



ISSN 0975-413X
CODEN (USA): PCHHAX

Der Pharma Chemica, 2022, 14(8): 1-7
(<http://www.derpharmachemica.com/archive.html>)

Antimicrobial Activity of Extraction of Adhatoda Vasica and its Combination Along with Antibiotics

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Received: 24-Mar-2020, Manuscript no: DPC-22-39102, Editor assigned: 07-Jul-2022, PreQC No: DPC-22-39102, Reviewed: 18-Jul-2022, QC No: Dpc-22-39102, QI No: Dpc-22-39102, Revised: 25-July-2022, Manuscript No: Dpc-22-39102, Published: 25-Aug-2022, DOI: 10.4172/0975-413X.14.8.5

ABSTRACT

Plant extracts have showed effective antimicrobial activity against bacteria and Fungi. Adhatoda Vasica is an important medicinal plant used as medicines for various ailments since ancient times. Combination of herbal extracts and antibiotics are used to decrease side effect of Allopathy drugs and also used to increase sensitivity of plant extracts. Day by day, bacteria regaining antibody against traditional antibiotics and becoming more powerful to stop their regenerating power, combination of plant extracts and antibiotics provide one of the best results in such direction. If nontoxic plant extracts have been taken in suitable doses, it may prove best supplementary remedies for patient. This is in vitro study and such combination must be followed by toxicity test and in vivo tests to determine its therapeutic application against the test organisms.

Keywords: Adhatoda vasica; Antibiotics; Antimicrobial activity.

INTRODUCTION

Nature provides various things against various diseases for thousands of years. [1] In Present time people are more attracted to word the natural medicine for against several diseases. 'Rasayana' has always play important role in living animals. World health organization (WHO) estimated that more than 80 percent of the living organism uses medicinal plant as medicine for primary health care requirements. [2] In some countries, the use of medicinal plants is often associated with witchcraft and superstition, because people do not have the scientific insight to explain and predict the curative action of plants. Antibiotics working today will not work tomorrow new drugs must be examined, with fewer resistance. [3] As a resistance to the spread of old antibiotics, new immune agents are rapidly developed if the problem is solved. However, past records of rapid and emergent emergence of new antimicrobial agents resonate indicate that new family of antimicrobial agents will have short life expectancy. [4] The emergence of bacterial confrontation to antibiotics and it giving out is a major modern universal public health problem, important to healing drawbacks for a huge number of drugs. In recent time medicine in surgery, cancer chemotherapy, and limb transplantation is credited to the use of antibiotics. [5] Antifungal activity of A. vasica was observed by Guleria et al [6]. The extract containing the alkaloid vascinol and 20 % vasicine inhibited ovalbumin-induced allergic reaction by about 37 % at a concentration of 5 mg [7]. A chemical constitutes of A. alkaloids, vasicine, produces bromhexine and ambroxol-two widely used mucolytic. Both chemicals have a pH depended progress inhibitory result on Mycobacterium tuberculosis. Indirect effects of asodha on tuberculosis include increased lysozyme and rifampicin levels in bronchial secretions, lungs tissue and sputum, suggesting that it may play an important role in the treatment of tuberculosis [8]. In laboratory experiments on cats and dogs, A. Vasica was found to increase bile activity when the animals were given an intravenous dose of 5 mg/kg in dogs, the amount of excreted bile increased by 40%-100%. The animals also showed an increase in bilirubin excretion Figure 1 [7].

Common name:

English: Malabar nut, adulsa

Hindi: Adosa, adalsa, vasaka

Gujarati: Arduasi, Adusi

Marathi: Vasuka

Plant anatomy

Kingdom: Plantae

Division: Angiosperm
Class: Dicotyledonae
Subclass: Gamopetalae
Series: Bicarpellatae
Order: Personales
Family: Acanthaceae
Genus: Adhatoda.
Species: Vasica



Figure 1: Malabar nut, adulsa.

METHODOLOGY

The leaves of the Adhatoda Vasica were collected from the local area. The earliest stages of studying medicinal plants are preparation of plant samples, which is to preserve biomolecules in plants before extraction. The leaves were completely washed with tap water and then washed with distilled water to remove dust and sand particles. The leaves were dried in the dark at room temperature for a few days and then powder by mortar. And it is extracted by using soxhlet extraction.

Qualitative phytochemical analysis

Qualitative phytochemical analysis was done using the following methods for different phytochemicals.

(a) Test for alkaloids:

Plant Extractions were dissolved in dil. HCl and filtered.

