

## AN ANALYTICAL STUDY OF *SHATAPUSHPA PHALA* (*Anethum sowa*) WSR TO ITS PHYSICOCHEMICAL COMPOSITIONS

Akhil Jain<sup>1</sup>, Nidhi Garg<sup>2</sup>

<sup>1</sup>Associate Professor Dept. of Shalakyta Tantra, <sup>2</sup>Assistant Professor Dept. of Agad Tantra, Ch. Devi Lal Ayurvedic Medical College, Haryana, India

### ABSTRACT

According to *Ayurveda* Drug does its action on basis of its *Rasa, Guna, Veerya, Vipaka* whereas in modern concepts drug acts because of its chemical composition. *Shatapushpa Phala Churna* widely recommended and traditionally used for gynecological disorders including dysmenorrhoea. The aim of study is Pharmaceutical and Analysis of *Shatapushpa Phala Churna* prepared and analysis was carried out through two major procedures incorporating physicochemical tests and advanced instrumental techniques TLC. *Shatapushpa Phala Churna* was evaluated for total ash, acid insoluble ash, water soluble ash, water soluble extractives, alcohol soluble extractive, moisture content, pH value of and total percentage of foreign matter. Sample of *Shatapushpa Phala Churna* was evaluated for presence or absence of various constituents like Carbohydrates, alkaloids, proteins, non reducing sugar, tannins, flavanoides, phenols, steroids, lipids, oils, anthocyanins, glycosides and saponin etc.

**Keywords-** *Shatapushpa Phala Churna*, Carbohydrates, alkaloids, proteins

### INTRODUCTION

*Shatapushpa* bearing qualities like *Ushnavirya, Katuvipaka, Kaphavatashamaka, Pittavardhaka, Katu, Tikta Rasa, Laghu, Snigdha, Teekshana Guna, Vajikara*<sup>1</sup>, *Raktagulmanashaka Agnideepaka, Stanyavardhaka, Rochaka, Atisaranashaka*<sup>2</sup>, *Jwaraghana*<sup>3</sup>, *Netrya, Vranaghna, Medhya, Shoolaghna*<sup>4</sup>, *Dahashamaka, Trishnashamaka, Vamananashaka, Arshoghna*<sup>5</sup>, *Aamatisaranashaka, Yonishoolaghna*<sup>6</sup> and also indicated in *Shukrado-sha*<sup>7</sup>, *Raktagulma, Agnimandya, Stanyakshaya, Aruchi, Atisara*<sup>8</sup>, *Jwara*<sup>9</sup>, *Netra Roga*<sup>10</sup>, *Vrana, Smritinasha, Shoola*<sup>11</sup>, *Daha, Trishna, Vamana, Arsha, Aamatisara, Yonishoola*<sup>12</sup>. 100 gm of Dill weeds powder has energy value of 180 KJ (43 kcal), carbohydrate 7 g, dietary fiber 2.1 g, fat 1.1 g, protein 3.5 g, Vitamin A- 7717(154 %) IU, Thiamine (B<sub>1</sub>) 0.1 mg (9%), Riboflavin (B<sub>2</sub>) 0.3 mg (25%), Nia-

cin B<sub>3</sub> 1.6 mg (11%), Pantothenic acid B<sub>5</sub> 0.4mg (8%), Vitamin C 85 mg (102%), Minerals- Ca (21%) 208 mg, Iron (51%) 6.6 mg, Magnesium (15%) 55mg, Manganese (62%) 1.3 mg, Phosphorus (9%) 66 mg, Potassium (16%) 738 mg, Sodium (4%) 61 mg, Zinc (9%) 0.9 mg and copper 0.14 mg (7%). The Analytical study on *Shatapushpa Phala Churna* showed total ash (43.5%w/w), acid insoluble ash (47.0%w/w), water soluble ash (20.0%w/w), water soluble extractives (38.8%w/w), alcohol soluble extractive (43.7%w/w), and moisture content (6.7%), pH of 10 % aqueous solution (6.5) and total percentage of foreign matter (0.92%). Carbohydrates, alkaloids, proteins, non reducing sugar, tannins, flavanoides, phenols, steroids, lipids and oils were present in sample of *Shatapushpa Phala Churna*. While anthocyanins, glyco-

sides, saponin were absent in sample of *Shatpushpa Phala Churna*

**Materials and methods-** The study was designed under the following heading,

- 1) Preparation of the drug.
- 2) Physicochemical analysis of *Shatpushpa Phala Churna*.

