

## Antibacterial Activity of *Acorus calamus* (Linn.), *Vitex negundo* (Linn.) and *Adhatoda vesica* (Nees.)

Anand Sagar\* and Shelly Rana

Department of Biosciences, Himachal Pradesh University, Shimla -171 005, India.

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There is worldwide interest in searching for the safe and effective novel antibacterial compounds of plant origin for the control of human pathogenic bacteria. In this study an attempt was made to determine the *in-vitro* antibacterial activity of leaves of *Adhatoda vesica*, *Vitex negundo* and rhizome of *Acorus calamus* against human pathogenic bacteria *Staphylococcus aureus* and *Escherichia coli*. The preliminary antibacterial activity was performed by agar well diffusion method. All test samples of aqueous extract showed inhibitory effect on both of test pathogens. Maximum activity against pathogen was found to be in *A. vesica* followed by *A. calamus* and *V. negundo*. The results obtained in the present study suggest that *A. vesica*, *A. calamus* and *V. negundo* can be used in treating diseases caused by these test organisms.

**Key words:** *Adhatoda vesica*, *Vitex negundo*, *Acorus calamus*, antibacterial activity.

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India has been using crude plants as medicine since Vedic period. A major part of total population in developing countries still uses traditional folk medicines obtained from the plant resources. Biological active compounds present in medicinal plants have always been of great interest to scientists working in this field. In recent years this interest to evaluate plants possessing antibacterial activity for various diseases is growing (Panthi and Chaudhary, 2006).

Infectious diseases are the main cause of human death world wide. Antibiotic resistance of infectious agents has become a global concern

.The clinical efficacy of many existing antibiotics is being threatened by the emergence of multidrug pathogens. Many infectious diseases are known to be treated with herbal remedies. Natural products as pure compounds or as standard plant extracts offer unlimited opportunities for new drugs because of unmatched availability of chemical diversity. Therefore, researches are increasingly turning our attention to folklore medicines, looking for new leads for developing better drugs against microbial infections. The increasing failure of chemotherapeutics coupled with antibiotic resistance exhibited by pathogenic microbial infectious agents has lead to screening of several medicinal plants for their potential antimicrobial activity (Srinivasan *et al.*, 2010). Hence the present investigations were undertaken to check the *in-vitro* antibacterial activity of three traditional medicinal plants (*Adhatoda vesica*, *Vitex negundo* and *Acorus calamus*) against two human pathogenic bacteria (*Staphylococcus aureus* and *Escherichia coli*).

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\* To whom all correspondence should be addressed.  
E-mail: as\_bio@reddifmail.com

## MATERIALS AND METHODS

### Collection and identification of traditional medicine plants

Fresh leaves of *Adhatoda vesica*, *Vitex negundo* were collected from village Kaloha of district Kangra (H.P.) whereas the rhizome of *Acorus calamus* were collected from village Kaleser of district Kangra and identified by Professor M.K. Seth, Department of Biosciences, Himachal Pradesh University, Shimla.

### Preparation of extracts

Fresh leaves of *Vitex negundo*, *Adhatoda vesica* and rhizomes of *Acorus calamus* were washed thoroughly for 4-5 times with tap water to remove dust and other foreign matter from surface and then with 2% mercuric chloride. Again it was washed 2-3 times with distilled water and sterilized water. Water was decanted off softly and material was kept in between filter paper to soak the excess of water from external surface. Plant material was weighed 100 g, chopped into small pieces and fine slurry of plant was prepared with known volume of sterile distilled water (1:1 w/v) using sterile mortar and pestle at room temperature. The resultant slurry was squeezed with using sterile muslin cloth to get extract. It was then filtered with Whatman No 1 filter paper to have clear solution. It was considered as 100% concentration (Srivastva, 2008).

### Preparation of media and determination of antibacterial activity

Screening of leaf extract of *Vitex negundo*, *Adhatoda vesica* and rhizome extract of *Acorus calamus* was done using Agar well diffusion method. Nutrient Agar Medium (Beef extract 1g, Yeast extract 2g, Sodium chloride 1g, Peptone 5g, Agar 20g, Distilled water 1 Lt) was used throughout the investigation for the growth of microorganisms. The medium was autoclaved at 121.6°C for 30 minutes. The plates were left overnight at room temperature to check for any contamination to appear. The bacterial test organisms were grown in nutrient broth for 24 hours. A 100 µl Nutrient broth culture of each bacterial organism was used to prepare bacterial lawns. 100 µl of bacterial suspensions were spread on Nutrient agar plates. Agar well of 8 mm diameter was prepared with the help of sterilized stainless steel cork borer. One well was prepared in the agar plates. The well in each plate was loaded with 15%, 30%, 50%, 100%

concentration prepared separately by dissolving extract in requisite amount of water. The plates containing bacterial extract were incubated at ±37 for 24 hrs (*E. coli*, *Staphylococcus aureus*) in incubation chamber. All the tests were repeated in triplicates. Diameter of bacterial colonies of treatment and control sets were measured in mutually perpendicular direction on second day. Percentage inhibition of bacterial/fungal microorganisms was calculated after subtracting the value of control from the value of extracts using control as standard (Hemashenpagam and Selvaraj, 2010).

