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# PHARMACOGNOSTICAL AND PHYTOCHEMICAL EVALUATION OF A POLYHERBAL ANTIHYPERTENSIVE AYURVEDIC FORMULATION [NIA/DG/2015/01]

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**ABSTRACT**

Introduction: Hypertension is a complex clinical illness with a frequency of 40.8 percent in the urban Indian population and 17.9 percent in the rural population. Multiple antihypertensives are used to treat hypertension. However, many have adverse effects and are hazardous. NIA/DG/2015/01 is a polyherbal antihypertensive *ayurvedic ghan* formulation, containing *Arjuna (Terminalia arjuna* Roxb.)*, Ashwagandha (Withania somnifera* Linn.)*, Jatamansi (Nardostachys jatamansi* DC.)*, Shankhpushpi (Convolvulus pluricaulis* Choisy.)*, Punarnava (Boerhavia diffusa* Linn.)*.* Aim and objective: To evaluate a polyherbal antihypertensive *Ayurvedic* formulation [NIA/DG/2015/01] in terms of pharmacognosy, physiochemistry, phytochemistry and chromatography. Materials and methods: A polyherbal antihypertensive ayurvedic formulation [NIA/DG/2015/01] was evaluated for purity, safety, and quality using pharmaco-gnostical, physiochemical, phytochemical, and chromatographic methods. Observations and Results: The pharmaco-gnostical, physiochemical, phytochemical, and chromatographic results were within normal limits per API. Conclusion: The present sample of a polyherbal antihypertensive ayurvedic formulation [NIA/DG/2015/01] met quality standards for purity, safety and authenticity.

**Keywords:** Pharmacognosy, Phytochemicals, Ashwagandha.

## INTRODUCTION

Hypertension, often known as high or rising blood pressure, is a disorder in which the blood vessels' pressure remains elevated over time. The vessels transport blood from the heart to all regions of the body. The heart pumps blood into the vessels every time it beats. The force of blood pushing against the walls of blood vessels (arteries) as pumped by the heart causes blood pressure. The greater the blood pressure, the harder it is for the heart to pump.[1]Hypertension was projected to be present in 40.8 percent of urban Indians and 17.9 percent of rural Indians[2] The prevalence of hypertension was 29.0 percent in 2015–2016, and it rose with age: 7.5 percent in the 18–39 age group, 33.2 percent in the 40–59 age group, and 63.1 percent in the 60+ age group. [3] Although numerous antihypertensives are on the market, they all come with dangers and long-term side effects.

Ayurveda has a holistic approach to medicine, which makes sense for complex conditions such as hypertension. NIA/DG/2015/01 is a polyherbal antihypertensive *Ayurvedic ghan* formulation, containing *Arjuna (Terminalia arjuna* Roxb.)*, Ashwagandha (Withania somnifera* Linn.)*, Jatamansi (Nordostachys jatamansi* DC.)*, Shankhpushpi (Convolvulus pluricaulis* Choisy.)*, Punarnava (Boerhavia diffusa* Linn.). All these drugs are very well known for *Hridya, Mootral, Rasayana* effect and pharmacological actions like cardioprotective[4], anti-hypertensive[5], antioxidant[6], antimicrobial[7], antifungal[8], antidepressant or anxiolytic[9], anti-inflammatory[10], psycho-immunomodulatory effect[11] and acetyl-cholinesterase inhibitory activity[12] etc. Although all medicinal plants include phytochemicals or bioactive substances that play a critical role in their therapeutic action, most medications have variances in these phytochemicals, resulting in significant differences in quality and efficacy.

Furthermore, due to globalisation and rising demand for *Ayurvedic* treatments, authentic and high-grade drugs that meet quality criteria are scarce. Pharmaco-gnostical investigations aid in correctly identifying samples and, as a result, authenticate the drug's purity, safety, and efficacy. Phytochemical investigations validate the naturally existing chemical substances in plants that contribute to their colour, taste, smell, activities, and other characteristics, and they also aid in the discovery of the bioactive profile of medicinal plants. Organic and inorganic materials in a plant, like alkaloids, tannins, saponins, phenols, flavonoids, etc., are tested to understand their pharmacodynamics.[12]

**Aim and objective:** To evaluate a polyherbal antihypertensive ayurvedic formulation [NIA/DG/2015/01] in terms of pharmacognosy, physiochemistry, phytochemistry, and chromatography.

