



E-ISSN: 2321-2187
P-ISSN: 2394-0514
IJHM 2019; 7(6): 51-56
Received: xx-09-2019
Accepted: xx-10-2019

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Pharmacognostical and phytochemical screening of *Chrysanthemum indicum* leaf extract

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Abstract

Chrysanthemum indicum is a perennial herb belonging to the family Asteraceae. It usually blooms from August to October. *Chrysanthemum indicum* is an erect, aromatic, perennial plant producing a clump of stems 25-100 cm tall from procumbent rhizomes. The whole plant is blood tonic, depurative, febrifuge and vulnerary. It is used for detoxifying, and dissipating blood stasis. The leaves are depurative. They are used in China in the treatment of migraine. The Photomicrographic evaluation revealed interesting microscopic characteristic. The transverse section of leaf is dorsiventral consists of midrib and lamina. Calcium-oxalate crystals were found in spongy parenchyma. *Chrysanthemum indicum* leaf surface contains simple, unicellular covering trichomes and paracytic type of stomata. Covering trichomes are more in number as compared to glandular trichomes. Midrib contains centrally located vascular bundle which is collateral surrounded by some parenchymatous cell. Phytochemical screening of aqueous of leaf showed the presence of glycoside, and ethanolic extract of leaf showed the presence of glycosides, tannins and flavonoids. % Loss on drying of the powder was found to be 33%. The ash value of powder of *Chrysanthemum indicum* leaf was determined as total ash, water soluble ash and acid insoluble ash and was found to be 9.20%, 2.05%, 8.71% respectively. The acid soluble extractive value and water soluble extractive value was found to be 16.8% and 13.9% respectively. Phytochemical investigation of extracts of *Chrysanthemum indicum* shows that aqueous extract contains glycosides, while ethanolic extract shows presence of glycosides, flavonoids and tannins.

Keywords: *Chrysanthemum indicum* leaf, extract, ash value, extractive value, powder

1. Introduction

Chrysanthemum indicum is a perennial herb belonging to the family Asteraceae. Chrysanthemums, often called mums or chrysanthus, are of the genus (*Chrysanthemum*) constituting approximately 30 species of perennial which is native to Asia [1]. Traditionally, *Chrysanthemum indicum* was used as a folk remedy to treat the deterioration of bone and muscle, ocular inflammation, and headache. Furthermore, tea of *Chrysanthemum indicum* has been used to treat anxiety by facilitating relaxation and curing insomnia. Recently, some studies have suggested that *Chrysanthemum indicum* has anti-inflammatory effects and anti-apoptotic effects *in vitro* and *in vivo* [2]. It is used in the traditional treatment of several infectious diseases such as pneumonia, colitis, stomatitis, cancer, fever, sores, and it is also used to treat vertigo, and hypertensive symptoms. The active molecules in *Chrysanthemum indicum* are glycosides. The plant has the ability to act as antibiotic to many species of bacterial pathogens [3]. *Chrysanthemum indicum* possesses anti-bacteria, anti-virus, anti-oxidant and immunomodulatory properties with high efficacy and low toxicity [4]. Natural products are one of the major sources of new drug molecules today. Hence an attempt has been made to investigate the active constituents present in *Chrysanthemum indicum*.

2. Material and Method

2.1 Collection of Plant material

The leaves of *Chrysanthemum indicum* were collected from the Satara district, Maharashtra, during the month of July and authenticated by Dept. of Botany, Y. C. I. S, Satara, Maharashtra, India. Specimen voucher was deposited in the college herbarium for future reference. Fresh drug obtained were dried and coarsely powdered and passed through sieve 100 mesh sizes and stored in air - tight containers for further use.

2.2 Preparation of Extract [5-8]

The pulverized dried *Chrysanthemum indicum* leaves were extracted with ethanol using Soxhlet apparatus. The powder of *Chrysanthemum indicum* leaves were also macerated with water. Ethanolic and water extracts were filtered & evaporated to dryness.

