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PHYSICOCHEMICAL AND PHYTOCHEMICAL EVALUATION OF *DURVA* (*CYNODON DACTYLON* LINN.)

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ABSTRACT
We find many references in Ayurveda regarding Durva in management of epistaxis, hematuria, scabies, fever, burning sensation, chronic diarrhea, dysentery, anasarca, dropsy catarrhal opthalmia, dysuria, bleeding piles, eye infection, epilepsy, hysteria, insanity[1] bleeding, wound healing and other purposes. It is used as ingredient of several important preparations like, *Durvadi tailam, Durvadi ghrutam, manaasmitravatakam*. Standardization of herbs is essential to assess the quality of drugs. This study reports on physicochemical standardization of *durva. Durva churna* was prepared by proper drying. Its methanol extract, ethanol extract, chloroform extract prepared by soxhlet technique and water extract prepared by cold maceration technique. It standardized on the basis of organoleptic characters, physical characteristics, Physico-chemical, properties and thin layer chromatography (TLC) methods. Foreign matter nil, Total Ash 8.287% w/w, Acid insoluble Ash 4.466% w/w, Water Soluble extract 15.596% w/w, Alcohol Soluble Extractive 11.04% w/w, Methanol Soluble Extractive 12.828% w/w, chloroform Soluble Extractive 3.740% w/w. Thin Layer chromatographic analysis (TLC) showed 3 and 6 picks at 254nm and 366nm respectively. This study was aimed at analytical parameters of *Cynodon dactylon.* These parameters help in standardizing the durva and give us an idea of photochemistry of durva according to extracts.

KEYWORDS: *Durva*, extract, *physicochemical.*

INTRODUCTION
We find many references in Ayurveda regarding Durva in management of epistaxis, hematuria, scabies, fever, burning sensation, chronic diarrhea, dysentery, anasarca, dropsy catarrhal opthalmia, dysuria, bleeding piles, eye infection, epilepsy, hysteria, and insanity1. It has different synonyms such as *Sitalata, Golomi*[2], *Ananta, Bhargavi, Sahasraveerya, Ruha, Shatavirya, Lohadravani, Ananta, Shataparva, Gndira, Shishira, rochani Shataparva, Bhargavi, Vijaya, Mangalya Shataparvika*[3], *Shadwala, Durmata*[4], *Shitakari Golomi*. Botanical Name of *durva* is *Cynodon dactylon* Linn. and English name is Bermuda grass. Its Family is Poeceae.

Morphologically its blades are a grey green colour and are short; usually 4-15cm long with rough edges with long stem, slightly flattened, and often tinged purple in colour. The seed heads are produced in a cluster of 3 to 7 spikes together at the top of the stem. It has a deep root system. In drought situations with penetrable soil, the root system can grow to over 2m deep, though most of the root mass is less than 60 cm under the surface. The grass creeps along the ground and root wherever a node touches the ground, forming a dense mat. It reproduces through seeds, through runners and rhizomes.

There are three varieties (*Durvatrayam*) namely *Niladurva* with bluish or greenishstems. *Swetadurva* with whitish stem and branches and *Ganda durva* with nodulosestems are mentioned. It is used as ingredient of several important preparationslike, *Durvadi tailam*[5], *Durvadi ghrutam*[6], *manaasmitravatakam.*[7]

According to WHO norms, botanical Standards are proposed as a protocol for the analysis of the herbal drug. The present study deals analytical study of durvaby incorporatingbasic concepts of Ayurveda and modern pharmaceutical techniques. The Phytochemicalstudies of drugs done by makinguse of various parameters inauthentication andstandardizing the drug.

MATERIALS AND METHODS

Analytical study was carried out in AYUSH Approved drug testing laboratory CRF, KAHER's Shri B.M.K Ayurved Mahavidyalaya, Shahpur, and Belagavi.