## MATERIAL AND METHODS

Sample preparation: Dried aqueous extracts of all five herbal ingredients mentioned in the table were obtained by crude drug extraction. (Table 1)

**Pharmacognostical Study:** It was carried out by the naked eye and magnifying lens as an organoleptic study for colour, odour, taste, and texture**.**

**Powder Microscopy:** To identify powdered drugs, microscopic powder inspection of medicinal plants is essential. For this, various chemicals were used to treat the powdered medication. When used in conjunction with other analytical procedures, a microscopic study may not always give invaluable supporting information.

**Table 1: Ingredients of sample preparation of NIA/DG/2015/01**

| Ingredient | Family | Part Used | Quantity |
|---|---|---|---|
| *Convolvulus pluricaulis* Choisy. | *Convolvulaceae* | Whole plant | 1 part |
| *Withania somnifera* Linn. | *Solanaceae* | Root | 1 part |
| *Terminalia arjuna* Roxb. | *Combretaceae* | Bark | 1 part |
| *Boerhavia diffusa* Linn. | *Nyctaginaceae* | Whole Plant | 1 part |
| *Nardostachys jatamansi* DC. | *Valerianaceae* | Rhizome | 1 part |

The ground NIA/DG/2015/01 *ghan* was sieved using a vibrio sifter.

Chemical reagents used for staining of the powder samples will be as follows:

- Safranin
- Dilute Ferric chloride
- Methylene blue
- Sudan red 3
- Iodine
- Dilute HCl

**Physiochemical Analysis:** The physiochemical parameters of the NIA/DG/2015/01ghan were analysed for the following:

**Determination of Moisture Content**

Moisture content was determined by placing 5 gm of NIA/DG/2015/01ghan in the oven at 105 °C for 5 hours. The weight of the sample was calculated every 30 minutes until it came out to be constant, or no variation of weight was recorded. This sample was allowed to cool to room temperature in a desiccator before weighing. [13]

**Calculations**

Weight of an empty Petri dish = W1gm
Weight of the drug sample = X gm
Weight of the Petri dish with the drug before drying (W3) = (W1 + X)
Weight of Petri dish after drying = W2gm
Loss on drying in % = W3-W2 x 100/X

**Determination of pH**

The pH of an aqueous solution of NIA/DG/2015/01ghan was measured by using a digital pH meter. It essentially denotes a quantitative measure of a solution's acidity or basicity.

- A digital pH meter was used to determine the pH of a particular solution.
- pH meter was standardised firstly; tablets having different pH were taken, and each tablet was diluted with 100 ml of distilled water for preparing solutions of different pH.
- The device was turned on before using various pH solutions and remained on for some time.
- The electrode was dipped in the buffer solution, which was stored in the beaker.
- A 10% aqueous sample solution was taken, the electrode was dipped, and the observations were registered. [14]

**Determination of Extractive values**

**Determination of Water Soluble Extractive**

5 gm of NIA/DG/2015/01ghan was macerated with 100 ml of distilled water of the specified strength in a closed flask and allowed to stand for twenty-four hours. Then, it was continuously shaken for six hours in a rotary shaker and then let stand for eighteen hours. Finally, the contents were then filtered using filter paper. The filtrate was transferred to a pre-weighed flat-bottomed dish and evaporated to dryness on a water bath; then, the dish was kept in an oven at 105 ºC to constant weight and finally weighed.

**Calculations**

Weight of the drug material = X gm
Weight of the empty petri dish = W1gm
Weight of the petri dish with dried extract = W2gm
Percentage of extractive value =W2-W1x100/X
The procedure was repeated three times, and the mean value was calculated.

**Determination of Alcohol Soluble Extractive**

The procedure of water-soluble extractive is the same as that of water-soluble extractive value but proceeded with alcohol instead of distilled water.

**Determination of Petroleum-Ether Soluble Extractive**

5 gm of NIA/DG/2015/01ghan was macerated with 100 ml of petroleum ether and set in a continuous extraction apparatus for 6 hours. Finally, the contents were filtered using filter paper. The filtrate was transferred to a pre-weighed flat-bottomed dish and evaporated to dryness on a water bath; then, the dish was kept in an oven at 105 ºC to constant weight and finally weighed. [14]

**Calculations**

Weight of the drug material = X gm
Weight of the empty petri dish = W1gm
Weight of the petri dish with dried extract = W2gm
Percentage of extractive value= W2-W1x100/X

**Determination of Ash value**

**Determination of Total Ash value**

5 gm of powdered NIA/DG/2015/01ghan was put in a silica crucible. This crucible was placed in a muffle furnace after spreading the sample evenly into a thin layer. The furnace temperature was set at 450 °C for about 6 hours or more until the ash was carbon-free. The crucible with the ash was then allowed to cool to the temperature in a desiccator and then weighed to constant weight.

