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## ANTI-TUSSIVE ACTIVITY OF ETHYL ACETATE AND METHANOL EXTRACTS OF *ADHATODA VASICA NEES*

Sweta Srivastava\* and G. P. Choudhary

Department of Pharmacognosy, School of Pharmacy, DAVV, Indore, Madhya Pradesh, India

### Keywords:

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### Correspondence to Author:

**Sweta Srivastava**

Department of Pharmacognosy,  
School of Pharmacy, DAVV, Indore,  
Madhya Pradesh, India.


**Email:** sweta.koka@gmail.com

**ABSTRACT:** The present study was carried out to evaluate anti-tussive activity of ethyl acetate and methanolic extract of leaves of *Adhatoda vasica* Nees. As cough is a natural reflex expulsive defense mechanism of the body, it is the most common symptom of respiratory disease. Ammonium hydroxide and Sulphur dioxide induced cough models in mice were used for evaluation of antitussive activity of ethyl acetate and methanolic extracts of leaves *Adhatoda vasica*. The ethyl acetate and methanolic extract of leaves *Adhatoda vasica* was orally administered at the dose levels of 500 mg/kg b.w. showed maximum inhibition of cough by 82% and 81% respectively. The standard anti-tussive drug Codiene phosphate (10mg/kg b.w.) showed maximum inhibition of cough by 84%. It was found that both extracts of *Adhatoda vasica* showed anti-tussive activity and obtained percentage inhibition of cough reflex is approximately comparable as standard drug.

**INTRODUCTION:** Plant and animal materials have been used successfully for the treatment of human diseases since ancient times. Every country in the world has enlisted various indigenous herbal remedies according to the diseases and human requirements. Ayurveda is an original holistic system of diagnosis and treatment involving nutrition, hygiene and rejuvenation, developed and perfected in India<sup>1</sup>. Cough is a normal physiological response to an irritation of the laryngo-tracheo-bronchial system caused by mechanical or chemical stimulation. It may be painful and fatiguing and require suppression.

Anti-tussive agents are used mainly to suppress dry and painful coughs. The most frequently used anti-tussive drugs in clinical conditions are from a group of narcotic analgesics. Their anti-tussive action is very effective at doses below those required for pain relief. However, these cause various adverse effects, like depression of the respiratory center, decreased secretion in the bronchioles and inhibition of ciliary activity. Their administration can lead to increased sputum viscosity, decreased expectoration, hypotension and even constipation. All of these adverse effects lead to look for other substances, which might prevent the pathological cough.

There are many types and causes of coughing which call for a variety of remedies. Thus, the treatment of cough is one area where the use of certain herbal remedies remains common today<sup>2, 3, 4</sup>. *Adhatoda vasica* Nees. (vasaka) is an indigenous medicinal plant used traditionally in the treatment

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of cold, cough, whooping cough and chronic bronchitis and asthma, as sedative expectorant, antispasmodic and anthelmintic<sup>5</sup>. The drug is employed in different forms such as fresh juice, decoction, infusion and powder; also given as alcoholic extract and liquid extract or syrup<sup>6</sup>. The leaves of the plant contain an essential oil and alkaloids vasicine, N-oxides of vasicine, vasicinone, deoxyvasicine and maiontone. The roots are known to contain vasicinolone, vasicol, peganine and 2' - hydroxy - 4 - glucosyl - oxychalcone. The flowers contain b-sitosterol-D-glucoside, kaempferol, itsglycosides and quereetin. Vasicine<sup>7,8</sup>.

## MATERIAL AND METHODS:

### Collection of Plant material:

Leaves of *Adhatoda vasica* Nees. were collected from Botanical garden, Indore (M.P) and identified and authenticated at Department of Botany, Govt. Agriculture College, Indore. A voucher specimen has been kept in the herbarium of our department for future references.

