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Research Article

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Development of Quality Standards of *Adhatoda Vasica* Nees

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ABSTRACT

The present study deals with the scientific evaluation and standardization of the Ayurvedic medicinal value of Adhatoda vasica Nees. The obtained value (ranges of physico-chemical) can be adopted to day down new pharmacoepial standards to be followed for traditional preparation of drugs of Adhatoda vasica. It is an antispasmodic and expectorant and has been used for centuries with much success to treat asthma, chronic bronchitis and other respiratory conditions. These plants have varieties of medicinal and pharmacological activities.

Keywords: *Adhatoda vasica*; Quality standards; Physiochemical analysis

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INTRODUCTION

Ayurveda aptly called the ‘’Science of Life’’ had been catering to the health demands of the people in the past, is catering to this need presently and will cater to the needs of the mankind in the future also. *Charaka Samhita* provides inexhaustible information regarding all the eight branches of *Ayurveda* (*Astanga of Ayurveda*).Greater stress is laid on the branches of *Kayacikista*, which pertains with the understanding and management of diseases which afflict our body. For the student and practitioners of *Ayurveda*, various text written centuries ago have been perennial sources of knowledge. Among these classics, 3 important classics, viz *Caraka Samhita*, *Susruta Samhita* and *Astanga Sangraha*, known as *brihatrayi*, are the most important oceans of information. It is a system of traditional *Hindu* medicine. It is native to the Indian Sub continent. The origin of *Ayurveda* has been traced back to around 3000 B.C when they originated as an oral tradition. Many people carry impressions that medicines derived from natural plants are harmless. Although a natural medicine induces fewer side effects than conventional drugs, there are plants that cause severe side effects.

Adosa (*Adhatoda vasica* Nees) belonging to family Acanthaceae, commonly known as Adosa is a small evergreen shrub found in major regions of India and throughout the world. *Adhotada vasica* is most well known for its effectiveness in treating respiratory system. The leaves of *Adhatoda vasica* shows stimulant effect on respiratory system. It has been used for centuries with much success to treat asthma, chronic bronchitis and other respiratory conditions [1-3].The plant is a shrub, 1 to 3m in height rarely found in higher altitudes of Western Ghats of Kerala, Uttar Pradesh, Madhya Pradesh and Tamil Nadu. Vasicine demonstrated bronchodilatory, respiratory stimulant, uterine stimulant and moderate hypotensive response. Chemical compounds found in Adhatoda leaves includes essential oils, fats, sugar, resin, gums, amino acids, proteins and vitamin C. At high concentrations, vasicine offered significant protection against histamine induced bronchospasm in guinea pig. Vasicinone, the auto oxidation product of vasicine has been reported to cause bronchodilatory effects both *invivo and invitro*. of the two alkaloids, vasicinone was found to be more potent than vasicine with potential antiasthmatic activity comparable to the disodium cromoglycate [4-7].

METHODOLOGY

Method of preparation of *curna*

Plant was collected from the forest of Chitrakoot region and these were washed, dried and grind and passed with 180um sieve. It was stored in an airtight container to protect from light and container. The sample of *Adhatoda vasica* were (prepared at research laboratory Ayurveda sadan, Chitrakoot) studied.

Physico-chemical parameter

Physico-chemical parameters i.e. loss on drying at 105^0C, total ash, water and alcohol soluble extractive were checked out in triplicate according to the prescribed Standard methods in Indian Pharmocoepia [8].

Loss on drying (1050C)

Take the weighed the empty petridish and pour 2gm of accurately weighed sample on each petridish. Kept the petridish on hot air oven for 5 hours at 105^0C. After 5 hours, place the peridish on desiccators for 30 mints for cooling and note down the reading. Again place the petridish on hot air oven for 30mints. and finally note down the final reading (the procedure is based on WHO guidelines) [9].

Extractive values

Water soluble extractive values:

Take 2gm of sample and add 100ml of distilled water and kept the conical flask on rotatary shaker for 5hrs. and kept the sample for 12hrs.without stirring. Now take the weight of empty petridish and pour 10ml of filtered water extract on these separate petridish. Kept the petridish on vacuum evaporator for complete dryness. Kept the petridish on desiccator for 15mints. and note down the reading [9].

Alcohol Soluble extractive value:

Take 2gm of sample and add 100ml of alcohol and kept the conical flask on rotatary shaker for 5hrs. and kept the sample for 12hrs.without stirring. Now take the weight of empty petridish and pour 10ml of filtered water extract on these separate petridish. Kept the petridish on vacuum evaporator for complete dryness. Kept the petridish on desiccator for 15mints. and note down the reading (All these procedures are given by as per WHO guidelines) [9].