Collection of drug

The raw drug *Durva* was collected from natural source in and around Belagaviregion. Identification and Authentication was done in AYUSH Approved drug testing laboratory CRF, KAHER's Shri B.M.K Ayurved Mahavidyalaya, Shahpur, Belgavi. The naturally collected durva was washed thoroughly with tap water and made free from mud and dust or any other foreign materials and dried on clean plastic sheet inroom. After proper drying durva was weighed (940grams) and stored in a labelled air tight plastic bag.

Preparationof durvachurna

The dried *panchanga of Durva* was made in to small pieces. These small pieces were pulverized and made in to coarse powder. The powdered *Durva churna* was sieved through Shifter using mesh no 40. The prepared *churna was* kept in air tight container. Quantity of raw material durva was 940gm .Quantity of *churna* was 846gms.

Preparation of extracts

Aqueous extracts of *durva coarse powder* was prepared with cold maceration technique and ethanol, methanol and chloroform extracts were prepared soxhlet extraction technique.

Organoleptic study

Appearance, colour, odour, taste, were noted through sensory organs. These characters are useful for having a primary idea about the quality of different formulations without using chemical tests.

Physico chemical Analysis[8]

Total Ash value :- 2 to 3 gram accurately weighed durva churna was taken in silica dish and incinerated at temperature not exceeding 450^0c until free from carbon and made it cool. Weight was taken and percentage of ash with reference to the air drug was calculated.

Acid insoluble ash - To the crucible containing total ash, 25 ml of dilute hydrochloric acid was added. The insoluble matter on an ashless filter paper (Whatman 41) was collected and washed with hot water until the filtrate became neutral. This filter paper kept to the crucible, desiccated on a hot-plate and ignited to steady weight. The residue was kept for cooling in suitable desiccators for 30 minutes and weight was taken without delay.

Test for inorganic components

Prepared ash of the durva was added with 50% of v/v HCl. The filtrate was then subjected to analyse the inorganic element such as calcium, magnesium and sodium etc.

Total liquid chromatography (TLC): TLC of alcoholic extract of durva was carried out as per the standard method with TLC Silica gel 60 F254 materials. The mobile phase selected was Toluene: Ethyl acetate: 9:1.

Phytochemical analysis[9]: - Phytochemical analysis of water extract, chloroform extract, ethanol extract and methanol extract of *duva* churna was done.

Test for carbohydrates**:** Molish's test**:** To 2-3 ml extract, few drops of alpha-naphthol solution in alcohol was added, shaked and added conc. H2SO4 from sides of the test tube.

Test for Reducing Sugars

a) Fehling's Test: 1 ml Fehling's A and 1 ml Fehling's B solutions was boiled for one minute. Equal volume of test solution was added and heated in boiling water bath for 5-10 min. First a yellow, then brick red precipitation as observed.

b) Benedict Test: Equal volume of Benedict's reagent and test solution in a test tube were mixed and heated in boiling water bath for 5 min. The solution appeared yellow depending on the amount of reducing sugar present in the test solution.

Test for Pentose Sugars: - a) Bial'sorcinol Test: To boiling Bial's reagent, few drops of test solution was added. Green colouration appeared.

Tests for Hexose Sugars: - Selwinoff's Test(for Ketohexose like fructose): 3 ml Selwinoff's reagent and 1 ml test solution was heated in boiling water bath for 1 to 2 minutes. The red colour was seen in aqueous & alcoholic soluble extract.

Test for Proteins:- Biuret Test: To 3 ml, T.S., 4% NaOH and few drops of 1% CuSO4 solution was added and violet colour appeared.

Test for Amino Acids :- Ninhydrin Test**:** 3 ml, T.S. heated in water bath and 3 drops 5% Ninhydrin solution was added, purple or bluish colour did not appear.

Test for alkaloids: - Dragendorff's test: To 2-3 ml test solution, few drops Dragendroff's reagent was added, orange brown ppt was formed. Wagner's Test**:** To 2-3 ml of filtrate, few drops of Wagner's reagent were added, the solution showed a reddish brown precipitate.