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2.3 Macroscopic Characteristic ^[9-11]

The macroscopy of fresh leaves were studied according to standard methods.

2.4 Microscopic characteristics ^[12, 13]

For microscopy hand section of leaf was taken, stained & mounted following usual micro-techniques.

2.5 Fluorescence Analysis ^[14, 15]

Many drugs shows fluorescence when their powder is exposed to ultraviolet radiation. It is important to observe all materials on reaction with different chemical reagents under U.V. light. The fluorescence characteristics of powdered drug were studied under U.V. light after treating with different chemical reagents is reported. Fluorescence analysis was carried out according to the method of Chase, Pratt and Kokoski.

2.6 Phytochemical Screening ^[17, 18]

The dried leaves were extracted with ethanol and water and preliminary chemical tests for ethanolic and aqueous extracts.

2.7 Chromatographic Studies ^[18-20]

Thin Layer Chromatography studies were carried out for extracts to confirm the presence of different phytoconstituents in these extracts. Thin Layer Chromatography is a mode of liquid chromatography, in which the extract is applied as a small spot or band at the origin of thin sorbent layer supported on a glass. The mobile phase migrates through the stationary phase by capillary action. The separation of solutes takes place due to their differential absorption/ partition coefficient with respect to both mobile and stationary phases. Each separated component has same migration time but different migration distance. The mobile phase consists of a single solvent or a mixture of solvents. Although, a number of sorbent like silica gel, cellulose, polyamide, alumina, chemically modified silica gel etc. are used, silica gel (type 60) is most commonly used sorbent. Handmade plates are prepared by using techniques like pouring, dipping or spraying. The retardation factor (Rf) is calculated using following formula,

$$Rf = \frac{\text{Distance travelled by sample from baseline}}{\text{Distance travelled by solvent from baseline}} \quad (1)$$

2.8 Thin Layer Chromatography

The extracts were subjected to thin layer chromatography for the presence of phyto-constituents. In this technique, the Silica gel-GF254 (for TLC) was used as an adsorbent and plates were prepared by spreading technique, then air dried for an overnight and activated for one hour at 110°C and used, carried out according to the standard procedures described by Kokate and Horborne.

2.8.1 Preparative Thin Layer Chromatography

A thick layer of silica gel GF-254 was coated on the plate and activated at 110 °C for one hr. The broad band (2 mm width) of extracted sample was applied on the plate.

2.9 Physico-chemical Analysis

2.9.1 Determination of ash value

Ash value are helpful in determining the quality and purity of a crude drug, especially in the powdered form. The objective of ashing vegetable drug is to remove all traces of organic

matter, which may otherwise interfere in an analytical determination. On incineration, crude normally roots an ash usually consisting of carbonates, phosphate and silicate of sodium, potassium, calcium and magnesium. The total ash of a crude drug reflects the care taken in taken in its preparation.

2.9.1.1 Total ash value

Weighed accurately about 2 g of the powdered drug in a tarred silica crucible. In cinerated at a temperate not exceeding 450 °C for 4 hr, until free from carbon cooled and weighed. Calculate the percentage of ash with reference to air-dried drug using following formula,

$$\text{Total ash value} = \frac{\text{Initial weight} - \text{Final weight}}{\text{Initial weight}} \times 100 \quad (2)$$

2.9.1.2 Water soluble Ash

The total ash obtained above was boiled with 25 ml of distilled water for 5 min. The insoluble matter was collected on an ash less filter-paper, washed with hot water and ignited to constant weight at low temperature. The weight of the insoluble matter was subtracted from the weight of total ash, represents the water soluble ash. The percentage of water soluble ash was calculated with reference to the air dried drug. The result was calculated with reference to the air dried drug.

