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.
(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 serval 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

**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

**Dragendorff’s Test**
Plant extract + Potassium Bismuth Iodide solution (Dragendorff’s Reagent) → Orange Precipitate
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     | FeCl3 Test       | +               | +               | +               |
| Saponins      | Foam Test        | -               | +               | +               |
| Tannins       | Gelatin 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 | 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 | Ac extract + Amo | EtOH extract + Amo | 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  | 32   31  30   29  |
| B. S     | 30   28  28   27  | 26   30  30   29  | 31   31  26   25  |
| P. A     | 1    11  9    9   | 8    13  11   10  | 14   12  11   10  |
| E. C     | 1    14  13   11  | 10   14  13   12  | 16   14  13   12  |

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

| Bacteria | Ac extract + Cip | EtOH extract + Cip | 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  | 29   28  27   26  |
| B. S     | 27   29  28   27  | 26   30  29   27  | 31   30  29   28  |
| P. A     | 27   33  31   30  | 28   35  32   30  | 36   35  32   30  |
| E. C     | 27   32  30   29  | 28   34  32   32  | 35   33  31   30  |

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

| Bacteria | Ac extract + Cef | EtOH extract + Cef | 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   |
| B. S     | 1    8   6    6   | 5    9   7    6   | 10    8   7    6   |
| P. A     | 5    13  11   10  | 9    16  14   12  | 19    17  14   13  |
| E. C     | 16   22  20   19  | 18   26  25   23  | 27    24  22   19  |
Table 6. Antibacterial activity of Adhatoda Vasica with Erythromycin

| Bacteria | Ery | Ac extract +Ery | EtOH extract + +Ery | MeOH extract +Ery |
|----------|-----|----------------|---------------------|-------------------|
|          |     | Concentration (µg/ml) |                  |                   |
|          | 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 | MeOH extract |
|---------|------------|--------------|--------------|
|         | Concentration (µg/ml) |                  |              |
|         | 1000 | 500 | 250 | 125 | 1000 | 500 | 250 | 125 | 1000 | 500 | 250 | 125 |
| A. N    | 8    | 7   | 6   | 5   | 9    | 8   | 7   | 6   | 10   | 9   | 9   | 5   |
| 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 | MeOH extract +Amp |
|-------|-----|----------------|-------------------|-------------------|
|       |     | Concentration (µg/ml) |                  |                   |
|       | 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 | MeOH extract + Flu |
|-------|-----|----------------|-------------------|-------------------|
|       |     | Concentration (µg/ml) |                  |                   |
|       | 1000| 500 | 250 | 125 | 1000 | 500 | 250 | 125 | 1000 | 500 | 250 | 125 |
| 25 µl | 25 µl| 25 µl | 25 µl | 25 µl | 25 µl | 25 µl | 25 µl | 25 µl | 25 µl | 25 µl | 25 µl | 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.

Ceftazidine which is also an antibacterial antibiotic also gives effective results in methanol when it is 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 than only plant and antibiotics. It also suggests that methanolic extract with higher concentration gives best results than others.

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**Abbreviations**

Amo: *Amoxicillin*

Cip: *Ciprofloxacin*

Cef: *Ceftazidime*

Ery: *Erythromycin*

Amo: *Amoxicilline*

Flu: *Fluconazole*

A. N: *Aspergillus Niger*

C. A: *Candida Albicans*

Ac: Acetone (CH$_3$COCH$_3$)

EtOH: Methanol (CH$_3$OH)

MeOH: Ethanol (CH$_3$CH$_2$OH)