**Calculation**

Wt. of Empty Silica Crucible = A1 gm
Wt. of Sample (X) = X gm
Wt. of the Crucible with Ash = A2 gm
Percentage of Total Ash = [A2 - A1 /X] x 100”

**Determination of Acid Insoluble Ash**

The total ash obtained was boiled with 25 ml of 2 M hydrochloric acid for 5 minutes. The insoluble matter was collected in a Gooch crucible, washed with hot water, ignited for 15 minutes at a temperature not exceeding 450 °C, cooled at room temperature in a desiccator, and weighed.

**Calculation**

Wt. of drug sample - X gm
Wt. of Crucible = G1 gm
Wt. of Crucible with insoluble Ash = G2 gm

Wt. of insoluble ash (G3) = G2-G1
Percentage of acid insoluble ash = G3/X×100"

**Determination of Water-soluble ash**

The total ash was boiled for 5 minutes with 25 ml of water. The insoluble matter was collected in a Gooch crucible, washed with hot water and ignited for 15 minutes at a temperature not exceeding 450 °C. The weight of the insoluble matter was subtracted from the weight of the ash; the difference in weight represented the water-soluble ash.

**Calculation**
Wt. of drug sample - X gm
Wt. of total ash – A gm
Wt. of Crucible - G1 gm
Wt. of Crucible with insoluble Ash - G2 gm
Wt. of insoluble ash (G3) = G2-G1
Water soluble ash (G4) = Wt. of total ash (A gm)- Wt. of insoluble(G3) Percentage of water-soluble ash = A – [(G3)/X] x 100

**Phytochemical Study**
Qualitative Phytochemical evaluation of both aqueous and alcoholic extracts was conducted for various phytochemicals as follows[15]:

**Tests for Carbohydrates**

**Molisch's test:** 2 ml of test solution taken in a test tube added with 2 ml of Molisch's reagent, shaken carefully and then about 1 ml of conc. $H_2SO_4$ is poured from the side of the test tube and allowed to stand for 1 minute. The formation of a purple colour ring at the junction of the two layers will indicate the presence of carbohydrates.

**Benedict's test:** It is used for detecting reducing sugars and is mainly composed of copper sulphate and sodium hydroxide. To 4 ml of aqueous drug solution, 1 ml of Benedict's solution was added and heated almost to boiling. Formation of green, yellow, orange, red and brown colours in order of increasing concentrations of simple sugar due to the formation of cuprous oxide.

**Fehling solution test**: It is generally used for detecting reducing sugars and is composed of two solutions, which are mixed in situ: Fehling solution A (0.5% copper sulphate) and Fehling solution B (Sodium Potassium Tartrate). Equal volumes of Fehling A and Fehling B solutions were mixed (1 ml each), and 2 ml of aqueous drug solution was added and then boiled for 5-10 minutes in a water bath.

**Tests for Alkaloids**

**Dragendorff's reagent test:** 2 ml of test solution was taken in a test tube in which 2 ml of Dragendorff's reagent (mixture of Potassium Iodide and Bismuth sub-nitrate solution) was added. The formation of an orange precipitate indicates the presence of alkaloids.

**Wagner's Test:** Drug solution, when added with a few drops of Wagner's reagent (dilute Iodine solution), reddish-brown precipitate formation indicates alkaloids.

**Hager's Test:** A saturated aqueous solution of picric acid was used for this test. It was added to the test sample. The formation of an orange-yellow precipitate will indicate the presence of alkaloids.

**Test for Amino acids**
**Ninhydrin test:** It is used to detect the presence of alpha-amino acids and proteins containing free amino groups. When heated with ninhydrin molecules, protein solution forms a complex between two ninhydrin molecules and nitrogen of free amino acid. This gives a characteristic deep blue or pale yellow colour.

**Tests for Proteins**

**Biuret test:** A few mg of the residue was taken in water, and 1 ml of 4% sodium hydroxide solution was added to it, followed by a drop of 1% solution of copper sulphate. The development of violet or pink colour indicates the presence of proteins.

