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Pharmacognostical and Physico-chemical Standardization of Root of *Eranthemum roseum*. (Vahl) R.Br.

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ABSTRACT

The present paper deals with preliminary pharmacognostical standardization of a well-known folklore remedy for leucorrhoea and ulcer i.e. *Eranthemum roseum* (Vahl) R.Br. The standardization is carried out on the basis of Physico-chemical and Phytochemical studies including parameters such as fluorescence characteristics and analysis of inorganic constituents. The study contributes to the development of standardization parameters of herbal drugs used in Indian system of medicine.

KEY WORDS: *Eranthemum roseum*, Standardization, Pharmacognosy, Physico-Chemical

INTRODUCTION

India has a rich cultural heritage of traditional medicines which chiefly comprised the two widely flourishing systems of treatments i.e. Ayurvedic and Unani systems since ancient times. The crude drugs being always available easily in abundance,comparatively cheaper, with negligible side effects and have frequently been prescribed to patients of all age groups. The multiple therapeutic action and uses of these drugs are sufficiently described in classical literature on indigenous medicines in many medicinal plant books and pharmacopoeias (1-2).

Various species of Genus *Eranthemum* are being used traditionally for wide varieties of ethno medicinal purposes. The *Eranthemum roseum* (Vahl) R.Br. (Acanthaceae) commonly known as Gulsham or Dasamuli (3), is up to 2 m height and found in tropical and subtropical parts of Asia that is western and southern parts of India like Chatthisgarh, and Satpuda valley in Maharashtra. This shrub is cultivated in Indian gardens for its attractive foliage and flowers. Flowers are blue when fresh, rose colored afterwards and brown on drying. Ethno botanically, root of *Eranthemum roseum* boiled with milk is a popular remedy for leucorrhoea. Roots are also given to pregnant cattle to promote the foetus growth (4). Few ayurvedic manufacturing units have formulated the products by using this plant for body massage oil and as mother tonic. TANUMARDAN massage oil and MOTHERTONE tonic are typical commercial products available in Indian market.

A Literature survey and screening of scientific data revealed that a large number of indigenous drugs have already been investigated as regards their botany and chemistry is concerned, however a systematic standardization including Pharmacognostical and physico-chemical study is still lacking. The present investigation of *Eranthemum roseum* (vahl) R.Br.(Acanthaceae) is therefore taken up to establish certain botanical and chemical standards which would help in crude drug identification as well as in checking adulteration, if any. Further, the study will greatly help in quality assurance of finished products of herbal drugs.

MATERIALS AND METHODS

Roots of *Eranthemum roseum* were collected from Satpuda valley, Nandurbar district (Maharashtra). The plant was authenticated at Department of Botany, S.S.V.P.S's College of science, Dhule (Maharashtra).The voucher specimen was kept at department of Pharmacognosy. The roots of the plant was powdered and passed through 40 mesh size and stored in an airtight container for further use.

Macroscopy

Color: Yellowish brown, Odour:Aromatic, Taste: Slightly bitter, Texture: Smooth, slippery, Size: 5-30 cm length, 0.5-1.5 cm in thickness.Shape: Spindle shaped tuberous root with tapering end.

Microscopy

Transverse section of *Eranthemum roseum* root indicates the presence of stratified cork, cortex, and endodermis with small pith. Epiblema observed without cuticle and stomata. Multilayered cortex made

of thin walled parenchymatous cells along with abundant lignified stone cells and phloem fibers. Endodermis was well developed followed by single layered pericycle cells. 7 to 8 number of radial lignified vascular bundles were present towards the endodermis (5).

