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Research Article

PHARMACOGNOSTICAL AND PHYTOCHEMICAL EVALUATION OF A NEW ANTI-HYPERTENSIVE AYURVEDIC FORMULATION [NIA/DG/2020/01]

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ABSTRACT

Hypertension, also known as high or raised blood pressure, is a multifactorial disease with multiple causes and multiple treatments. The prevalence of hypertension in the urban Indian population was estimated to be 40.8% and in the rural population was 17.9%. Though there are multiple treatment of disease still it is challenge to manage this disease effectively with lower side effects as all available treatments are full of side effects and toxic effects. NIA/DG/2020/01 is a new anti-hypertensive Ayurvedic *ghan* formulation, containing *Arjuna (Terminalia arjuna* Roxb.), *Ashwagandha (Withania somnifera* Linn.), *Jatamansi (Nordostachys jatamansi* DC.), *Shankhpushpi (Convolvulus pluricaulis* Chois.), *Punarnava (Boerhavia diffusa* Linn.), *Gojihwa (Onosma Bracteatum Wall.), Guduchi (Tinospora cordifolia* Willd.*), Mukta Shukti (Margarita)* and *Praval Pisti (Corrallium Rubrum).* All these components are very well known for *Hridya, Mootral, Rasayana, Pitta shamak* effect and pharmacological actions like cardioprotective, anti-Hypertensive, antioxidant, antimicrobial, antifungal, Antidepressant or anxiolytic, anti-inflammatory, psycho-immunomodulatory effect and acetyl-cholinesterase inhibitory activity etc. **Aim and Objective:** To perform pharmacognostical, physio-chemical, phytochemical and chromatography evaluation of a new anti-hypertensive Ayurvedic formulation [NIA/DG/2020/01]. **Material and Methods:** This study involves pharmacognostical, physio-chemical, phytochemical and chromatography evaluation of a new anti-hypertensive Ayurvedic formulation [NIA/DG/2020/01] to ensure its purity, safety and quality. **Observations and Results:** All the findings of the pharmacognostical, physio-chemical, phytochemical and chromatography evaluation were within the standards of quality. **Conclusion:** The present sample of a new anti-hypertensive Ayurvedic formulation [NIA/DG/2020/01] was found to be rich in quality and was safe, pure and authentic.

INTRODUCTION

Hypertension is a condition in which the blood vessels have persistently raised pressure. It is a multifactorial disease with multiple causes and multiple treatments. Blood is carried from the heart to all parts of the body in the vessels. Each time the heart beats, it pumps blood into the vessels. Blood pressure is created by the force of blood pushing against the walls of blood vessels (arteries) as it is pumped by the heart.

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The higher the pressure the harder the heart has to pump.[1] The prevalence of hypertension in the urban Indian population was estimated to be 40.8% and in the rural population was 17.9%.[2] During 2015–2016, the prevalence of hypertension was 29.0% and increased with age: age group 18–39, 7.5%; 40–59, 33.2%; and 60 and over, 63.1%.[3] Though there are lots of anti-hypertensives available in market but they are associated with risks and long term side-effects. Ayurveda adopts a holistic approach which logically suits better for multifactorial diseases like hypertension. NIA/DG/2020/01 is a new anti-hypertensive ayurvedic formulation, containing *Arjuna (Terminalia arjuna* Roxb.), *Ashwagandha (Withania somnifera* Linn.), *Jatamansi (Nordostachys jatamansi* DC.), *Shankhpushpi (Convolvulus pluricaulis* Chois.), *Punarnava (Boerhavia diffusa* Linn.), *Gojihwa (Onosma*