**1) Preparation of the drug-** *Shatpushpa* fruits was collected from the market. Before the collection, *Shatpushpa* fruits were properly identified with the help of experts.

#### **Drying and Powdering:**

The work was carried out at Pharmacy of Shri D.G.M Ayurvedic Medical College, Gadag. The drug was dried and subjected to powdering using pulverizer and 20 number sieves were used to get coarse powder of *Shatpushpa* fruits. Fine powder of *Shatpushpa* fruits was prepared by using 120 number sieves.

#### **Preservation of Sample:**

The Sample *Shatpushpa* (*Anethum sowa*) seeds powder was preserved in an air tight container

#### **Physicochemical Analysis<sup>13</sup> of *Shatpushpa Phala Churna*:**

**Place of work:** Physicochemical analyses of *Shatpushpa* fruits powder was carried out at Biogenics, Research and Training Center in Biotechnology, Hubli, Karnataka.

#### **a) Determination of Total ash:**

**Materials:** Silica crucible, Physical balance, Desiccators, Bunsen burner.

**Method:** Weight of the empty crucible was noted down. 2 grams of the sample was taken in the previously weighed crucible and was heated on a Bunsen burner until it turned into ash. It was then cooled in desiccators and weighed.

**% Ash = difference in weights X 100/ weight of the sample.**

#### **b) Determination of Acid insoluble ash:**

**Materials:** Digital balance, silica crucible, muffle furnace, desiccators, and total ash, ash less filter paper, funnel, electric Bunsen burner.

**Method:** 25ml of dilute hydrochloric acid was added to the total ash obtained and stirred well for 15 minutes. It was filtered through an ash less filter paper to separate the insoluble matter. The residue was washed twice with hot water. The filter paper with residue was put into pre-heated, weighed silica crucible. This was ignited in an electric Bunsen till the fumes cease to appear from the silica dish. Transferred to Muffle furnace and ignited for an half an hour at 450°C. Heating was continued till constant weight of the dish was obtained. The percentage of the acid insoluble ash of the crude drug with reference to the air dried sample of the crude drug calculated.

**Calculation:** Wt. of the residue = a gram (Acid insoluble ash). y g of the air-dried drug gives – a g of acid insoluble ash. Therefore 100gm of the air-dried drug gives  $100 \times \frac{a}{y}$  g of acid – insoluble ash. Acid insoluble ash value of the sample =  $100 \times \frac{a}{y}$  %.

#### **c) Determination of Water soluble ash:**

**Materials:** Total ash, digital balance, muffle furnace, desiccators, ash less filter paper, electric Bunsen, funnel, silica crucible.

**Method:** To the total ash obtained, 25ml of water was added and boiled for 5 minutes. It was filtered through an ash less filter paper to separate the insoluble matter. The residue along with filter paper was taken in a pre-heated, weighted silica dish. Transferred to muffle furnace and ignited for 15 minutes at the temperature not exceeding 450°C. The dish was cooled in a desiccators & weighed again.

Heating was continued till constant weight of the dish was obtained. The weight of the insoluble matter from the weight of the ash was subtracted. The percentage of water soluble ash with reference to the air dried drug was calculated

**d) Determination of moisture content:**

**Materials:** Powdered drug, digital balance, porcelain dish, desiccators, hot air oven.

**Method:** Accurately weighed 5g of the coarsely powdered drug was taken in a dried, weighed porcelain dish. Dish was kept in hot air oven at 105°C for five hours. Dish was taken out, cooled in desiccators and weighed. Drug was weighed at each one hour interval. Drying was continued till constant weight was obtained. Percentage of moisture content (loss on drying) with reference to the air dried drug was calculated.

