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**ANTIBACTERIAL ACTIVITY OF LEAF EXTRACTS OF SELECTED  
MEDICINAL PLANTS *PIPER NIGRUM*, *PIPER LONGUM* AND  
*ADHATODA VASICA***

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**ABSTRACT**

The present study aimed at evaluating the antibacterial activity of ethanolic extracts of leaves of *Piper nigrum*, *Piper longum* and *Adhatoda vasica* against *Streptococcus mutans*, *Staphylococcus aureus*, *Bacillus subtilis*, *Escherichia coli*, *Klebsiella pneumoniae*. The experiment was carried out by disc diffusion method and antibiotic sensitive method. The results revealed that the ethanolic extract of leaf presented the highest zone of inhibition in *Bacillus subtilis*, *Streptococcus mutans*. Other species show significant zone of inhibition.

**KEYWORDS:** *Piper nigrum*, *Piper longum* and *Adhatoda vasica*, antibacterial activity and zone of inhibition.



## INTRODUCTION

The development of resistance in the microorganisms is due to the use of antibiotics has created an emergency to unravel the antibacterial compounds from the plants. A medicinal plant is any plant which, in one or more of its organs contains substances that can be used for therapeutic purposes or as a chemo pharmaceutical semi synthesis. In order to improve the efficiency and ethics of modern medical practice, researchers are increasingly turning their attention to folklore medicines as a source of new drugs (Swiader *et al.*, 1997). Bioactivity of essential oils of temperate aromatic plants were revealing antibacterial, antioxidant, anti-inflammatory and other related pharmacological activities (Svoboda *et al.*, 1998). The antiseptics qualities of aromatic and medicinal plants and their extracts have been recognized antiquity, several attempts to characterize these properties in the laboratory date back (Martindale, 1910). The phytochemicals are used in the antimicrobial drugs which is been tested in humans (Clark, 1996). The plant volatile oils were used for their antibacterial properties against 25 genera of bacteria, using an agar diffusion technique (Deans *et al.*, 1987). Antimicrobials have the significant clinical value in the

treatment of resistant microbial strains. Therefore in the present study *Piper nigrum*, *Piper longum* and *Adhatoda vasica* was screened for their antibacterial activity against selected strains.

## MATERIALS AND METHODS

The leaves of *Piper nigrum*, *Piper longum* and *Adhatoda vasica* were collected from “Centre for Indian Medical Plant Heritage”, Palakkad district of Kerala state. The leaves were prepared by grinding fresh leaves with absolute alcohol (100%) in a sterile mortar and pestle in the concentration of 1g/ml. This homogenate was then centrifuged and filtered using sterile Whatmann No: 1 filter paper.

### Test microorganisms

Five clinical strains used for study were *Streptococcus mutans*, *Staphylococcus aureus*, *Bacillus subtilis*, *Escherichia coli*, *Klebsiella pneumoniae*. These cultures were collected from “Kovai Medical Centre and Hospital” Coimbatore. The stock cultures were stored in nutrient agar medium at 37°C.

### Antibacterial activity

Antibacterial activity of ethanol extract of *Piper nigrum*, *Piper longum* and *Adhatoda*



*vasica* was studied using agar disc diffusion method. Petridishes containing 20ml of nutrient agar medium were selected with 24hrs culture of a selected bacterial strain. Whatmann No: 1 filter paper discs containing 50 $\mu$ g / disc of leaf extract were placed on the surface of the medium. The petriplates were incubated at 37°C overnight. The antibacterial activity was based on the measurement of zone of inhibition observed around the discs.

#### **Diffusion method to check Antimicrobial Activity**

Nutrient agar media was prepared; 20ml of the medium was poured into sterile petriplates and allowed it to solidify. A well with diameter 0.5cm was made in the centre of the sterile nutrient agar plate using cork borer. The culture was then swabbed on the nutrient agar plates with sterile cotton swabs. A volume of 100 $\mu$ l of the various leaf extracts were added into the well and incubated at 37°C overnight. Control maintained by using absolute alcohol (100%) instead of leaf extract in the same plate.