Test for total Ash

Incinerate about 2 to 3 g accurately weighed, of the ground drug in a tarred platinum or silica dish at a temperature not exceeding 650^0C until free from carbon, cool and weigh. If a carbon free ash can not be obtained in this way, exhaust the charred mass with hot water, collect the residue on an ash less filter paper, incinerate the residue and filter paper, add the filtrate, evaporate to dryness, and ignite at a temperature not exceeding 650^0C. Calculate the percentage of ash with reference to the air-dried drug [9].

Phytochemical analysis

The phytochemical analysis of this plant was performed for the detection of active constituent's i.e alkaloids, protein, saponin, resin, tannin, carbohydrate, flavonoid and steroid.

Alkaloid

Dragendorff's test:

Take 2ml of alcoholic or aqueous extracts of sample in 5ml of distilled water in a test tube. Now add 2ml of 1N HCL until a complete acidic reaction occurs. Add few drops of dragendorff reagent. Appearance or formation of orange colour indicates the presence of alkaloid [10-11].

Wagner test:

Take 1ml of alcoholic extract of sample in a test tube and add 3-4 drops of wagner reagent. Brown colour indicates the presence of alkaloid [10-11].

Mayer's test:

Take 1ml of alcoholic extract extract of sample and add few drops of Mayer's reagent in a test tube. Pale yellow colour indicates the presence of alkaloid [10-11].

Carbohydrate

Anthrone test:

Add 0.5ml of aqueous extract of drug in 2ml of anthrone reagent. Green or blue colour indicates the presence of carbohydrate [10-11].

Fehling test:

To 1ml aqueous extract of drug, add 1ml of each of equal part of Fehling solution A and Fehling solution B. Boil the content for 5 minutes. Formation of brick red colour indicates the presence of carbohydrate [10-11].

Molisch test:

To 1ml of aqueous extract of drug, add 2-3 drops of alpha napthol after it add few drops of conc. sulphuric acid. Red violet colour ring appears which gradually disappear on addition of excess of alkali which shows the presence of carbohydrate [10-11].

Flavonoid

To 0.5ml of an alcoholic extract of drug, add 5-6 drops of dilute HCL and few pieces of Magnesium metal. Pink or brown colour indicates the presence of flavonoid [10-11].

Protein

Bieuret test:

To 1ml of alcoholic extract of drug, add 1.5% sodium hydroxide solution and add 1or 2 drops of 5% copper sulphate solution. Violet colour indicates the presence of protein.

Millons test:

To 1ml of alcoholic extract of drug add 5-6 drops of millons reagent which result in formation of white precipitate which turns red on heating [10-11].

Resin

Add 1ml of alcoholic or aqueous extract in 2ml of acetone and add 1ml of distilled water. Turbidity indicates the presence of resins [10-11].

Saponin

Pour 1ml of alcoholic or aqueous extract of drug in 1ml of sodium bicarbonate. Honey comb like froth forms indicate the presence of saponin [10-11].

Steroid

To 1ml of alcoholic extracts of drug, add 2ml of chloroform and 1ml of sulphuric acid from the side wall of test tube. Formation of red colour ring on chloroform layer indicates the presence of steroid [10-11].

Tannin

To 1ml of alcoholic or aqueous extract of drug, add 3-4 drops of ferric chloride. Brown colour indicates the presence of tannin and green colour indicates the presence of gallactotanin [10-11].

Quantitative estimation of protein estimation (UV spectrophotometer)

Principle:

The blue colour developed by the reduction of phosphomolybdicphosphotungstic components in the folin ciocalteu's reagent by the amino acid tyrosine and tryptophan present in the protein plus the colour developed by the biuret reaction of the protein with the alkaline cupric tartarate as measured in the Lowry method (UV-Vis spectrophotometer (Model-UV-1800, Shimadzu Corporation, Japan) [12-13].

Reagents

2% sodium carbonate in 0.1N sodium hydroxide, 0.5% copper sulphate in 1% potassium sodium tartarate, alkaline copper solution and folin reagent.

Protein stock solution
Weigh accurately 50mg of Bovine Serum albumin (fraction v) and dissolve in distilled water and make upto 50ml in standard flask. Working standard: Dilute 10ml of stock solution to 50ml with distilled water in a standard flask. 1ml of this solution contains 200ug protein.

