

**IN VITRO ANTIMICROBIAL AND PHYTOCHEMICAL ANALYSIS OF  
VARIOUS EXTRACTS OF ADHATHODA VASICA LEAVES****SHIJI THOMAS\* AND DR.FATHIMATHU ZUHARA.K***Department of Life Sciences, University of Calicut, Kerala- 673635, India.***ABSTRACT**

*Adhathoda vasica* is a well-known herb used in Ayurvedic system of medicine which possesses diverse pharmacological properties. In the present study leaves of *Adhathoda vasica* were extracted using four different solvents of increasing polarities (chloroform, methanol, ethanol and water). The efficacy of these extracts was tested against various bacterial strains through agar well-diffusion method. The antibacterial activity was evaluated using ten clinical and four standard bacterial strains. Minimum Bactericidal Concentration (MBC) of the extracts which exhibited greater antimicrobial potential in agar well diffusion assay was performed using broth dilution techniques. The methanolic extract of *Adhathoda vasica* was subjected to qualitative phytochemical screening for identification of various classes of active chemical constituents such as phenols, flavonoids, saponins, terpenoids, glycosides and alkaloids. According to the findings of the present antibacterial assay, among the four solvents used for extraction, the chloroform, ethanol and methanol extracts exhibited significant antimicrobial activity. This study provides scientific understanding to further explore other pharmacological properties of the plant.

**KEY WORDS:** *Adhathoda vasica*, Cold method, Soxhlet method, Agar well diffusion, Minimum Bactericidal Concentration (MBC), Phytochemical analysis.

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## INTRODUCTION

Drug discovery has been an essential pursuit of mankind since ancient times. Antibiotic resistance has been spreading at an alarming rate during the last few decades. The success story of chemotherapy lies in the continuous search for new drugs to counter the challenge posed by resistant strains<sup>1</sup>. Medicinal plants contain a large amount of biologically active substances applied in traditional medicine and are an inexhaustible sources of substances for various pharmaceutical industries. Biologically active compounds from natural sources have always been a great interest for scientists working on infectious diseases<sup>2</sup>. Several distinct chemicals derived from plants are important drugs currently used in different parts of the world. The WHO reported that 80% of the world's population rely chiefly on traditional medicine and a major part of the traditional therapies involve the use of plant extracts or their active constituents<sup>3</sup>. *Adhathoda vasica* is a plant used in folk medicine for the treatment of various diseases and disorders, particularly for the respiratory tract infections. *Adhathoda vasica* (family Acanthaceae) is a shrub 1-2.5 m height with opposite ascending branches. It is commonly known as Vasaka or Malabar nut. The Pharmacologically most studied chemical component in *Adhathoda vasica* is a bitter quinazoline alkaloid, vasicine which is present in the leaves, roots and flowers<sup>4,5</sup>. Phytochemical studies reveal that this plant mainly possesses pyrro- quinazoline alkaloids which comprise vasicine, vasicol, vasicinone, peganine along with other minor constituents<sup>6</sup>. Vasicinone formed by oxidation of vasicine at C-8 position, is one of the major alkaloids of *Adhathoda vasica* and is known to possess interesting biological activities<sup>7</sup>. Broad spectrum antimicrobial activity of alkaloids isolated from *Adhathoda vasica* has been demonstrated by many researchers<sup>8,9</sup>. These alkaloids inhibit the activity of *Mycobacterium tuberculosis* beta-ketoacyl acyl carrier protein synthase III (mt FabH) thereby preventing the initial step of fatty acid biosynthesis<sup>6</sup>. The present study focuses on the *in vitro* antimicrobial activity of various extracts of *Adhathoda vasica* leaves prepared using four different solvents against ten clinical and four standard strains.

## MATERIALS AND METHODS

### **Plant material collection and processing**

Fresh leaves of *Adhathoda vasica* Nees free from disease were collected from Botanical garden, Calicut University. The plant material was washed with water and rinsed with sterile distilled water, shade dried and powdered using mechanical grinder. The powdered material was then extracted using four different solvents (chloroform, methanol, ethanol and water). Dried extract was stored in air tight container and maintained at 4°C in a refrigerator.

### **Extraction of plant material**

#### **a) Cold method<sup>10</sup>**

b) The method described by Harbone (1989) was used with slight modification in this study. 10 g powder of the dried plant material was taken and mixed with 100 ml of the solvent. It was mixed well and kept on an orbital shaker at 200 rpm for 15 to 20 hours at room temperature. The extract was then filtered using whatman no.1 filter paper. The procedure was repeated for three times or until clear supernatant solvent was formed. The supernatant was collected and evaporated to dryness. The residue thus obtained was weighed and used for further analysis.