Physico-chemical standards

Physico-chemical parameters of the powdered drug such as ash value, extractive value, loss on drying and crude fiber content were performed according to the method (6-8). Extracts were prepared by various solvents by standard methods and percentage of dry extract was calculated in terms of air-dried root powder. (Table 1, 2, 3)

Fluorescence characteristics

When physical and chemical parameters are inadequate as it often happens with the Powdered drugs, the plant material may be identified from their adulterants on basis of fluorescence study (9-10). (Table 4)

Behaviour of root powder with different chemical reagents

Behaviour of root powder of *Eranthemum roseum* with different chemical reagents were performed to detect the occurrence of phytoconstituents along with color changes under ordinary daylight by standard method (11). (Table 5)

Metal analysis of the Ash

Root ash was prepared by taking 1.0 gm of sample and keeping in muffle furnace at 150 degree centigrade till constant weight was obtained. The major inorganic constituents of ash was determined quantitavely by Atomic absorption spectrophotometer (12). (Table 6)

Quantitative Standards

Total Carbohydrate content in roots of *Eranthemum roseum* by Phenol-Sulphuric acid method (13) was estimated to be 14.31%w/w. Similarly protein content & calorific value (14), was found to be, 0.741%w/w 3.34 k.cal/gm respectively.

(Table 7)

Determination of Saponin

According to the results obtained from positive foaming test and high foaming index (15) of roots of *Eranthemum roseum* further study was carried out for estimation of total saponin content (16-17) (Table 7) and its haemolytic activity (18-19). (Table 8 & fig 1).

Estimation of Total Sapogenin content in roots of *Eranthemum roseum* by acid hydrolysis(20).

Drug powder was extracted with petroleum ether (60-80^{0}C) by refluxing for half hour. Marc obtained, was again refluxed with 90 % v/v methanol for half an hour. The methanol extract was distilled off under reduced pressure to obtain semi-solid residue. The soft extract, left after distillation of methanol, was partitioned between distilled water and n-butanol (1:1).Aqueous and butanol fraction were separated and aqueous fraction was again partitioned with n-butanol 2-3 times. Combined n-butanol fraction was then evaporated under reduced pressure to yield semi-solid mass. For hydrolysis of saponin the semi-solid mass was then refluxed with 2N HCl for 8 hours. After cooling the contents were partitioned for 2-3 times with chloroform. Combined chloroform was evaporated and dried to constant weight and total saponin content was calculated.

Preliminary Phytochemical Investigation

The Qualitative chemical test of various extracts of *Eranthemum roseum* was carried out using standard procedure (21). Saponin glycoside, Carbohydrate and Protein was found to be present in aqueous and methanolic extracts and steroidal sapogenin were found to be in petroleum ether & chloroform extract.

HPTLC fingerprinting of different Extracts of *Eranthemum roseum* (22)

1) Butanolic extracts

10µl of 1mg/mL solution of butanolic extract in methanol was applied on the silica gel GF_{254} HPTLC Plates (10x10). Chloroform: Acetone: Formic Acid (9:2:1) was used as the mobile phase. After development the plates were scanned in ultraviolet range at 254 nm and 366nm and then the plates were derivatized by using 20% ethanolic sulphuric acid. After spraying four spots were observed at R_f 0.32, 0.49, 0.59 and 0.87.

2) Sapogenin extracts

10µl of 1mg/ml solution of sapogenin extract in acetone was applied on the silica gel GF_{254} HPTLC Plates (10X10).Chloroform: Methanol (9.4:0.6) was used as the mobile phase. After development the plates were scanned in ultraviolet range at 254 nm and 366nm. Six spots with R_f 0.27, 0.38, 0.51, 0.57,0.75 and 0.81 were observed under 366nm.