Sample preparation
Extraction is usually carried with buffers used with enzyme assays. Weigh 0.5-1gm of the sample and grind well with pestle and mortar in 10-20ml of buffer. Centrifuge and use the supernatant for protein estimation.

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. Ehen 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.

Quantitative estimation of alkaloid
Reagents
Dragendorff reagent, Standard bismuth nitrate solution, Thiourea 3% and Disodium sulfide.

Stock solution of alkaloid:
10mg of each pure alkaloid was dissolved in 10ml of methanol.

Sample preparation:
10gm of coarsely plant material was Soxhlet extracted with 50ml of methanol.

Procedure of calibration curve:
The calibration curve was obtained with Bismuth nitrate pentahydrate stock solution. Series dilutions of the stock solution were made by pipetting out 1, 2, 3, 4, 5, 6, 7, 8 and 9ml stock solution into separate 10ml standard flask and diluting to volume with distilled water. 1ml amount of this solution was taken and 5ml thiourea solution was added to it. The absorbance value of the yellow solution was measured at 435nm against colorless reagent blanks.

Procedure for assay of alkaloid and plant extracts
A 5ml amount of the extract/solution was taken and the pH was maintained at 2-2.5 with dilute HCL. A 2ml amount of DR was added to it, and the precipitate formed was centrifuged. The centrifuged was checked for complete precipitation by adding DR. After centrifugation, the centrifugate was decanted completely and meticulously. The precipitate was further washed with alcohol. The filterate was discarded and the residue was then treated with 2ml disodium sulfide solution. The brownish black precipitate formed was then centrifuged. Completion of precipitate was checked by adding 2drops of disodium sulfide. The residue was dissolved in 2ml concentrated nitric acid, with warming if necessary. This solution was diluted to 10ml in a standard flask with distilled water. 1ml was then pipetted out and 5ml thiourea solution was added to it. The absorbance was measured at 435nm against the blank containing nitric acid and thiourea [14,15].

High Performance Thin Layer Chromatography
The dried fresh leaves of *Adhatoda vasica* were prepared in methanolic extract. Extract 2g of powdered drug with methanol by warming on a water bath. The extract was collected and place in vacuum evaporator for complete evaporation. The sample was carefully scratch and again dissolved in methanol having AR grade and used for chromatographic fingerprinting. The solvent system used for *Adhatoda vasica* leaves was Toluene: Ethyl acetate (7:3). Apply 5ul of test solution on precoated silica gel 60F254 (E.Merck) of uniform thickness of 0.2mm. Develop the plate in the solvent system to a distance of 8cm. Observe the plate under UV 254nm, 366nm and white r and spray the plate with 5% methanolic sulphuric. Note the R_f value and colour of resolved bands [16-17].

RESULTS AND DISCUSSION

In present study the leaf of *Adhatoda vasica* were evaluated for its physicochemical and phytochemical aspects. Organoleptic parameters revealed that the powder of leaves of *Adhatoda vasica* are green in color, with the characteristic odour, astringent and better taste and fine and hard texture (Table 1). The results of preliminary phytochemical analysis in the ethanolic and water extracts of the drugs showed the presence of carbohydrates, steroids, alkaloids, resin, saponins and tannins (Table 2) which could make the drug useful for treating different ailment as having a potential of providing useful drugs for human use.

Table 1: Organoleptic characters of *Adhatoda* vasica leaf

S.N.	Parameters	Observation
1	Color	Green
2	Odour	Characteristic
3	Taste	Astringent and bitter
4	Texture	Fine

Table 2: Phytochemical analysis of Adhatoda vasica leaf

Parameters	Tests methods	Results	
		Ethanol/ aqueous extract	Observation
Carbohydrates	Anthrone test	-	Brown colour appears
	Fehling test	+	Brick red colour precipitate appears
	Molisch test	+	Red violet colour ring appears
Resin	Acetone	+	Turbidity appears
Saponin	Foam tests	+	Honey comb like froth appears
Alkaloids	Dragendorff's test	+	Orange colour appears
	Wagners test	+	Brown colour precipitate appears
	Mayers test	+	Pale yellow colour appears
Steroids	Salkowski tests	+	Red violet colour ring appears
Flavanoids	Shinoda test	-	Pale yellow colour appears
Tannins	5% $FeCl_3$	+	Green colour appears (galltannin)
Proteins	Biurate test	-	Brown colour appears
	Mellons test	-	Greeen colour appears

Note: + (Present) and – (Absent)

Physicochemical investigations for all parts of drug powder were performed for loss on drying, total ash content, water soluble extractive and alcohol soluble extractive the results were tabulated in (Table 3). The extractive values names water soluble and alcohol soluble indicates the amount of active constituents in given amount of plant material when extracted with respective solvent. The loss on drying value obtained is an indicative of amount of moisture content present in the drug.