**e) Determination of Foreign matter:**

**Materials:** Sand particle, piece of sticks, paper, lens.

**Method:** 2 g of fruits material spread on a thin layer of paper. To sort into different groups of foreign matters, it has to be examined by using a magnifying 10x and foreign matters are picked out and the percentage is recorded. Foreign matter = difference in weights X 100 / weight of the sample.

**f) Determination of Alcohol soluble extractive values:**

**Procedure:** Accurately weighed 5gms of drug was transferred to a dry 250ml iodine flask. The 100ml of alcohol (90%) was added to the iodine flask. The iodine flask was closed with a stopper. Frequent shaking of this mixture was done for 6 hrs. It is kept undisturbed for 18 hrs. After 24 hours it was filtered into a 50 ml cylinder. 25 ml of filtrate was transferred to a dry, weighed porcelain dish. It was evapo-

rated on a water bath and completely dried in oven at 100°C. It was cooled in desiccators and weighed. The percentage (w/w) of extractive value was calculated with reference to air dried

**Calculation:** 25ml of alcohol extraction gives – x of residue. 100ml of alcohol extract gives – 4x g of residue. 5g of air dried drug gives – 4x g of alcohol soluble residue. 100g of air dried drug gives – 80x g of alcohol soluble residue. Extractive value of the sample – 80x%

**Chemical investigations of Shatapushpa Phala Churna:**

Qualitative chemical tests were conducted for Shatapushpa to identify the various constituents. The various tests and reagent used are given below and observations are recorded.

**Equipment:** Test tube, holder, stand, spirit lamp, pipette, glass rods, beaker 50 ml to 250 ml, conical flask, water bath.

**Methods:**

**Test for Proteins:**

**A) Millon's test:** Millon's test is used to detect the presence of proteins. Millon's reagent is a solution of mercury nitrate and nitrous acid. This test is specific for testing the presence of tyrosine residues in protein. Tyrosine is an aromatic amino acid with a hydroxyl group. Millon's reagent reacts with the tyrosine residues and gives a red coloration to the solution. 2 ml of test solution was taken in a test tube, a few drops of Millon's reagent were added to test solution and which was then heated gently, a reddish brown coloration or precipitate indicates the presence of tyrosine residue which occurs in nearly all proteins.

**B) Biuret test:** 2 ml of 10% copper sulphate solution was added to 2 ml. of test solution, mixed well and 2 drops of 1% CuSO<sub>4</sub> solution was added. Violet or pink color indicates the

presence of two or more peptide bond of proteins.

**C) Ninhydrin test:** 1 ml. of 0.1% freshly prepared ninhydrin solution was added to 4 ml. of the test solution, which should be neutral pH. The contents were mixed and boiled for a minute and was allowed to cool. Violet or purple colored solution indicates the presence of amino acids and proteins.

**D) Xanthoproteic test:** 1 ml. of conc. HNO<sub>3</sub> was added to 5 ml. of the solution. The contents were boiled and cooled. Appearance of yellow color indicates the presence of nitro derivatives of aromatic-amino acids. To this solution 40% of NaOH was added. A deep orange color solution indicates the presence of sodium salts of nitro derivatives of aromatic amino acids.

**E) Hopkins-Cole test:** 2 ml of glacial acetic acid was added to 2 ml of the test solution and mixed well. To this 2 ml conc. H<sub>2</sub>SO<sub>4</sub> was added carefully along the sides of the test tube. The formation of violet ring in the junction of two liquids indicates the presence of indole group of tryptophan.

**F) Sulphur test:** Two ml of 40% NaOH solution and 10 drops of 2% lead acetate solution were added to the 2 ml of solution and the contents were boiled for a minute and cooled back. Precipitate indicates the presence of sulphur containing amino acids of proteins.

#### **Tests for Carbohydrates:**

**A) Molisch's test:** Two drops of Molisch's reagent was added along the sides of the test tube. At the junction of two liquids a red cum violet colored ring indicates the presence of carbohydrates.

