

A Review on Concept of Malabar Nut

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Abstract:

Justicia adhatoda L., also known as *adhatodavasic* [L.] Nees, is a member of the *acanthaceae* family and is regarded as the most important plant in the entire globe. It is found throughout India and the world and is also referred to as *vasaka*, *vasica*, *adosa*, or *malbur nut*. It is a widely used herb in the unani and ayurvedic medical systems. For more than 2,000 years, this herb has been part of India's traditional medical system. The plant is extremely valuable medicinally and is used to treat a wide range of illnesses, most commonly respiratory conditions such as chronic bronchitis, asthma, TB, and common cold symptoms. Medicines are made from every component of the plant. This plant's primary chemical component, *vasicine*, has a number of therapeutic uses and is used into several ayurvedic formulas. Additionally, it possesses a number of pharmacological qualities that have been recorded, including antispasmodic, sedative, expectorant, antitussive, oxytocic, antibacterial, anti-diabetic, wound healing, abortifacient, antiasthma, and anti-pyorrhea benefits.

Keywords: Vasapatra, Ayurveda, Rasapanchak, Pharmacological properties.

INTRODUCTION:

Since ancient times, plants have been used as a variety of therapeutic treatments. Numerous scientific investigations and research projects worldwide have been sparked by the understanding of Indian medicinal plants and their use in the Ayurvedic and Unani medical systems. For much longer than 50 years, studies on Indian medicinal herbs have been conducted. Many of the active components that have been extracted from plants are crucial to contemporary medicine. Numerous pioneers conducted extensive research on India's medicinal flora, which ultimately provided a vast array of active ingredients and plant drugs for contemporary medicine. Organic item Drug materials encompass a wide range of products, from plant parts to basic extracts to isolated active ingredients. The term includes a broad variety of naturally occurring compounds that are significant for their medicinal properties or as adjuvants in pharmaceutical products. ^[1]

India is known as the botanical garden of the world and is the world's largest producer of medicinal herbs. The country officially recognizes over 3000 plants for their medicinal value, and it is generally estimated that over 6000 plants are in use in traditional, folk, and herbal medicine, representing about 75% of the medicinal needs of the Third World countries. India is sitting on a gold mine of well-recorded and traditionally well-practiced knowledge of herbal medicine. *Adhatodavasic* is a small, three-meter-tall perennial shrub that is evergreen. Its branches are rising and opposite. The broad, leathery leaves are 4 cm

wide and 10 to 15 centimeters long. They are occasionally used as an insecticide. With a lighter green top and a darker green bottom, they are pubescent. [2]

The leaves grow in an opposite orientation, are entirely lanceolate, and short petiolate. They taper towards the base and tip. When the leaves are dry, they take on a brownish-green color and smell and taste bitter, like strong tea. It makes good charcoal with a soft stem. Massive, dense terminal spikes featuring attractively big, white petals with a purple tinge on the lower lip. The fruit is a tiny capsule with four globular seeds within that is clavate and has longitudinal channels. Treatments for tuberculosis, bronchitis, and other lung and bronchiole problems can be achieved with Adhatodavasica. Cough & other cold symptoms may be alleviated by making a decoction from the leaves of the Vasaka plant. [3]

Table 1: Vernacular names for Adhatodavasica

Hindi	Adosa, adalsa, vaska
Sanskrit	Shwetavasa, vasa, vaska, vidyamataasinghee
Bengali	Basak
Tamil	Adatodai
Marathi	Vasuka
Telugu	Adasaram
Malayalam	Ata - lotakam
Gujarati	Aradusi, adusa
Punjabi	Bansa, basui, bhekkar
English	Malabur nut
China	Ya- Zui-Hua
Manipuri	Nonmangkha-agouba
Kannada	Adusoge
Arabic	Adusha

Table 2: Botanical Classification of Adhatodavasica

Taxonomical Rank	Taxon
Kingdom	Plantae
Division	Angiosperms
Class	Eudicots
Order	Lamiales
Family	Acanthaceae
Genus	Justicia
Species	J.adhatoda
Common name	Adulsa (Vasaka)

Distribution of adhatodavasika:

- Botanical description of Adhatodavasica-**

A member of the Acanthaceae family, Adhatodavasica is a tiny, dense, evergreen shrub with many branches that grows throughout the year. This plant can grow up to 1-3 or 6 meters in height. [4] It has long, opposing branches. The stem has an adaxial side that is herbaceous and an abaxial side that is woody.

The flowers are bisexual, zygomorphic, huge, dense, terminal spikes with large bracts, tiny, irregular, hypogynous, and have an appearance of white, pink, or purple. They measure 1.9–2.2 cm in length and 2.2–0.8 cm in width.^[5,6] The plant has a harsh, unpleasant taste and smell.^[7] The plant has simple, dark green leaves with a tapering base that are reticulate, opposite, short peduncled, elliptic-lanceolate or ovate-lanceolate, hairy, and about 4–7 cm in width and 7–19 cm in length.^[8] This plant produces a tiny, clavate fruit that is longitudinally capsulated and contains four globular seeds that are 5–6 mm long.^[9]



- **Geographical distribution of Adhatodavasica :**

A shrub that is native to the Indo-Malayan region, Adhatodavasica is evergreen. Plants such as these can be found in the Malay Peninsula, the Indonesian Archipelago^[10,11] Nepal, Germany, Pakistan, Burma, Malaysia, India, and Southern China. It is mostly grown in tropical and subtropical areas of India, particularly at an elevation of 1350 meters in the lower Himalayan region. Punjab, Bengal, Manipur, and Kerala are the states where it is most prevalent.^[12-13-14]