2.9.1.3 Acid Insoluble Ash

The total ash obtained was boiled with 25 ml of dilute hydrochloric acid for 5 min. The insoluble matter was collected on tarred grouch crucible, washed with hot acidulated water, ignited, cooled and weighed. The percentage acid insoluble ash was calculated with reference to the air dried drug.

2.9.1 Loss on Drying

Weight about 1.5 gm of the powdered drug into weighted flat and thin porcelain dish. Dry in the oven at 100°C or 105°C, until two consecutive weighings do not differ more than 0.5mg. Cool in a desiccators and weigh. The loss in weight is usually recorded as moisture. It can be calculated using formula,

$$\% \text{ Loss on Drying} = \frac{\text{Loss in weight of the sample}}{\text{Initial weight of the sample}} \times 100 \quad (3)$$

2.9.3 Determination of Extractive Values ^[21]

This method determines the amount of active constituents extracted with solvents from a given amount of medicinal plant material.

2.9.3.1 Water Soluble Extractive Value

Place about 5gm of coarsely powdered air-dried material, accurately weighed, in a glass-stoppered conical flask. Add 100ml of water. Shake the flask frequently during first 6 hr. Keep it aside without disturbing for 18 hr and then filter. Pipette out 25 ml of the filtrate and evaporate to dryness in a weighed shallow flat-bottomed dish on a water bath. Calculate the percentage of water soluble extractive.

2.9.3.2 Alcohol Soluble Extractive Value

Place about 5gm of coarsely powdered air-dried material, accurately weighed, in a glass-stoppered conical flask. Add 100ml of alcohol (90%). Shake the flask frequently during first 6 hr. Keep it aside without disturbing for 18 hr and then

filter. Pipette out 25 ml of the filtrate and evaporate to dryness in a weighed shallow flat-bottomed dish on a water bath. Calculate the percentage of alcohol soluble extractive. Extractive values were calculated by using this formula,

$$\text{Extractive value} = \frac{\text{Initial weight} - \text{Final weight}}{\text{Initial weight}} \times 100 \quad (4)$$

2.10 Phytochemical Tests ^[16]

2.10.1 Test for Glycosides

2.10.1.1 Keller-Killiani Test: To 2 ml extract, glacial acetic acid, one drop 5% FeCl₃ and conc. H₂SO₄ were added. Reddish brown appears at junction of the two liquid layers and upper layer appears bluish green indicates the presence of glycosides.

2.10.1.2 Glycosides Test: To small amount of extract was mixed with 1 ml water and was shaken well. Then aqueous solution of NaOH was added. Yellow colour appeared that indicates the presence of glycosides.

2.10.1.3 Concentrate H₂SO₄ Test: To 5ml extract, 2ml glacial acetic acid, one drop 5% FeCl₃ and conc. H₂SO₄ were added. The appearance of brown ring indicates the presence of glycosides.

2.10.1.4 Molisch's Test: To 1ml of extract, 2 drops of Molisch's reagent was added in test tube and 2 ml of concentrate H₂SO₄ was added carefully keeping the test tube slightly curved. Formation of violet ring at junction indicated the presence of glycosides.

2.10.2 Test for Tannins

2.10.2.1 Ferric chloride test: To 2 ml of test solution, a few drops of 5% ferric chloride solution was added. Formation of blue color indicated the presence of hydrolysable tannins.

2.10.2.2 Lead acetate test: To 5 ml of extract, a few drops of 10 % lead acetate solution was added. Formation of yellow or red precipitate indicated the presence of tannins.

2.10.2.3 Acetic Acids Solution: To 5ml extract, a few drop of Acetic Acid solution was added. Formation of Red color solution.

2.10.2.4 Potassium Dichromate Test: To 5 ml extract of few drop of Potassium dichromate solution was added. Formation of red ppt.

2.10.2.5 Dilute Iodine Solution: To 5 ml extract of few drops of dilute iodine solution was added. Formation of transient red color.

