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EVALUATION OF PHARMACOGNOSTICAL END POINTS AND PRELIMINARY PHYTOCHEMICAL SCREENING OF VITEX NEGUNDO *(SINDHUWAR)* AND CORDIA DICHOTOMA *(SLESHMATAKA)*- A RESEARCH ARTICLE

Dr. Anamika Tuli*[1], Dr. Bhawana Mittal[2], Dr. Ramesh Chandra Tiwari[3] and Gaurav Sharma[4]

PG Scholar*[1], Assistant Professor[2], Prof. and Head[3]

[1,2,3]P.G. Department of Agad Tantra Evum Vidhi Vaidayak, Uttarakhand Ayurved University, Rishikul Campus, Haridwar, Uttarakhand, India.

[4]Pharmacologist, Bilwal Medchem and Research Laboratory Pvt. Limited, Reengus, Sikar, Rajasthan.

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***Corresponding Author**
Dr. Anamika Tuli
PG Scholar, P.G. Department of Agad Tantra Evum Vidhi Vaidayak, Uttarakhand Ayurved University, Rishikul Campus, Haridwar, Uttarakhand, India.

ABSTRACT

Ayurveda is a 'SCIENCE OF LIFE' pointed out concept of positive health means metabolically well balanced human-beings. Its eternality is related to its preventive aspects and treatment principles described to treat illness. Numerous new diseases are emerging with new titles. **Introduction:** In the recent time, the Traditional System of Medicine is giving competitive aspect to the Modern medicine as it's having fewer side effects. **Material and Methods:** The present investigation is aimed to evaluate the pharmacognostical parameters and Phytochemical screening to find out the bioactive compounds present in Vitex negundo belonging to family Lamiaceae and Cordia dichotoma belonging to family Boraginaceae. **Discussion:** The study of the selected plant consisted of checking for drug contamination and investigating the various phytochemicals present in the extract at a preliminary level using the aqueous and ethanolic extract. The following study will provide detailed reference.

KEYWORDS: Vitex negundo, Cordia dichotoma, pharmacognostical, Phytochemical, *Sindhuwar, Sleshmataka.*

INTRODUCTION

Nature has always been a storehouse of remedies to cure all mankind's ailments. Since time immemorial, people have used natural substances derived from plants in medicines to alleviate and treat ailments. Herbal medicines are currently in high demand as primary health care in developing countries. This is basically due to the popular belief that herbal medicines have less side effects, are cheap and available locally. The World Health Organization (WHO) estimates that up to 80% of people still rely primarily on traditional medicines. This study was conducted to evaluate the pharmacological and phytochemical parameters of roots, bark and leaves of Vitex negundo *(Sindhuwar)* and fruits and bark of Cordia dichotoma *(Sleshmataka).* Phytochemical analysis is also important for finding the presence of active ingredients.[1]

PLANT DESCRIPTION[2,3,4]

STUDY DRUGS	LATIN NAME	FAMILY	PHARMACOLOGICAL ACTION
SINDHUWAR	*Vitex negundo*	*Lamiaceae*	Antioxidant Anti Bacterial Anti-inflammatory Anti-Fungal
SLESHMATAKA	*Cordia dichotoma*	*Boraginaceae*	Anti-inflammatory Anti-Fungal & Anti-Bacterial Antioxidant

AYURVEDIC PROPERTIES[2]

Study Drugs	***Rasa***	***Guna***	***Virya***	***Vipaka***	***Prabhav***	***Prayojya-Anga***	***Sarvdeihikkarma***
Sindhuwar	*Katu* *Tikta*	*Laghu* *Ruksha*	*Ushna*	*Katu*		*Patra* *Mool*	*Kushthahar Keshya*
					Vishaghna	*Beej*	*Shoolhar* *Shothahar*
							Krimighan
							Kaasjit
Sleshmataka	*Madhura*	*Snigdha* *Guru*	*Sheeta*	***Fruit-*** *Madhur*	*Vishaghna*	*Twak* *Phala*	*Vishahar* *Sfothahar*
		Picchila		***Bark-***			*Vranhar*
		Bark-		*Katu*			*Kushthahar*
		Kashaya					*Keshya*
		Tikta					*Krimighan*

MATERIAL AND METHODS

MATERIALS

Chemical and Consumable

Lead acetate, Mayer's reagent, Barium chloride, Ferric Chloride, Benzene, Perchloricacid, Sodium sulphide, Acetone, Potassium bismuth- iodide, Sodium potassiumtartarate, Vanillin sulphuric acid, Ammonium chloride, Methanol, Ethanol, Chloroform, Millon reagent, Molisch's reagent, Iodine solution, Pyridine, Ninhydrin, Seliwanoff's reagent, Copper sulphate, Picric acid etc.