**B) Bradford's test:** To 0.5 ml of test sample, 2ml of Bradford's reagent is added and blue color is observed.

**C) Fehling's test:** One ml of Fehling's solution "A" and one ml of Fehling's solution "B" were added to 1 ml of test solution. The contents were mixed well and boiled for a minute. Yellow or brownish-red precipitates indicate the presence of the reducing sugars.

**D) Benedict's test:** Two ml of Benedict's reagent was added to five drops of the test solution. Boiled for a minute in a water bath and cooled, yellow, red or green color precipitate indicates the presence of reducing sugars.

#### **Test for Non-reduction sugar such as sucrose:**

**A) Benedict's test:** Benedict's test showing no characteristic color formation indicates the presence of non-reducing sugars in the test solutions.

#### **Test for Tannins:**

**A) Ferric chloride test:** Test solution treated with few drops of ferric chloride solution gives dark color.

**B) Gelatin test:** The solution was evaporated to dryness and the residue was dissolved in gelatin 2%. To this, salt solution (10% NaCl) was added. A white precipitate was obtained which indicates the presence of tannins.

#### **Test for Anthocyanins:**

**A) Aqueous NaOH test:** 1 ml of aqueous NaOH solution was added to the 1 ml test solution, formation of blue to violet color indicates the presence of anthocyanins.

**B) Conc. H<sub>2</sub>SO<sub>4</sub> test:** 1 ml of Conc. H<sub>2</sub>SO<sub>4</sub> was added to the 1 ml test solution, formation of yellow to orange color indicates the presence of anthocyanins.

#### **Test for Glycosides:**

##### **A) Molisch's test:**

1 ml of Molisch's reagent and 1 ml of Conc. H<sub>2</sub>SO<sub>4</sub> was added to the test solution, forma-

tion of reddish violet color ring at the junction of the two liquids indicates the presence of glycosides.

**B) Conc. H<sub>2</sub>SO<sub>4</sub> test:**

1 ml of Conc. H<sub>2</sub>SO<sub>4</sub> solution was added to the 1 ml of solution and was allowed to stand for 2 minutes. Formation of reddish color indicates the presence of glycosides.

**C) Keller-kiliani test:** The test solution was dissolved in glacial acetic acid boiled for a minute and cooled. To this solution 2 drops of ferric chloride solution was added, the contents were transferred to a test tube containing 2 ml. of concentrated sulphuric acid. A reddish brown color ring was observed at the junction of two layers indicating the presence of glycosides.

**Test for Saponin:**

**A) Foam test:** Extract is shaken vigorously with distilled water in a test tube. Honey comb like foam produced, persists for few minutes. It confirms the presence of Saponin.

**B) Froth test-** Water extract is obtained by boiling on the water bath. The extract is transferred into a test tube and shaken vigorously then is left to stand for 10 minutes and the result is noted. A thick persistent froth indicates Saponin.

**Test for Flavanoides:**

**A) Shinoda test (Mg/HCl)** This test is applied in the same way as Zn/HCl, but magnesium powder was used instead of Zinc. The development of a deep-red or magenta color of the solution is an indication for the presence of flavone dihydroflavanol. Dihydrochalcones and other flavanoides do not react with the reagent (Dey and Harborne, 1989).

**B) Aqueous NaOH solution-** 1 ml of aqueous NaOH solution was added to the 1 ml test solution. Formation of yellow color indicates the presence of flavanoides.

**C) Conc. H<sub>2</sub>SO<sub>4</sub> test-** 1 ml of Conc. H<sub>2</sub>SO<sub>4</sub> was added to 1 ml test solution, formation of red color indicates the presence of flavanoides.

**Test for Phenols:**

**A) Phenol test-** When 0.5 ml of FeCl<sub>3</sub> solution was added to 2 ml of test solution, formation of an intense color indicates the presence of phenols.