Dragendroff's test:

- Reaction of plant extract with Potassium Bismuth Iodide solution (Dragendroff's Reagent) gives Orange Colored precipitate which indicates presence of Alkaloids.

Hagers's test:

- Reaction of plant extract with Picric Acid (Hagers's Reagent) gives Yellow Colored precipitate which indicates presence of Alkaloids.

Mayers's test:

- Reaction of plant extract with Potassium Mercuric Iodide solution (Mayers's Reagent) gives Cream Colored precipitate which indicates presence of Alkaloids.

Wagner's test:

- Reaction of plant extract with Iodine in Potassium Iodide (Wagner's Reagent) gives Red-brown Colored precipitate which indicates

presence of Alkaloids.

(b) Test for flavonoids

Test with Alkaline

- Plant extract with Sodium Hydroxide gives formation of intense yellow color which become colorless on further adding Dil. acid which indicates presence of Flavonoids.

Test with lead acetate

- Treat plant extract with few drop of Lead Acetate gives Yellow precipitate which indicates presence of Flavonoids.

Shinoda test:

- In dried powder of plant add 5 ml of 95% ethanol and few drops of conc. HCl along with 0.5 g magnesium turnings gives Pink Colored precipitate which indicates presence of Flavonoids.

(c) Test for glycosides

Keller-kiiani test

Treat Plant extract with glacial acetic acid and few drops 5% FeCl₃. Add concentrated. sulfuric acid to the extract in it. The Formation of blue color in acetic layer conform test for glycosides.

Legal test

Dissolve pyridine in the plant extract up on add sodium nitroprusside solution to it and made alkaline, Pink or red Color produce which indicates presence of Glycosides.

(d) Test for phenolics

Ferric Chloride Test

Gives blue color with addition of aqueous ferric chloride in plant extract.

(e) Test for saponins

Foam test

Small Amount of extract was shaken in test tube with a little quantity of water, the foam produced persisted for 10 minutes. This confirms the presence of saponins.

(f) Test for tannins

Gelatin test

To the extract few drops of 1% solution of gelatin containing 10% sodium chloride was added, White precipitate was observed which indicates presence of Tannins.

Ferric chloride test

Plant extract was taken in a test tube and Ferric Chloride solution was added drop by drop, blue-black precipitate was observed which indicates presence of Tannins.

(g) Test for Terpenoids

Salkowski test:

Treat the extract with few drops of concentrated sulphuric acid formation of yellow colored at lower layer which indicates presence of Terpenoids.

Preparation of combination (antimicrobial analysis)

Antimicrobial activities of different plant extracts and their combination were determined by zone of inhibition method. Antimicrobial activities of plant extracts against four pathogenic bacteria and two pathogenic fungi were investigated by agar diffusion method. Antimicrobial activities were prepared by using agar cup method. Each purified extract was dissolved in dimethyl sulfoxide, sterilized antibiotics were used by filtration and stand at 40 C. A standard antibiotic was used for comparison of the results. All the extracts were screened for their antibacterial activities against B. Subtilis EC, Aeruginosa and Aureus, and antifungal activities against A Niger and C. albicans. Plant extract were prepared in Acetone, methanol and ethanol solvents Muller-Hinton agar plates were seeded with indicator bacterial and fungal strains, and allowed to incubate at

370°C for 24 hours. The sensitivity of microorganism species to the plant extracts were determined by measuring the size of inhibitor zone on agar surface.

RESULTS AND DISCUSSION

Discussion

Table 1 and 2 shows antibacterial activity of Adhatoda Vasica extract by using solvents like acetone, ethanol and methanol for 25 µl. Results show that A. Vasica extract is more powerful against Pseudo aeruginosa and against E. coli. Methanol extract has highest zone of inhibition against E. coli. Here with increasing concentration from 125 µg/ml to 1000 µg/ml, zone of inhibition increases. This is used to decide dosage of plant extract for human body.

Table 1: Qualitative phytochemical analysis.