Equipment's

Digital balance, Rotary Shaker, Hot air oven, Heating Mantle, Silica Crucible, Grinder, Water bath, Muffle furnace, TLC chamber, Vacuum pump, U.V./ Vis. Spectrophotometer, Common glass wear etc.

METHODS[5]

ANALYSIS OF SINDHUWAR AND SHLESHMATAKA

A) **Macroscopic Study / Organoleptic Parameters –** The collected samples was studied organoleptically, with naked eye, magnifying lens and measuring tape. Pharmacognostical parameters i.e, appearance, colour, odour and taste and findings were recorded.

B) **Microscopic study –** Microscopy is a tool of sample identification.

- **Powder microscopy** –Powder microscopic inspection of medicinal plant materials is indispensable for the identification of broken or powdered materials; the specimen has to be treated with chemical reagents. An examination by microscopy alone cannot always provide complete identification, though when used in association with other analytical methods it can frequently supply invaluable supporting evidence. Comparison with a reference material will often reveal characteristics not described in the requirements which might otherwise have been attributed to foreign matter rather than normal constituents.

Procedure – For examining the characters of the powder take sufficient amount of powder in different chemical reagents on a slide and warm over a low flame for a short time. Put drop of glycerine on the slide, cover it with the cover slip and observe under the microscope.

Chemical reagents used for staining of the powder samples were as follows –

- ✓ Safranin
- ✓ Dilute Ferric chloride
- ✓ Eosine
- ✓ Methylene blue

C) Physicochemical Analysis – Physicochemical study is a vital step in the preparation of any formulation. It is the study of the relations between composition and physical properties of the elements.

- ➢ **Determination of Moisture Content[6]:-** Moisture content is a water holding capacity of sample, higher moisture content in sample show that it may decrease stability. Moisture content was determined by placing weighed sample of 5gm of drug in oven at 105° for 5 hours, and calculated weight of sample for every 30 minute, until the weight of the sample came out to be constant, no variation of weight was recorded. This sample was allowed to cool at room temperature in a desiccator for 1 hour before weighing.

CALCULATION

Weight of the empty petridish = W_1gm

Weight of the drug sample = X gm

Weight of the petridish with drug before drying= (W_3) (W_1 +X)

Weight of petridish after drying= W_2gm

Loss on drying in %-W_3-W_2x100/X

- ➢ **Determination of pH –**The pH value of an aqueous liquid may be defined as the common reciprocal of the hydrogen ion concentration expressed in gram per litre. It practically means the quantitative indication of the acidity or basic nature of a solution. The pH of a given solution is measured by using digital pH meter.

- First Standardized the pH meter. Tablets of different pH were taken and each tablet was dissolved in 100 ml of distilled water to prepare solutions of different pH.
- The instrument was switched on and left for some time until required different pH solutions appeared.
- Buffer solution was taken in the beaker and the electrode was dipped in it. Same procedure was repeated for the other buffer solution after washing the electrode thoroughly with distilled water.

- The sample was taken (10% aqueous solution) and electrode was dipped in it and the value of pH was noted.

➢ **Determination of Extractive value** – It is a gravimetric analysis (Maceration Process), the extraction of any crude drug with a particular solvent yields a solution containing different phyto-constituents. The composition of these phyto -constituents in that particular solvent depends upon the nature of the drug and Solvent used.

❖ **Determination of Alcohol Soluble Extractive**

5 g coarsely powdered air dried drug was macerated with 100 ml of Alcohol of the specified strength in a closed flask for twenty-four hours. It was then continuously shaken for six hours using rotary shaker and allowed to stand for eighteen hours. The content was 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 oven at 105º, to constant weight and weighed.

❖ **Determination of Water Soluble Extractive:-** Procedure was same as that of alcohol soluble extractive value and it was proceeded using distilled water instead of alcohol.

➢ **Determination of Total Ash[7]:-** Ash is a quantity analysis technique for determining siliceous material and inorganic substance in sample. Acid Insoluble Ash shows siliceous material and heavy metals. Water Soluble Ash shows quantity of water in organic Substance. The total ash method is designed to measure the total amount of material remaining after ignition. This includes both physiological ash which is derived from the plant tissue itself and non-physiological ash which is the residue of the extraneous matter (e.g. sand and soil) adhering to plant surface. Silica Crucible was cleaned, dried well, labeled with glass pencils and then weighed to constant weight. 5gm of powdered drug sample was put in the Silica crucible. The drug was spread evenly into a thin layer. This crucible was placed in a muffle furnace and ignited at a temperature of 450°C for about 6 hrs or more until the ash was totally free from Carbon. The crucible containing the ash was allowed to be cooled in desiccators and subsequently weighed to constant weight. The percentage of ash with reference to the air dried drug was calculated.

