

## Biological Effect on Adhatoda Vasica Extract and its Combination with Antibiotics

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**Extract of many plants proved potent antimicrobial interest against bacteria and Fungi. Adhatoda Vasica use as a therapeutic plant for various diseases since prehistoric eras. Mixture of plant extracts and antibiotics were used to increase sensitivity of plant extract and lower side effect of allopathy tablets. Gradually, bacteria salvage antibody towards traditional antibiotics and becoming greater powerful. To prevent their stimulating power, combination of plant extracts and antibiotics suggest one of the high-quality consequences in such direction. If dependable plant extracts had been taken in proper doses, it is able to prove pleasant ancillary therapies for patient. This is in vitro look at and such mixture must be trailed with the aid of toxicity check and in vivo assessments to determine its therapeutic usage against the test organisms.**

**Keywords:** Adhatoda Vasica, Antimicrobial Study, Antibiotics.

Nature gives several things against several diseases since last many decades. (Samuelsson *et al.*, 2017) Adhatoda Vasica is a perpetual plant having a place with family Acanthaceae, generally known as Vasaka. (Claeson UP, *et al.*, 2000) It is a small evergreen, perpetual bush dispersed all through India. It has been utilized for the treatment of different infections and clutters, especially for the respiratory tract illnesses. It is recognized with an upsetting smell and bitter taste. (Maurya S, *et al.*, 2010) Vasicine and vasicinone alkaloids are the significant chemical constituents of the plant and are liable for its solid respiratory energizer activity. (Rastogi RP, *et al.*, 1999) The drug for the most

part contains fresh or dried leaves however the flowers, foods grown from the ground are likewise broadly utilized for different illnesses. Different measurement types of leaf-like powder, fresh juice, decoction, alcoholic and aqueous extract, and so on., are portrayed to be utilized for different diseases. (Anonymous, 1985)

A. Vasica was especially utilized as a home-grown medication for treating respiratory grievance including cold, hack, incessant bronchitis, asthma and as antispasmodic. (Singh B, *et al.*, 2013) It has been accounted for antibacterial, injury mending, hypoglycemic, abortifacient, antitussive, anti-inflammatory and hepatoprotective activities.

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(Ahmad S, *et al.*, 2009) Most part contains phytochemical likes pyrroquinazoline alkaloids, flavonoids, triterpenes, (Atta-Ur-Rahman, *et al.*, 1997) steroids, tannins, saponins and glycosides. (Yusuf M, 2016) Therefore, influencing the bacterial pathogenic potential through majority detecting restraint is one of the new methodologies that are utilized to battle the microbial resistance. (Bhardwaj K, *et al.*, 2013) In laboratory experiments on cats and dogs, *A. Vasica* increases bile activity when animals are given a dose of 5mg /kg. in dogs, the amount of excreted bile increases by 40-100%. Animals also showed increased bilirubin excretion. (Gangwar *et al.* 2014)

In recent time medicine in surgery, cancer chemotherapy, and limb transplantation is credited to the use of antibiotics. (Livermore *et al.*, 2002) Antibiotics working today will not work tomorrow New drugs must be examined, with fewer resistance (Sarkar *et al.*, 2003) As a resistance to the spread of old antibiotics, new immune agents are rapidly developed. Yet, records of the rapid emergence and emergence of new antimicrobial agents suggest that a new family of antimicrobial agents will have a shorter life span. (Coates *et al.*, 2002) For that much chemical contained the *Adhatoda Vasica* is effective against several disease. Combination of plant extracts and antibiotics were used to increase sensitivity of plant extract and lower side effect of allopathy tablets.

#### Common Name

English : Malabar nut, *Adulsa*

Hindi : *Adosa*, *Adalsa*, *Vasaka*



**Fig. 1.** *Adhatoda Vasica* Plant

Gujarati : *Ardusi*, *Adusi*

Marathi : *Vasuka*

#### Plant Anatomy (Flora of Gujarat state, 1978)

Kingdom : Plantae

Division : Angiosperm

Class : Dicotyledonae

Series : Bicarpellatae

Order : Personales

Family : Acanthaceae

Genus : *Adhatoda*

Species : *Vasica*

## MATERIALS AND METHODS

### Material Use for Study

#### *Adhatoda Vasica* Plant

Antibacterial Antibiotics : Amoxicilline, Ciprofloxacin, Ceftazidime, Erythromycin

