

**Pharmacognostic & Phyto-chemical study of *Kushmand* fruit
(*Benincasa hispida* Linn.)**

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Abstract:-

Kushmand (fruit of *Benincasa hispida* Linn.) is one of the easily available medicine mentioned as Antiepileptic in Ayurvedic literature. Pharmacognostic and phytochemical studies of *Kushmand fruit* has not been done yet, hence this study was undertaken to find out pharmacognostic nature and phytoconstitute of *Kushmand fruit*. Phytochemical study showed presence of carbohydrates, Steroides, alkaloides. protiens, starch present in swarasa but absent the tannins. carbohydrates, Steroides, alkaloides starch present in aqueous extract but absent the tannins and proteins. Carbohydrates, alkaloids and protein in alcohol extract but absent the Steroids, tanines and starch. Pentose sugars (Bials orchinal Test) absent in all samples. In TLC, the Rf values of 0.02,0.03,0.06 were recorded for Aqueous extract and 0.05,0.19,0.37 for Alcoholic extract. In HTLC, the Rf values of Phyto-

constituents present in Aqueous extract are 0.11, 0.14, 0.61, 0.73, 0.81 while the Rf of Phyto- constituents present in Alcoholic extracts are: 0.14, 0.74 @ 254 nm. Rf values of Phyto-constituents present in Aqueous extract are 0.04, 0.09: while the Rf of Phyto-constituents present in Alcoholic extracts are: 0.04, 0.07, 0.09, 0.77 @ 366 nm. Rf values of Phyto-constituents present in Aqueous extract are 0.05,0.09,0.28,0.31,0.39,0.64,0.90; while the Rf of Phyto-constituents presen in Alcoholic extracts are: 0.04,0.07,0.14,0.31,0.38,0.45,0.54,0.71,0.80,0.90 @ 580 nm. after derivatization.

Keywords:- *Kushmand fruit*, Pharmacognostic & Phyto-chemical study

Introduction:-

Nature has been a source of medicinal agents for thousands of years. The widespread use of herbal remedies

and healthcare preparations has been described in ancient texts like the Vedas and the Bible. In fact, plants produce a diverse array of bioactive molecules, making them a rich source of diverse type of medicines ⁽¹⁾. Thus, natural products with pharmacological or biological activities still play a very important role in medicine ^(2,3). World Health Organization (WHO) described plant as a plant with one or more organs which contain substances that can be used for therapeutic purposes or which are precursors for the synthesis of useful drugs ⁽⁴⁾.

Benincasa hispida (Thunb) COGN. is employed traditionally to treat disorders such as dry-cough, fever, urethral discharges, biliousness, thirst. It acts as brain tonic and also possesses anti-helminthic property. In China, it is used in the treatment of appendicitis. Oil from seeds is soporific, good for the brain and liver and effective in the treatment of syphilis. (Nadkarni's Indian Materia Medica, 1995; Kirtikar and Basu, 1985).

Pharmacognostic and phytochemical studies of *Kushmand fruit* has not been done yet, hence this study was undertaken to find out pharmacognostic nature and phytoconstitute of *Kushmand fruit*. Hence by considering its therapeutic importance and pharmaceutical application in various Karma. It is essential to understand Pharmacognostical and Physico-chemical property of *Kushmand fruit*.

Aims and Objectives: Pharmacognostical study and Physico-chemical analysis of *Kushmand*.

Place of work :

All pharmacognostic and phytochemical study is carried in *Dravyaguna* lab and Q.C. lab of Shri B.M.K. Ayurveda Mahavidyalaya, Belgaum.

Materials & Methods:

Pharmacognostical Evaluation:

Raw drugs were identified and authenticated by the Pharmacognosy department. The identification was carried out based on the morphological features, organoleptic features and powder microscopy of the individual drugs. The microphotographs were also taken under the microscope.

2. Macroscopic Study

The macroscopic study refers to the physical evaluation of the drug in terms of size, shape, surface, fracture, etc. The sample was subjected to the macroscopic study with the help of simple microscope and magnifying glass.

3. Method of microscopic examination of fruit:

This method allows more detailed examination of a drug in relation to their histological characters.