$$\% \text{ of growth inhibition} = \frac{\text{Control-Test}}{\text{Control}} \text{ (Kannen et al., 2009)}$$

### Test bacteria

Pathogenic strains of *E. coli* and *Staphylococcus aureus* were procured from Department of Microbiology, Indira Gandhi Medical College, Shimla. The bacteria were maintained on nutrient broth at 4 and sub cultured periodically on fresh medium.

### Statistical analysis

The results of antibacterial activity of replicates were expressed as mean ± standard deviation (SD) and data were subjected to examine by analysis of variance using (SPSS) software.

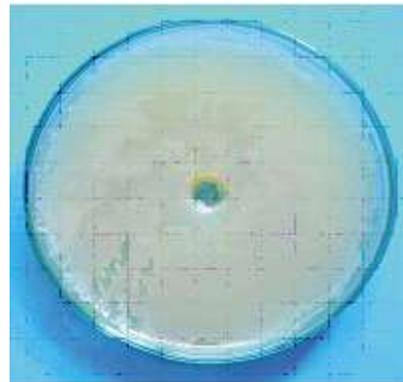
## RESULTS AND DISCUSSION

Medicinal plants are considerably useful and economically essential. They contain active constituents that are used in treatment of many human diseases (Stary and Hans 1998). Plant produces diverse range of diverse molecules, making them rich source of different medicines. Most of the drugs today are from natural sources or semi-synthetic derivatives of natural products and used in traditional system of medicine so, logic approach in drug discovery is to screen traditional natural products (Sukanya et al., 2009).

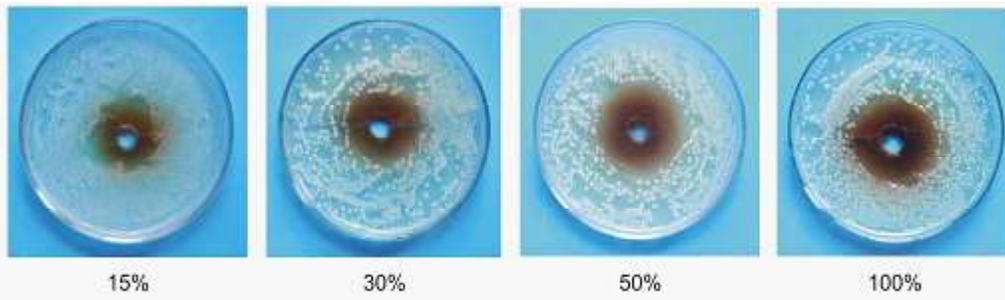
Considering these, as a first step in the present investigation, leaf extracts of the local medicinal plants *A. vesica*, *V. negundo* and rhizome extract of *A. calamus* was screened *in-vitro* for antibacterial activity against two pathogenic bacteria (*E. coli* and *S. aureus*). Comparative analysis in Table 1 shows results of antibacterial



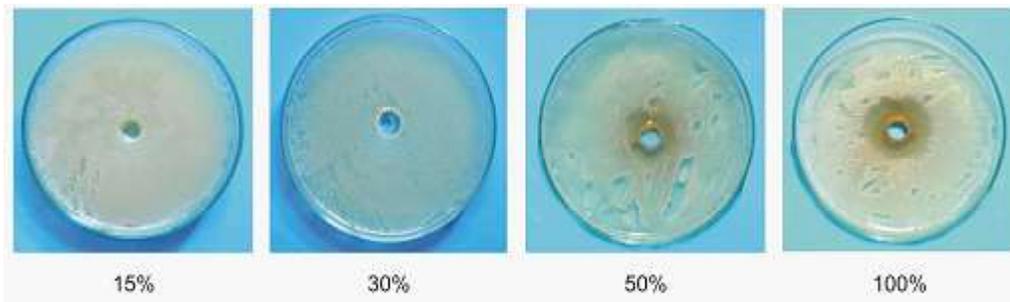
**Plate 1.** Pure culture of *Escherichia coli*