#### **b) Soxhlet method<sup>11</sup>**

Soxhlet method makes use of high temperature extraction. In this method 10 g of the powdered plant material was taken and wrapped in a good quality filter paper and put in a soxhlet apparatus. The extract was collected and evaporated to dryness.

### **Antimicrobial activity screening**

#### **a) Microorganisms**

Ten clinical strains and four standard strains were analysed in the present study. The clinical strains were obtained from Calicut Medical College. These were *Escherichia coli*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Acinetobacter baumannii*, *Proteus vulgaris*, *Proteus mirabilis*, *Staphylococcus aureus*, *Methicillin resistant Staphylococcus aureus*, *Salmonella typhi* and *Shigella flexnerii*. *Escherichia coli* MTCC 1687,

*Pseudomonas aeruginosa* MTCC 1688, *Staphylococcus aureus* MTCC 737 and *Klebsiella pneumoniae* MTCC 109 were the standard strains used in the present study. These standard strains were collected from Institute of Microbial Technology (IMTECH), Chandigarh, India. The identification and maintenance of cultures were performed by using classical diagnostic microbiology procedures<sup>12</sup>.

### b) Agar well diffusion method<sup>13</sup>

This method was employed to determine the antimicrobial activities of plant extracts. The media used for bacteria was Mueller-Hinton agar. Wells (6 mm in diameter) were cut from agar plates using sterilized stainless steel borer. Fifty microliters of extracts of leaves at a concentration of 1 mg/ml were used against the test microorganisms.

### c) Minimum Bactericidal Concentration(MBC)<sup>14</sup>

Quantitative antimicrobial activity assay was carried for extracts which exhibited greater antimicrobial activity in agar well diffusion method. The initial concentration of the plant extract was diluted using double fold serial dilution and having obtained the different concentrations of the extracts, each concentration was inoculated with 0.1ml of the standardized bacterial cell suspensions and incubation was done at 37°C for 24 hours. The growth of the inoculum in the broth is indicated by turbidity or cloudiness of the broth. The

lowest concentration of the extracts that inhibits growth of the organisms, as detected by lack of visual turbidity, was designated as the minimum inhibitory concentration (MIC). Control tubes were maintained for each test batch. For calculation of minimum bactericidal concentration (MBC) of extracts, inoculum from each of the tubes of broth was sub-cultured on Nutrient agar plates.

### Phytochemical analysis

The methanolic extract of *Adhathoda vasica* was subjected to qualitative phytochemical screening for the identification of various classes of active chemical constituents such as phenols, flavonoids, saponins, terpenoids, glycosides and alkaloids. The phytochemical analysis was done according to standard methods<sup>15,16,17,18</sup>.

## RESULTS AND DISCUSSION

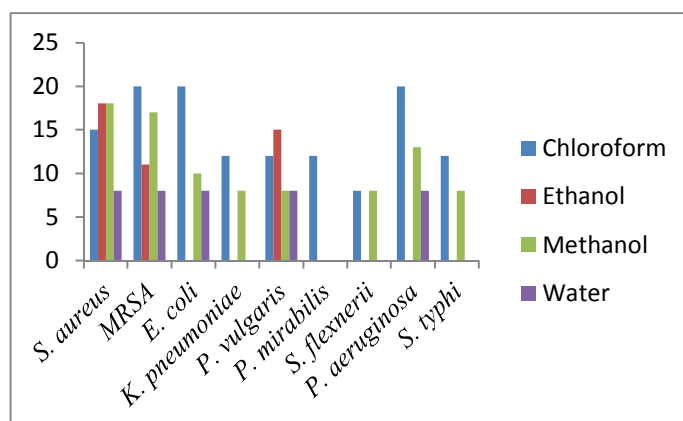
In the present investigation, the antimicrobial activity of plant extracts from *Adhathoda vasica* was evaluated *in vitro* against ten bacterial pathogens and four standard strains. The plant extracts were prepared using four different solvents i.e. Chloroform, Ethanol, Methanol and water. DMSO serves as a negative control. Table 1 illustrates zone of inhibition of different extracts against the ten clinical strains using agar well diffusion method.

**Table I**  
**Antibacterial activities of various extracts of *Adhathoda vasica* leaves against clinical strains using agar well diffusion method.**

Microorganism	Zone of Inhibition of plant Extracts(in mm)				
	Chloroform	Ethanol	Methanol	Water	DMSO
<i>S. aureus</i>	15	18	18	8	-
MRSA	20	11	17	8	-
<i>E. coli</i>	20	-	10	8	-
<i>K. pneumoniae</i>	12	-	8	-	-
<i>P. vulgaris</i>	12	15	8	8	-
<i>P. mirabilis</i>	12	-	-	-	-
<i>S. flexnerii</i>	8	-	8	-	-
<i>P. aeruginosa</i>	20	-	13	8	-
<i>S. typhi</i>	12	-	8	-	-
<i>A. baumannii</i>	8	-	-	-	-

(-)- No activity.