2.10.2.6 Dilute HNO₃: To 2ml of extract of few drops of Dilute HNO₃ solution was added. Formation of reddish to yellow color.

2.10.3 Test for Flavonoids

To Small quantity of residue, add lead acetate solution. Yellow colored precipitate is formed. Addition of increasing amount of sodium hydroxide to the residue shows coloration, which decolorizes after addition of acid.

3. Result and discussion

3.1 Preliminary Pharmacognostic Characteristics

In present study, the leaves were investigated for its

macroscopic characteristics. Results are presented in Table 1.

Table 1: Preliminary Pharmacognostic Characteristics of *Chrysanthemum indicum* Leaf

S. No.	Parameters	Observation
1.	Colour	Green
2.	Odour	Characteristics
3.	Taste	Bitter
4.	Shape	Oval-Orbicular
5.	Size	8cm length/6cm Width

3.2 Microscopy of Leaf

3.2.1 Microscopic Characteristics of *Chrysanthemum indicum* Leaf

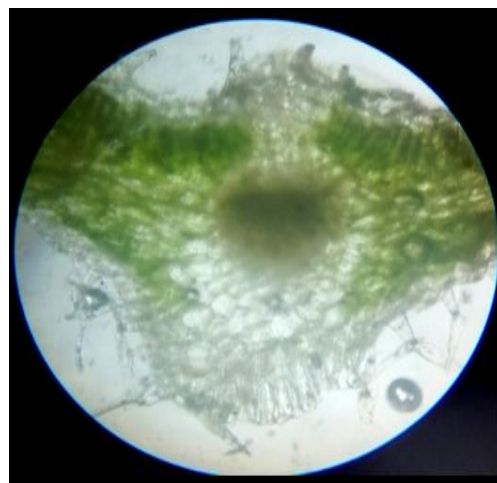


Fig 1: Transverse section of *Chrysanthemum indicum* Leaf

As shown in Fig. 1, the transverse Section of leaf is dorsiventral consists of Midrib and Lamina.

3.2.1.1 Midrib

It consist of single layered closely arranged elongated cells externally covered with striated cuticle on either sides of leaf named as upper and lower epidermis. Leaf surface contains simple, unicellular covering trichomes and paracytic type of stomata. Below the upper epidermis 3-4 layers of well developed more or less isodiametric collenchymatous tissues were observed.

Midrib contains centrally located vascular bundle which is collateral surrounded by some parenchymatous cells. Xylem is well developed and the phloem consists of strands of sieve tubes and small celled parenchyma. Lower epidermis consist of single layer of elongated cells with cuticle and just above the lower epidermis 2-3 layers of parenchymatous cells followed by the layers of collenchymatous cells were present.

3.2.1.2 Lamina

It consists of Dorsi-ventral structure with single layered upper and lower epidermis. Below upper epidermis single layered palisade cells followed by 5-7 layers of mesophyll parenchyma which are rounded in shape and are devoid of intracellular spaces. Calcium-oxalate crystals were found in spongy parenchyma. Covering trichomes are more in number as compared to glandular trichomes.

3.3 Observation of Fluorescence Analysis

The fluorescence characteristics of powdered drug were studied under U.V. light after treating with different chemical reagents is reported in Table 2.

Table 2: Fluorescence Analysis

S. No.	Test	Fluorescence Observed	
		At 254nm	At 360nm
1.	Powder+ Methanol	Light Green	Green
2.	Powder+ Ethanol	Light Green	Green
3.	Powder+ 1N HCl	Light Green	Green
4.	Powder+ 50% HNO ₃	Light Green	Green
5.	Powder+ 5% KOH	Dark Green	Green
6.	Powder+ 1N NaOH	Dark Green	Green
7.	Powder+ n Hexane	Light Green	Green

3.4 Physico-chemical Analysis

The Loss on drying, Ash values (Total Ash, Water soluble ash, Acid insoluble ash), Extractive values of leaf powder are given in Table 3.