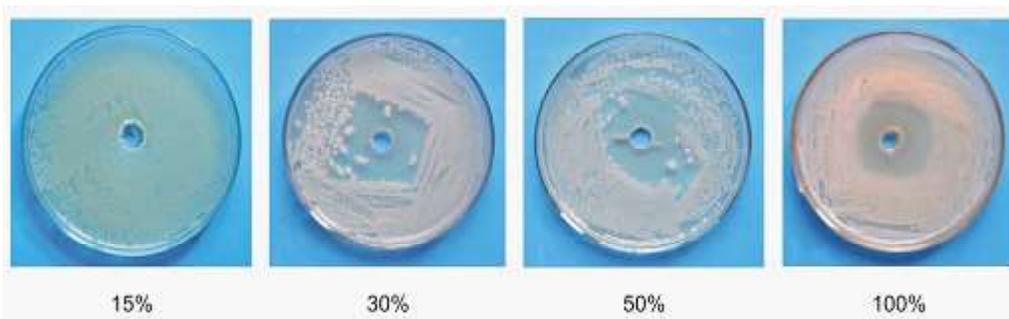
**Plate 2.** Pure culture of *Staphylococcus aureus*



**Plate 3.** Percentage of inhibition of growth of *E. coli* by *Adhatoda vesica* leaf extract at different concentrations



**Plate 4.** Percentage of inhibition of growth of *E. coli* by *Acorus calamus* rhizome extract at different concentrations



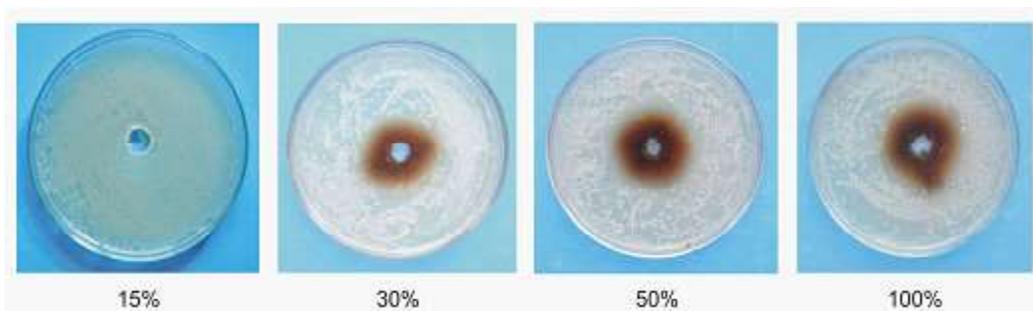
**Plate 5.** Percentage of inhibition of growth of *E. coli* by *Vitex negundo* leaf extract at different concentrations

screening of leaf extract of *A. vesica* against two above mentioned bacterial *sp.* The screening revealed that leaf extract of *A. vesica* was most effective in inhibition against *S. aureus* (17% at 100%) followed by (11.66% at 50%), (6.66% at 30%) and (3.66% at 15%), whereas in the case of *E. coli* inhibition was (14.55% at 100%) followed by (9.55% at 50%), (6.33% at 30%) and (NIL at 15%).

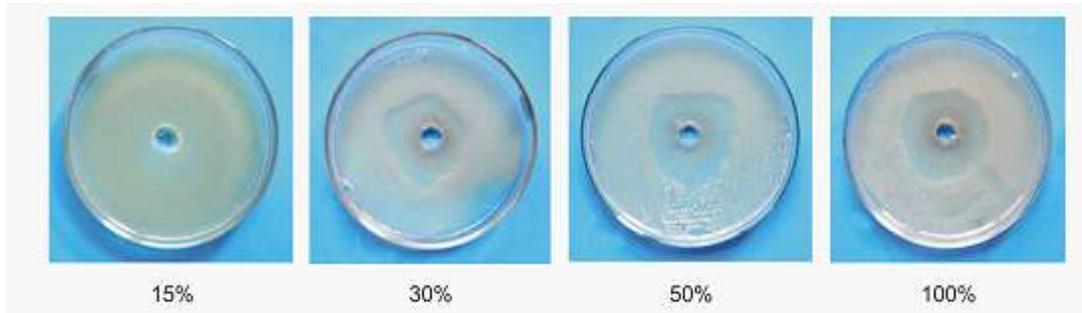
Aqueous rhizome extract of second medicinal plant *A. calamus* was screened *in-vitro* for antibacterial activity against two pathogenic bacteria (*E. coli* and *S. aureus*). Comparative

analysis in Table 1 shows the results of antibacterial screening of rhizome extract of *A. calamus* against two above two mentioned bacterial *sp.* Rhizome of second plant was found to be most effective against *S. aureus* (16% at 100%) followed by (9.77% at 50%), (6% at 30%) and (0.00% at 15%), whereas in *E. coli* inhibition was (14% at 100%), (8.11% at 50%), (4.66% at 30%) and (0.00% at 15%) concentrations respectively.