- **Phytochemical constituents of Adhatodavasica:**

Adhatodavasica is a plant that contains a variety of chemical constituents, including essential oils, fats, sugar, gum, resins, amino acids, proteins, and vitamin C.^[15] The analysis results revealed that J. adhatoda leaves contain phenols, flavonoids, alkaloids, anthraquinone, saponins, and reducing sugar.^[16] In terms of pharmacology, the most studied phytochemical constituent is bitter quinazoline alkaloid, Vasicine (1, 2, 3, 9-tetrahydropyrrole [2, 1-b] quinazoline-3-ol, C₁₁H₁₂N₂O), which is found in flowers, roots, and leaves.^[17] The synthesis of Vasicine involves adding 2- amino benzylamine to vicinylvasicinone tricarbonyl reagent.^[18] Aerial portions of Adhatodavasica Nees also include two more chemicals, epitaraxerol and peganidine, along with the other triterpenoid 3-hydroxyl-D-friedoolean-5-ene. As part of elemental analysis, atomic absorption spectrometry is used to find major trace elements like K, Na, Ca, and Mg as well as minor trace elements like Zn, Cu, Cr, Ni, Co, Cd, Pb, Mn, and Fe.^[19]

Leaves:

Vasicine (0.85%) and vasicinone (0.027%), which are found in the leaves and roots of this plant, are the two main alkaloids.[20] Other alkaloid compounds include hydroxypeganine, adenosine, adenosine, adenosineone, anisotine, and vaccinone are also present in the plant's leaves. It also includes trace amounts of crystalline acid, betaine, steroids, alkanes, and essential oil. [21-22-23]

Flower:

In addition to alkanes and 4-dihydrochalcone-4-glucoside, it contains triterpenes (alpha-amyrin), flavonoids (Astragalin, Kaempferol, Quercetin, Vitexin, and Apigenin). [24-25-26]

Root:

The root portion has the following chemical composition: Vitamin C (5.2%), lipids (2.5%), carbohydrates, alkanes, alkaloids (such as vascine (7.5%), vasicinal, vasicinolone, and vasicinone 3.5%), fiber (5.2%), and adhatonine. The plant's roots also include extracts of sitosterol, deoxyvasicine, and β -glucoside-galactose. [27]

Seeds :



Deep yellow oil, comprising glycerides of arachidic acid 3.1%, lignoceric acid 10.7%, oleic acid 49.9%, cerotic 5%, and linoleic acid 12.3%, makes up 25.8% of the oil in seeds. β -sitosterol (2.6%), and behenic 11.2%. [28]

Table 3: Morphology of Adhatoda Vasica:

Parameters	Properties
Colour	Light green
Size	10 – 13 cm long
Apex	Acuminate
Shape	Ovate-lanceolate
Odour	Characteristic
Taste	Bitter
Margine	Slightly crenate to entire
Base	Symmetric
Texture	Leathery
Venation	Pinnate

A. Cultivation:

A variety of conditions, such as breathing difficulties, coughs, colds, nasal congestion, sore throats, asthma, bronchitis, and other upper respiratory tract infections, bleeding issues, and so forth, can be effectively treated with the herb plant Justicia Adhatoda.

Although it is also found in Nepal, Sri Lanka, Pakistan, Malaysia, Indonesia, and China, Adulsa leaves are indigenous to India. The golden bark, crossbow-shaped leaves, white and purple flowers, and pubescent, club-shaped capsular fruits are characteristics that set this Vasaka plant apart. Your Adulsa plant can be placed in the balcony garden.

Table 4: Plants specification of adhatodavasika

Plant height	20-30 cm
Maximum reachable height	Upto 14 feet
Difficulty level	Easy to grow

- **Here is a step-by-step guide to growing:-**

Adusi Plant:**1. Obtain seeds or cuttings:**

Starting with seeds from a reliable nursery or garden center is a good place to start. As an alternative, throughout the growing season, you can take cuttings from a healthy Vasaka plant.

2. Choose right location:

Adusi Plants like bright, partly shaded areas. It may require shade on hot summer afternoons, but it can withstand full sun as well.

3. Prepare the soil:

Vasaka favors soil with lots of organic materials and good drainage. Garden soil and compost or well-rotted manure can be combined to prepare the soil. It is recommended to have a pH between 6.0 and 7.5, which is slightly acidic to neutral.

4. Planting:

In the event that seeds are being used, plant them straight in the prepared soil, about 1/4 inch (0.6 cm) deep, and lightly cover with earth. If you're planting cuttings, snip off a healthy stem that has a few leaves and plant it straight into the ground.

5. Watering:

Maintain a constant moisture content in the soil, but do not overwater it. The Adusi plant prefers to be watered frequently, particularly in dry seasons. To avoid root rot, however, ensure that the soil has adequate drainage.

6. Sunlight:

A bright, indirect light source is ideal for your Adulsa plant. For a healthy growth, Vasaka plants require three to six hours of direct sunlight every day, with the best light coming from the sun in the morning.

7. Mulching:

Plants might benefit from having a layer of organic mulch applied around them to help preserve soil moisture and inhibit weed growth. As mulch decomposes, it also enriches the soil with nutrients.

8. Fertilizing:

Once every two weeks during the growing season (spring and summer), fertilize the Vasaka plant with a balanced, water-soluble fertilizer. During the winter, you can lessen how often you fertilize.

9. Pruning:

Frequent trimming promotes bushier growth and more prolific flowering. After the flowering season, you can prune the plant to keep it in shape.