Phytochemical	Name of test	Acetone extract	Ethanol extract	Methanol extract
Alkaloids	Dragendroff's Test	-Ve	+Ve	+Ve
	Hagers's Test	-Ve	+Ve	+Ve
	Mayers's Test	-Ve	+Ve	+Ve
	Wagner's Test	-Ve	+Ve	+Ve
Flavonoids	Test with Alkaline	-Ve	+Ve	+Ve
	Test with Lead Acetate	-Ve	+Ve	+Ve
	Shinoda test	-Ve	+Ve	+Ve
Glycosides	Keller-Kiiani Test	-Ve	-Ve	+Ve
	Legal Test	-Ve	+Ve	+Ve
Phenolics	Ferric Chloride Test	+Ve	+Ve	+Ve
Saponins	Foam Test	-Ve	+Ve	+Ve
Tannins	Gelatin Test	-Ve	-Ve	-Ve
	Ferric Chloride Test	+Ve	+Ve	+Ve
Terpenoids	Salkowski Test	+Ve	+Ve	+Ve

Antimicrobial analysis adhatoda vasica, antibiotics and their combination for 25 µl

Table 2 to 6 shows antibacterial activity of combination of Adhatoda Vasica extract with antibiotics respectively Amoxicilline, Ciprofloxacin, Ceftazidime and Erythromycin for 25 µl. Here synergic effect observed for combination. Further methanol and ethanol extract are little stronger compare to acetone extract. By increasing concentration from 125 to 1000 µg/ml, value of zone of inhibition increased.

Table 2: Table shows antibacterial activity of Adhatoda Vasica extract for 25µl.

Bacteria	Acetone extract				Ethanol extract				Methanol extract			
	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
S. aureus	8 mm	7 mm	6 mm	5 mm	9 mm	8 mm	7 mm	6 mm	10 mm	9 mm	8 mm	7 mm
B. subtilis	7 mm	6 mm	6 mm	5 mm	8 mm	7 mm	7 mm	6 mm	9 mm	8 mm	8 mm	7 mm
P.aeruginosa	11 mm	10 mm	9 mm	8 mm	12 mm	11 mm	10 mm	9 mm	13 mm	12 mm	11 mm	10 mm
E. coli	13 mm	12 mm	11 mm	10 mm	14 mm	13 mm	12 mm	11 mm	15 mm	14 mm	13 mm	12 mm

Table 3: Table shows antimicrobial activity of Adhatoda Vasica with Amoxicilline for 25 µl.

Bacteria	Amox	Acetone extract + Amoxicilline				Ethanol extract + Amoxicilline				Methanol extract + Amoxicilline			
		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
S. aureus	28 mm	30 mm	29 mm	29 mm	28 mm	31 mm	30 mm	29 mm	29 mm	32 mm	31 mm	30 mm	29 mm
B. subtilis	30 mm	28 mm	28 mm	27 mm	26 mm	30 mm	30 mm	29 mm	28 mm	31 mm	31 mm	26 mm	25 mm
P.aeruginosa	1 mm	11 mm	9 mm	9 mm	8 mm	13 mm	11 mm	10 mm	9 mm	14 mm	12 mm	11 mm	10 mm
E. coli	1 mm	14 mm	13 mm	11 mm	10 mm	14 mm	13 mm	12 mm	10 mm	16 mm	14 mm	13 mm	12 mm

Table 4: Table shows antimicrobial activity of combination of Adhatoda Vasica with Ciprofloxacin for 25 µl.

Bacteria	Cipro	Acetone extract + Ciprofloxacin				Ethanol extract + Ciprofloxacin				Methanol extract + Ciprofloxacin			
		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
S. aureus	24 mm	26 mm	25 mm	25 mm	24 mm	30 mm	29 mm	27 mm	22 mm	29 mm	28 mm	27 mm	26 mm
B. subtilis	27 mm	29 mm	28 mm	27 mm	26 mm	30 mm	29 mm	27 mm	27 mm	31 mm	30 mm	29 mm	28 mm
P.aeruginosa	27 mm	33 mm	31 mm	30 mm	28 mm	35 mm	32 mm	30 mm	29 mm	36 mm	35 mm	32 mm	30 mm
E. coli	27 mm	32 mm	30 mm	29 mm	28 mm	34 mm	32 mm	32 mm	28 mm	35 mm	33 mm	31 mm	30 mm

Table 5: Table shows antimicrobial activity of combination of Adhatoda Vasica with Ceftazidime for 25 µl.

Bacteria	Cefta	Acetone extract + Ceftazidime				Ethanol extract + Ceftazidime				Methanol extract + Ceftazidime			
		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
S. aureus	1 mm	9 mm	7 mm	6 mm	5 mm	10 mm	8 mm	7 mm	5 mm	12 mm	10 mm	9 mm	7 mm
B. subtilis	1 mm	8 mm	6 mm	6 mm	5 mm	9 mm	7 mm	7 mm	6 mm	10 mm	8 mm	7 mm	6 mm
P.aeruginosa	5 mm	13 mm	11 mm	10 mm	9 mm	16 mm	14 mm	12 mm	10 mm	19 mm	17 mm	14 mm	13 mm
E. coli	16 mm	22 mm	20 mm	19 mm	18 mm	26 mm	25 mm	23 mm	21 mm	27 mm	24 mm	22 mm	19 mm

Table 6: Table shows antimicrobial activity of combination of Adhatoda Vasica with Erythromycin for 25 μ l.

