

## Physico-Chemical Standardisation of *Chrysanthemum cinerariaefolium* Trev.

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### Abstract

Mother tincture prepared from the flower heads of *Chrysanthemum cinerariaefolium*, mostly used in Pediculosis and Scabies. Physico-Chemical standardization of raw drug as well as mother tincture was carried out to lay down pharmacopoeial standards. The formulation, weight/ml., total solids, alcohol content, PH, UV absorbance, preliminary phytochemical screening, besides TLC, HPLC and HPTLC profiles of mother tincture is provided, which help in identification and authentication of the drug.

**Key words:** *Chrysanthemum cinerariaefolium*, Homoeopathic medicine, Physico-chemical standardization.

### Introduction

*Chrysanthemum cinerariaefolium* belonging to the family compositae is used in treatment of Pediculosis and Scabies<sup>1</sup>. Earlier workers reported the presence of Pyrethral I, Pyrethrin II, Cinerin II<sup>2</sup>, Jasmolin II<sup>3</sup>, Sesquiterpenoids<sup>4</sup>, Polyphenolids<sup>5</sup>, Carotenoids<sup>6</sup>, Sesquiterpene lactone<sup>7</sup>. Hitherto no detailed physico-chemical standardization pertaining to homoeopathic mother tincture is available in literature. Hence the present study is taken to lay down Physico-chemical standards for identification and authentication of raw drug as well as mother tincture.

### Materials and Methods

*Chrysanthemum cinerariaefolium* flower heads supplied by Survey of Medicinal Plants and Collection Unit, CCRH Udagamandalam, Tamilnadu, were air dried coarsely powdered and used for the studies. Various parameters viz.,

- 1) Moisture content (Loss on drying),
- 2) Ash values,
- 3) Extractive values in different solvents,
- 4) Preliminary phytochemical screening of the extracts,
- 5) Physical constants of mother tincture viz. weight/ml, pH, total alcohol, total solids,

U.V. absorbance are determined following recommended methods<sup>8,9</sup>,

- 6) TLC plates coated with ACME silica gel (0.2mm thickness) and activated at 110°C for one hour used. Various solvent systems were used for separation of constituents of different extracts and the best separation achieved for each of the solvent system is recorded<sup>10,11,12</sup>.
- 7) HPLC run was made on E Merk Lachrom with flow rate of 1.0ml/min, c18/c15 µm column size using methanol: water(50:50) as mobile phase.
- 8) HPTLC run of mother tincture was made on Camag. (5 µl, 10 µl, and 15 µl, sample concentrations are applied). TLC plates (Silica gel 160F 254 EM) were used as stationary phase and Chloroform: Acetic acid: Methanol: Water (6.3 : 2.1 : 2 : 0.8) as mobile phase and the detection was done with Camag TLC scanner 3. Chromatograms are photo documented before and after derivitization. All the reagents and solvents used are Analar (S.D.) grade and the values reported are average of two experiments.

### Results and Discussion

Physico-chemical standards are of great significance in assuring the quality and authenticity of the drug which determines the efficacy of the drug. Detailed investigations related to ash values, extractive values in different solvents which help in determining foreign matter (inorganic content) of raw drug are depicted in Table I. Table II incorporates the results of preliminary phytochemical screening, except ethanol extract all extracts including mother tincture answered positive to Liebermann burchard's reagent. All the extracts gave positive test with ethanolic KOH. Table III presents the formulation of mother tincture. Table IV incorporates physical constants of mother tincture including u.v. absorbance (fig 1). Table V depicts TLC profiles of various extracts and mother tincture with Rf value of each constituent, detecting reagent and solvent system. HPLC & HPTLC chromatograms of mother

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tincture before and after derivatisation are provided (fig 2 and 3 a to i), which serve as finger prints of the authentic sample.

The results of the above qualitative and quantitative standards of raw drug and mother tincture help in determining quality of the drug.