**Test for Steroids:**

**A) Salkowski test:** Wine red color was developed when conc. H<sub>2</sub>SO<sub>4</sub> was added to the test solution, it indicates the presence of Steroidal nuclei.

**B) Liberman and Burchard test:** The blue-green color was developed in the test solution when treated with 50% H<sub>2</sub>SO<sub>4</sub> and acetic anhydride to the test solution indicates positive reaction for steroids.

**Test for Alkaloids:** The various extracts were mixed well with ammonia and then extracted with chloroform; the chloroform solution is then extracted with 0.1N HCl and filtered. The filtrate was used for further test.

**A) Mayer's test:** The filtrate when mixed with few drops of Meyer's reagent gives creamy white precipitate.

**B) Wagner's test:** The filtrate when mixed with few drops of Wagner's reagent gives reddish brown colored precipitate.

**C) Dragendroff's test:** The filtrate when added with few drops of Dragendroff's reagent gives orange red color.

**Tests for fixed oils and fats:**

A small quantity of petroleum ether and chloroform extracts was pressed separately between two filter papers. Appearance of oil stain on the paper indicates the presence of oils and fats.

### Test for triterpenoid:

Ten mg of the extract was dissolved in 1 ml of chloroform; 1 ml of acetic anhydride was added following the addition of 2 ml of Conc.H<sub>2</sub>SO<sub>4</sub>. Formation of reddish violet color indicates the presence of triterpenoids.

### Identification by T.L.C

**Drug:** Extraction of sample (Pet. ether) which is treated with 1:10 ml solute; solvent like ethyl alcohol with dilution method.

**Equipment:** Silica gel, TLC kit, hot air oven, standard glass, watt man glass plate, beakers, sprayer.

**Chemicals:** Dragendroff's reagent, Silica gel, ethyl alcohol.

### Method:

T.L.C. of petroleum ether extract of the sample was carried out as follows.

The silica gel powder mixed with water and made thin slurry, and then with the help of glass slide, the silica gel was spread on glass plates uniformly. After some times the air dried plate were kept in a hot oven at 110-1200o C. The samples were loaded on one end of the plate with the help of capillary tubes, leaving 5 cm from the edge. The spots were carefully done without allowing them to spread. The spots were air dried and the spot-

ted plate was gently immersed in pre saturated closed, TLC chamber for development. The development was stopped when solvent front reached to 3/4th of the plate. The plate was then removed from TLC chamber and the solvent front was immediately marked with a pencil line. Then the plate was air dried and observed under UV trans- illuminator to note the fluorescing spots. Then Dragendroff's solution is sprayed on the plates.

Rf value of the spots were found out by using the formula,

$$R_f = \frac{\text{Distance traveled by the Solute}}{\text{Distance traveled by the solvent}}$$

## RESULTS & DISCUSSION

### Physicochemical Analysis

The results of the study are grouped as follows.

1. Results pertaining to analysis of *Shatpushpa* for physical constants.
2. Results pertaining to chemical analysis of *Shatpushpa*.
3. Observation of Thin layer chromatography (TLC)

### Results pertaining to analysis of *Shatpushpa* for physical constants

**Table 1: Physical constants of *Shatpushpa* fruits powder**

S. No.	Parameters	Results %
1.	Total Ash (% w/w)	43.5
2.	Water soluble ash	20.0
3.	Acid-insoluble ash (% w/w)	47.0
4.	Water-soluble extractive (% w/w)	38.8
5.	Alcohol-soluble extractive (% w/w)	43.7
6.	Moisture Content	6.7
7.	pH (10 % aqueous solution)	6.5
8	Total % of Foreign matter	0.92

**Table 2: Results of chemical analysis of powder of *Shatapushpa* fruits:**

**Carbohydrates test**

Sl. No	Test	Observation
a) Molisch's test	Purple violet ring at the junction of two layers	Presence of carbohydrates
b) Fehling's test	Orange red precipitates	Presence of carbohydrates
c) Bradford test	Yellow, red or Green precipitates	Presence of carbohydrates
d) Benedict's test	Green color	Presence of carbohydrates