### Preparation of extracts:

250 gm of coarsely powdered leaves were subjected to successive solvent extraction using Petroleum ether, Chloroform, Ethyl acetate, Methanol as a solvent. All the four extract obtained were filtered, concentrated on water bath, dried in vacuum and stored in refrigerator for further experiment. Since main phytoconstituents flavonoids and alkaloids were found in ethyl acetate as well as in methanolic extract thus these two extract were taken for the further studies with ethyl acetate and methanol as solvent using soxhlet apparatus<sup>9</sup>.

### Experimental animals:

Swiss albino mice of either sex (20-30g) were used in the study. The animals were housed in polypropylene cages under standard conditions (12 h light; 12 h dark cycle; 25± 5°C; 35-60% humidity). They were fed with standard pellet diet (Pranav Agro Ltd, Dehradun) and water *ad libitum*. The experimental protocol was approved by the Institutional Animal Ethical Committee (IAEC/PCP/2014/49).

### Anti-tussive activity:

#### Sulphur Dioxide induced cough:<sup>10</sup>

Swiss albino mice were divided into four groups, each group containing six mice. The control group was treated with distilled water orally, and the positive control was treated with Codiene Phosphate. The remaining groups were treated with the ethyl acetate and methanol extract at doses of 500mg/Kg body weight respectively.

The experimental model was performed as reported. Cough response of all the groups are observed (0 minute) by placing the animals in desiccators. A certain amount, 5ml sulfur dioxide gas is induced into the dessiccator. After a minute of introducing the gas, the animal is taken out of the dedicator and frequency of cough is observed for five minutes in an un-ended filter funnel with a stethoscope at the tip in which mice is confined. In the same fashion the frequency of cough are observed for all the animal groups after every 30 minutes for 2 hrs. The percentage frequency of cough reflex was calculated by the formula

$$\% \text{ frequency of cough reflex} = (1 - T / C) \times 100$$

Where, T= Cough reflex in tested drug treated in mice; C= Cough reflex in control group treated mice.

#### Ammonium hydroxide induced Cough:<sup>11</sup>

Swiss albino mice were divided into four groups, each group containing six mice. The control group was treated with distilled water orally, and the positive control was treated with dexamethorphan. The remaining groups were treated with the ethyl acetate and methanolic extract at doses of 500 mg/Kg body weight respectively. Anti-tussive activity was investigated on a classical mouse cough model induced by ammonia liquor. Each mouse was placed in a 300 ml special glass chamber and exposed to 40µl 25% NH<sub>4</sub>OH. The cough frequency produced during 2 min exposure period was counted. The cough frequency and latent period of cough were also recorded. The percentage frequency of cough reflex was calculated by the formula

$$\% \text{ Frequency of Cough Reflex} = (1 - T / C) \times 100$$

Where, T= Cough reflex in tested drug treated in mice; C= Cough reflex in control group treated mice.

**RESULTS:** The antitussive activity of the ethyl acetate and methanolic extract of leaves of *Adhatoda vasica* Nees was investigated for its effect on a cough model induced by sulphur dioxide gas and ammonium hydroxide in mice and found to have significant anti-tussive activity when compared with control and the standard drug

Codiene phosphate and Dextromethaphan. The antitussive effect of ethyl acetate and methanolic at doses 500 mg/kg are shown in **Table 1-4**. The decrease in cough frequency was statistically significant ( $P<0.01$ ), when compared to the control group. The Methanol and ethyl acetate extract at doses 500 mg/kg of the selected herbs produced a significant inhibition of cough reflex. Ethyl acetate extract showed slight better results in the comparison of methanol extract.