3) Petroleum ether extracts

10µl of 1mg/ml solution of petroleum ether extract in acetone was applied on the silica gel GF_{254} HPTLC Plates (10X10). Petroleum ether: ethylacetate: acetone (9:0.5:0.5) was used as the mobile phase. Six spots at R_f 0.27, 0.38, 0.51, 0.57, 0.75 and 0.81 were observed under visible after spraying the plates with 20% ethanolic acid reagent. (fig 2)

CONCLUSION

Table 1: Ash values

Sl. no.	Type of ash	Results
1.	Total ash	15.89% w/w
2.	Acid insoluble ash	0.94 % w/w
3.	Water soluble ash	2.15 % w/w

Table 2: Extractive values, Percentage yield and color of extracts

Solvent used	Percentage yield	Color of extract
Petroleum ether (60-80°C)	1.16	Yellow
Ethanol	14.18	Reddish brown
Water	24.68	Brownish black

Table 3: Loss on drying

Loss on Drying	8.72% w/w

Table 4: Fluorescence characteristics of powder root of Eranthemum roseum

Powder +Reagent	Color observed in Ordinary light	Color observed under Ultraviolet light	
		Short (254nm)	Long (365nm)
Powder	Brownsh black	Green	Green
Powder + 1N NaOH in methanol.	Green	Green	Green
Powder + 1N NaOH in water.	Brownish yellow	Green	Black.
Powder + 1 N HCl.	-	Green	Black
Powder + 50% HNO3.	Slight brownish yellow	Light green	Black.
Powder + 50 % H2SO4.	Slight brown	Green	Black.
Powder + Methanolic NaOH, dried + nitrocellulose in acetic acid.	Yellowish brown	Dark green	Black.
Powder +1N NaOH + nitrocellulose in acetic acid.	Dark brown	Light green	Greenish black
Powder + 1N HCl + nitrocellulose in acetic acid	Brownish Black	Light green	Dark green.

Table 5: Behaviour of root powder of Eranthemum roseum with different chemical reagents

Reagent	Color/ppt	Constituent
Powder as such	Yellow	-
Powder + conc.H_2SO_4	Reddish brown	Steroids present
Powder + Aqs. Ferric chloride	No Black color	Tannins absent
Powder + Iodine solution	No blue color	Starch absent
Powder + Aqs. Mercuric chloride	No brown color	Alkaloids absent
Powder + Picric acid	No change	Alkaloids absent
Powder + magnesium HCl	No change	Flavonoids absent
Powder + Aqs. Silver nitrate	Precipitates	Protein present
Powder + ammonia solution	No change	Anthraquinone glycosides absent
Powder + Aqs. KOH	No change	Anthraquinone glycosides absent

Table 6: Metal analysis of ash of roots of E. roseum using Atomic absorption spectrometry

S. No.	Parameter	Value in mg/kg	
		In ash	In Whole Plant
1.	Zinc	128.12	20.36
2.	Copper	60.94	9.68
3.	Magnesium	112.50	17.88
4.	Lead	39.06	6.21
5.	Iron	89.06	14.15
6.	Aluminum	2950.00	468.76
7.	Nickel	54.68	8.69
8.	Chromium	15.62	2.48

Table 7: Results of Quantitative estimations of roots of E. roseum

S. No.	Estimation		Results
1.	Foaming index		More than 1000
2.	Total Saponin content	Method I Method II	9.2% w/w 10.1% w/w
3.	Total Sapogenin content		2.40% w/w
4.	Total Carbohydrates		14.31% w/w
5.	Total Proteins		0.741% w/w
6.	Total Crude Fibers		5.50%w/w
7.	Calorific Value		3.34 k.cal/gm

Table No. 8: Results of Haemolytic activity of different extracts of roots of E. roseum

S. No.	Conc. µg/ml	% Haemolytic activity		
		Butanolic extract	Methanolic Extract	Aqueous Extract
1	1000	100.59±0.75	100.07±0.17	17.24±0.32
2	500	99.97±0.30	73.34±0.56	3.68±0.25
3	250	99.51±0.15	42.76±0.30	2.07±0.35
4	100	89.85±0.86	32.81±0.49	1.28±0.21
5	50	73.14±0.25	25.32±0.63	0.23±0.30
6	25	33.37±0.39	13.04±0.28	-0.56±0.32
7	10	8.44±0.39	10.25±0.50	-1.15±0.15
8	5	0.46±0.36	-0.19±0.66	-1.57±0.37
HD_{50}		39.32 µg/ml	153.09 µg/ml	Ns
Saline		7.91±0.150		
Distilled Water		100.03±0.496		