**Test for Alkaloids**

a) Mayer's test	Creamy white precipitates	Presence of Alkaloids
b) Wagner's test	Reddish brown color	Presence of Alkaloids
c) Dragendorff's test	Orange red color	Presence of Alkaloids

**Test for protein**

a) Biuret test	Blue color observed	Presence of proteins
b) Ninhydrin test	Violet or purple color solution	Presence of proteins
c) Xanthoproteic test	Yellow precipitates	Presence of proteins
d) Hopkins test	Violet ring at junction of two liquids	Presence of proteins
e) Bradford test	Blue color observed	Presence of proteins
f) Sulphur test	Presence of precipitates	Presence of proteins

**Test for Non reducing sugars such as sucrose**

a) Benedicts test	No characteristic color formation	Presence of non-reducing sugar
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**Test for Anthocyanins**

a) Aqueous NaOH test	No Blue to violet color	Absence of anthocyanins
b) Conc. H <sub>2</sub> SO <sub>4</sub>	No formation of Yellow to orange color	Absence of anthocyanins

**Test for Tannin**

a) Ferric chloride test	Blue, Black, Dark color observed	Presence of tannin
b) Gelatin test	White precipitates	Presence of tannin

**Test for Glycosides**

a) Molisch's test	No reddish violet ring at the junction of two layers	Absence of glycosides
b) Con H <sub>2</sub> SO <sub>4</sub>	No reddish color	Absence of glycosides

**Test for Flavanoides**

a) Shinoda test	Deep red color formation	Presence of Flavanoides
b) Aqueous NaOH	Formation of yellow color	Presence of Flavanoides
c) Conc. H <sub>2</sub> SO <sub>4</sub> test	Red color formation	Presence of Flavanoides

**Test for phenols**

a) Phenol test	Formation of intense color	Presence of phenols
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<b>Test for saponin</b>		
a) Foam test	No honey comb like foam seen	Absence of saponin
b) Froth test	No honey comb like froth seen	Absence of Saponin
<b>Test for steroids</b>		
a) Liberman Burchard test	Blue green color formation	Steroids present
b) Salkowski test	Wine red color formation	Steroids present
<b>Test for lipids</b>	Original iodine color disappears	Lipids present
<b>Test for oils</b>	Clear greasy spot was observed	Oils present

**Result of TLC:**

Sample – Ethanol

Mobile Phase – Chloroform: Methanol (80:20)

Stationary Phase – TLC Plates Prepared with silica gel

Spraying agents – Dragendroff’s reagent

**Table 3: Rf values in TLC of ethanol extracts of powder of *Shatapushpa* fruits**

Extract	Rf values of bands at 365 nm	Rf values of bands at 365 nm with spraying reagent
Ethanol	0.93	0.93

**CONCLUSION**

Sample of *Shatapushpa Phala Churna* showed total ash (43.5%w/w), acid insoluble ash (47.0%w/w), water soluble ash (20.0%w/w), water soluble extractives(38.8%w/w), Alcohol soluble extractive(43.7%w/w) Moisture content(6.7%), pH(10 % aqueous solution) 6.5 and total percentage of foreign matter(0.92%). Carbohydrates, alkaloids, proteins, non reducing sugar, tannins, flavanoides, phenols, steroids, lipids and oils were present in sample of *Shatapushpa Phala Churna*. While anthocyanins, glycosides, saponin were absent in sample of *Shatapushpa Phala Churna*.

Dill is a plant with wide range of chemical constituents with many pharmacological effects and not only in gynecological disorders. It delays the process of aging because of flavanoides, it is very rich in vitamins and minerals like Ca, K, Na which have effect on memory and brain development, Vit-A, niacin, Mg, P are responsible for goodness of skin, eye, hair, bones.

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#### **CORRESPONDING AUTHOR**

**Dr. Nidhi Garg**

Asst. Prof., Agad Tantra Dept, Ch. Devi Lal Ayu. College

**Email:** [nidhi.garg9795@gmail.com](mailto:nidhi.garg9795@gmail.com)

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