Bracteatum Wall.), Guduchi (Tinospora cordifolia Willd.), Mukta Shukti (Margarita) and *Praval Pisti (Corrallium Rubrum).* All these components are very well known for *Hridya, Mootral, Rasayana, pitta shamak* 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. Usually, all medicinal plants contain phytochemicals or bioactive compounds which play the key role in its therapeutic action, still most of the drugs show variations in these phytochemicals which results in severe variations of its quality and efficacy. Also, the globalization and increasing demand for Ayurveda drugs resulted in unavailability of authentic and quality drugs in market which meets the quality standards. Pharmacognostical studies helps in providing the correct identification of the samples and there by authenticate the purity, safety and efficacy of the drug. Phytochemical studies confirm the naturally occurring chemical compounds in plants which contribute to its colour, taste, smell, actions and other properties and it also helps to discover the bioactive profile of the plants of therapeutic importance. The organic and inorganic substances present in a plant like alkaloids, tannins, saponins, phenols, flavonoids etc are tested to understand its complete pharmacodynamics.[12]

AIM AND OBJECTIVE

To perform pharmacognostical, physio-chemical, phytochemical and chromatography evaluation of a new anti-hypertensive ayurvedic formulation [NIA/DG/2020/01].

MATERIAL AND METHODS

Sample preparation- Dried aqueous extracts of all seven herbal ingredients mentioned in table were obtained by crude drug extraction method and two mineral contents i.e., *Mukta Shukti* and *Praval Pisti* were added to dried extract.

Table-01-Ingredients of sample preparation of NIA/DG/2020/01

Sr. No.	Ingredient	Family	Part Used	Quantity
1.	***Convolvulus pluricaulis* Chois.**	Convolvulaceae	Whole plant	**3 parts**
2.	***Withania somnifera* Linn.**	Solanaceae	Root	**3 parts**
3.	***Terminalia arjuna* Roxb.**	Combretaceae	Bark	**3 parts**
4.	***Boerhavia diffusa* Linn.**	Nyctaginaceae	Whole Plant	**3 parts**
5.	***Tinospora cordifolia* Willd.**	Menispermaceae	Stem	**3 parts**
6.	***Nordostachys jatamansi* DC.**	Valerianaceae	Rhizome	**2 parts**
7.	***Onosma bracteatum* Wall.**	Boraginaceae	Aerial Part	**3 parts**
8.	***Margarita/Pearl oyster***	-	-	**1/8 part**
9.	***Corrallium rubrum***	-	-	**1/4 part**

The grinded NIA/DG/2020/01 *Ghan* was sieved using a vibro sifter. The testing procedures included-

Pharmacognostical Study: It was carried out by naked eye and magnifying lens as organoleptic study for color, odor, taste, texture.

Powder Microscopy: For identification of powdered drug, microscopic powder inspection of medicinal plants is important. For this, various chemical 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.

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/2020/01 *Ghan* was analysed for the following:

Determination of Moisture Content/Total Soluble Solids [13]

Moisture content was determined by placing 5gm of NIA/DG/2020/01 *Ghan* in oven at 105°C for 5 hours. The weight of the sample was calculated in 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.

Calculations

Weight of an empty petridish = W1gm

Weight of the drug sample = X gm

Weight of the petridish with drug before drying (W3) = (W1 + X)

Weight of petridish after drying = W2gm

Loss on drying in % = W3-W2 x 100/X"

Determination of pH[14]

The pH of aqueous solution of NIA/DG/2020/01 *Ghan* was measured by using digital pH meter. It essentially denotes a quantitative measure of a solution acidity or basicity.

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

Determination of Extractive values[15]

• Determination of Water Soluble Extractive

5gm of NIA/DG/2020/01 *Ghan* was macerated with 100ml 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 allowed to 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°, to constant weight and finally weighed.

Calculations

Weight of the drug material = X gm

Weight of the empty petridish = W1gm

Weight of the petridish with dried extract = W2gm

Percentage of extractive value =W2-W1x100/X

The procedure was repeated three times and the meanvalue was calculated."

•Determination of Alcohol Soluble Extractive

Procedure of water soluble extractive is same as that of water soluble extractive value but it was proceeded with alcohol instead of distilled water.