Table 3: Physico-chemical Analysis

S. No.	Physical constant	Results
1.	Ash values (%w/w)	
	• Total Ash	9.20%
	• Water soluble Ash	2.05%
	• Acid Insoluble Ash	8.71%
2.	Loss on Drying(%w/w)	33%
3	Extractive values(% w/w)	
	• Acid Soluble Extractive Value	16.8%
	• Water Soluble Extractive Value	13.9%

3.5 Phytochemical investigation

Phytochemical investigation of extracts of *Chrysanthemum indicum* shows that aqueous extract contains glycosides, while

ethanolic extract shows presence of glycosides, flavonoids and tannins. All results are represented in Table 4.

Table 4: Phytochemical Investigation

S. No	Name of The Test	Aqueous extract	Ethanolic extract
1.	Test for Steroids	-	-
2.	Test For Glycosides	+	+
3.	Test For Carbohydrate	-	-
4.	Test For Alkaloids	-	-
5.	Test For Flavonoids	-	+
6.	Test For Tannins	-	+
7.	Test For Saponins	-	-

3.6 Observation of micro chemical Test

Table 5 represents micro chemical tests performed on leaf powder of *Chrysanthemum indicum*.

Table 5: Observation of Micro chemical Test

S. No	Test	Observation	Inference
1.	Phloroglucinol+ Conc. HCl (1:1)	Pink	Vessels and Pericyclic Fibres
2.	Sulphuric acid (60% w/w)	Calcium sulphate crystals	Calcium oxalate
3.	Sudan Red III	Red	Oil globules

3.7 Microscopically Characteristic of Powdered Drug

All results of microscopical characteristic of powdered drug are presented in Table 6 and Fig. 2.

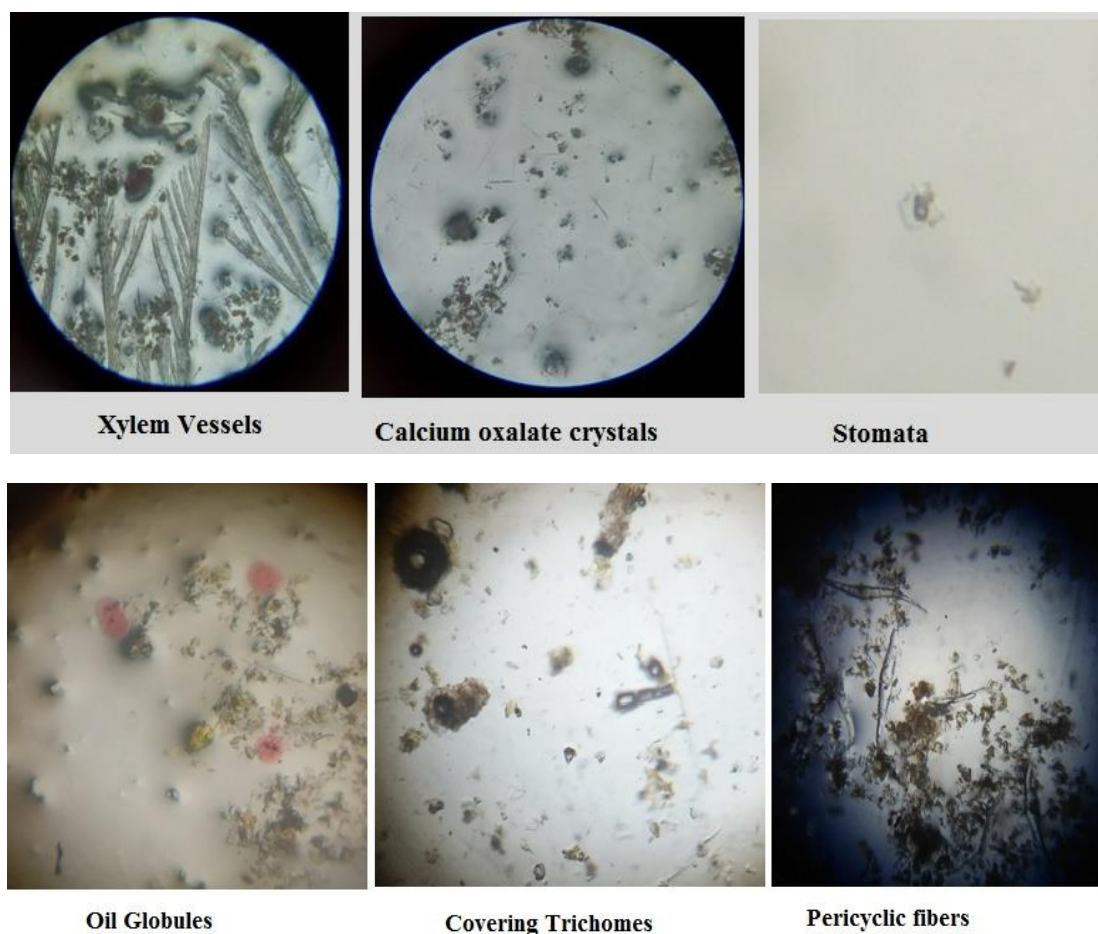
**Fig 2:** Powder Characteristic of powdered drug

