



## PHYTOCHEMICAL SCREENING AND DETERMINATION OF QUINAZOLINE ALKALOID IN *ADHATODA VASICA*

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### ABSTRACT

*Adhatoda vasica* plant shows various pharmacological activity like antispasmodic, fever reducer, anti-inflammatory, anti bleeding, bronchodilator, antidibetic, disinfectant, anti-jaundice, oxicotic and expectorant. Phytochemical analysis of its leaf extracts shows the presence of alkaloids, sugar, tannins, sterols, phenols and flavonoids. The extracts were chemically analysed for glycosides, saponin, proteins and amino acids also. The  $R_f$  values of vasicine and vasicinone were found as 0.54 and 0.62 by detecting under ultraviolet light at wavelength of 254 nm.

**Keywords:** *Adhatoda vasica*, antispasmodic, bronchodilator, anti-inflammatory, vasicine, vasicinone.

### INTRODUCTION

*Adhatoda vasica* (L.) Nees is a shrub having height of 1.0-2.5 m, with opposite ascending branches. The drug contains stem, leaf, flower, fruit and seeds. The leaves are simple, opposite, petiolate and exipulate 7-19 cm long and 4-7 cm wide and shape is lanceolate. The margin is crenate with acuminate apex. There are 8-10 pairs of lateral veins. Taste is bitter and odor is characteristic<sup>1</sup>. The older stem is greyish-green, warty and woody. Flowers are white, pink or purple. The flowers are 2-lipped, creamy-white with purple streaks on the lower lip of the flowers. They are arranged on a dense leafy spike. *Vasaka* produces its flowers and then prune-shaped fruits through the months of August to November. The fruit are compressed capsule. The capsule small, clavate and longitudinally channelled, containing four globular seeds. Seed are orbicular compressed<sup>2</sup>.

*Vasaka* is seen in all types of climates. It prefers loamy soils with good drainage and high organic content. This shrub grows on the plains of India and in the lower Himalayans up to an altitude of 1000 m. This plant is also cultivated in other tropical areas. It will grow well in low moisture areas and dry soils. Commercial propagation is by using 15-20 cm long terminal cuttings. This is either grown in polybags first, then in the field or planted directly. The cuttings are planted during April-May into the beds at a spacing of 30x30 cm. Regular irrigation and weeding are necessary. Harvesting is always done at the end of second or third year. Roots are collected by digging the seedbeds. Stem are cut 15 cm above the root. Stems and roots are dried and stored<sup>3</sup>.

*Adhatoda vasica* is useful in treatment of cold, cough, whooping cough and chronic bronchitis and asthma as sedative expectorant, antispasmodic and anthelmintic. It is an official drug and is mentioned in the India Pharmacopoeia (1966). 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. The leaf juice is stated to cure diarrhoea, dysentery and glandular tumor (Wealth of India 1985). *Vasaka* is a bitter quinazoline alkaloid and its major alkaloids are vasicine and vasicinone which is present in all parts of the plant. The leaves, roots and young plants of *Adhatoda vasica* contain the quinazoline alkaloids (vasicine, 7-hydroxyvasicine, vasicinolone, 3-deoxyvasicine, vasicol, vasicoline, vasicolinone, adhatodine, anisotine) betaine, steroids carbohydrate and alkanes. In the flowers triterpenes (a-amirine), flavonoids (Apigenin, astragalin, kaempferol, quercetin, vitexin) have been found<sup>4,5</sup>.

### MATERIALS AND METHODS

**Collection of Plant Material:** Leaves of *Adhatoda Vasica* Nees, a member of family Acantheace, was used for the experiments. It was collected from medicinal garden of Faculty of Pharmacy, Integral University, Lucknow.

**Authentication of Plant Material:** Sample of plant material was given to NBRI Lucknow, India for identification and taxonomic authentication. The test report from CIF, NBRI, Lucknow, conformed the taxonomic authentication of plant material sample. Specification No.: NBRI-SOP-202, Receipt no. & date: 19/76, 27-2-2009

#### **Chemicals and Instruments**

TLC plate, silica gel, development tank, acetic acid, petroleum ether, chloroform, ethanol, soxhlet apparatus.

