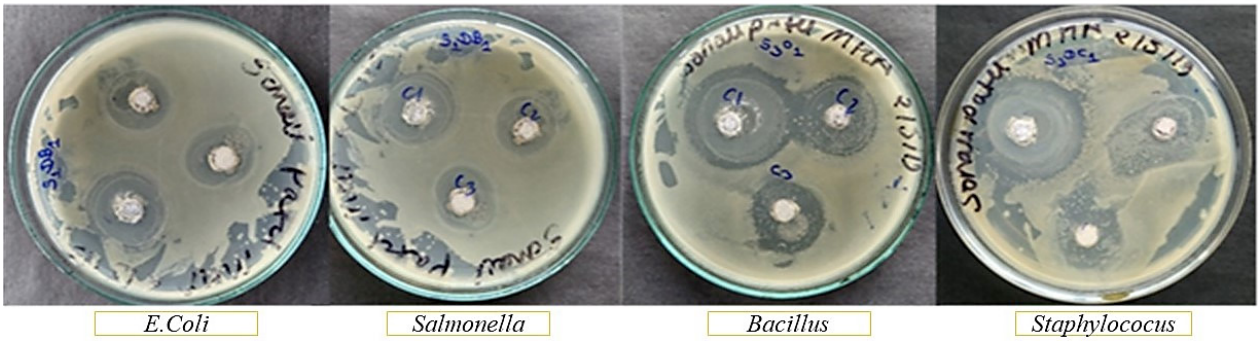

Fig. 3: Antimicrobial activity of plant leaf extracts against pathogens

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 inhibiter of bacterial enteropathogens. 100 mg/ mL of *Ocimum* leaf extract gives 15, 16, 14, and 16.5 mm zone of inhibition against pathogen *E. coli, Salmonella sp., B. cerus*, and *S. aureus,* respectively (Table 2, Figs. 2 and 3). Goyal *et al*.,2011 also shown that leaf extract of tulsi can inhibit gram-positive and gram-negative bacteria.

Carom leaf or ajwain leaf is used as an edible and flavoring agent. Traditionally this plant is used in gastric problems, the plant having as rich flavonoids and phenolic compounds (Rajeshwari *et al*., 2011). This study finding reveals that the extract of ajwain leaf extract also gives good inhibition against all four enteropathogens. Results show maximum antibacterial activity was found as the concentration of 100 mg/mL, which 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.

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.

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