10. Pest & disease control:

Adusi Plants are comparatively resistant to illnesses and pests. Aphids and mites, for example, are frequent garden pests to be aware of. If infestations need to be controlled, apply neem oil or organic insecticidal soap.

11. Additional care:

As Adulsa plants are particularly susceptible to root rot at this point, cut back on the amount of hydration you provide them. Two frequent pests to be aware of are mealybugs and spider mites.

12. Harvesting:

As Adulsa plants are particularly susceptible to root rot at this point, cut back on the amount of hydration you provide them. Two frequent pests to be aware of are mealybugs and spider mites. ^[29]

B. Collection:

Adhatoda vasica Nees stem cuttings were obtained from the Medicinal and Aromatic Research Center and propagated using the conventional vegetative method. To determine the vasicinone content, fragile stems at a comparable growth stage and newly grown, completely opened 60-day-old leaves were collected.

In order to initiate and multiply axillary shoots in vitro, the in vivo-collected nodal-segment explants were injected into Murashige and Skoog (1962] (MS] media supplemented with 1.1 mg/l of N 6-benzyladenine, in accordance with Panigrahi and Patel's (2014] technique. The in vivo-collected nodal segments were simultaneously injected in MS media supplemented with 1 mg/l of 2,4-dichlorophenoxy acetic acid for the purpose of in vitro callus induction. For HPTLC analysis, vasicinone was used as a standard and the leaves and stems of in vivo plants as well as in vitro-regenerated leaves, stems, and induced callus were utilized. [30,31].

C. Extraction :

a) Preparation of extract:

Adusa leaves were dried in an oven at 45 0C. Using a grinder, the dried leaves were powdered. A hydroalcoholic solution was used to extract the resulting powder. In accordance with the experimental design, 40g of the drug sample powder was extracted using 320 ml of hydro alcohol solvent at various ethanol concentrations and temperatures. [32]

Table5: Boundaries of the experimental domain and spacing of the compositional variable leaves for Adusa leaf

Independent variables	Symbol code	Low variables	High variables
Temperature(c]	A	60	80
Concentration of ethanol (%)	B	30	70
Time	C	6	8

To improve the quantity and purity of extracted components from A. vasica plant materials, the uniformly powdered samples underwent double extraction utilizing the Soxhlet extraction and column chromatographic extraction technique. Hexane, toluene, ethyl acetate, acetone, and methanol were the solvents used for the extraction process depending on their polarity. Using 600 ml of solvent and 4 g of powdered material, this process was performed. After six or eight hours of initial extraction and six rounds of the solvent being boiled, the extraction process was completed. Every chemical and solvent used was analytical grade, bought from Sigma-Aldrich, and utilized exactly as supplied, requiring no additional purification. [33]

b) Ethanolic extraction: [34]

The drug followed by proper particle size reduction [mills]

↓
Then placed in Soxhlet apparatus

↓
Extracted with ethanol efor 48 hrs

↓
After that a band tube was used to concentrate the ethanolic extraction and recover the Soxhlet



The ethanol extract is refrigerated for faster crystallization



To carry out further phytochemical screening concentrated ethanolic extraction was used.

• **Microscopic study:**

A basic microscope was used to conduct morphological investigations. We looked at the leaf powder's shape, apex, base, margin, taste, and smell. Studying under a microscope: A free-hand transverse section of the leaves and stem was obtained, stained with double-stained differential staining, and then mounted in DPX (Johanson,1940). Using a camera lucida, the cellular and anatomical illustration was created, and a digital camera was used to help take some pictures. To analyze the trichomes of the upper and lower epidermis as well as the stomata, the leaf was peeled off. The stem was macerated with Jeffrey's fluid for the vascular study, dyed with 1% aqueous saffranin, mounted in glycerine, and made semipermanent by ringing with DPX mountant. To find lignin, chloroglucinol and HCl were applied to the powdered leaves. Starch grains and calcium oxalate crystals were identified using glycerin and iodine solutions, respectively. Using fresh plant leaves, the numbers for the stomata, stomatal index, vein islet, and vein termination were ascertained using quantitative microscopy [Kokate, 1997] ^[35]



Fig. 1 : T. S. of Stem

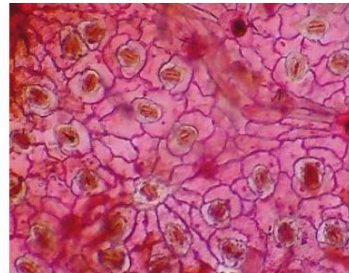


Fig 3 : Stomata Lower epidermis

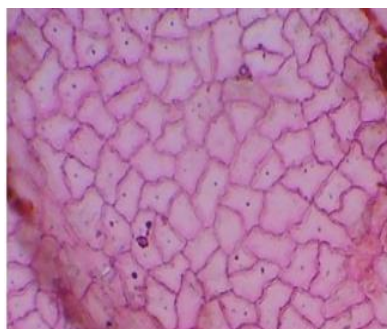


Fig 2: Stomata upper epidermis



Fig. 4 : Stem Vessel

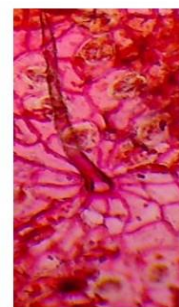


Fig- 5 : Trichomes-upper epidermis

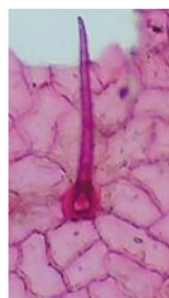


Fig 6: Trichomes-lower

• **Moisture content :**

It is important to keep a drug's moisture content as low as possible to avoid chemical or microbiological contamination or the breakdown of crude drugs. Another sign of excess moisture is that the buyer is paying a premium for water that is not needed. It is possible to calculate the loss on drying or heating to constant weight for materials devoid of volatile chemicals at the drying temperature [17]. After precisely weighing about 2 g of the sample, it was put into a weighing vial that had already been weighed. After removing the loose stopper, the bottle was baked for 30 minutes at 105°C. The bottle was weighed until a consistent weight was reached after being dried and allowed to cool at room temperature in a desiccator.