**Xanthoproteic test:** 2 ml of the test sample in a test tube is added with 0.5 ml of concentrated nitric acid. The development of a yellow colour indicates the presence of proteins.

**Mellon's test:** A small quantity of test sample was taken, and 2 to 3 ml of millions reagent was added. The white precipitate slowly turns to pink, indicating the presence of proteins.

**Test for saponin**

**Foam test:** A small quantity of the test sample was taken in a test tube and shaken vigorously with a small amount of sodium bicarbonate and water. A stable, characteristic honeycomb-like foam indicates the presence of saponins.

**Test for glycosides**

**Bontrager's Test:** 1 ml of Benzene and 0.5 ml of dilute ammonia solution were added to the ethanolic extract and were observed for the formation of a reddish-pink colour.

**Test for Phenolic Compound**

**Phenolic test:** The extract was taken in water and warmed; to this, 2 ml of ferric chloride solution was added and observed for green and blue colour formation.

**Test for Steroids**

**Salkowski reaction:** A few mg of extract was taken in 2 ml of chloroform, and 2 ml of concentrated sulphuric acid was added from the side of the test tube. The test tube was shaken for a few minutes. The development of red colour indicates the presence of steroids.

**Test for Tannins**

**Ferric chloride solution:** A 5 percent ferric chloride solution in 90% alcohol was prepared. A few drops of this solution were added to the test sample. The appearance of dark green or deep blue colour indicates the presence of tannins.

**Lead acetate:** A 10 percent w/v solution of basic lead acetate in distilled water was added to the test filtrate. The development of precipitate indicates the presence of tannins.

**Pot. Dichromate:** A solution of potassium dichromate was added to the filtrate. The appearance of dark colour indicates the presence of tannins.

**Chromatographic Study**

Chromatography is a technique to separate a mixture of substances into components based on their molecular structure

and composition. TLC-thin layer chromatography is used to separate the mixture and identify its chemical constituent.
The plates utilised were T.L.C. plates covered with a 0.25 mm layer of silica gel 60 F254 with fluorescent indicator. (Each plate measures 10 cm in length and 2 cm in breadth.) [16]

**Activation of pre-coated Silica gel 60 F254**: Plates were dried for one and a half hours in a hot oven at 105 $^0$C.

**Preparation of mobile solution:** Chloroform: Methanol: Acetic Acid: Ethyl Acetate: Toluene(3:1:1:2:3).

**Test solution:** Alcoholic Extract

**Visualisation:** In Iodine vapours

**Rf Value**: The distance of each spot from the place of application was measured and recorded, and the Rf value was computed by dividing the distance travelled by the spots by the distance travelled by the front of the mobile phase.

**Calculation of Rf Value:** Distance travelled by solute from origin line /Distance travelled by solvent from origin line

## RESULTS AND OBSERVATION

The observations and the results of the present study are tabulated below.

### Pharmacognostical Analysis

**Table 2: Organoleptic characters of dried NIA/DG/2015/01ghan**

| Parameters | Observations |
|---|---|
| Colour | Grey |
| Odour | Characteristic |
| Taste | Bitter |
| Texture | Fine powder |

**Powder microscopy**: The presence of fibre, starch, crystals, oil glands and parenchyma was observed as shown in Figure 2[17].