#### **Preparation of the extracts**

**(a) Ether extract:** The leaves of *adhatoda vasica* was dried under shade and then crushed in to powder with a mechanical pulveriser. The powdered plant material (20 gm of *adhatoda vasica* leaves) was extracted with 250 ml of petroleum ether for 12



hours, reflux at 20°C. After filtering and evaporating to dryness, the crude extracts were obtained.

- (b) **Ethanol extract:** The leaves of *adhatoda vasica* was dried under shade and then crushed in to powder with a mechanical pulveriser. The powdered plant material (20 gm of *adhatoda vasica* leaves) was extracted with 250 ml of ethanol for 18 hours, reflux at 50°C. After filtering and evaporating to dryness, the crude extracts were obtained.
- (c) **Chloroform extract:** The leaves of *adhatoda vasica* was dried under shade and then crushed in to powder with a mechanical pulveriser. The powdered plant material (20 gm of *adhatoda vasica* leaves) was extracted with 250 ml of chloroform for 10 hours, reflux at 60°C. After filtering and evaporating to dryness, the crude extracts were obtained.
- (d) **Aqueous extract:** The leaves of *adhatoda vasica* was dried under shade and then crushed in to powder with a mechanical pulveriser. The powdered plant material (20 gm of *adhatoda vasica* leaves) was extracted with 250 ml of water for 16 hours, reflux at 70°C. After filtering and evaporating to dryness, the crude extracts were obtained<sup>6</sup>.

#### Phytochemical Screening

##### Test for Alkaloids:

- (i) **Dragondroff's test:** The prepared dragondroffs reagent was sprayed on watmann no.1 filter paper and the paper was dried. The test filtrate after basification with dilute ammonia was extracted with chloroform and the chloroform extract was applied on the filter paper, impregnated with Dragondroff's reagent, with the help of a capillary tube. Development of an orange red colour on the paper indicated the presence of alkaloids.
- (ii) **Mayer's Test:** The mayer's reagent was prepared by adding 1.36 gm of mercuric chloride and 600 ml of distilled water. Both the solutions were mixed and diluted to 100 ml with distilled water. Small amount of the test filtrate was taken in a watch glass and a few drops of the above reagent were added. Formation of cream coloured precipitate showed the presence of alkaloids.
- (iii) **Hager's reagent:** A saturated aqueous solution of picric acid was employed for this test. When the test filtrate was treated with this reagent, an orange yellow precipitate was obtained, indicating the presence of alkaloids.
- (iv) **Wagner's test:** 1.27 g of iodine and 2g of potassium iodide were dissolved in 5 ml of water and the solution was diluted to 100 ml with water. When few drops of this reagent were added to the test filtrate, brown flocculent precipitate was formed indicating the presence of alkaloids.

##### Test for Sugars:

- (i) **Molisch's test:** The Molisch's reagent was prepared by dissolving 10g of  $\alpha$ - naphthol in 100 ml of 95% alcohol. Few mg of the test residue was placed in a test tube containing 0.5 ml of water, and it was mixed with 2 drops of Molisch's reagent. 1 ml of conc. sulphuric acid was added from the sides of an inclined test tube, so that the acid formed a layer beneath the aqueous solution without mixing with it. Appearance of red brown ring at the common surface of the liquids shows sugars are present.
- (ii) **Burford's test:** This reagent was prepared by dissolving 13.3 gm of crystalline neutral copper acetate in 200ml of 1% acetic acid solution. The test residue dissolved in water and heated with a little amount of the reagent. Red precipitate of cuprous oxide within two minutes shows the presence of monosaccharide.
- (iii) **Fehling's reduction test:** when the solution of carbohydrate is added with Fehling A and Fehling B, brick red precipitate is obtained after heating.

##### Test for Amino Acids:

**Ninhydrin test:** The ninhydrin reagent is 0.1% w/v solution of ninhydrin in n-butanol. A little amount of the ninhydrin reagent was added to the test extract. Violet colour shows that amino acids are present.