•Determination of Petroleum-Ether Soluble Extractive

5gm of NIA/DG/2020/01ghan was macerated with 100ml of petroleum ether and set in a continuous extraction apparatus for 06 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°, to constant weight and finally weighed.

Calculations

Weight of the drug material = X gm

Weight of the empty petridish = W1gm

Weight of the petridish with dried extract = W2gm

Percentage of extractive value= W2-W1x100/X

Determination of Ash value

•Determination of Total Ash value

5gm of powdered NIA/DG/2020/01ghan was put in a Silica crucible. This crucible was placed in a muffle furnace, after spreading the sample evenly into a thin layer. The temperature of the furnace was set at 450°C for about 6 hrs or more until the ash was totally free from Carbon. The crucible with the ash was then allowed to cool to the temperature in 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 25ml of 2M hydrochloric acid for 5 minutes. The insoluble matter was collected in a Gooch crucible, washed with hot water and ignite 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 25ml of water. The insoluble matter was collected in a Gooch's crucible, washed with hot water and ignite 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[16]

Qualitative Phytochemical evaluation of both aqueous and alcoholic extracts were conducted for various phytochemicals as follows:

Tests for Carbohydrates

- **Molisch's test-** 2ml of test solution taken in a test tube added with 2ml of the Molisch's reagent,

shaken carefully and then about 1ml of conc. H_2SO_4 is poured from side of the test tube and allowed to stand for 1 minute. Formation of purple colour ring at the junction of the two layers will indicate the presence of Carbohydrate.

- **Benedict's test-** It is used for detecting reducing sugars and is mainly composed of Copper sulphate and sodium hydroxide. To 4ml of aqueous solution of drug, 1ml 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 formation of cuprous oxide.
- **Fehling Solution Test**- It is generally used for detecting reducing sugars and composed of two solutions, which are mixed in situ. Fehling solution A (0.5% of copper sulphate) and Fehling solution B (Sodium Potassium Tartrate). Equal volumes of Fehling A and Fehling B solutions were mixed (1ml each) and 2ml of aqueous solution of drug was added and then boiled for 5-10 minutes on water bath.

Tests for Alkaloids

- **Dragondroff's reagent test-** 2ml of test solution was taken in a test tube in which 2ml of the Dragondroff's reagent (mixture of Potassium Iodide and Bismuth sub nitrate solution) was added. Formation of orange precipitate indicates presence of alkaloids.
- **Wagner's Test-** Drug solution when added with few drops of Wagner's reagent (dilute iodine solution) a formation of reddish-brown precipitate indicates presence of 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. Protein solution when heated with ninhydrin molecules, it results in formation of complex between two ninhydrin molecule 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 1ml of 4% sodium hydroxide solution was added to it, followed by a drop of 1% solution of copper sulphate. Development of violet or pink colour indicates the presence of proteins.
- **Xanthoprotic test-** 2ml of test sample in test tube is added with 0.5ml of concentrated nitric acid. Development of yellow colour indicates presence of proteins.
- **Millon's test-** A small quantity of test sample was taken and 2 to 3ml of millions reagent was added. The white precipitate slowly turning to pink, indicate 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 froth indicates the presence of saponins.

Test for glycosides

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

Test for Phenolic Compound

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

Test for Steroids

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

Test for Tannins

- **Ferric chloride solution-** A 5 percent solution of ferric chloride in 90% alcohol was prepared. Few drops of this solution were added to the test sample. 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. Development of precipitate indicates the presence of tannins.
- **Pot. Dichromate-** A solution of potassium dichromate was added to the filtrate. Appearance of dark colour indicates the presence of tannins.

Chromatographic study[17]

Chromatography is a technique to separate mixture of substances into components on the basis of their molecular structure and molecular composition. TLC-thin layer chromatography, is used for separation of mixture and identification of its chemical constituent.

The plates utilized 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.)"