FRUIT MICROSCOPY ⁽⁵⁾ :

Mature fruit shows cuticularised epicarp consisting of single layered, squarish or slightly tangentially elongated cells of epidermis, outer tangential walls of epidermis

thickened and cuticularised; a few epidermal cells divide periclinally and become 2 or 3

layered; mesocarp has a heterogeneous structure consisting of multilayered hypodermis

composed of tangentially elongated, thin-walled, parenchymatous cells; immediately

within this is a zone of thick-walled, multilayered, lignified sclereids with the outer one

to three layers thicker than the inner 2 to 6 or more layers; beneath this zone, thin walled tangentially elongated, parenchymatous cells present, their size gradually increasing from those at periphery to those inside of mesocarp, the latter becoming circular having conspicuous intercellular spaces; vascular bundles poorly developed, bi-collateral, found scattered throughout mesocarp.

4. Methods for powder analysis

Powder preparation-

POWDER MICROSCOPY ⁽⁵⁾:

Dirty brown; shows numerous fragments of thin-walled, tangentially elongated and circular parenchymatous cells, numerous sclereids in groups and singles and a few fragments of xylem vessels having spiral thickenings.

5. Methods for determination of foreign matter. Weight 100-500 gm of the drug sample to be examined or minimum quantity prescribed in the monograph, and spread it out in a thin layer. The foreign matter should be detected by inspection with the unaided eye or by the use of lens 6x: separate and weigh it and calculate the percentage.

PHYSICO-CHEMICAL STUDY ⁽⁵⁾:

In physical methods quantitative standards like total ash, acid insoluble

Ash, alcohol soluble extract, water-soluble extract, Acid value, etc. are determined.

These are determined by following procedures

7) Preliminary phytochemical scereening ⁽⁶⁾:

Qualitative chemical tests were conducted for Swarasa, aqueous and alcohol extracts of *Kushmanda (Benincasa hispida* Thumb) to identify the various phyto constituents. The various tests and reagent used are given below and observations are recorded.

1. TEST FOR REDUCING SUGARS -Benedict's test
2. TEST FOR MONOSACCHARIDES - Barfoed's test
3. TEST FOR PENTOSE SUGARS - Bial's Orcinol test
4. TEST FOR HEXO SUGARS- Selwinoff's test
5. TEST FOR PROTEINS- Biuret Test (General Test)
6. TESTS FOR TANNINS AND PHENOLIC COMPOUNDS:

- a. To 2 – 3 ml of aqueous or alcoholic extracts, add few drops of following reagents: 5% FeCl₃ solution deep blue-black colour.

7. TESTS FOR STEROIDS - Salkowski reaction
8. TEST FOR ALKALOIDS - Wagner's Test

TEST FOR INORGANIC ELEMENTS:

Tests for Inorganic Elements:

Test for Iron –To 5 ml test solution add few drops of 5% ammonium thiocyanate. solution turns blood red.

Test for Sulphate – To 5ml filtrate, add few drops of 5% BaCl₂ solution white crystalline BaSO₄ ppt. Appears that is insoluble in HCl.

Test for Chloride –To 3 ml test solution prepared in NH₄OH add few drops of 10% AgNO₃ soln. White precipitate. Of

AgCl₂ observes which is soluble in dilute ammonia solution.

Test for Sodium- To 10 ml filtrate add 2 ml of potassium pyroanthlollate gives white precipitate.

Test for Calcium- Filtrate with solution of ammonium carbonate gives white precipitate which is insoluble in ammonium chloride solution.

Test for Potassium- To 2-3 ml test solution, add few drops of sodium cobalt nitrite solution. Yellow precipitate of potassium cobalt nitrite observed.

Test for Magnesium- Filtrate with ammonium carbonate solution gives white precipitate but not with ammonium chloride solution.

T.L.C is one of the most widely used techniques for rapid identification of drugs and its formulations. It is equally applicable to the drugs as raw material state and pure state.

Chromatographic conditions:

The samples were subjected for thin layer chromatography as follows,

PREPARATION OF TLC:

SAMPLE PREPARATION: 2grams of *Benincasa hispida* Thumb coarse powder was macerated with 25ml of Alcohol for 1 hour filtered and made up to volume of 50ml and used.