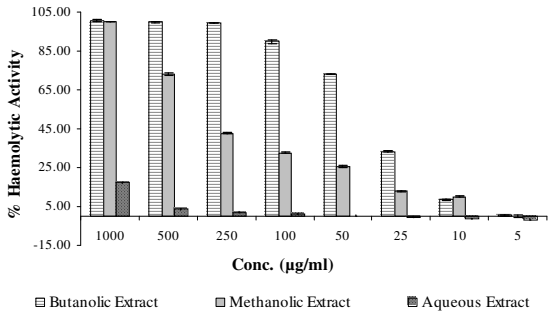
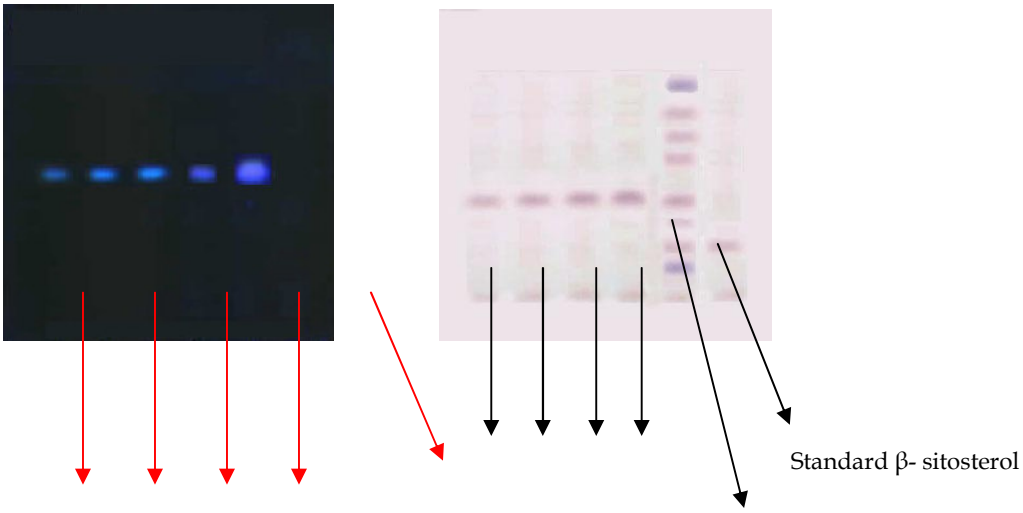


Fig 1: Haemolytic Activity of different extracts of roots of *E. roseum*

***Fig 2: HPTLC of Pet.Ether Extract*:**

Figure A **Figure B**



The present study on pharmacognostical characteristics and preliminary phytochemical screening of *Eranthemum roseum* provide useful information, which may help in authenticating the genuine plant along with nature of phytoconstituents present in it.

DISCUSSION

In the present days of modernization, Ayurveda no longer can afford to remain confined to use of conventional conservative norms of medication. It has to accept the new challenges and be prepared to answer the queries of the modern man about the quality and efficacy of the herbal drugs administered to him and also how they are collected, processed, preserved and used. To meet this new thrust inquisitiveness, standardization of Ayurvedic drugs is mandatory which may help in understanding and solving some of the controversies with regard to their therapeutically active ingredients and action. The above studies provide information in respect of their identification, chemical constituents and physico-chemical characters which may be useful for pharmacognostical study and standardization of herbal drugs of folk medicinal practice of present era and enrichment of Ayurvedic Pharmacopoeia. It will also determine therapeutic diagnostic tools for the scientists who are keen and sincere to evaluate the herbal medicine of indigenous resources.

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