Antifungal Antibiotics : Amphotericin-B, Fluconazole

Bacteria : *Bacillus subtilis* (MTCC 441), *Escherichia coli* (MTCC 1687), *Pseudomonas aeruginosa* (MTCC 1688), *Staphylococcus aureus* (MTCC 737)

Fungi : *Aspergillus Niger* (MTCC 1344), *Candida albicans* (MTCC 81)

#### Method

Leaves of the *Adhatoda Vasica* was conglomerate from local area of north Gujarat in June 2018. Identification of the plant was done by Dr. N. K. Patel, head of the botany department, Sheath M. N. Science college, Patan. The first stages of the study of medicinal plants are to prepare plant samples, which is to store biomolecules in plants before extracted. The leaves were thoroughly wash with tap water and then washed with deionized water to remove particles of dust and sand. The leaves were dried in the dark at room temperature for several days and then powder with mortar. And it is extracted by using Soxhlet extraction.

#### Qualitative Phytochemical Analysis

Following methods were used for analysis of different type phytochemicals.

#### Test for Alkaloids

Plant Extractions were dissolved in dil. HCl and filtered

#### Dragendroff's Test

Plant extract + Potassium Bismuth Iodide solution (Dragendroff's Reagent) → Orange Precipitate

**Hagers’s Test**

Plant extract + Picric Acid (Hagers’s Reagent) → Yellow Precipitate

**Mayers’s Test**

Plant extract + Potassium Mercuric Iodide solution (Mayers’s Reagent) → Cream precipitate

**Wagner’s Test**

Reaction of plant extract + Iodine in Potassium Iodide (Wagner’s Reagent) → Red-brown precipitate

**Test for Flavonoids**

**Test with Alkaline**

Plant extract + Sodium Hydroxide → intense yellow color

which become colorless on further adding Dil. acid

**Test with Lead Acetate**

Treat plant extract + few drops of Lead Acetate → Yellow color precipitate

**Shinoda Test**

Dried powder of plant + 5 ml of 95% CH<sub>3</sub>OH + Few drops of conc. HCl along + 0.5 g magnesium → turnings Pink precipitate

**Test for Glycosides**

**Keller-Kiiani Test**

Plant extract + gla. CH<sub>3</sub>COOH + few drops 5% FeCl<sub>3</sub> + concentrated sulfuric acid → formation of blue color in acetic layer.

**Legal Test**

Dissolve plant extract in pyridine + sodium nitroprusside solution + made alkaline → Pink or red Color produce

**Test for Phenolics**

**FeCl<sub>3</sub> Test**

Plant extract + aqueous FeCl<sub>3</sub> → blue color

**Test for Saponins**

**Foam Test**

Plant extract in test tube with a little quantity of water ? foam produced persisted for 10 minutes.

**Test for Tannins**

**Gelatin Test**

Plant extract + 1% Gelatin Solution containing 10% sodium chloride → White precipitate

**FeCl<sub>3</sub> Test**

Plant extract + FeCl<sub>3</sub> → Blue-black precipitate

**Test for Terpenoids**

**Salkowski Test**

Extract + Con. H<sub>2</sub>SO<sub>4</sub> → formation of yellow colored at lower layer

**Preparation of Combination**

Activities of various extract and their combination were resolute by the zone of ??the inhibition method. It was investigated by agar diffusion method using agar cup method. Purified extract was diluted in dimethyl sulfoxide and purified antibiotics were used by purification; and stand at 40 C. For compare slandered results we used standard antibiotic. Antimicrobial activity of all extracts and their combination were tested against for some bacteria and fungi which was prepared in Acetone (CH<sub>3</sub>COCH<sub>3</sub>), Methanol (CH<sub>3</sub>OH) and ethanol (CH<sub>3</sub>CH<sub>2</sub>OH)

**Table 1.** Qualitative Phytochemical Analysis

Phytochemical	Name of Test	Acetone extract	Ethanol Extract	Methanol Extract
Alkaloids	Dragendroff’s Test	-	+	+
	Hagers’s Test	-	+	+
	Mayers’s Test	-	+	+
	Wagner’s Test	-	+	+
Flavonoids	Test with Alkaline	-	+	+
	Test with Lead Acetate	-	+	+
	Shinoda test	-	+	+
Glycosides	Keller-Kiiani Test	-	-	+
	Legal Test	-	+	+
Phenolics	FeCl <sub>3</sub> Test	+	+	+
Saponins	Foam Test	-	+	+
Tannins	Gelatin Test	-	-	-
	FeCl <sub>3</sub> Test	+	+	+
Terpenoids	Salkowski Test	+	+	+

(+) shows the Positive result and (-) show Negative Result of the Test

solvents. Muller-Hinton agar plates were seeded with indicator bacterial and fungal strains, and incubated for 24 hours at 37°C. The sensitivity of microbial species to plant extracts was determined by measuring the resistance zone size on the agar surface.