**Figure 1: NIA/DG/2015/01**

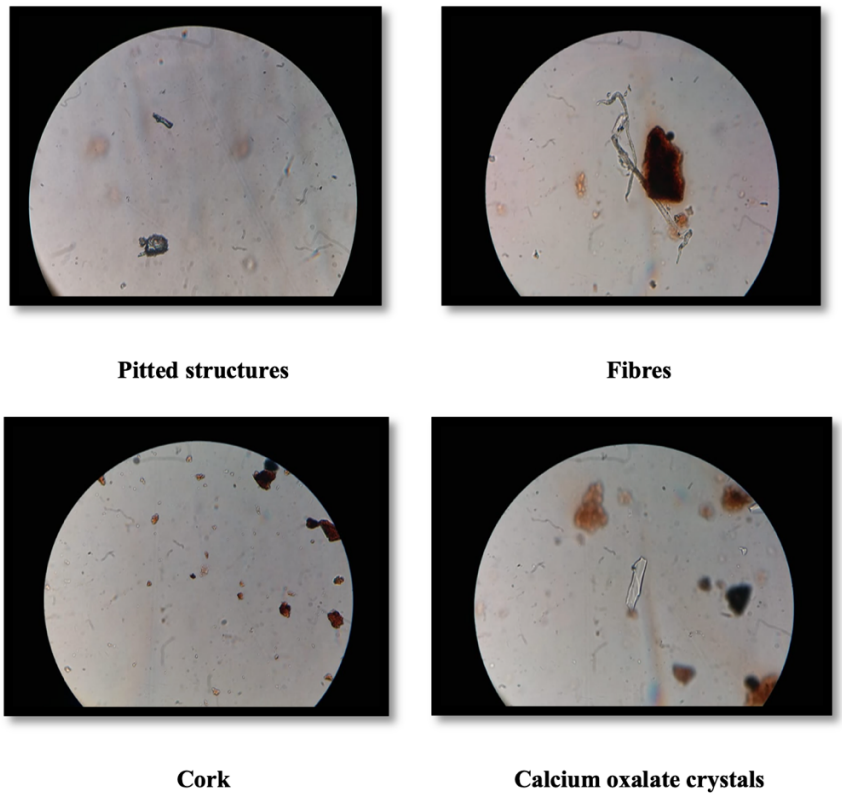


**Figure 2: Powder Microscopy of NIA/DG/2015/01**

**Physiochemical Analysis**

**Table 3: Observations of Physiochemical parameters**

| Physiochemical parameters | Observations |
|---|---|
| Moisture Content | 8.5 % |
| pH | 7.5 |
| Alcohol soluble extractive value | 27.53 % |
| Water soluble extractive value | 55.21 % |
| Petroleum ether soluble extractive value | 6.0 % |
| Ash value | 7.61 % |
| Acid insoluble ash value | 0.82 % |
| Water–soluble Ash | 6.7 % |

**Phytochemical Study**

**Table 4: Observations of Phytochemical parameters**

| Phytochemicals | Tests | Aq. Ext of NIA/DG/2015/01 | Al. Ext of NIA/DG/2015/01 |
|---|---|---|---|
| **Carbohydrates** | Molisch test | + | + |
| | Benedict test | + | + |
| | Fehling test | + | + |
| **Alkaloids** | Dragendorff's test | + | + |
| | Wagner test | + | + |
| | Hager test | + | - |
| **Amino acids** | Ninhydrins test | + | + |
| **Proteins** | Biuret test | + | - |
| | Xanthoproteic test | + | + |
| | Mellon test | + | + |
| **Saponin** | Foam test | + | - |
| **Glycosides** | Borntrager's test | - | - |
| **Phenolic Compound** | Phenolic test | + | + |
| **Steroids** | Salkowski test | + | + |
| **Tannins** | Fecl3 | + | + |
| | Lead acetate | + | + |
| | Pot. Dichromate | + | + |

**Figure 3: Phytochemical of NIA/DG/2015/01**

**Chromatography Study**

The visualisation was done under normal light and Iodine.

**Table 5: Results of TLC of NIA/DG/2015/01**

| Distance of solvent | Distance of spot(cm) | R F Value | Image | Image in iodine vapour |
|---|---|---|---|---|
| 5.0 | 4.9 | 0.98 |  |  |
| | 4.5 | 0.90 | | |
| | 4.2 | 0.84 | | |
| | 4.0 | 0.80 | | |
| | 3.5 | 0.70 | | |
| | 3.3 | 0.66 | | |
| | 3.0 | 0.60 | | |
| | 2.5 | 0.50 | | |
| | 2.0 | 0.40 | | |
| | 1.5 | 0.30 | | |
| | 1.0 | 0.20 | | |

## DISCUSSION

**Pharmacognostical Study:** The sample is organoleptically within the limits of API. Figures 1-4 show the presence of fibres, starch, crystals and oil glands in the sample. [18]

**Physiochemical Analysis:** The sample is stable as it has a normal moisture level. The ash value, which is within the standard limits as per API, indicates the authenticity and purity of the present sample. Extractive values within the standards indicate the sample's absence of exhausted or adulterated drugs. [13,14]

**Phytochemical Study:** The water extract of the sample showed positive results for the presence of carbohydrates, alkaloids, amino acids, proteins, saponins, phenolic compounds, steroids and tannins. The alcohol extract shows the presence of carbohydrates, alkaloids, amino acids, proteins, phenolic compounds, steroids and tannins. [16]

**Chromatography Study:** TLC of the Alcohol extract of NIA/DG/2015/01 shows bands at Rf-0.98, 0.90, 0.84, 0.80, 0.70, 0.66, 0.60, 0.50, 0.40, 0.30 & 0.20. [17]

## CONCLUSION

Based on the observations, results and discussions, it has been concluded that the present NIA/DG/2015/01 sample is within all the quality standards as per API. All the pharmacognostic, physiochemical, phytochemical and thin-layer chromatography studies helped identify and authenticate the sample of NIA/DG/2015/01.