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Table I

#### Physical constants of Flower heads

1. Loss of moisture at	=	13% (w/w)
2. Ash value	=	14.24 gm%
3. Acid in soluble ash	=	2.6% (w/w)
4. Water soluble ash	=	8.4% (w/w)
5. Successive extractive values in different solvents (Soxhlet extraction)		
a. n- Hexane	=	0.43% (w/w)
b. Benzene	=	0.52% (w/w)
c. Chloroform	=	0.66% (w/w)
d. Ethyl acetate	=	0.48% (w/w)
e. Ethanol	=	0.56% (w/w)

Table II

#### PHYTO-CHEMICAL SCREENING

Reagent	n-Hexane extract	Benzene extract	Chloroform extract	Ethyl acetate extract	Ethanol extract	Mother tincture
10% Iodine	No change	No change	No change	No change	Turned blue	No change
10% FeCl <sub>3</sub>	No change	No change	No change	No change	Turbed black	Turned black
50% NaOH	No change	No change	No change	No change	No change	No change
Liebermann Burchard's	+	+	+	+	-	+
Millon's	+	-	-	+	-	+
Ninhydrin	-	-	-	-	-	+
Ethanolic KOH	+	+	+	+	-	+
Mayer's	-	-	-	-	-	-
Wagner's	-	-	-	-	-	-
Spot test	+	-	-	+	-	+
Foam test	-	-	-	-	-	-

Table III

#### Formulation of Mother tincture

Drug strength	=	1/10
<i>Chrysanthemum cinerariaefolium</i> coarse Powder	=	120 gm.
Purified water	=	340 ml.
Strong alcohol	=	680 ml.
(to make one thousand millilitres of the mother tincture)		
Method of preparation - percolation		

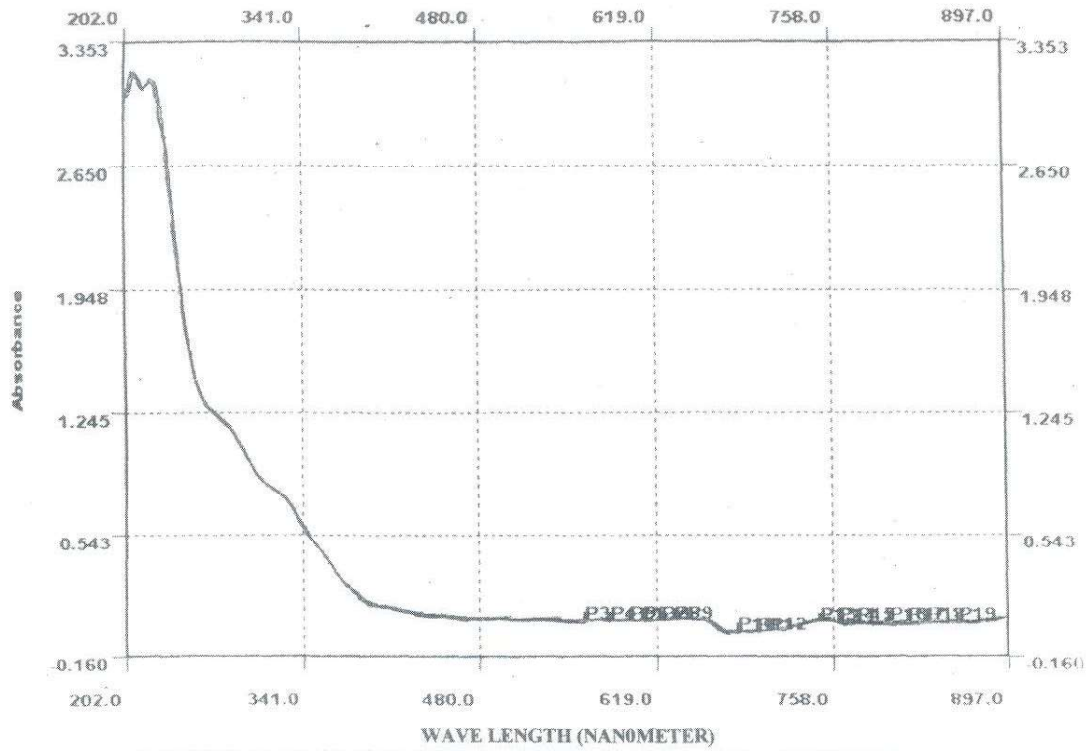
Table IV

#### Physical constants of Mother tincture

1. Organoleptic characters		
a) appearance	=	Clear non viscous
b) Colour	=	Light brown
c) Odour	=	Characteristic
2. Sediments	=	Absent
3. Specific gravity	=	0.8900
4. Total solids	=	0.32% v/w
5. Total Alcohol	=	58-63 v/v
6. pH at room temperature	=	5.5-6
7. $\lambda$ max Ethanol	=	254nm, 266nm