CALCULATION

Wt. of Empty Silica Crucible = A_1 gm

Wt. of Sample(X) = X gm

Wt. of the Crucible with Ash = A_2 gm

Percentage of Total Ash =[A_2-A_1/X] x100

- **Determination of Acid Insoluble Ash[8]:-** Acid insoluble Ash value determined as per Pharmacopoeia of India, 1996. Boiled the total ash with 25 ml of 2 M hydrochloric acid for 5 minutes, collected the insoluble matter in a Gooch crucible or on an ash less filter paper, washed with hot water, ignite, cool in a desiccators and weighed. Calculate the percentage of acid-insoluble ash with reference to the air-dried drug.

Calculation

Wt. of drug sample - X gm

Wt. of Crucible= G1gm

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[9]:-** Water–soluble ash value determined as per Pharmacopoeia of India 1996. Boiled the total ash for 5 minutes with 25 ml of water; collected the insoluble matter in a Gooch‘s Crucible or on an ash less filter paper, Washed with hot water and ignite for 15 minutes at a temperature not exceeding 450 C. Subtract the weight of the insoluble matter from the weight of the ash; the difference in weight represented the water – soluble ash. Calculate the percentage of water soluble ash with reference to the air-dried drug.

Calculation

Wt. of drug sample - X gm

Wt. of total ash–Agm

Wt. of Crucible-G1gm

Wt. of Crucible with insoluble Ash - G2 gm

Wt. of insoluble ash(G3)=G2-G1

Water soluble ash (G4) = Wt. of total ash gm- Wt. of insoluble ash (G3)

Percentage of water soluble ash=A –[(G3)/X] x100

D) Phytochemical Screening[10]

Tests for Carbohydrates

- **Molisch's Test:** 2 ml of test Solution was taken in a test tube and 2 ml of the Molisch's reagent was added and shaken carefully and then about 1ml. of conc. H_2SO_4 is poured from side of the test tube and allowed to stand for one 1 minute. A Purple colour ring at the junction of the two layers if formed indicated the presence of Carbohydrate.
- **Benedict's test:** It is used for reducing sugars and composed of mainly Copper sulphate and sodium hydroxide. To the 4 ml of aqueous solution of drug, 1 ml of Benedict's solution was added and heated almost to boiling. Formation of green, yellow, orange, red or brown colour in order of increasing concentrations of simple sugar in the test solution, due to formation of cuprous oxide.
- **Barfoed'stest:** The test sample was dissolved in water and heated with a little of the Barfoed's reagent. Formation of red precipitate of cuprous oxide within two minutes indicates the presence of monosaccharides.
- **Fehling solution test:** It is generally used for reducing sugars and composed of two solutions, which are mixed in situ. Fehling solution A composed of 0.5% of copper sulphate where as Fehling solution B composed of Sodium Potassium Tartarate. Equal volumes of Fehling A and Fehling B solutions were mixed (1 ml each) and 2 ml of aqueous solution of drug was added followed by boiling for 5-10 minutes on water bath.

Tests for Alkaloids

- **Mayer's reagent test:** 2 ml of test Solution was taken in a test tube to which 2 ml of the Mayer's reagent (Potassium Mercury Iodide solution) was added. A White or Pale Yellow precipitate if formed indicated presence of Alkaloids except with Alkaloids of the Purine groups and few others.
- **Dragendroff's reagent test:** 2 ml of test Solution was taken in a test tube in which 2 ml of the Dragon Droff's reagent (Mixture of Potassium Iodide and Bismuth subnitrate solution) was added. An orange precipitate if formed indicate presence of Alkaloids.
- **Wagner's Test:** Drug solution + few drops of Wagner's reagent (dilute Iodine solution), formulation of reddish-brown precipitate indicates the presence of alkaloids.
- **Hager's Test:** 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 which indicates the presence of alkaloids.

Test for Amino acids

- **Ninhydrin test:** The Ninhydrin test isused to detect the presence of alpha-amino acids and proteins containing free amino groups. Protein solution when heated with ninhydrin molecules, it gives characteristic deep blue or pale yellow colour due to the formation of complex between two ninhydrin molecule and nitrogen of free amino acid.