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

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

Test solution: Alcoholic extract

Visualization- 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

Figure 1: Macroscopic Study of NIA/DG/2020/01

Table 2: Organoleptic characters of dried NIA/DG/2020/01 *Ghan*

S. No	Parameters	Observations
1	Color	Whitish Grey
2	Odor	Charecteristic
3	Taste	Bitter
4	Texture	Fine powder

Powder Microscopy[18]: The presence of fibers, starch, crystals, oil glands and parenchyma were observed as shown in Figure.

Figure 2: Powder Microscopy of NIA/DG/2020/01

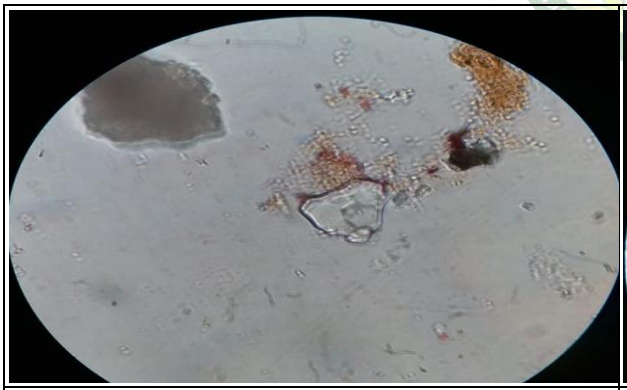


Fig: It shows pitted structures

Fig: Fibres are seen

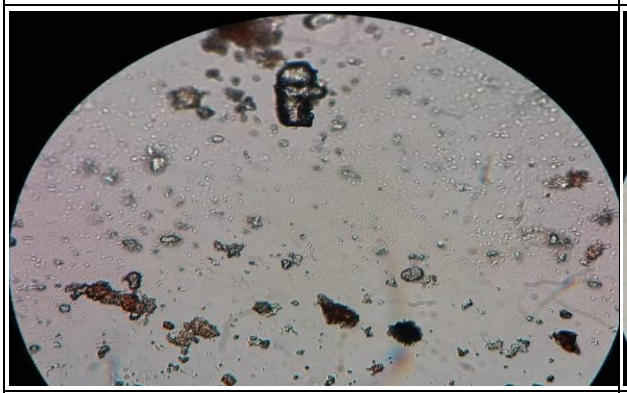


Fig: Cork is seen

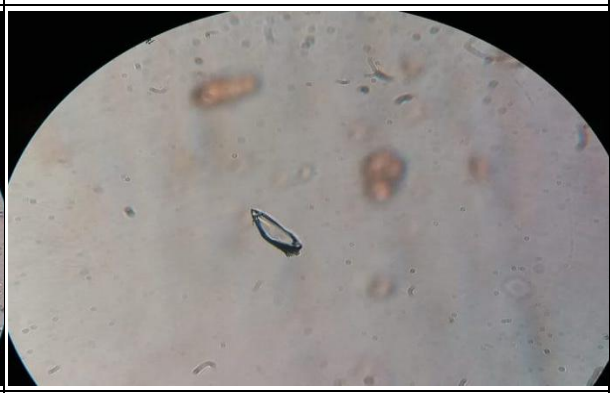


Fig: Calcium oxalate crystals are seen

Physiochemical Analysis

Moisture Content of Sample

Figure 3: Moisture content of sample of NIA/DG/2020/01

Table 3: Moisture content of sample of NIA/DG/2020/01

S.NO.	Weight of sample	Weight of container	Weightafter drying with container	Weightafter drying without container	Value%
1.	4.9803 gm	60.8575 gm	65.5630 gm	4.7055 gm	5.5%