**Graph I**  
**Antibacterial activities of various extracts of *Adhathoda vasica* leaves against clinical strains using agar well diffusion method.**



**Table II**  
**Antibacterial activities of various extracts of *Adhathoda vasica* leaves against standard strains using agar well diffusion method.**

Microorganism	Zone of Inhibition of plant extract (in mm)				
	Chloroform	Ethanol	Methanol	Water	DMSO
<i>S. aureus</i> 737	8	8	8	-	-
<i>E. coli</i> MTCC 1687	8	8	8	8	-
<i>K. pneumoniae</i> MTCC 109	8	8	8	-	-
<i>P. aeruginosa</i> MTCC 1688	-	8	8	-	-

(-)- No activity.

The plant extracts prepared using four different solvents (Table I and II) exhibited varying degrees of antimicrobial activity on the microorganisms tested. The antimicrobial activity was more prominent in chloroform, ethanol and methanol extracts when compared to water extracts. Water extract of *Adhathoda vasica* exhibited negligible antimicrobial activity.

**Table III**  
**Antibacterial activities of methanol extract (Soxhlet) of *Adhathoda vasica* leaves against clinical strains using agar well diffusion method.**

Microorganism	Zone of Inhibition (in mm)	
	Plant extract	DMSO
<i>S. aureus</i>	18	-
MRSA	18	-
<i>E. coli</i>	8	-
<i>K. pneumoniae</i>	8	-
<i>P. vulgaris</i>	-	-
<i>P. mirabilis</i>	-	-
<i>S. flexnerii</i>	-	-
<i>P. aeruginosa</i>	13	-
<i>S. typhi</i>	-	-
<i>A. baumannii</i>	-	-

(-)- No activity.

**Table IV**  
**Antibacterial activities of methanol extract (Soxhlet) of *Adhathoda vasica* leaves against standard strains using agar well diffusion method.**

Microorganism	Zone of Inhibition (in mm)	
	Plant extract	DMSO
<i>S. aureus</i> 737	8	-
<i>E. coli</i> MTCC 1687	8	-
<i>K. pneumoniae</i> MTCC 109	-	-
<i>P. aeruginosa</i> MTCC 1688	8	-

(-)- No activity.

**Table V**  
**Minimum Bactericidal Concentration (MBC) of various extracts of *Adhathoda vasica* against clinical bacterial strains.**

Microorganism	Plant extract	MBC(mg/ml)
<i>E.coli</i>	Chloroform	230
<i>MRSA</i>	Chloroform	230
<i>S .aureus</i>	Methanol	250
<i>MRSA</i>	Methanol	250
<i>S.aureus</i>	Methanol(Soxhlet)	250
<i>MRSA</i>	Methanol(Soxhlet)	250

Though, the mechanism of the action of these plant constituents is not yet fully known it is clear that the effectiveness of the extracts largely depends on the type of solvent used. The organic extracts provided more powerful antimicrobial activity as compared to aqueous extracts. Similar observations are reported by many researchers<sup>19</sup> & <sup>20</sup>. The preliminary phytochemical screening carried out on leaf

extract of *Adhathoda vasica* revealed the presence of phytoconstituents such as phenols, flavanoids, terpenoids, saponins, glycosides and alkaloids<sup>21</sup>. These phytochemical constituents and several other aromatic compounds of plants serve as a defence mechanism against predation by many microorganism, insects and herbivores<sup>22</sup>.

**Table V**  
**The preliminary phytochemical analysis of chloroform extract of *Adhathoda vasica* leaves**

Type of the extract	Phenols	Flavanoids	Terpenoids	Saponins	Glycosides	Alkaloids
Methanol	+	+	+	+	+	+

## CONCLUSION

The present study reveals the presence of active antimicrobial components in the medicinal plant *Adhathoda vasica* which could be exploited in designing new plant based antimicrobial compounds. The study also emphasizes that organic solvents could be a better medium for extracting antimicrobial compounds from *Adhathoda vasica* since more consistent antimicrobial activity was observed in those extracts. Comparison of the hot soxhlet extract and cold extracts of different solvents also proved

that high temperature has no influence on the active antimicrobial component of the plant. Various extracts of *Adhathoda vasica* exhibited antimicrobial activity against both gram positive and negative organisms. On the basis of present finding, it is proven that *Adhathoda vasica* leaves possess broad spectrum antimicrobial activity and this plant could be used as a potential source for developing new antimicrobial compounds.