Plate/Stationary phase-silica gel G

Solvent front run up to 15 cms

TABLE NO-1

PARTICULARS	Powder	Swarasa	Aqueous extract	Alcohol extract
Colour	Blackish	Whitish	Reddish brown	Yellow
Smell	Not distinct	Characteristic Odor	Fragrant	Fragrant
Taste	Not distinct	Sweet,	Sweet	Sweet
Touch	Rough	Sticky or Viscous	Moderately thick	Moderately thick

Sample loading – Capillary

Solvent system (Mobile phase) –

Toluene : Ethyl acetate (9 : 1)

SCANNING AND DOCUMENTATION

The H.P.T.L.C. equipments are supplied with computer and data recording and storing devices. The development of H.P.T.L.C. plates scanned at selected UV regions wavelength by the instruments and the detected spots are seen on computers in the form of peaks. The scanner converts bond into peak and peak heights or area is related to the concentration of the substance on the spot. The peak heights and the area under the spot are measured by the instrument and are recorded as percent on the printer.

Place of work:

Physico- Chemical Studies and Preliminary Phytochemical investigation of Swarasa and extracts of *Kushmand* is carried out in the Quality control Laboratory KLE'S Shri B.M.K.Ayurved College Belgaum. Thin layer Chromatography was carried out in Quality control Laboratory KLE'S Shri B.M.K.Ayurved College Belgaum.

OBSERVATION AND RESULTS:

ORGANOLEPTIC RESULTS

PHYSICO-CHEMICAL ANALYSIS
TABLE NO –2

Sl.No	Name of the Taste	API Standards	Obtained Values
1	Foreign Matter	Not more than 1	Nil
2	Total ash value	Not more than 12	7.0582 %
3	Acid Insoluble ash value	Not more than 1	0.9368 %
4	Water Insoluble ash	-	2.2211 %
5	Sulphated ash	-	8.7912 %
6	Total % of moisture	-	13.875 %
7	Water soluble extract	Not less than 24	56.417 %
8	Alcohol soluble extract	Not less than 10	34.3538 %

TABLE NO –3

Sl.No	Name of the Taste	Values
1	pH of aqueous ext.	4.5
2	pH of Swarasa	4.95
3	Specific gravity of aqueous ext	1.0107
4	Specific gravity of Swarasa	1.0105
5	Acid soluble ash	6.121 %
6	Water soluble ash	4.8374 %

PRELEMINARY PHYTOCHEMICAL SCREENING
TABLE N0-4

TEST FOR	Swarasa	Aqueous extract	Alcohol extract
Reducing test (Benedict's Test)	Present	Present	Present
Monosaccharide's (barfoeds test)	Present	Present	Present
Pentose sugars (Bials orchinal Test)	Absent	Absent	Absent
Hexosugars (Selwinoffs test)	Present	Present	Absent
Steroids (Salkowski reagent)	Present	Present	Absent
Alkaloids (Wagner's Reagent)	Present	Present	Present

Tannins (FeCl₃ (5%)	Absent	Absent	Absent
Protiens (Millions test)	Present	Absent	Present
Starch (Tannic acid for starch)	Present	Present	Absent

TEST FOR INORGANIC COMPONENTS: TABLE NO-5

Sl.No.	Test	Test drug
01	Test for Iron: a) Test soln. + Ammonium thiocyanate	Present
02	Test for Sodium: a) Test soln. + Potassium ferrocynaide	Present
03	Test for Chlorides: a) Test soln. + Silver nitrate	Present
04	Test for Calcium: a) Test soln. + Ammonia + Potassium ferrocynide	Present
05	Test for Potassium: a) Test soln. + Sodium cobalt nitrate	Present
06	Test for Sulphate: a) Test soln. + 5 % barium Chloride	Absent
07	Test for Magnesium: a) Test soln. + ammonium carbonate	Present

FLUORESCENCE ANALYSIS UNDER U.V. LIGHT: TABLE NO-6

Sr. No.	Sample	Fluorescent
01	Aqueous extract	Slight Black greenish
02	Alcohol extract	Greenish