The air-dried sample was utilized as a reference to calculate the drying loss. The extracted materials were subsequently put through a battery of qualitative tests aimed at identifying different plant the components. [36]

Physicochemical parameters of adhatoda vasika leaf

Parameters	Results
Loss on drying at 105°C(%w/w)	7.2
Total ash value(%w/w)	14.46
Water soluble extractive value(%w/w)	34.5
Alcohol soluble extractive value(%w/w)	9

• **Quantitative estimation :**

Principle :

The Lowry method determines the blue color produced by the protein's biuret reaction with the alkaline cupric tartarate, as well as the color produced by the reduction of phosphomolybdicphosphotungstic components in the folin ciocalteu's reagent by the amino acids tyrosine and tryptophan.

A]Protein estimation :

Reagent :

0.1N sodium hydroxide, 0.5% copper sulfate copper solution, 2% sodium carbonate, and folin reagent.

Protein stock solution :

Accurately weigh 50 mg of bovine serum albumin (fraction v], dissolve it in distilled water, and then fill a standard flask to the brim with the remaining 50 milliliters. Operational criteria: Use a standard flask filled with distilled water to dilute 10 milliliters of stock solution to 50 milliliters. There are 200 ug of rotein in this mixture.

Sample preparation :

Usually, buffers used in enzyme assays are employed for extraction. Weigh the sample to 0.5–1 grams, then thoroughly ground it. in 10–20 ml of buffer using a pestle and mortar. After centrifuging, measure the amount of protein in the supernatant.

Estimation of protein :

1. Pipette out 0.2, 0.4, 0.6, 0.8 and 1ml of working solution into a series of test tubes
2. Pipette out 0.1ml and 0.2ml of sample extract in two other test tubes.
3. Make up the volume to 1ml in all the test tubes. Tubes with 1ml of distilled water serve as a blank
4. Add 5ml of reagent C in each tube including blank. Mix well and allow to stand for 10 minutes.
5. Then add 0.5ml of reagent D, mix well and incubate at room temperature in the dark for 30minutes. Blue colour is developed.

6. Take the reading at 660nm
7. Draw a standard graph and calculate the amount of protein in the sample. [37,38]

B) Alkaloid Estimation :

Reagent :

Dragendorff reagent, Standard bismuth nitrate solution, Thiourea 3% and Disodium sulfide.

Stock solution :

10ml of methanol was utilized to dissolve 10 milligrams of each pure alkaloid.

Sample preparation :

50ml of methanol was employed to Soxhlet extract 10g of coarsely ground plant material.

Estimation of alkaloid :

1. The stock solution of bismuth nitrate pentahydrate was used to obtain the calibration curve.
2. The stock solution was diluted in series by pipetting off 1, 2, 3, 4, 5, 6, 7, 8, and 9 milliliters into individual 10 milliliter standard flasks and diluting with distilled water to volume.
3. An ounce of this solution was taken, and five milliliters of thiourea solution were added
4. Measured at 435 nm against a colorless reference, the absorbance value of the yellow solution. [39,40]

Estimation of alkaloid content and protein content in adhatoda vasika leaf

Parameter	Result
Alkaloid	567.04µg/ml
Protein	12.71 µg/ml

• Phytochemical & Chromatographic studies :

A) Thin layer chromatography :

TLC plate :

Alumina, cellulose, kieselghur, polyamide, and silica gel G have been the only stationary phases used in TLC traditionally. These phases have been coated onto glass, plastic, or aluminum foil. Plates are used for the chromatography, which might be pre-coated or prepared using following methods.

1. Spraying procedure
 2. Spreading procedure
 3. Emulsion procedure
- TLC is performed in various steps, which are as follows:

1. Pre-conditioning Plates :

Plates may need to be cleaned before being separated. The migration of suitable solvents can do this, or the plate can be impregnated. If needed, the plates can be activated at the time of usage by heating them to 100–105 degrees Celsius for an hour.

2. Chromatographic chamber :

A chromatographic chamber including a flat bottom and a twin trough made of clear, inert material that fits the size of the plates to be used, all secured with a tight-fitting cover. In addition to having a trough for the mobile phase, the horizontal development chamber also incorporates a device that guides the mobile phase into the stationary phase.

3. Sample applicators :

A better resolution can be achieved by using methods such as micro-pipettes, micro syringes, calibrated disposable or linomat applicator system [HPTLC] capillary tubes for the correct application of samples or solutions onto the plates.

4. Development :

Initially, TLC was as easy as putting the plate in a glass chamber with the right amount of solvent and letting it move the necessary distance to get the desired separation. A wide range of advancement strategies have been developed to enhance the type of separation that the fundamental TLC approach provides. These techniques include multiplication and its instrumented variants of programmed multiple development [PMD] and automated multiple development [AMD], centrifugal development, over pressurized TLC, two dimensional TLC, triangular development, and continuous multiple development [which combines the continuous and multiple development techniques].