## REFERENCES

- Gibbons A, Exploring New Strategies to Fight Drug-Resistant Microbes. Science, 257(5073): 1036-1038, (1992).
- Thenmozhi K, Saradha M, Manian S and Paulsamy S, *In vitro* antimicrobial potential of root extracts of the medicinal plant species, emilia sonchifolia (linn.) dc. Asian journal of pharmaceutical and clinical research,6(3):149-151, ( 2013 ).
- World Health Organization. Summary of WHO guidelines for the assessment of herbal medicines, Herbal Gram, 28:13-4 (1993).
- Sandeep Dhankhar, Ramanjeet Kaur, Ruhil S, Balhara M, Seema Dhankhar and Chhillar AK, A review on *Justicia adhatoda*: A potential source of natural medicine. African Journal of Plant Science, 5(11):620-627, (2011).

5. Rashmi PA, Reshma John and Linu Mathew, Isolation and characterisation of vasicine from *in vitro* cultures of *Justicia adhatoda* L. Int J Pharm Bio Sci 3(3):58-64, (2012).
6. Jha DK, Panda L, Lavanya P, Ramaiah S and Anbarasu A, Detection and confirmation of alkaloids in leaves of *Justicia adhatoda* and bioinformatics approach to elicit its anti-tuberculosis activity. Appl Biochem Biotechnol,168(5): 980-990, (2012)
7. Srivastava S, Verma R K, Gupta M M, Singh S C, & Kumar S, HPLC determination of vasicine and vasicinone in *adhatoda vasica* with photo diode array detection. Journal of Liquid Chromatography & Related Technologies, 24(2): 153-159, (2001).
8. Sawant CS, Save SS and Bhagwat AM, Antimicrobial activity of alkaloids extracted from *Adhatoda vasica*. International Journal of Pharma and Bio Sciences, 4(3): 803-807,(2013).
9. Rashmi Pa, Linu Mathew, Antimicrobial activity of leaf extracts of *Justicia adhatoda* L. in comparison with vasicine. Asian Pacific Journal of Tropical Biomedicine, S1556-S1560,(2012).
10. Harbone JB, Methods in plant biochemistry, In: Plant phenolics: Academic Press, London, UK, (1989).
11. Dhiman A, Nanda A, Ahmad S and Narasimhan B, In vitro antimicrobial activity of methanolic leaf extract of *Psidium guajava* L. J Pharm Bioallied Sci 3(2) : 226-229, (2011)
12. Pelczar Michael J, Chan E C S, Krieg Noel R, Microbiology, 5<sup>th</sup>Edn, McGraw-Hill, New York: 140-141, (1986).
13. Arora-Daljit S, Kaur J, Antimicrobial activity of spices. Int. J. Antimicrob. Agents. 12:257-262, (1999).
14. Mendoza MT, What's new in antimicrobial susceptibility testing?. Philipp. J. Microbiol. Infect. Dis. 27(3): 113-115.(1998).
15. Singleton VL, Orthofer R, Lamuela-Raventós RM, Analysis of total phenols and other oxidation substrates and antioxidants by means of Folin–Ciocalteu reagent. Methods Enzymol. 299 : 152-17,(1999).
16. Vogel AI, A textbook of practical organic chemistry. London: Longman. 90–92,(1958).
17. Sofowara A, Medicinal plants and traditional medicine in Africa, Spectrum Books Ltd., Ibadan, Nigeria, 289, (1993).
18. Odebiyi OO and Sofowora A, Antimicrobial alkaloids from Nigeria chewing sticks. Planta Medica. 36 (3): 204, (1979).
19. Meignanalakshmi S, Vinoth Kumar S, Deepika J and Farida Begum I, Evaluation of antibacterial activity of methanol extract of leaves of *Adhatoda vasica* on mastitis pathogens. Hygeia.J.D.Med. 5 (1) : 1-4, (2013).
20. Jalander V and Gachande BD, Antibacterial activity of some important medicinal plants. International Journal of Food, Agriculture and Veterinary Sciences, 4 (2) : 61-63, (2014).
21. Karthikeyan A, Shanthi V, Nagasathaya A, Preliminary phytochemical and antibacterial screening of crude extract of the leaf of *Adhatoda vasica* . L. Int J Green Pharm, 3:78-80, (2009).
22. Anubha arora, Phytochemical analysis of methanolic extracts of leaves of some medicinal plants. Biological Forum – An International Journal, 5(2): 91-93, (2013).