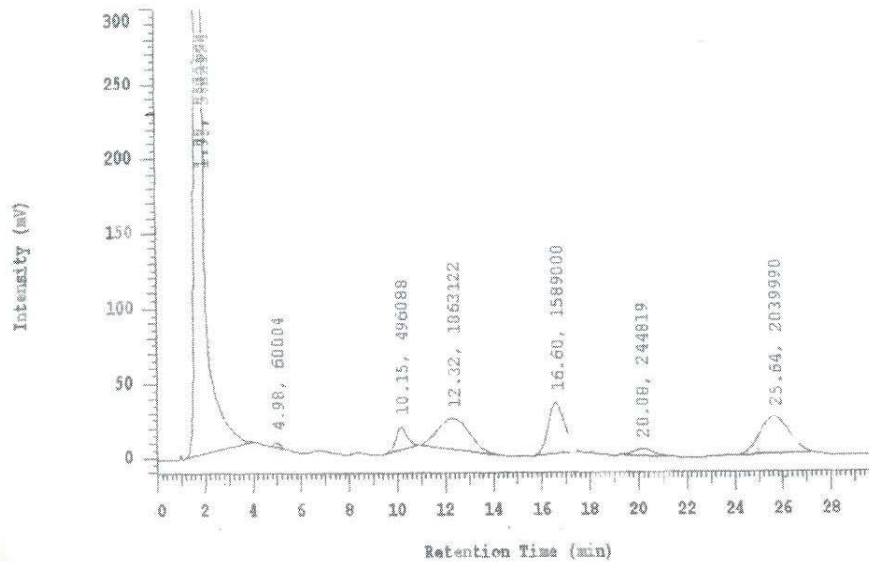


Fig.1



VISIBLE/U.V.SPECTRA (ETHANOL) OF MOTHER TINCTURE OF *CHRYSANTHEMUM CINERARIAEFOLIUM*

Fig.2



H.P.L.C. PROFILE OF MOTHER TINCTURE OF *CHRYSANTHEMUM CINERARIAEFOLIUM*

**Table V**  
**TLC Profiles of various extracts and mother tincture**

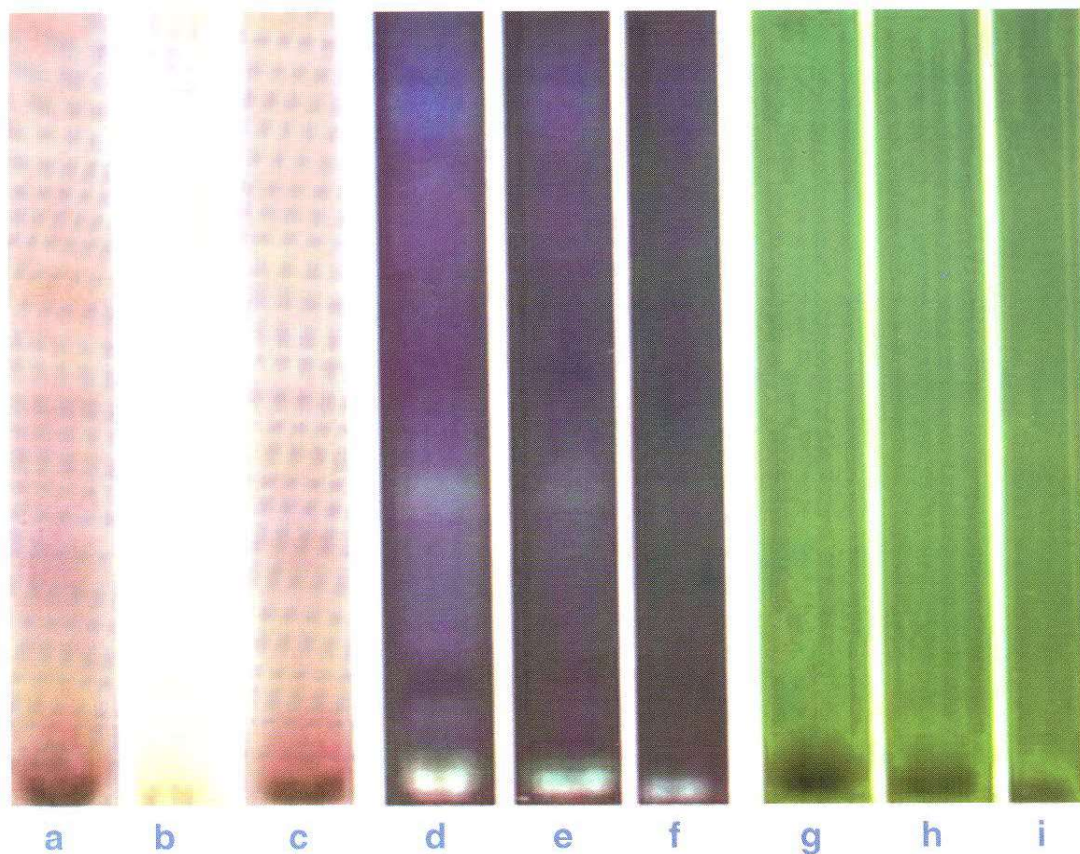
Extracts	No. of spots	Rf values	Solvent system	Spraying reagent
Hexane	9	0.98, 0.90, 0.73, 0.62 0.45, 0.39, 0.34, 0.22 0.09	Benzene	10% H <sub>2</sub> SO <sub>4</sub>
Benzene	7	0.97, 0.86, 0.73, 0.67, 0.17, 0.11, 0.08	Chloroform	10% H <sub>2</sub> SO <sub>4</sub>
Chloroform	5	0.92, 0.75, 0.50, 0.08, 0.05	Chloroform	10% H <sub>2</sub> SO <sub>4</sub>
Ethylacetate	9	0.87, 0.72, 0.57, 0.37, 0.25, 0.17, 0.1, 0.06, 0.03	Chloroform: 50 Ethylacetate: 5	10% H <sub>2</sub> SO <sub>4</sub>
Ethanol	9	0.92, 0.75, 0.43, 0.22, 0.2, 0.17, 0.1, 0.06, 0.03	Chloroform: 50 Methanol: 5	10% H <sub>2</sub> SO <sub>4</sub>
Mother tincture	10	0.82, 0.32, 0.21, 0.17, 0.15, 0.11, 0.09, 0.07 0.05, 0.04	Chloroform: 50	10% H <sub>2</sub> SO <sub>4</sub>
Mother tincture	11	0.8, 0.43, 0.3, 0.2, 0.21, 0.14, 0.11, 0.09, 0.05, 0.03, 0.02	Chloroform: 50	10% H <sub>2</sub> SO <sub>4</sub>
Mother tincture	1	0.08	Chloroform: 50 Ethylacetate: 5	15% FeCl <sub>3</sub>
Mother tincture	1	0.90	Chloroform: 50 Ethylacetate: 5	SbCl <sub>3</sub>