Separation of chemical constituents by thin layer chromatography :

Prepare the TLC plate by using distilled water



Keep the plate in oven at 105°C for 30 minutes



Developed the developing tank by using the different ratio of the mobile phase



Ratio of the mobile phase used(ethylacetate: methanol: ammonia (8: 0.5: 0.2))



spot the sample on TLC plate using capillary tube



Keep the plate in developing tank



When mobile phase run 3/4th of the TLC plate, Plate is removed from the developing tank



Keep the TLC plate for air dry for to two minutes



Spray the Dragendorff's reagent on TLC plate



Two spot was observed

▪ **Solvent system:**

1. Toluene: methanol: dioxane: ammonia
2. Ethyl acetate: methanol: ammonia

▪ **Detecting agent:**

1. UV 254 nm.
2. Spraying with perchloric acid .
3. Spraying with dragendroff's reagent.
4. Spraying with antimony trichloride in hydrochloric acid.
5. Spraying with sulphuric acid. ^[41]



• **PHYTOCHEMICAL SCREENIN**

Preliminary test of adhatodavasika:

Test	Observation	Inference
Colour	Green	Leaf drug
Odour	Specific	Aromatic crude drug
Taste	Astringent	Drug contain tannin
Texture	Fine	--

Preliminary Phytochemical Screening of leaves Powder of Adhatodavasika:

Phytochemical	Test
Alkaloid	+
Glycoside	-
Flavonoid	+
Tannin	+
Reducing sugar	+
Thlobatannins	-
Saponins	+
Terpenoid	-
Anthraquinones	+
Cardiac glycoside	-

A] Test for alkaloid: ^[42]

Test	Observation	Inference
Mayer's test[potassium mercuric iodide solution]: 1 ml of Mayers reagents was added to 1 ml of extract.	White, yellow or creamy precipitate forms	Alkaloids are present
Wagner's test[Iodine potassium iodide solution]: 1 ml of wagners reagents was added to 1 ml of extract.	Reddish brown precipitate	Alkaloids are present
Hager's test[Saturated solution of picric acid]: 1 ml of hager's reagents was added to 1 ml of extract.	Yellow precipitate	Alkaloids are present

B] Test for steroids:

Test	Observation	Inference
Libermannburchard test: To 1ml of the extract 2ml of acetic anhydride Concentrated sulfuric acid (2 ml) was added.	Violet to green or blue	Steroids are present

C]Test for terpenoids: ^[45]

Test	Observation	Inference
Salkowski test: to 1ml of extract 2ml of chloroform along with a few drops of sulfuric acid	Reddish brown ring	Terpenoids are present

D]Test for flavonoids: ^[46]

Test	Observation	Inference
Alkaline reagent test: A few drops of strong hydrochloric acid and a few drops of diluted ammonia solution were added to 1 ml of extract.	Yellow colour produced	Flavonoids are present
Shinoda test: A small quantity of the powdered substance was boiled, combined with alcohol, and then filtered. After adding a few magnesium turns and a few drops of strong hydrochloric acid, the alcoholic solution was heated for five minutes.	Purple colour produced	Flavonoids are present

Zinc hydrochloride test: A solution of strong hydrochloric acid and zinc dust was added to the alcoholic extract.	Red colour produced	Flavonoids are present
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E]Test for saponins: [45]

Test	Observation	Inference
Froth Test: 20ml water+ 0.5gm powder+ boil for 2 minutes+filter+cool+ water+ shake	Foams are observed	Saponins are present

F]Test for phenols: [45]

Test	Observation	Inference
Lead acetate test: 1ml of lead acetate solution was added to 1ml of extract.	Development of precipitate	Phenols are present

G]Test for tannins: [45]

Test	Observation	Inference
Lead acetate test: 1ml of lead acetate solution was added to 1ml of extract.	White precipitate	Tannins are present
Ferric chloride test: A few drops of ferric chloride solution were added to the powdered drug aqueous solution	Bluish black colour observed	Tannins are present

H]Test for carbohydrates: [43,44]

Test	Observation	Inference
Molish test: 100 ml of 95% ethanol were used to dissolve 10 gm of naphol to create this reagent. In order to hydrolyze the extract (heated with dil HCl over a water bath), the reagent was applied to both alcoholic and aqueous extracts.	Purple tint or colour	Carbohydrates are present
Barfoed test: 1 ml of Barfoed's reagent was added to the 2 ml of extract, and the mixture was heated for a few minutes.	Reddish brown precipitate	Carbohydrates are present

I]Test for amino acids: [45]

Test	Observation	Inference
Millon's test: Add around 2 ml of the million reagents to the test solution.	White precipitate	Amino acids are present
Ninhydrin test: Add 3 to 4 drops of ninhydrin solution to 1ml of sample, then boil for ten minutes in a water bath.	Purple or Blue colour	Amino acids are present

J]Test for reducing sugar: [45]

Test	Observation	Inference
Fehling's test: Equal amounts of Fehling's solutions A and B were boiled and added to 1 ml of extract.	Brick red precipitate	Reducing sugar present

K]Test for Glycoside: [47]
a) General Test

Test	Observation	Inference
Test A: A 5% sodium hydroxide solution was used to neutralize and filter 200 mg of the powdered medication that had been heated on a water bath and extracted with 5 ml of diluted sulfuric acid. After that, two minutes of water bath heating and the addition of 0.1 ml of Fehlings solutions A and B made it alkaline.	Red precipitate	Glycosides are present
Test B: 5ml of water were used in place of sulfuric acid to extract 200 mg of the powdered medication. In place of the sodium hydroxide solution, an equal volume of boiled water was added. After that, two minutes of water bath heating and the addition of 0.1 ml of Fehlings solutions A and B made it alkaline.	Red precipitate	Glycosides are present

b)Cardiac Glycoside:

Test	Observation	Inference
Keller killiani Test: 10 ml of 70% alcohol were added to 1gm of powdered leaf, which was then heated for two minutes, cooled, and filtered. 10 ml of water and 5 drops of lead subacetate solution were added to the filtrate, which	Reddish brown layer acquiring bluish green colour after standing was observed	Cardiac glycosides are present

was then filtered. After adding chloroform to the filtrate, the layer of chloroform was separated and dried out by evaporation. 3ml of glacial acetic acid with a hint of ferric chloride were used to dissolve the residue.		
Raymond Test: Methanolic alkali that was heated was added to the leaf's alcoholic extract.	Violet colour was produced	Cardiac glycosides are present
Legal's Test: Pyridine and an alkaline sodium nitro prusside solution were added to the drug's alcoholic extract in powder form.	Red colour was produced	Cardiac glycosides are present

• **ADHATODA VASIKA IN AYURVEDA:**

The Ayurvedic pharmaceutical system uses Adhatodavasica, which is regarded as the primary herb, to treat a variety of ailments. According to Ayurveda, this plant is mostly used to treat respiratory conditions like bronchitis, asthma, coughing, and cold symptoms. It is used to treat bronchitis and is referred to as Vasaka or Vasa in the Indigenous medical system. ^[49,50] Many Ayurvedic doctors prescribe the J. adhatoda plant because of its many medicinal qualities in treating conditions like pitta and kapha-caused malaria, internal hemorrhage, cough, asthma, chronic fever, leprosy, skin conditions, and piles. ^[51]

Ayurvedic Formulation:

More than 20 products, including Vasarishta, Mahatikataghrita, Triphalaghrita, Vasavaleha, Vasakasava, Mahatriphalaghrita, Panchatiktaghritaguggulu, and Panchatiktaghrita, are made from the leaf juice of the Vasaka plant [Vasa Swarasa[52,53] Additional preparations of the Vasa plant include Vasa ghrita [clarified butter made from A. Vasica leaves], Vasa Avaleha [sugar formulation of A. Vasica leaves], and Vasa Asava/Arista [alcoholic preparation of A. Vasica leaves].

RASAPANCHAK [properties of adhatodavasika in ayurveda]:

KARMA [actions]:

1. Hridya: It act against heart diseases
2. Kaphapittahara: It act against digestion problem, heartburn, arthritis
3. Raktasangrahika: It helps in blood circulation.
4. Kasaghna: It is used against cough and cold

•Properties of Vasaka plant:

1. Raktapitta : It is used to cure hemorrhagic disorder/purpura.
2. Kasa : It is used against cough and cold.
3. Jwara : It is used to cure fever.
4. Kshaya : It is used in the treatment of Phthisis.
5. Rajayakshma : It is used to cure tuberculosis.

6. Parshvashula : It is used to cure pain in flanks.
7. Hritshula : It is used to treat cardiovascular diseases like angina pectoris.
8. Shotha: It is used to cure oedema.

Sanskrit/English	Sanskrit/English
Virya/potency	Sheeta/cold
Vipaka [metabolic property]	Katu/astringent, laghu/light
Guna [physical property]	laghu/light
Rasa [taste]	Pikta/bitter,Kashaya,astringent

➤ **Pharmacological and therapeutic uses:**

Anti asthmatic and bronchodilator activity:

Adhatodavasica contains two alkaloids that are powerful respiratory agents in medicine: vasicine and vasicinone. Many lung conditions, including as bronchial diseases, bronchitis, cough, and cold, can be treated with Adhatodavasica leaf and root extracts. Adhatoda leaf decoction can be used as an expectorant and has a soothing effect that helps relieve sore throats [54]. have examined the effects of Adhatodavasica powdered leaves on guinea pigs induced to stifle their airways using histamine and acetylcholine, as well as in vitro studies on isolated guinea-pigileum, in order to determine the plant's anti-asthmatic properties. A dose-dependent suppression of bronchial construction has been demonstrated by the extract. [55,56]

Anti bacterial activity:

By extracting the leaves of Adathodavasica using different solvents and evaluating them on a Petri plate containing microorganisms, the antibacterial qualities of the plant were ascertained. Using either the well method or the diffusion method, the extract was deposited onto the Petri dishes, and they were then incubated at 37°C for a full day. It has been demonstrated that certain gram-positive and gram-negative bacterial strains are sensitive to the extract. An activity that involves the healing of wounds is called wound healing. The plant's ability to heal wounds was found to exist throughout. Once the animals had made wounds on their spinal columns, they were given an alcoholic extract of the entire plant and contrasted with a control group that did not receive any treatment.

The groups that received Adathosavasica treatment had considerably greater wound healing activity in comparison to the control group (Bhargava etal, 1988). Gv and Sundar (2010a) studied the Adhatodavasica plant's ability to heal wounds in Wistar albino mice. The plant was extracted, followed by diethyl ether, methanol, and chloroform, and then turned into an ointment. The animals were given an incision, and the wound was treated with ointment. Out of all the extracts, it was found that the methanolic extract had the strongest ability to cure wounds. wound was treated with ointment. Out of all the extracts, it was found that the methanolic extract had the strongest ability to cure wounds. [57]

Anti-tussive activity:

The leaves of Adhatodavasika were extracted using ethyl acetate and methanol by Srivastava and Choudhary (2016), who then compared the results to the standard of inducing cough using sulphur dioxide and ammonium hydroxide (codeine phosphate and dextromethorphan).The suppression of cough revluxes

was observed at a dose of 500 mg/kg for both ethyl acetate and methanol extracts; however, ethyl acetate demonstrated slightly superior results than methanol. ^[58]