Table 3: Physicochemical parameters of Adhatoda vasica leaf

S.No.	Parameters	Results
1	Loss on drying at 105^0C (%w/w)	7.2
2	Total ash value (%w/w)	14.46
3	Water soluble extractive value (%w/w)	34.5
4	Alcohol soluble extractive value (%w/w)	9

The results for alkaloids content (AC) and protein content (PC) in the leaves of *Adhatoda vasica* are presented in the table 4, AC and PC was expressed in µg/ml. The results showed that the leaves extract of *A. vasica* showed highest alkaloid content (567.04 µg/ml) and protein content (12.71 µg/ml).

Table 4: Determination of alkaloids content and protein content in Adhatoda vasica leaf

S.N.	Parameters	Results
1	Alkaloids, µg/ml	567.04 µg/ml
2	Protein, µg/ml	12.71 µg/ml

The TLC plate were examine under ultra violet light at 254 nm; at 366 nm; at visible for both before and after derivetisation with 5% methanolic-sulphuric acid reagent (Fig. 1-4). The R_f values and colours of the bands obtained were recorded. It shows major spots at visible light R_f 0.33 (black), 0.40, 0.61,0.73 (all spots yellow), 0.79 (yellow),

0.93 (yellow); at 254nm R_f 0.33, 0.40, 0.78, 0.92 (all spots black) and at 366nm R_f 0.14 (brown), 0.25 (light pink), 0.36 (red), 0.41 (black), 0.42 (pink), 0.53, 0.59, 0.66, 0.72, 0.80 (all spots red). After spray the plate shows major spots at visible light R_f 0.35, 0.43 (Faint purple), 0.60 (light brown), 0.80, 0.97 (all spots brown).

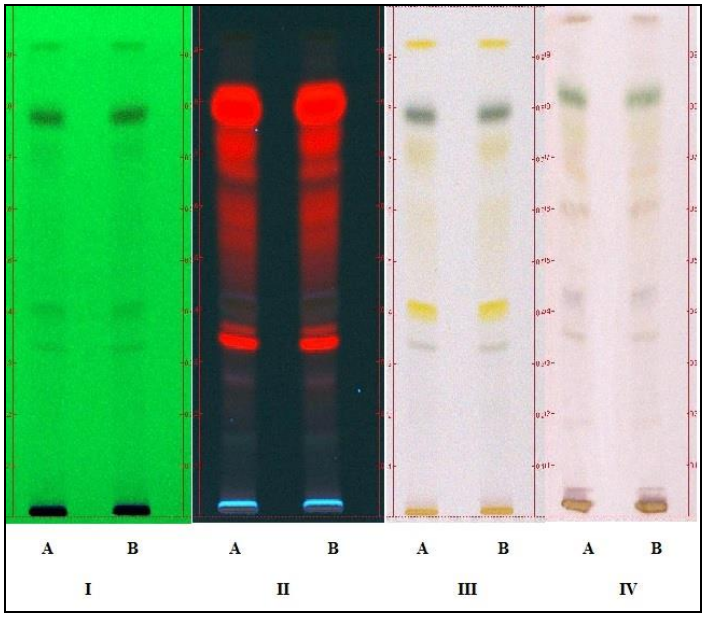

Figure 1: TLC Finger prints profiles of *Adhatoda vasica* leaf I-A and B track both duplicate, at 254nm (before derivatization); II at 366 nm (before derivatization); III- at visible light (before derivatization) and IV- at visible light (after derivatization)

CONCLUSION

The Ayurvedic system of medicine is prevalent in India since the Vedic period and as early as the dawn of human civilization. Though, Ayurveda has undergone many changes in the course of its long history, it still remains the mainstay of medical relief to a large section of population of the nation. Due to urbanization and dwindling of forest, the *Vaidya* by and large is no longer a self contained unit collecting and preparing his own medicines as before. He has now to depend on newly developed agencies like one collecting and supplying the crude drugs and the other undertaking mass production of medicines in the Ayurvedic pharmaceutical units run on the commercial scale. The Pharmacognostical features of *Adhatoda vasica* studied in the present study have been utilized in developing standards of this plant which will be useful in the detection of its identity and authenticity. The parameters such as physiochemical analysis, preliminary phytochemical test, fluorescence analysis, quantitative estimation (alkaloids & protein) and HPTLC studied add to its quality control and quality assurance for proper identification.

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