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. Development of violet or pink colour indicates the presence of proteins.
- **Xanthoproteic test** A small quantity of test sample was taken with 2 ml of water and 0.5 ml of concentrated nitric acid was added to it. Development of yellow colour indicates the presence of proteins.
- **Millon's test:** A small quantity of test sample was taken and 2 to 3 ml of millons 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

- **Borntrager'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

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

Test for Flavonoids

- **Shinoda test:** A small quantity of test sample was dissolved in 5 ml ethanol (95%v/v) and reacted with few drops of concentrated hydrochloric acid and 0.5gm of magnesium metal. Appearance of pink, crimson or magenta colour with in a minute or two indicates the presence of flavonoids.

Test for Steroids

- **Salkowski reaction:** Few mg of extract was taken in 2 ml of chloroform and 2 ml 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 a little of the above filtrate. 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.

E) Chromatograph[11]

Chromatography plates

T.L.C plate coated with 0.25mm layer of silica gel 60F254 with fluorescent indicator was Used. (Each plate dimension is 10 cm long and 2 cm width).

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

Test solution: **Alcoholic Extract**

Preparation of mobile solution: Toluene: Ethyl Acetate : Formic acid (6:3:1)

Visualization: Vanillin sulphuric acid Spray

Rf Value- Measured and recorded the distance of each spot from the point of its application and calculated Rf value by dividing the distance travelled by the spots by the distance travelled by the front of the mobile phase.

Calculation of Rf Value

$$Rf = \frac{\text{Distance travelled by solute from origin line}}{\text{Distance travelled by solvent from origin line}}$$

OBSERVATIONS AND RESULTS

Table No. 1: Results of organoleptic characters of *Sindhuwar and Shleshmataka.*

S. No	Organoleptic	*Sindhuwar*	*Sleshmataka*
1	**Colour**	Light Ochre	Dark Brown
2	**Odour**	Characteristics	Characteristics
3	**Appearance**	Coarse Powder	Coarse Powder
4.	**Taste**	Madhur, Kashaya	Tikta Kashaya

Table No. 2: Results of Physicochemical Analysis of *Sindhuwar* and *Shleshmataka.*

S.No	Tests	*Sindhuwar*	*Sleshmataka*	Test method
1	Loss on drying (%)	6.67	6.14	A.P.I, Part II, Vol-I, Appendices- 2.2.10
2	Aqueous Extractive Value (%)	23.44	16.58	A.P.I, Part II, Vol-I, Appendices- 2.2.8
3	Alcoholic Extractive Value (%)	10.24	11.07	A.P.I, Part II, Vol-I, Appendices- 2.2.7
4	Total Ash (%)	7.89	8.85	A.P.I, Part II, Vol-I, Appendices- 2.2.3
5	Acid Insoluble Ash (%)	2.36	3.86	A.P.I, Part II, Vol-I, Appendices- 2.2.4
6	Water Soluble Ash (%)	5.79	6.48	A.P.I, Part II, Vol-I, Appendices- 2.2.5
7	pH	6.8	7.8	A.P.I, Part II, Vol-II, Appendix-3.3

Table No. 3: Results of Phytochemical Screening of *Sindhuwar and Shleshmataka.*

Name of Test	Vitex negundo		Cordia dichotoma	
	Aqueous Extract	Ethanolic Extract	Aqueous Extract	Ethanolic Extract
Carbohydrate				
Molish test	+ *ve*	+ *ve*	+ *ve*	+ *ve*
Benedict test	+ *ve*	- *ve*	- *ve*	+ *ve*
Fehling test	- *ve*	+ *ve*	+ *ve*	- *ve*
Alkaloids				
Dragendorff test	- *ve*	+ *ve*	+ *ve*	- *ve*
Wagner's test	- *ve*	- *ve*	- *ve*	+ *ve*
Hager's test	- *ve*	- *ve*	- *ve*	+ *ve*
Amino acids				
Ninhydrine	- *ve*		+ *ve*	- *ve*
Protein				
Biuret test	- *ve*	+ *ve*	+ *ve*	+ *ve*
Xenthoprotic test	+ *ve*	+ *ve*	- *ve*	- *ve*
Saponin				
Foam test	+ *ve*	- *ve*	+ *ve*	- *ve*
Glycosides				
Borntrager's test	- *ve*	+ *ve*	+ *ve*	- *ve*