##### Test for Tannins:

- (i) **Ferric chloride reagent:** A 5% w/v solution of ferric chloride in 90% alcohol was prepared. Few drops of this solution were added to little amount of the test filtrate. Deep blue colour shows that tannins are present.
- (ii) **Lead acetate test:** A 10% w/v solution of basic lead acetate in distilled water was added to the test filtrate. Precipitate shows that tannins are present.
- (iii) **Potassium dichromate test:** On addition of solution of potassium dichromate in a test filtrate, dark colour is developed, it shows that tannins are present.
- (iv) **Gelatine solution test:** 1% w/v solution of gelatine in water, containing 10% sodium chloride was prepared. A little amount of this solution was added to the filtrate. White precipitate shows that tannins are present.
- (v) **Bromine water test:** Bromine solution was added to the test filtrate. Decolourization of bromine water shows that tannins are present.

##### Test for Proteins:

- (i) **Biuret test:** Few mg of the residue was taken in water and 1ml of 4% sodium hydroxide solution was added to it. This was followed by a drop of 1% solution of copper sulphate. Formation of violet colour shows that proteins are present.



- (ii) **Xanthoproteate test:** Little amount of residue was taken in 2ml of water and 0.5ml of concentrated nitric acid was added to it. Appearance of yellow colour shows that proteins are present.
- (iii) **Millon's test:** Millon's reagent was prepared by dissolving 3 ml of mercury in 27 ml of fuming nitric acid, keeping the mixture well cooled; this solution was then diluted with equal quantity of distilled water. Aqueous solution of the residue was taken and 2 to 3 ml of Million's reagent was added to it. The white precipitate which slowly turns to pink shows that proteins are present.

#### Test for Glycosides:

- (i) **Keller Kiliani test:** 1 ml of glacial acetic acid containing traces of ferric chloride and one ml of concentrated sulphuric acid were added to the extract. Formation of reddish brown colour at the junction of two layers and the upper layer turns bluish green shows the presence of glycoside.
- (ii) **Borner's test:** 1 ml of benzene and 0.5 ml of dilute ammonia solution were added to the ethanolic extract. Formation of reddish pink colour shows the presence of glycosides.
- (iii) **Legal test:** Concentrated ethanolic extracts were made alkaline with few drops of 10% sodium hydroxide solution and then freshly prepared sodium nitroprusside solution were added to the solution. Formation of blue colour was observed.

#### Test for Flavonoids:

**Shinoda test:** A small quantity of residue was dissolved in 5 ml ethanol (95%v/v), few drops of concentrated hydrochloric acid and 0.5 g of magnesium metal. Developed of the pink crimson colour, within a minute, shows that flavonoids are present.

#### Test for Saponins:

**Foam test:** A few mg of the test residue was taken in a test tube and shaken vigorously with a small amount of sodium bicarbonate and water. Saponins are present because honeycomb like froth is obtained.

#### Test for Sterols:

- (i) **Salkowski reaction:** Few mg of the residue of each extract was taken in 2ml of chloroform and 2ml of conc. sulphuric acid was added from the side of the test tube. The test tube was shaken for few minutes. The development of red colour in the chloroform layer indicated the presence of sterols.
- (ii) **Liebermann's test:** Few mg of the residue in a test tube, few ml of acetic anhydride was added and gently heated. The contents of the test tube were cooled and few drops of concentrated sulphuric acid were added from the sides of the test tube. A blue colour gave the evidence of presence of sterols.

- (iii) **Liebermann's Burchards reaction:** Few mg of residue was dissolved in chloroform and few drops of acetic anhydride were added to it, followed by concentrated sulphuric acid from sides of the test tube. A transient colour development from red to blue and finally green indicated the presence of sterols.

#### Test for Phenolic Compounds:

- (i) **Ferric chloride solution:** The extracts were taken in water and warmed, then 2ml of ferric chloride solution was added and observed for the formation of green and blue colour.
- (ii) **Lead Acetate solution:** To the extract (2ml) lead acetate solution was added and observed for the formation of precipitate.

#### Thin Layer Chromatography (TLC) of *Adhatoda vasica*

Thin layer chromatography is a thin (0.25 mm) layer of silica gel (SiO<sub>2</sub>) used as inert material. Layer of silica gel made from a slurry of powder in water. The slurry is spread over a flat surface (glass) and dried.