Anti-tubercular activity:

According to Narimanian et al. (2005), the production of ambroxol and bromhexine from vascine is responsible for the *Adhatodavasica*'s potent anti-tubercular action against the *Mycotuberculosis* tuberculosis strain. Ignacimuthu and Shanmugam (2010) evaluated a powdered leaf extract against a strain of *Mycobacterium tuberculosis* using hexane, ethyl acetate, and methanol. Isoniazid (0.2 gm/ml) and rifampicin (2 gm/ml) are used as a baseline. The results were statistically significant when 100 g/ml of the extract was compared to other concentrations. ^[59,60]

Anti-microbial activity:

Adhatodavasica leaves were extracted using n-hexane, methanol, and water by Prasad et al. (2011), who then tested the extracts' antibacterial and antifungal properties against strains of bacteria and fungi. The research employed ciprofloxacin and fluconazole as reference medications for their antibacterial and antifungal properties. According to Pradhan & Pradhan (2015) and Dymock et al. (1890), all three extracts yielded satisfactory results, although the methanolic extracts showed greater promise. *Adhatodavasica* extracts were synthesised and tested against bacterial strains of *Klebsiella pneumonia*, *Staphylococcus aureus*, *Streptococcus pyogenes*, *Proteus vulgaris*, *Escherichia coli*, and *Pseudomonas aeruginosa* in methanol, ethanol, acetone, chloroform, and water by Sheebab and Mohan (2012). Though all extracts have demonstrated improved performance, diethyl extract has demonstrated significantly more encouraging results. ^[61]

1. Antioxidant & Radical Scavenging Activity:

Pant et al. (2015) prepared an ethanolic extract of *Adhatodavasica* leaves using soxhlet extraction, and they subsequently carried out a number of tests to gauge its antioxidant and radical-scavenging capabilities. The DPPH, ABTS, superoxide anion, and hydroxyl radical scavenging activities of the extract were assessed. TBA procedure, lipid peroxidation assay, reducing power assay, and NO scavenging activity. There were dose-dependent results from every experiment. ^[62]

2. Anti-ulcer activity:

Using Sprague-Dawley rats, Shrivastava et al. (2006) created an *Adhatodavasica* leaf extract and examined its anti-ulcer effects. The ulcer was caused by two different methods: pylorus ligation combined with aspirin dose, and ulcer produced by ethanol. As a baseline, the effects of the extract are contrasted with a formulation that is sold commercially. The results of the extracts were significant when compared to the reference chemical (Shrivastava et al., 2006). Vinothapooshan and Sundar (2011) used methanolic, chloroform, and diethyl ether extracts to study the anti-ulcer efficacy of *Adhatodavasica* leaf extracts in ulcer-induced Wistar albino rats. The ulcer was caused by alcohol and aspirin. The effects of the extracts and the prescription medication ranitidine were contrasted. Comparing the 200 mg/kg dosage of methanolic extract to the standard has yielded significant findings. ^[63]

Anti-inflammatory activity:

One of the chemicals found in *Adhatodavasica* is called vasicine, and it has anti-inflammatory qualities. A modified hen's egg chorioallantoic membrane assay is used to measure the anti-inflammatory activity of the plant extracts. The methanolic extract of the plant exhibits promising results. ^[64,65]

Anti-diabetic activity:

Adhatodavasica leaves were prepared into methanol extracts using the soxhlet apparatus by Sathyamurthy

(2017). The extract was then filtered and dried to produce the dry residue. We looked at the anti-diabetic effects of Adhatodavasica using the residue. Utilising Adipocyte 3T3L1 cell lines derived from a 19-day-old Murine Swiss mouse embryo, the study was conducted. Adhatodavasica's antidiabetic activity is assessed using a deoxyribose test and contrasted with quercetin as a reference. The extract has shown encouraging results when compared to the reference medication. [66,67]

Anti-allergic:

According to reported research, when tested on mice, rats, and guinea pigs, the vasicinone component of the *J. adhatoda* plant possesses anti-allergic properties. When tested on guinea pigs, the plant's methanolic extract showed anti-allergic and anti-asthmatic properties when inhaled or given at a dosage of 6 mg per animal or 2.5 gm/kg, respectively. [68,69]

Abortifacient and uterotonic activity:

The goal of the in vitro and in vivo investigations was to identify *J. adhatoda* plant's uterotonic activity, which is comparable to that of methylergometrine and oxytocin. The Vasicine component was also said to have abortifacient effect. Hamsters, rabbits, guinea pigs, and rats were used in the study. Vasicine was discovered to have abortifacient properties and to function by releasing prostaglandin hormones. [70,71] Studies were conducted in vitro on synthetic compounds of vasicine and vasicinone, which showed oxytocic action at doses more than 1 µg/ml [82]. The aqueous leaf extract demonstrated 100% abortifacient efficacy when administered at a dose of 175 mg/kg in the albino rat model. [72,73]

Expectorant activity:

The investigations that have been reported indicate that the leaves petroleum ether extract, when administered at a dose of 50 mg/kg, has expectorant properties. [74]

Hepatoprotective activity:

When administered at doses of 50–100 mg/kg to Swiss albino rats that had liver damage caused by CCL4, the ethyl acetate extract of Adhatodavasica exhibited hepatoprotective action. [75]

Radiomodulator activity:

Research conducted on Swiss albino mice's peripheral blood revealed that the plant's leaf extract exhibited radio modulation activity against radiation-induced hematological change. The study's findings indicated that while blood alkaline phosphatase significantly increased, irradiated animals fed with leaf extract had lower acid phosphatase activity for the whole duration of the study. [76]

Immunomodulator activity:

Diethyl ether, chloroform, and methanolic extract extracted from Adhatodavasica leaves have all been shown in numerous experimental investigations to contain the immunomodulatory function. In order to determine the immunomodulatory action, male Wistar rats were given an oral dosage of 400 mg/kg, which increased the host's immunity and the percentage of neutrophil adherence to nylon fibers [77].