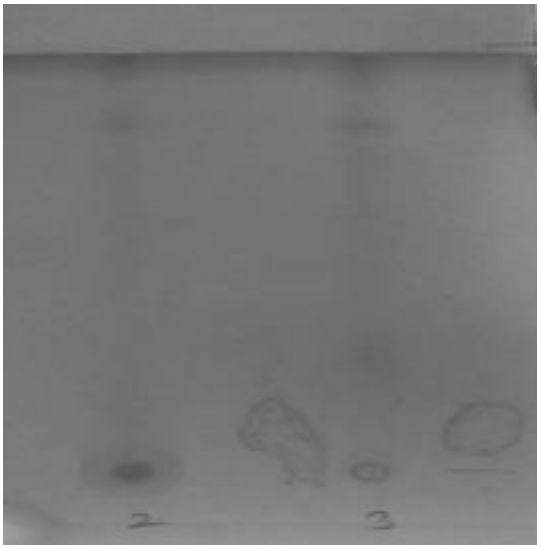
Phenolic compound				
Phenolic test	+ *ve*	+ *ve*	+ *ve*	+ *ve*
Steroids				
Salkowaski	- *ve*	+ *ve*	- *ve*	+ *ve*
Tannins				
$Fecl_3$	+ *ve*	+ *ve*	+ *ve*	+ *ve*
Lead acetate	+ *ve*	- *ve*	+ *ve*	- *ve*
Pot. Dichromate	+ *ve*	- *ve*	- *ve*	- *ve*

4. AFLATOXIN

S.No	Aflatoxin	*Sindhuwar*	*Sleshmataka*	Reference	Test method
1	**Aflatoxin B1**	Not Detected	Not Detected	**0.5 PPB**	**A.P.I, Part II, Vol-I, Appendix - 2.7**
2	**Aflatoxin B2**	Not Detected	Not Detected	**0.1 PPB**	
3	**Aflatoxin G1**	Not Detected	Not Detected	**0.5 PPB**	
4	**Aflatoxin G2**	Not Detected	Not Detected	**0.1 PPB**	

5. THIN LAYER CHROMATOGRAPHY

R_f Value	Test method
Sindhuwar:	**Stationary Phase:**
0.44	Silica gelG60F254
0.57	
0.83	**Mobile solution**
Sleshmataka:	Toluene : Ethyl Acetate (6:4)
0.27	
0.73	**Visualization:**
0.85	Iodine Vapours
0.92	



(Vitex negundo) (Cordia dichotoma)

DISCUSSION

The following research was done to standardise the Vitex negundo (leaves, roots, and bark) as well Cordia dichotoma (Fruits and bark). Before conducting any tests, a macroscopic and microscopic description of a plant is the first stage in determining the identification and level of purity of such materials. Organoleptic study indicates that colour of *Sindhuwar* is Light Ochre and *Sleshmataka* is Dark brown having Characteristics odour. Moisture content is a water holding capacity of sample, higher moisture content in sample shows that it may decrease stability. Loss on drying of *Sindhuwar* is 6.67% and *Sleshmataka* is 6.14%. Although, Acid insoluble ash defines the amount of silica and Carbonates. Acid insoluble ash value of *Sindhuwar* is 2.36% and *Sleshmataka* is 3.86 %. Total ash evaluation allowed us to

identify the amount of organic and inorganic substances present in the sample. Acid insoluble ash value of *Sindhuwar* is 2.36% and *Sleshmataka* is 3.86%. We were able to establish the amount and kind of active phytoconstituents in the extract by looking at the percentage of extractives in various solvents. Aqueous extractive value of *Sindhuwar* and *Sleshmataka* is 23.44% and 16.58% and Alchoholic extractive values of *Sindhuwar* and *Sleshmataka* is 10.24% and 11.07%. TLC Identification is used for semi-quantitative analysis of these extracts. Rf value of *Sindhuwar* is 0.44, 0.57, 0.83 and *Sleshmataka* is 0.27, 073, 0.85 and 0.92. To determine the presence of phytoconstituents, a preliminary phytochemical study was performed on the extracts produced by consecutive solvent extractions. Quantitative analysis for the presence of various functional group showed the presence of Carbohydrates, Alkaloids, Amino acids, Proteins, Saponins, Glycosides, Phenolic compounds, Flavonoids, Steroids and Tannins.

CONCLUSION

The pharmacognostic evaluation which comprises of macromorphology and microscopic characters, the estimation of physicochemical parameters and the phytochemical and TLC profile are constant features of a plant which are highly essential for raw drugs or plant parts used for preparation of phytomedicine The phytochemical examination of a plant involves the selection, collection, identification and authentication, extraction of the plant material. The present study may be useful to provide information with regards to its identification and standardization and also in carrying out further research of its use in the *Ayurvedic* System of Medicine.

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