Mother tincture	2	0.03, 0.12	Chloroform: 50 Ethylacetate: 5	Ethanolic KOH
Mother tincture	9	0.02, 0.10, 0.30, 0.38, 0.43, 0.51, 0.62, 0.72, 0.8	Chloroform: 50 Ethylacetate: 5	Liebermann Burchards

The No. of spots and Rf values incorporated in the table help to evaluate the chromatograms developed. The no. of spots are characteristic for given extract in a particular solvent and a particular species of a plant and are diagnostic in nature. The Rf values are useful in comparative TLC to determine the authenticity of plant material. Identification can be effected by observation of spots of identical Rf value and equal magnitude obtained respectively with an unknown and a reference sample chromatographed on the same plate. The TLC spots can be used as fingerprints for identifying plant material.

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### HPTLC Chromatograms

- a,b,c : Chromatograms at 264 nm before derivatisation.
- d,e,f : Chromatograms at 366 nm before derivatisation.
- g : Chromatogram after derivatisation with Methanolic Sulphuric acid.
- h : Chromatogram after derivatisation with Methanolic KOH.
- i : Chromatogram after derivatisation with L.B. Reagent.

Sample Concentration a and b : 5  $\mu$ .lit,  
 b and e : 10  $\mu$ .lit, c,f,g,h and i : 15  $\mu$ .lit