TLC PROFILE: TABLE NO: 7

Sr. No.	SAMPLE	Rf VALUES
01	Aqueous extract	0.02, 0.03,0.06
02	Alcohol extract	0.05,0.19,0.37

HPTLC ANALYSIS PROFILE

All Samples @254nm

TABLE NO:8

Sr. No.	SAMPLE	Rf VALUES
01	Aqueous extract	0.11, 0.14, 0.61, 0.73, 0.81
02	Alcohol extract	0.14, 0.74

HPTLC ANALYSIS PROFILE

All Samples @366nm

TABLE NO:9

Sr. No.	SAMPLE	Rf VALUES
01	Aqueous extract	0.04, 0.09
02	Alcohol extract	0.04, 0.07, 0.09, 0.77

HPTLC ANALYSIS PROFILE

All Samples @580nm

TABLE NO:10

Sl No	SAMPLE	Rf VALUES
01	Aqueous extract	0.05,0.09,0.28,0.31,0.39,0.64,0.90
02	Alcohol extract	0.04,0.07,0.14,0.31,0.38,0.45,0.54,0.71,0.80,0.90

Discussion:

Collection of *Kushmanda* fruit was done from natural habitat. As the fruit of *Kushmanda* are personally collected directly from natural habitat, thus no foreign matter was found.(physical impurity) like mud, sand, grass etc. Fruit micro and macroscopic descriptions were as per Ayurvedic Pharmacopeia of India. The all parameters as moisture content (13.875%), total ash (7.0582%), acid soluble (6.121%) and insoluble ash (0.9368%), water soluble (4.8374 %) and insoluble ash (2.2211) within API standards which shows standard quality of the drug. **The aqueous & alcohol extractive values** were 56.417%, 34.353%, respectively. It shows more ingredients extracted in the water as compared to alcohol. **pH values** swrasa and aqueous extract are 4.95 and 4.5 respectively, it shows sample is more acidic in nature. Anurasa of *Kushmanda* fruit is also mentioned amla in classics.

1. Preliminary Phyto-chemical tests showed presence of carbohydrates, Steroides, alkaloides. protiens, starch present in swarasa but absent the tannins.

2.Preliminary Phyto-chemical tests showed presence of carbohydrates, Steroides, alkaloides starch present in Aqueous extract but absent the taanins and proteins. 3.Preliminary Phyto-chemical tests showed presence of carbohydrates, alkaloides and protein in alcohole extract but absent the Steroids, tanninas and starch.4. Pentose sugars (Bials orchinal Test) absent in all samples. In Thin Layer Chromatography in which Alcoholic extract Sample shows the Rf values 0.05, 0.19, 0.37 and aqueous extract sample show the Rf values 0.02, 0.03, 0.06 it indicates the same phytochemical is separated. •

HPTLC result: Rf values of Phyto-constituents present in Aqueous extract are 0.11, 0.14, 0.61, 0.73, 0.81 while the Rf of Phyto-constituents present in Alcoholic extracts are: 0.14, 0.74@ 254 nm. Rf values of Phyto-constituents present in Aqueous extract are 0.04, 0.09: while the Rf of Phyto-constituents present in Alcoholic extracts are: 0.04, 0.07, 0.09, 0.77 @ 366 nm. Rf values of Phyto-constituents present in Aqueous extract are 0.05,0.09,0.28,0.31,0.39,0.64,0.90; while

the Rf of Phyto-constituents present in Alcoholic extracts are: 0.04,0.07,0.14,0.31,0.38,0.45,0.54,0.71,0.80,0.90 @ 580 nm. after derivatization. It is observed that, Rf values of the phyto-constituents present in Alcoholic extracts are similar to those of phyto-constituents present in Aqueous extract. But some extra phyto-constituents are found to be present in aqueous extract.

Conclusions:

- ✓ After subjecting the *Kushmand* fruit through Pharmacognostic and phytochemical investigation as per Standard Operative Procedures (S.O.P) and after conducting Anti-epileptic study following Conclusions are drawn.
- ✓ Most of the phytochemical constituents are soluble in water; thus preferred formulation of *Kushmunda* should be of water base—like Swarasa, Aqueous extract etc.

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