Test for steroids: Salkowski reaction**:** To 2 ml of extract, 2 ml of chloroform and 2 ml concentrated H2SO4 was added and Chloroform layer did not give any colour change.

Tests for glycosides: Free sugar content of the extract was determined and the extract was hydrolized with mineral acid (di.Hel/dil H2SO4). Again the total sugar content of the hydrolyzed extract was determined.

Tests for Saponin (Glycosides):-Foam test: the drug extract was shaked vigorously and water persistent foam observed.

Test for Tannins and Phenolic Compounds**:** To 2-3 ml of extract, few drops of Lead acetate solution were added it gave white ppt.

Test for Flavonoids: To a small quantity of residue, lead acetate solution was added. The yellow coloured precipitate was formed.

RESULTS

Organoleptic study

Table 1: Organoleptic study of durva churna.

Sl No.	Parameters	*Durva churna*
1	Appearance	Powder form
2	Colour	Yellowish Greenish
3	Odour	Characteristic
4	Taste	Bitter, Astringent

Physicochemical Analysis of durva Churna

The standard protocols available for various procedures were adopted.

Table 2: Shows results of Physicochemical Analysis of durva churna.

Sl. No.	Parameters	*Durva Churna*	API Standards
1	Foreign matter	Nil	Not more than(NMT) 2%
2	Loss on Drying at 110o C (%w/w)	0.12%	NMT 2%
3	Total Ash (%w/w)	8.287%	NMT 9%
4	Acid insoluble Ash (%w/w)	4.466%	NMT 4.5%
5	Water Soluble Extractive (%w/w)	15.596	NLT9.5%
6	Alcohol soluble extractive (%w/w)	11.04%	NLT 3%
8	Methanol soluble extractive (%w/w)	12.828%	-
9	Chloroform soluble extractive (%w/w)	3.740%	-

The results are tabulated in Table no 3

Table 3: Shows the inorganic components present in durva churna.

Sl. No.	Parameters	*Durva*
1	Calcium	Absent
2	Magnesium	Absent
3.	Sodium	Absent
4	Potassium	Absent
5	Iron	Present
6	Chloride	Present
7	Nitrate	Absent
8	Sulphate	Present
9	Carbonate	Present
10	Phosphate	Absent

Phytochemical Analysis of *durva churna*

Table 4: Phytochemical analysis of water extract, chloroform extract, ethanol extract and methanol extract of *duva* churna.

S.No	Parameters	Test	*Durva Churna*			
			Water extract	*Chloroform extract*	*Ethanol extract*	*Methanol extract*
1	Reducing sugar	Benedicts test	Present	Present	Present	Present
2	Monosaccharides	Barford's test	Absent	Absent	Absent	Absent
3	Protiens	Biuret's test	Present	Present	Present	Present
4	Amino acids	Ninhydrin Test	Present	Absent	Present	Absent
5	Steroids	Salkowski reaction test	Absent	Present	Present	Present
6	Cardiac Glycosides	Legal's test	Absent	Present	Present	Present
7	Flavonoids	Lead acetate sol. test	Present	Present	Present	present
8	Alkaloids	Dragon doffs test	Absent	Present	Present	Present
9	Tannins & Phenolic compounds	Lead acetate sol. Test	Absent	Present	Present	Present

TLC

Result of TLC of alcoholic extract of durva mentioned below in table 4.

Table 5: Illustrates Rf values of phytochemicals separated during TLC from alcoholic ext. of durvawith solvent system Toluene: Ethyl Acetate (9:1).

	Rf- Short wavelength (254nm)	Rf- Long wave length (366nm)
Durva Churna	0.12 0.65 0.75	0.04 0.12 0.52 0.58 0.68 0.78

DISCUSSION

Nowadays importance of herbal medicines increases day by day as it is being used for health promotion and therapy purposes. In various Ayurvedic formulations durva is used as ingredient. Detailed Analytical study of *durva was* decided to undertake to bring out the salient diagnostic features which would help in s standardization of the quality of durva and generated data can also be used for the development of herbal formulation.