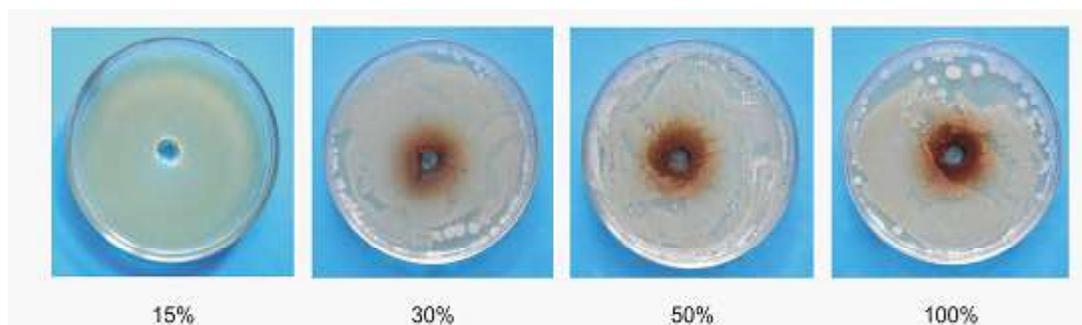
The third medicinal plant *V. negundo* was screened *in-vitro* for antibacterial activity against two pathogenic bacteria (*E. coli* and *S. aureus*).



**Plate 6.** Percentage of inhibition of growth of *Staphylococcus aureus* by *Adhatoda vesica* leaf at different concentrations



**Plate 7.** Percentage of inhibition of growth of *Staphylococcus aureus* by *Acorus calamus* rhizome leaf at different concentrations



**Plate 8.** Percentage of inhibition of growth of *Staphylococcus aureus* by *Vitex negundo* different concentrations

**Table 1.** Comparative analysis of Antibacterial screening of aqueous fruit extracts of *A. vesica*, *A. calamus* and *V. negundo* against two test bacteria namely *S. aureus* and *E. coli*.

Concentration (In %)	% inhibition of growth of test bacteria (mm ± S.E.)					
	<i>A. vesica</i>		<i>A. calamus</i>		<i>V. negundo</i>	
	<i>S. aureus</i>	<i>E. coli</i>	<i>S. aureus</i>	<i>E. coli</i>	<i>S. aureus</i>	<i>E. coli</i>
15%	3.66 ± 0.03	0.0 ± 0.00	0.0 ± 0.00	0.0 ± 0.00	0.0 ± 0.00	0.0 ± 0.00
30%	6.66 ± 0.46	6.63 ± 0.05	6.00 ± 0.05	4.66 ± 0.06	4.44 ± 0.11	0.0 ± 0.00
50%	11.66 ± 0.05	9.55 ± 0.06	9.77 ± 0.06	8.11 ± 0.05	8.00 ± 0.11	6.77 ± 0.05
100%	17.00 ± 0.33	14.55 ± 0.05	16.00 ± 0.33	14.00 ± 0.33	13.33 ± 0.11	12.44 ± 0.06

Comparative analysis in Table-1 shows the result of antibacterial screening of leaf extract of *V. negundo* against two mentioned bacterial *sp.* Aqueous leaf extract of this plant was also found to be most effective against *S. aureus* (13.33% at 100%), (8% at 50%), (4.44% at 30%) and (0.00% at 15%) where as in case of *E. coli* inhibition was (12.44% at 100%) followed by (6.77% at 50%), (0.00% at 30%) and (0.00% at 15%) concentrations respectively.

It was concluded from the above experimental observations that three medicinal plants (*A. calamus*, *A. vesica*, *V. negundo*) were more effective against *S. aureus* at all concentrations as compared to *E. coli*. Similar finding were drawn by Singh and Pandey (2009), Ramya *et al.* (2010), Pavia *et al.* (2003), Nair *et al.* (2005), Rai *et al.* (2010), Voravuthikunchai *et al.* (2004), Devi and Gangewala (2009) Khond *et al.* (2009).

Finding of present study confirm that the plant extract of *Vitex negundo*, *Acorus calamus*, *Adhatoda vesica* can be used as potential antimicrobial agents against pathogenic bacteria. Extracts of these plant requires further research formulation to control human diseases.

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