Table 6: Observation table for Microscopic characteristics

Microscopic characteristics	Explanation
Xylem vessels	Thick walled, Lignified vessels.
Calcium oxalate crystals	Prism shaped and clusters, isolated from Parenchymatous cells.
Stomata	Paracytic stomata. Long axis of two subsidiary cells is parallel to that of stomata.
Oil globules	Oil globules in the cell of cuticle.
Covering Trichomes	Three to Five celled.
Pericyclic fibers	Lignified, tapering ends, narrow lumen.

3.8 Thin Layer Chromatography

Results of Thin Layer Chromatography are presented in Table No 7.

Table 7: Thin Layer Chromatography Profile

Extract	No. of spots	Colour of spot	R _f value
Aqueous	1	Faint yellow	0.92
Ethanollic	1	Faint yellow	0.91

4. Discussion

The leaves were collected from Satara district, Maharashtra region and authenticated. The leaves were subjected for Pharmacognostic investigation which includes determination of physical constants such as ash value, extractive values, loss on drying and fluorescence analysis. The powder of leaves shows fluorescence at 254 nm and 360 nm. Macroscopic and microscopic characteristics of the leaf were studied. The microscopic study shows that it contains midrib and lamina portion. The lamina shows upper and lower epidermis, spongy parenchyma, palisade cell layer while midrib portion shows upper and lower epidermis, collenchyma, vascular bundles, etc., Powder characteristics shows presence of paracytic stomata, xylem vessels, calcium oxalate crystals, covering trichomes, oil globule and pericyclic fibers. The leaves of plant were subjected to extraction by using ethanol and water and these extracts were subjected to phytochemical investigation. Phytochemical investigation of extracts of *Chrysanthemum indicum* shows that aqueous extract contains glycosides. While ethanolic extract shows presence of glycosides, flavonoids and tannins. Chromatographic study of the extracts was carried out. Thin layer chromatography shows R_f value 0.92 and 0.91 for aqueous and ethanolic extract respectively.

5. Conclusion

As there is less information available on pharmacognostical work on leaves of *Chrysanthemum indicum*, hence morphological study, microscopical studies, physico-chemical parameters, fluorescence analysis and chemical tests performed will guide in the proper identification of the plant species as well as help in authentication of the purity of the plant. All these parameters also help to build up a suitable plant profile.

6. Acknowledgement

The Authors are very much thankful to the Arvind Gavali College of Pharmacy, Jaitapur, Satara for providing the Laboratory and other Facilities needed to carry out the project work.

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