pH Value of Sample

Figure 4: pH Value of Sample of NIA/DG/2020/01

Table 4: pH Value of Sample of NIA/DG/2020/01

S.NO.	Sample	pH
1.	NIA/DG/2020/01	6.9

Extractive value of sample

Figure 5: Extractive value of sample of NIA/DG/2020/01

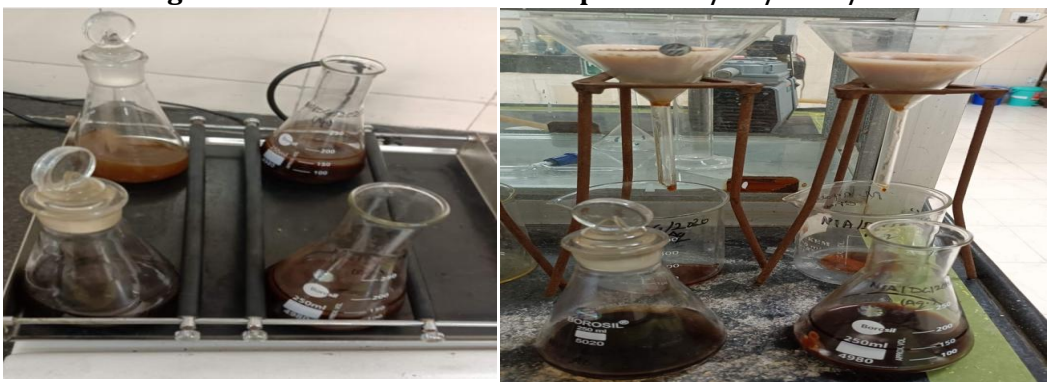


Table 5: Extractive value of sample of NIA/DG/2020/01

S.No.	Extractive values	Sample weight	Beaker weight	Beaker + extract weight	Extract weight	Extract value (%)
1.	Alcohol soluble extractive Value	5.0119gm	138.21gm	139.6194gm	1.4094gm	28.12%
2.	Water soluble extractive value	5.0120gm	146.023gm	148.6140gm	2.591gm	51.69%
3.	Petroleum ether soluble extractive value	5.0007gm	123.6397gm	123.9625gm	0.3228 gm	6.4%

Ash Value of Sample

Figure 6: Ash Value of Sample of NIA/DG/2020/01


Table 6: Total Ash value of Sample of NIA/DG/2020/01

S.NO.	A1	X	A2	Total ash (%)
1.	39.7840 gm	4.9770 gm	40.072 gm	5.8%

Table 7: Acid Insoluble Ash value of sample of NIA/DG/2020/01

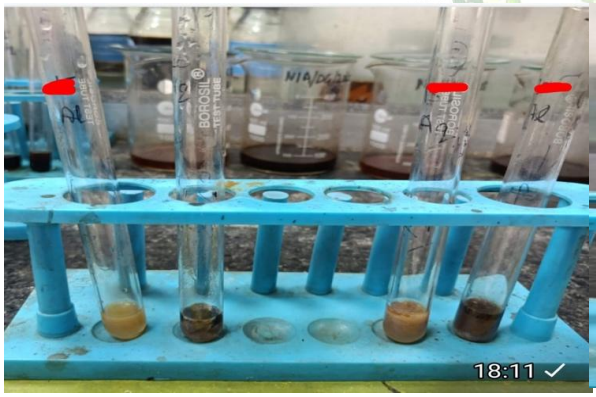
S.NO.	X	G1	G2	G3	Total ash (%)
1.	4.9770 gm	39.7840 gm	39.8143 gm	0.0303 gm	0.61%

Table 8: Water Soluble Ash value of sample of NIA/DG/2020/01

S.NO.	X	A	G1	G2	G3	Total ash(%)
1.	5.0058 gm	0.2903 gm	31.5600 gm	31.8373 gm	0.2773 gm	4.47%