## RESULT AND DISCUSSION

Antimicrobial Analysis *Adhatoda Vasica*, Antibiotics and Combination both for 25 µl.

\*Growth of the Bacteria and Fungi shown in table number 2 to table number 9 are in Millimeter (mm)

**Table 2.** Antibacterial activity of *Adhatoda Vasica*

Bacteria	Ac extract				EtOH extract Concentration (µg/ml)				MeOH extract			
	1000	500	250	125	1000	500	250	125	1000	500	250	125
S. A	8	7	6	5	9	8	7	6	10	9	8	7
B. S	7	6	6	5	8	7	7	6	9	8	8	7
P. A	11	10	9	8	12	11	10	9	13	12	11	10
E. C	13	12	11	10	14	13	12	11	15	14	13	12

**Table 3.** Antibacterial activity of *Adhatoda Vasica* with Amoxicilline

Bacteria	Amo	Ac extract + Amo				EtOH extract + Amo Concentration (µg/ml)				MeOH extract + Amo			
	1000	500	250	125	1000	500	250	125	1000	500	250	125	
S. A	28	30	29	29	28	31	30	29	29	32	31	30	29
B. S	30	28	28	27	26	30	30	29	28	31	31	26	25
P. A	1	11	9	9	8	13	11	10	9	14	12	11	10
E. C	1	14	13	11	10	14	13	12	10	16	14	13	12

**Table 4.** Antibacterial activity of *Adhatoda Vasica* with Ciprofloxacin

Bacteria	Cip	Ac extract + Cip				EtOH extract + Cip Concentration (µg/ml)				MeOH extract+ Cip			
		1000	500	250	125	1000	500	250	125	1000	500	250	125
S. A	24	26	25	25	24	30	29	27	22	29	28	27	26
B. S	27	29	28	27	26	30	29	27	27	31	30	29	28
P. A	27	33	31	30	28	35	32	30	29	36	35	32	30
E. C	27	32	30	29	28	34	32	32	28	35	33	31	30

**Table 5.** Antibacterial activity of *Adhatoda Vasica* with Ceftazidime

Bacteria	Cef	Ac extract + Cef				EtOH extract + Cef Concentration (µg/ml)				MeOH extract + Cef			
		1000	500	250	125	1000	500	250	125	1000	500	250	125
S. A	1	9	7	6	5	10	8	7	5	12	10	9	7
B. S	1	8	6	6	5	9	7	7	6	10	8	7	6
P. A	5	13	11	10	9	16	14	12	10	19	17	14	13
E. C	16	22	20	19	18	26	25	23	21	27	24	22	19

**Table 6.** Antibacterial activity of *Adhatoda Vasica* with Erythromycin

Bacteria	Ery	Ac extract +Ery			EtOH extract + +Ery Concentration (µg/ml)				MeOH extract +Ery				
		1000	500	250	125	1000	500	250	125	1000	500	250	125
S. A	16	22	22	21	19	22	21	24	23	23	21	20	19
B. S	22	24	24	23	21	26	25	24	23	25	24	23	21
P. A	1	13	11	9	8	14	11	10	9	15	13	11	10
E. C	5	17	17	16	15	19	17	17	16	19	17	16	15