#### Extraction of *Adhatoda vasica* Nees

Dried powdered leaves of *A. vasica* were extracted with ethanol for 24 hours; the extract was filtered and concentrated. The concentrated material was treated with 5% acetic acid and warmed for 15 minutes and filtered. The filtrate was defatted with hexane, basified with ammonia (pH 9.0), and then extracted with chloroform to yield of crude alkaloids<sup>7</sup>.

#### Techniques

Silica gel is used as coating material, which are also called as adsorbent in TLC. The slurry of silica gel is placed in an applicator. This is moved over the stationary glass plate. Drying the thin layer plate, for 30 minutes in air and then in an oven at 110°C for 30 minutes. This drying makes the adsorbent layer active. Capillary tube may be used for sample application. A drop of sample placed near one edge of the plate and its position is marked with a pencil. After the sample solvent has evaporated, the plate is placed in a closed container saturated with vapours of the developing solvents.

#### Solvent system

Toluene: methanol: dioxane: ammonia (2 : 2 : 5 : 1)

#### Development tank

The plate is placed in a development chamber at an angle of 45°. The bottom of the chamber is covered up to nearly 1mm by the solvent system. Three sides of tank are lined with solvent impregnated paper while top is covered tightly with the lid. The development chamber is saturated with solvent vapours. This is essential to avoid unequal solvent evaporation losses from the developing plate<sup>8</sup>. Observe the plate under UV 254 nm & spray the plate with dragendorff's reagent, then note the R<sub>f</sub> value.



## RESULTS AND DISCUSSION

Phytochemical analysis of leaf extracts of *Adhatoda vasica* shows presence of alkaloids, sugar, tannins, sterols, phenols and flavonoids (table 1). It was also observed that glycosides, saponin, proteins and amino acids compounds were not present in the extracts. The  $R_f$  values of vasicine and vasicinone were found as 0.54 and 0.62 (table 2) by detecting under ultraviolet light at wavelength of 254 nm.

**Table 1:** Phytochemical composition observation of different extracts of *Adhatoda vasica*

Test	Ether	Chloroform	Ethanol	Water
<b>Test for Alkaloids:</b>				
Dragendroff	+	+	+	+
Mayer	+	+	-	+
Hager	+	+	+	+
Wagner	+	-	+	+
<b>Test for Sugar:</b>				
Molish	+	+	-	-
Fehling	+	-	+	+
Burford	-	+	+	+
<b>Test for Amino acids:</b>				
Ninhydrin	-	-	-	-
<b>Test for Tannins:</b>				
5% FeCl <sub>3</sub>	+	+	-	+
Lead acetate solution	+	-	+	+
Gelatin solution	+	+	-	+
Bromine water	+	-	-	-
Potassium dichromate	-	+	+	-
<b>Test for Proteins:</b>				
Biuret	-	-	-	-
Xanthoproteate	-	-	-	-
Millon	-	-	-	-
<b>Test for Glycosides:</b>				
Borntager's	-	-	-	-
Legal	-	-	-	-
Keller kiliani	-	-	-	-
<b>Test for Flavonoids:</b>				
Shinoda	-	+	-	+
<b>Test for Saponin:</b>				
Foam test	-	-	-	-
<b>Test for Sterols:</b>				
Salkowaski	+	-	+	-
Liebermann	+	+	+	+
Liebermann-Burchard	+	+	-	+
<b>Test for Phenols:</b>				
Ferric chloride solution	+	-	+	-
Lead acetate solution	+	+	+	+

**Table 2:**  $R_f$  values by TLC of vasicine and vasicinone (Detection of Components)

S.No.	Compound	$R_f$ value
1	Vasicine	0.54
2	Vasicinone	0.62

## CONCLUSION

The leaves were extracted in petroleum ether, ethanol, chloroform and water. Chemical analysis of leaf extracts of *Adhatoda vasica* shows presence of alkaloids, sugar, tannins, sterols, phenols and flavonoids. The extracts were chemically analysed for glycosides, saponin, proteins and amino acids also. It was observed that these compounds were not present in the leaf extracts of *Adhatoda Vasica*. Thin layer chromatographic (TLC) was used to detect the vasicine and vasicinone. The  $R_f$  values of vasicine and vasicinone were found as 0.54 and 0.62 by detecting under ultraviolet light at wavelength of 254 nm.

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