#### **Antibiotic Sensitivity Assay: Kirby-Bauer method**

Using sterile aseptic technique a swab was moistened with the broth culture of the organism. It is uniformly spread over the

surface of the agar plate by moving the swab back and forth in all directions. Sterile antibiotic discs were used Forceps dipped in 70% ethyl alcohol and flamed. An antibiotic disc was placed on the surface of the swabbed plates. The Petri plates were incubated at 37°C overnight. On incubation, resistance and susceptibility were observed.

#### **a) Plant Extraction**

Ethanol extraction of medicinal plant leaves was taken by grinding fresh leaves, stems, with absolute alcohol (100%) using mortar and pestle in the concentration 1g/ml. This homogenate was then centrifuged and filtered to a sterile screw cap test tube.

#### **b) Compound Analysis**

All medicinal plants extraction and antibacterial compound analyzed from Ind-Ag Inspection and Testing Laboratory, Coimbatore.

### **RESULTS AND DISCUSSION**

Laboratory experiment was conducted to study the antibacterial activity of *Piper longum* *Piper longum* and *Adhatoda vasica*. Stem and leaves of medicinal plants have been reported to contain a variety of Phenolics, Quinones, Flavones, Tannins, Coumarins,



Terpenoids, Alkaloid which have diverse biological properties namely antibacterial activities (Trillini *et al.*,1996).The ethanolic extract of leaves of *Piper nigrum* showed significant antibacterial activity against all the bacteria tested. The maximum zone of inhibition was found more in the leaf against *Bacillus subtilis* (1.1cm) and the ethanolic extract of *Piper longum* was analysed against bacteria and the zone of inhibition was more for *Streptococcus mutans* (1.4cm) and the ethanolic extract of *Adhatoda vasica* was

analyzed against different microorganisms (Gollapudi *et al.*, 1994; Tripathi, 1979). The zone of inhibition was more for *Bacillus subtilis* (1.6cm) than the other from gas chromatography study. The gas chromatography result showed an unknown peak which not corresponding with any of is known antimicrobial compounds. The result can be compared with these medicinal plants were active against Gram positive bacteria and moderately active against Gram negative bacteria.

**Table: 1 Antimicrobial activity of *Piper nigrum***

S. No	Name of the organism	Diameter of the zone (cm)(Z1)	Diameter of the well (cm)(Z2)	Zone of inhibition (cm)(Z) $Z=Z1-Z2$
1.	<i>Streptococcus mutans</i>	1.5	0.5	1.0
2.	<i>Staphylococcus aureus</i>	1.2	0.5	0.7
3.	<i>Bacillus subtilis</i>	1.6	0.5	1.1
4.	<i>Escherichia coli</i>	-	0.5	-
5.	<i>Klebsiella pneumoniae</i>	1.0	0.5	0.5

Table: 2 Antimicrobial activity of *Piper longum*

S. No	Name of the organism	Diameter of the zone(cm)(Z1)	Diameter of the well(cm)(Z2)	Zone of inhibition (cm)(Z) $Z=Z1-Z2$
1.	<i>Streptococcus mutans</i>	1.9	0.5	1.4
2.	<i>Staphylococcus aureus</i>	1.1	0.5	0.6
3.	<i>Bacillus subtilis</i>	1.7	0.5	1.2
4.	<i>Escherichia coli</i>	1.5	0.5	1.0
5.	<i>Klebsiella pneumoniae</i>	1.0	0.5	0.5

Table: 3 Antimicrobial activity of *Adhatoda vasica*

S. No	Name of the organism	Diameter of the zone(cm)(Z1)	Diameter of the well(cm)(Z2)	Zone of inhibition (cm)(Z) $Z=Z1-Z2$
1.	<i>Streptococcus mutans</i>	1.3	0.5	0.8
2.	<i>Staphylococcus aureus</i>	1.5	0.5	1.0
3.	<i>Bacillus subtilis</i>	2.1	0.5	1.6
4.	<i>Escherichia coli</i>	1.2	0.5	0.7
5.	<i>Klebsiella pneumoniae</i>	1.5	0.5	1.0



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