**TABLE 1: EFFECT OF ETHYL ACETATE AND METHANOL EXTRACT OF ADHATODA VASICA ON SO<sub>2</sub> INDUCE MODEL**

Treatment	Dose(mg/kg)	Cough frequency in minutes					
		N=6	0 min	30 min	60 mmin	90 min	120 min
Control			74.16±2.92	76.83 ±2.48	75.5±0.83	74.33±3.07	74.67±3.01
Dextromethapahan	10		76.33± 1.21**	34.83±2.63**	22.33±1.86**	14.5±2.42**	11.5±1.04**
Ethyl acetate AV	500		73.16±2.48**	62.33±1.86**	51.33±1.36**	32.34±0.816**	14.16±1.16**
Methanol AV	500		76.5±0.54**	65±1.67**	54.83±1.16**	35.66±1.63	14.83±1.94**

The frequency of cough was counted for 5min after the sulphur dioxide gas challenge; Data are expressed in mean ± S.E.M. (n=number of animals). \*\*: p<0.05; figures in parentheses indicate percentage inhibition of cough reflex

**TABLE 2: EFFECT OF ETHYL ACETATE AND METHANOL EXTRACT OF ADHATODA VASICA ON % INHIBITION OF COUGH REFLEX**

Treatment	Dose (mg/kg)	% Inhibition of Cough Reflex			
		30 min	60 mmin	90 min	120 min
Dextromethapahan	10	54%	70%	80%	84%
Ethyl acetate extract AV	500	18%	32%	56%	81%
Methanol extract AV	500	15%	27%	52%	80%

**TABLE 3: EFFECT OF ETHYL ACETATE AND METHANOL EXTRACT OF ADHATODA VASICA ON AMMONIUM HYDROXIDE INDUCE MODEL**

Treatment	Dose(mg/kg)	Cough Frequency in Minutes					
		N=6	0 Min	30 Min	60 Min	90 Min	120 Min
Control			77.38±1.58	78.13±1.57	76.88±	75.63±1.44	76.25±1.51
Codiene Phospahte	10		76.38±1.55**	31.75±1.31**	18.25±1.24**	13.88±1.30**	11.5±1.51**
Ethyl acetate extract AV	500		76.75±1.62**	60.13±1.53**	52.25±1.36**	34.88±1.22**	13.83±1.94**
Methanol extract AV	500		76.25±1.42**	67.50±1.59**	53.13±1.49**	36.75±1.63**	14.86±1.16**

The frequency of cough was counted for 5min after the sulphur dioxide gas challenge; Data are expressed in mean ± S.E.M. (n=number of animals). \*\*: p<0.05; figures in parentheses indicate percentage inhibition of cough reflex

**TABLE 4: EFFECT OF ETHYL ACETATE AND METHANOL EXTRACT OF ADHATODA VASICA ON % INHIBITION OF COUGH REFLEX**

Treatment	Dose (mg/kg)	% Inhibition of Cough Reflex				
		N=6	30 Min	60 Min	90 Min	120 Min
Codiene Phospahte	10		59%	76%	81%	84%
Ethyl acetate extract AV	500		23%	32%	53%	81%
Methanol extract AV	500		13%	30%	51%	80%

**DISCUSSION AND CONCLUSION:** Cough can be induced in experimental animals by chemical irritation, mechanical stimulation, by electrical stimulation of tracheal mucosa or by nerve stimulation. However, it is considered that cough produced by chemical stimulation is more

comparable to that in humans than is obtained with other tussigenic stimuli. In the present study, the antitussive activity of *Adhatoda vasica* leaves ethyl acetate and methanol extracts has been compared with that of control group against coughing induced in mice by chemical (Sulphur dioxide and

ammonium hydroxide gas) stimulation. The methanolic and ethyl acetate extracts showed marked antitussive effect, comparable to the standard drug (dextromethorphan and codeine phosphate). Thus the extracts might be acting via the central nervous system. The antitussive activity of the ethyl acetate extracts was better in comparison to the ethanolic extracts. This may be due to the presence of ethyl acetate soluble compounds like flavonoids and other poly phenolic compounds.

So from this study it can be concluded that the ethyl acetate and methanol extracts of *Adhatoda vasica*, produced a significant antitussive effect and may prove useful and an effective antitussive preparation in human.

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**CONFLICT OF INTEREST:** Authors declare that there is no conflict of interest is involved in this research study.

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