The raw drug *durva* was collected from natural source in Belgaum. Identification and Authentication was done. The raw material was crushed in to small size or made in to coarse powder. According to API reference, the particle size of Churna should be 40 to 60 mesh size for extraction process. The size reduction of raw materials is to rupture the cell structure of the plant material, so that, the medicinal compositions present in the plant materials are exposed to the solvent and dissolved in it. If the raw drugs are in fine state or powder form it settled at the bottom of the soxlet apparatus and leads to blockage of chamber of apparatus and hampers the proper functioning of apparatus.

Prepared powder of durva was gray in colour. The Quantity of raw material was 940gm and Quantity of prepared c*hurna* was 846gms. The lost weight durva during churna preparation was 94 gram. Durva churna was yellowish green in colour, bitter and astringent in taste and had characteristic odour.

Results for physicochemical analysis were found within normal limits. In durva churna inorganic components Iron, sulphates and carbonate present. The results of water extract, methanol extract, ethanol extract and chloroform extract of durva are 15.596%, 12.828%, 11.04%, 3.740% respectively. In Photochemical analysis monosaccharide, steroids, cardiac glycosidesalkaloids, tannin and phenolic compounds and alkaloids and steroids present in chloroform extract, methanol extract, ethanol extract but absent in water extract. These chemical constituents having wound healing and antimicrobial activity.[10] So, methanol extract, ethanol extract and chloroform extract of durva churna were more potent than water extract.

TLC of *alcoholic extract of durva churna* showed 3 bands under UV light at 254 nm and 6 bands under UV light at 365nm in mobile phase toluene and ethyl acetate 9:1. The reduction in the number of bands can be due loss of thermo liable components after drying the *durva*.[11]

Analytical Reports

SHRI B.M.K. AYURVEDA MAHAVIDYALAYA

A constituent unit KLE Academy of Higher Education & Research
Deemed-to-be-University
Central Research Facility

DRUG AUTHENTICATION REPORT

Submitted By: Dr. Shivanarayan Prasad Gupta
Submitted Date : 10/10/2018

Date of Issue: 15/10/2018

SL NO	Sample Name	Scientific Name	Family	Part submitted	CRF Code	Authenticated as				
						Ayurvedic Name	Scientific Name	Family	Part Authentieated	Remarks
1.	Durva	*Cynodon dactylon* (L)Pers.	Poaceae	Whole part	CRF/Auth/ 2018/166	Durva	*Cynodon dactylon* (L)Pers.	Poaceae	Whole part	
2.	Nisha	*Curcuma longa* Linn	Zingiberaceae	*Rhizome*	CRF/Auth/ 2018/167	Nisha	*Curcuma longa* Linn	Zingiberaceae	*Rhizome*	

Signature:

Authentication Expert Name: Mr.Ajit Lingayat

Date:15/10/2018

CRF SHAHAPUR, BELGAUM

Signature of Coordinator
ASU Drug Testing Laboratory

SHRI B M KANKANAWADI AYURVED MAHAVIDYALAYA

A Constituent Unit of KLE ACADEMY OF HIGHER EDUCATION & RESEARCH (DEEMED-TO-BE-UNIVERSITY)

(Re-Accredited 'A' Grade by NAAC (2nd Cycle) || Placed under Category 'A' by MHRD Gol)

CENTRAL RESEARCH FACILITY

(AYUSH Approved ASU Drug Testing Laboratory Lic. No.TL-8/2011)

Reference No:CRF/RM/184/18-19 Registration Dt.: 25/10/2018

Submited by :KLE Ayurved Pharmacy Requstion no: -----

Sample : Durva Batch No. : NA Part/Form : Whole plant

Product : Plant Sample Qty : 50gm Report Date : 07/11/2018

(* N/A - Not Available)

TEST REPORT

Form-50 [See Rule 160-D (f)]