**Table 7.** Antifungal activity *Adhatoda Vasica*

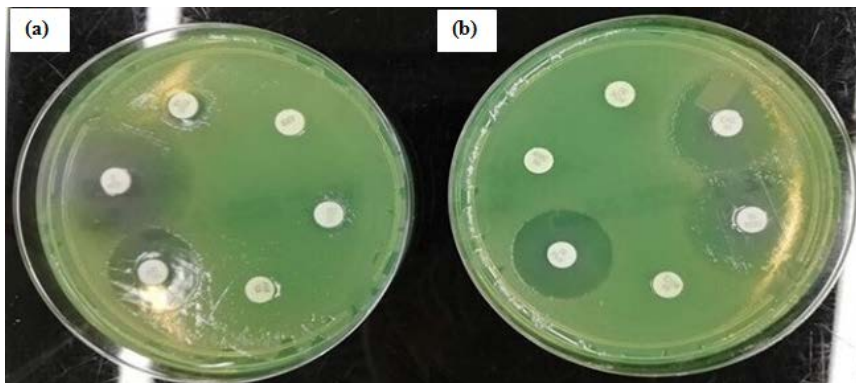
Fungi	Ac extract				EtOH extract Concentration (µg/ml)				MeOH extract			
	1000	500	250	125	1000	500	250	125	1000	500	250	125
A. N	8	7	6	5	9	8	7	6	10	9	8	7
C. A	7	6	5	4	9	7	5	4	9	8	7	5

**Table 8.** Antifungal activity of *Adhatoda Vasica* with Amphotericin B

Fungi	Amp	Ac extract +Amp			EtOH extract +Amp Concentration (µg/ml)				MeOH extract +Amp				
		1000	500	250	125	1000	500	250	125	1000	500	250	125
A. N	14	19	19	18	18	19	17	16	14	22	21	20	19
C. A	8	12	10	9	8	18	16	13	12	19	17	14	13

**Table 9.** Antifungal activity of *Adhatoda Vasica* with Fluconazole

Fungi	Flu	Ac extract + Flu				EtOH extract + Flu Concentration (µg/ml)				MeOH extract + Flu			
		1000 25 µl	500 25 µl	250 25 µl	125 25 µl	1000 25 µl	500 25 µl	250 25 µl	125 25 µl	1000 25 µl	500 25 µl	250 25 µl	125 25 µl
A. N	1	9	9	8	8	10	8	7	6	12	11	10	8
C. A	1	7	6	5	4	9	7	5	4	9	8	7	5



**Fig. 2.** Activity in *Adhatoda Vasica* extract (a) Antibacterial (b) antifungal

In the above table three different solvent were used for biological study. The study for each solvent were done with four different concentration. Table shows methanolic extract of in 1000 µg/ml concentration gives highest growth Which is used to decide dosage for human body.

Amoxicilline which is an antibacterial antibiotic is combined with the plant. The study shows the combination with 1000 µg/ml in MeOH extract gives highest zone of inhibition.

The mixture of Plant extract and ciprofloxacin in 1000 µg/ml gives good values in zone of inhibition of bacteria.

Ceftazidime which is also an antibacterial antibiotic also gives effective results in methanol when it mixed with plant

Combination of plant extract and an antibacterial antibiotics Erythromycin gives good activity in higher concentration.

In this table antifungal activity of Adhatoda Vasica extract was studied in Acetone, Methanol and Ethanol solvent. Biological study in two fungi, Aspergillus Niger and Candida Albicans fungi shows increasing of zone inhibition in higher concentration. Methanolic extract shows higher activity then other two solvent.

An antifungal antibiotic Amphotericin B when combined with Adhatoda Vasica extract gives very good activity in 1000 µg/ml concentration.

Fluconazole which is also an antifungal antibiotic also gives effective activity in higher concentration of the solvent.

### CONCLUSION

Plant drugs from Ayurvedic framework are being investigated globally. The consequences of the essential examination demonstrated that alongside its traditional helpful cases and some experimentally demonstrated pharmacological exercises, Adhatoda Vasica additionally has the possibility to be created as a powerful plant with combination of antibiotics. Combination of A. Vasica and antibiotics indicated pronounced antimicrobial activities with wonderful restraints. This work gives logical confirmations to medicinal uses of A. Vasica with commitment of a portion of the distinguished and tried phytoconstituents in the got organic impacts. From the study we find that the combination of plant extract with antibiotics

gives very effective activity then only plant and antibiotics. It also suggests that methanolic extract with higher concentration gives best results than others.

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### Abbreviations

Amo: *Amoxicilline*

Cip: *Ciprofloxacin*

Cef: *Ceftazidime*

Ery: *Erythromycin*

Amo: *Amoxicilline*

Flu: *Fluconazole*

A. N: *Aspergillus Niger*

C. A: *Candida Albicans*

Ac: Acetone ( $\text{CH}_3\text{COCH}_3$ )

EtOH: Methanol ( $\text{CH}_3\text{OH}$ )

MeOH: Ethanol ( $\text{CH}_3\text{CH}_2\text{OH}$ )