Bacteria	Eryth	Acetone extract + Erythromycin				Ethanol extract + Erythromycin				Methanol extract + Erythromycin			
		Concentration (μ g/ml)											
		1000	500	250	125	1000	500	250	125	1000	500	250	125
	25 μ l	25 μ l	25 μ l	25 μ l	25 μ l	25 μ l	25 μ l	25 μ l	25 μ l	25 μ l	25 μ l	25 μ l	25 μ l
S. aureus	16 mm	22 mm	22 mm	21 mm	19 mm	22 mm	21 mm	24 mm	23 mm	23 mm	21 mm	20 mm	19 mm
B. subtilis	22 mm	24 mm	24 mm	23 mm	21 mm	26 mm	25 mm	24 mm	23 mm	25 mm	24 mm	23 mm	21 mm
P.aeruginosa	1 mm	13 mm	11 mm	9 mm	8 mm	14 mm	11 mm	10 mm	9 mm	15 mm	13 mm	11 mm	10 mm
E. coli	5 mm	17 mm	17 mm	16 mm	15 mm	19 mm	17 mm	17 mm	16 mm	19 mm	17 mm	16 mm	15 mm

Table 7 shows antifungal activity of Adhatoda Vasica extract for 25 μ l. With increasing concentration, zone of inhibition increased. Methanol extract having little higher value of zone of inhibition. Methanol extract is further more effective against A. Niger.

Table 7: Table shows antifungal activity of combination of Adhatoda Vasica for 25 μ l.

Fungi	Acetone extract				Ethanol extract				Methanol extract			
	Concentration (μ g/ml)											
	1000	500	250	125	1000	500	250	125	1000	500	250	125
	25 μ l	25 μ l	25 μ l	25 μ l	25 μ l	25 μ l	25 μ l	25 μ l	25 μ l	25 μ l	25 μ l	25 μ l
A. Niger	8 mm	7 mm	6 mm	5 mm	9 mm	8 mm	7 mm	6 mm	10 mm	9 mm	8 mm	7 mm
C. Albican	7 mm	6 mm	5 mm	4 mm	9 mm	7 mm	5 mm	4 mm	9 mm	8 mm	7 mm	5 mm

Table 8 and 9 shows antifungal activity of combination of Adhatoda Vasica extract with Amphotericin B and Fluconazole respectively for 25 μ l. Results shows that with increasing concentration for combination for methanol extract zone of inhibition increased. Combination showed synergic effect against Aspergillus Niger and Candida Albican. But better resistive properties obtained for all extract against Aspergillus Niger.

Table 8: Table shows antifungal activity of combination of Adhatoda Vasica with Amphotericin B for 25 μ l.

Fungi	Amph	Acetone extract + Amphotericin				Ethanol extract + Amoxicilline				Methanol extract + Amoxicilline			
		Concentration (μ g/ml)											
		1000	500	250	125	1000	500	250	125	1000	500	250	125
	25 μ l	25 μ l	25 μ l	25 μ l	25 μ l	25 μ l	25 μ l	25 μ l	25 μ l	25 μ l	25 μ l	25 μ l	
A. Niger	14 mm	19 mm	19 mm	18 mm	18 mm	19 mm	17 mm	16 mm	14 mm	22 mm	21 mm	20 mm	19 mm
C. Albican	8 mm	12 mm	10 mm	9 mm	8 mm	18 mm	16 mm	13 mm	12 mm	19 mm	17 mm	14 mm	13 mm

Table 9: Table shows antifungal activity of combination of Adhatoda Vasica with Fluconazole for 25 µl.

Fungi	Fluco	Acetone extract + Fluconazole				Ethanol extract + Fluconazole				Methanol extract + Fluconazole			
		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. Niger	1 mm	9 mm	9 mm	8 mm	8 mm	10 mm	8 mm	7 mm	6 mm	12 mm	11 mm	10 mm	8 mm
C. Albican	1 mm	7 mm	6 mm	5 mm	4 mm	9 mm	7 mm	5 mm	4 mm	9 mm	8 mm	7 mm	5 mm

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