(The Drugs & Cosmetic Act 1940 and the rules there under)

Preliminary Phytochemical test

TESTS	METHANOL EXTRACT	CHLOROFORM EXTRACT
Test for Reducing sugar	Positive	Positive
Test for Monosaccharides	Negative	Negative
Test for Protiens	Positive	Positive
Test for Amino Acids	Negative	Negative
Test for Steroids	Positive	Positive
Test for Flavonoids	Positive	Positive
Test for Alkaloids	Positive	Positive
Test for Tannins	Positive	Positive
Test for Glycosides:		
A.Cardiac Glycosides	Positive	Positive

ANALYST

Shri B. M. Kankanwadi Ayurved Mahavidyalaya • K.L.E. University • CRF SHAHAPUR, BELGAUM

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CENTRAL RESEARCH FACILITY

(AYUSH Approved ASU Drug Testing Laboratory Lic. No.TL-8/2011)

Reference No:CRF/RM/184/18-19
Submited by :Dr. Shivanarayan Prasad Gupta
Sample : Durva
Product : PLANT
(* N/A - Not Available)

Batch No. : NA
Sample Qty : 50 gm

Registration Dt.: 25/10/2018
Requstion no: -----
Part/Form : Whole Plant
Report Date : 07/11/2018

TEST REPORT

Form-50 [See Rule 160-D (f)]

(The Drugs & Cosmetic Act 1940 and the rules there under)

TESTS	RESULTS
TLC (Alcoholic Extract)	**Rf Values**
Mobile Phase-Toluene : Ethyl acetate	Short Wave :0.12,0.65,0.75
Ratio - 9 : 1	Long Wave :0.04,0.12,0.52,0.58,0.68,0.78

ANALYST

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CENTRAL RESEARCH FACILITY

(AYUSH Approved ASU Drug Testing Laboratory Lic. No.TL-8/2011)

Reference No:CRF/RM/184/18-19
Submited by :Dr. Shivanarayan Prasad Gupta
Sample : Durva
Product : PLANT
(* N/A - Not Available in API)

Batch No. : NA
Sample Qty : 50 gm

Registration Dt.: 25/10/2018
Requstion no: -----
Part/Form : Whole Plant
Report Date : 07/11/2018

TEST REPORT

Form-50 [See Rule 160-D (f)]

(The Drugs & Cosmetic Act 1940 and the rules there under)

Description Macroscopic :

TESTS	LIMITS	RESULTS
PART	Whole plant	: Whole Plant
COLOUR	Yellowish green	: Yellowish green
TASTE	-	: Sweet mucilaginous (NA)
ODOUR	-	: Odourless (NA)

Physico Chemical Standards :

TESTS	LIMITS	RESULTS
Foreign Matter	Not more than 2%	:Nil
Ash Value	Not more than 9%	:8.287 %
Acid insoluble Ash	Not more than 4.5%	:4.466 %
Water soluble extractive	Not less than 9.5%	:15.596 %
Chloroform soluble extractive	-	:3.740%
Methanol soluble extractive	-	: 12.828%

(Standards reffered above are as per **API**)

* In my opinion the Sample is standard quality

ANALYST

CRF SHAHAPUR, BELGAUM

AUTHORISED SIGNATORY

Durva (*Cynodon dactylon* Linn.)

Durva (*Cynodon dactylon* Linn.) powder

Extraction of Durva (*Cynodon dactylon* Linn.)

Tests of *Durva* (*Cynodon dactylon* Linn.)

CONCLUSION

Durva was analysed by different analytical parameters. These parameters help in standardizing the durva and give us an idea of physicochemical and phytochemical evaluation of durva according to its extracts. Methanol, ethanol, chloroform extract of durva is more potent than aqueous extract of durva can be used for different formulations.

ACKNOWLEDGEMENT

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drug testing laboratory CRF, KAHER's Shri B.M.K Ayurveda Mahavidhyalaya, Shahpur, Belagavi.

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