Clinical Studies:

- a. **Oxytocic effect:** 24 human volunteers took a dose of 16 mg of vasicine hydrochloride as part of the safe trial. In a typical puerperium, vasicine hydrochloride was administered on days two through eight. Following Vasicine administration, it was seen that the uterus constricted and became hard, demonstrating the drug's oxytocic impact. [78]
- b. **Dyspepsia/gastric activity:** 20 patients with dyspepsia (amlapitta) participated in the experimental investigation. Each patient received a daily 60 ml dosage of *A. vasica* syrup (30 g of the crude medicine) in four divided doses over a period of six weeks. In 85% of the patients, it was found that

the amount of stomach acidity had decreased. [79]

- c. **Pyorrhea:** 25 pyorrhea patients underwent the test. For three weeks, the plant's leaf extract was applied twice daily to irritated gums. It was discovered that the gums' inflammatory and bleeding conditions have improved. [80]
- d. **Asthma and bronchitis:** A comparison study was carried out on 24 Shwasa patients in order to evaluate the effectiveness of Vasa-arishtha and Vasakaasava. The Vasa Arishta group showed better results overall, but the VasakaAsava treated group showed improvements. The subjects of the other study, which had 32 Shwasa patients, were separated into three groups: Vasa avleha, Vasa arishta, and Vasa ghrita. Vasa avleha was proven to produce more beneficial outcomes than the other two. Other clinical research revealed that, as compared to Vasa avleha prepared from Kwatha, Vasa avleha prepared from Swarasa yields more beneficial therapeutic results. [81]
- e. **Toxicity of vasapatra:** When used in experiments for research, the J. adhatoda plant often has no negative side effects. No information about this medication's side effects has been mentioned. All the information that could be obtained indicated that taking large amounts of it causes vomiting and diarrhea

➤ **Pharmacokinetics:**

The findings of studies on the absorption and distribution of vasicine in mice after intramuscular, intravenous, and subcutaneous administration are in line with those seen in rats. Vasicine (20 mg/kg) exhibited good absorption, with maximal concentrations of approximately 56 g/ml observed in the blood of pregnant and non-pregnant rats and 10 g/ml in the amniotic fluid (Atal, 1980). After an intravenous injection, rats and mice showed signs of high vasicine concentrations in their uteri after 5 minutes, reaching their peak level after 10 minutes.

Following intravenous therapy, the half-lives for intramuscular and subcutaneous injection were 1.5 and 2 hours, respectively, and 5 to 7 minutes. Reports state that the majority of vasicine and its metabolites are excreted in the urine. Vasicine was responsible for almost 18% of the excretory output in the first 24 hours following oral delivery, compared to approximately 55% in the first 18 and 22 hours following intravenous and intramuscular treatment, respectively. After oral therapy, the uterus had very little concentration. The liver uses vasicinone and other metabolites that are produced from vasicine to make additional metabolites that are important for removing vasicine and that also have first-pass effects. [82,83]

➤ **MARKETED PREPARATION:**



➤ **Result:**

Worldwide, the use of herbal remedies has grown in popularity. The selling of herbal goods rose between 2000 and 2008, according to some surveys, by 3% to 12% annually⁷⁰. The risk associated with herbal medicines increases as a result of the growing demand for herbal products. This risk arises from contaminated raw materials containing toxic metals, microbes, and other residues, adulteration (adding of synthetic or inferior plant material, orthodox drugs, or foreign material), and other factors that lead to poor quality raw materials and finished products.

The current need is to develop new dose formulations without changing the main component. The plant *J. adhatoda* is used to make a variety of formulations. *Vasa candy* is one of the plant's formulations; it is a more pleasant, more steady dosage made from this plant. Other formulations of this plant that are significant medications made without altering the fundamental structure are *Adusa cough syrup*, *Adusa tablet*.

Acute respiratory syndrome is a common side effect of the COVID-19 pandemic, which is now causing a significant death toll worldwide. Respiratory symptoms are common with COVID-19, and in some patients, there are serious repercussions for the heart and kidneys. This study looks into the potential of *Vasaka* (*Adhatodavasica* Nees) in the therapy and prevention of COVID-19 symptoms. In Ayurvedic medicine, *vasaka* is a well-known natural shrub with therapeutic benefits, particularly for respiratory issues. There has long been a search for natural viral inhibitors. One possible approach in the development of novel pharmaceuticals is to search for naturally occurring antiviral compounds in plants. Over the past century, numerous studies have been conducted in an effort to find phytochemicals that may prevent cancer. Over the past century, numerous studies have been conducted in an effort to create phytochemicals that can stop the spread of viruses. New bioactive plant compounds for therapeutic research and development may be discovered and developed as a result of ethnopharmacology. Strong antibacterial effectiveness of *Justicia adhatoda* alkaloids has been demonstrated against highly pathogenic pathogens such as *Salmonella typhi*, as well as highly resistant bacteria like *Staphylococcus aureus* and *Pseudomonas aeruginosa*.

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