Phytochemical study

Figure 7: Phytochemical study of NIA/DG/2020/01



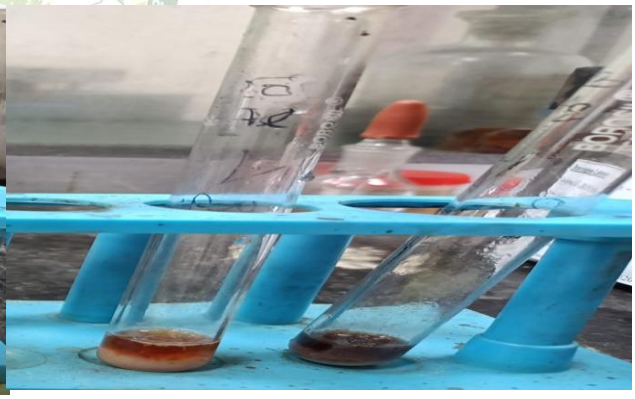


Table 9: Observations of Phytochemical parameters

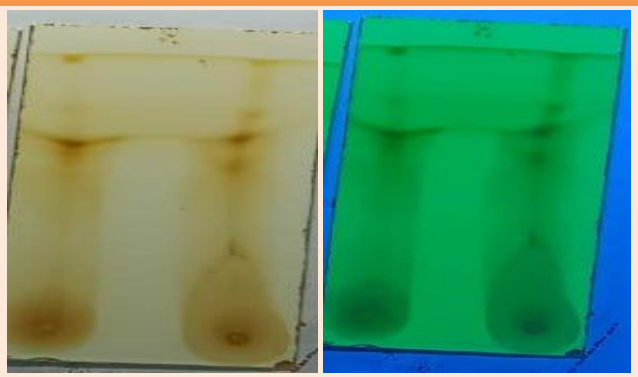
Phytochemicals	Tests	Aq. Ext of NIA/DG/2020/01	Al. Ext of NIA/DG/2020/01
Carbohydrates	1.1- Molish test	+	+
	1.2- Benedict test	+	+
	1.3-Fehling test	+	+
Alkaloids	2.1-Dragendorff test	+	+
	2.2-Wagner test	+	+
	2.3-Hager test	+	-
Amino acids	3.1- Ninhydrine test	+	+
Proteins	4.1-Biuret test	+	-
	4.2-Xanthoprotic test	+	+

	4.3- Millon test	+	+
Saponin	5.1-Foam test	+	-
Glycosides	6.1- Borntrager test	-	-
Phenolic Compound	7.1- Phenolic test	+	+
Steroids	8.1- Salkowaski test	+	+
Tannins	9.1-Fecl3	+	+
	9.2- Lead acetate	+	+
	9.3-Pot. Dichromate	+	+

Chromatography Study

Visualization was done under normal light and Iodine.

Table 10: Results of TLC

Distance of solvent	Distance of spot (cm)	R F Value	Image
5.0	4.9	0.98	
	4.0	0.80	
	3.7	0.74	
	3.4	0.68	
	2.7	0.54	
	1.7	0.34	
	1.2	0.24	
	0.8	0.16	

DISCUSSION

- **Pharmacognostical study:** Sample is organoleptically within the limits. Picture no: 02 shows the presence of fibers, starch, crystals and oil glands in the sample.
- **Physiochemical analysis:** Sample is stable as it has normal moisture level. The ash value which is within the standard limits is indicating the authenticity and purity of the present sample. Extractive values within the standards indicate the absence of exhausted or adulterated drugs in the sample.
- **Phytochemical Study:** The water extract of sample had shown positive results for the presence of carbohydrates, alkaloids, amino acids, proteins, saponins, phenolic compound, steroids and tannins. The alcohol extract shows presence of carbohydrates, alkaloids, amino acids, proteins, phenolic compounds, steroids and tannins.
- **Chromatography study:** TLC of the Alcohol extract of **NIA/DG/2020/01** shows bands at Rf- 0.98, 0.80, 0.74, 0.68, 0.54, 0.34, 0.24, 0.16.

CONCLUSION

On the basis of the observations, results and discussions it has been concluded that the present sample of NIA/DG/2020/01 is within all the standards of quality. All the pharmacognostical, physiochemical, phytochemical and Thin Layer Chromatography study helped in identification and authentication of the sample of NIA/DG/2020/01.

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