## REFERENCES


1. Beavers G, Lip GY, O'Brien E. ABC of hypertension: The pathophysiology of hypertension. BMJ.2001;322:912–6].
2. Misha Tannu *et al.,* Prevalence of hypertension in India: A meta-analysis World J Meta-Anal. 2013 August 26; 1(2): 83-89.
3. NCHS Data Brief No. 289(health e-stat by Centre for Disease Control and Prevention)https://www.cdc.gov/nchs/products/databriefs/db289.htm.
4. Das PK, Malhotra CL, Prasad K. Cardiotonic activity of *Ashwagandhine* and *Ashwagandhinine*, two alkaloids from *Withania ashwagandha*, kaul. Archives Internationales de pharmacodynamie et de thérapie 1964;150: 356.
5. Rizwan M, Khan AA. Assessment of efficacy of *Sankhahuli (Convolvulus pluricaulis* Choisy.) and *Gokhru (Tribulus terrestris* L.) in the management of hypertension. Indian Journal of Traditional Knowledge 2014;13(2): 313-318.
6. Ashok, Gajarmal A, Shende MB. A clinical evaluation of antistress activity of *Ashwagandha (Withania somnifera* Dunal) on employees experiencing mental stress at workplace. International Journal of Ayurveda and Pharma Research 2015;3:1.
7. A Gautam S, Navneet, Kumar S. Appraisal of Antibacterial Properties of *Onosma bracteatum* Wall Fruit Extract Against Respiratory Tract Pathogens. J Herbs Ethnomedi. 2015; 1:108-15.
8. Yasmin A, Kousar K, Anjum N, Farooq O, Ghafoor S. *In Vitro* Antibacterial and Antifungal Activity of Different Solvent Extracts of *Onosma Bracteatum* Leave. Kashmir Journal of Medical Sciences. 2018;11(3):451-453.
9. Bihaqi Waseem S, Singh AP, Tiwari M. *In vivo* investigation of the neuroprotective property of *Convolvulus pluricaulis* in scopolamine-induced cognitive impairments in Wistar rats. Indian journal of pharmacology. 2011;43(5): 520.
10. Kalpana Govind bhai Patel, Kirti Vinod rai Patel And Ricky Gandhi. Evaluation of The Effect of *Onosma bracteatum* Wall (Boraginaceae) On Bronchial Hyperreactivity In Sensitised Guinea Pigs. Iranian Journal of Pharmacology & Therapeutics, By Razi Institute for Drug Research (Ridr), 2008;7:35-41.
11. Badruddin, Fareed S, Siddiqui H, Haque Se, Khalid M, Akhtar J. Psycho-immunomodulatory Effects of *Onosma Bracteatum* Wall. *(Gaozaban)* On Stress Model in Sprague Dawley Rats. J Clin Diagn Res. 2012;6(7):1356-60.
12. Ashraf M, Ahmad K, Ahmad I, Ahmad S, Arshad S, Shah SMA, Nasim FH. Acetylcholinesterase and Oxidase Inhibitory Activity of Some Medicinal Plants. Journal of Medicinal Plants Research, 2011;5(10):2086-2089.
13. Laboratory guide for the analysis of Ayurveda and Siddha formulations. New Delhi; CCRAS, Dept. of AYUSH, ministry of health and family welfare, Govt. of India. p. 27.
14. Laboratory guide for the analysis of Ayurveda and Siddha formulations. New Delhi; CCRAS, Dept. of AYUSH, ministry of health and family welfare, Govt. of India. p. 29-30.
15. Laboratory guide for the analysis of Ayurveda and Siddha formulations. New Delhi; CCRAS, Dept. of AYUSH, ministry of health and family welfare, Govt. of India. p. 83-87.
16. Laboratory guide for the analysis of Ayurveda and Siddha formulations. New Delhi; CCRAS, Dept. of AYUSH, ministry of health and family welfare, Govt. of India. p. 89-92.

17. Dr. K. R. Khandelwal, Dr. Vrunda Sethi. Practical Pharmacognosy Techniques and Experiments. Pune; Nirali Prakashan; 2019. p. 20.3.

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