

Pharmacognostical and Phytochemical Investigation of *Calendula Officinalis***Neelesh Kumar*****Joni Sharma****Sakshy Sharma**Department of Pharmacognosy,
NKBR College of Pharmacy &
Research Centre, Meerut, INDIA***Corresponding Author:**neeshj15@gmail.com**ABSTRACT**

In the present investigation the leaves of *Calendula officinalis* Linn (Asteraceae) were studied for their identification, morpho-anatomical, phytochemical characteristics and standardization parameters. Various pharmacognostic parameters including examination of morpho-anatomical, ash value, crude fibre, extractive value and LOD were studied. Phytochemical evaluation including examination of preliminary physicochemical screening and TLC profile has been done. Preliminary phytochemical investigation of different extracts of leaves of *Calendula officinalis* shows the presence of alkaloids, carotenoids, flavonoids, saponin steroid, tannin and triterpenes

Keywords: *Calendula officinalis*, Phytochemical, Standardization Parameter**INTRODUCTION**

Calendula officinalis Linn (Asteraceae), commonly known as English garden marigold is aromatic annul herb, which is used in Traditional system of medicine to treat various diseases¹. The reported main constituents are flavonoids, flavonol, glycosides, and saponin². This plant is a native of Southern Europe and it is used as diuretic, diaphoretic, stimulant and also possesses spermicidal activity³. The plant is used because of its varied sources of biological activities like anti-inflammatory, antimutagenic, diuretic, and antispasmodic. It is also used in gastro-intestinal, gynecological, eye diseases, skin injuries and in some cases of burn⁴. So far no reports have been studied on the pharmacognostical aspect of the plant. Hence our studies were devoted in this direction for the botanical aspect of this plant.

MATERIALS AND METHODS**Plant Material:**

The leaves of *Calendula officinalis* were collected from the herbal garden of Allahabad and Meerut in the month of January, and were identified as *Calendula officinalis* Linn (Asteraceae) by comparison with a voucher. A voucher specimen was kept in the institute herbarium for further reference. The collected leaves were used freshly for the experiments.

Pharmacognostical studies

Morphological studies were done by using simple microscope to determine the shape, apex- acute, base, margin-dentate, nature-equal, size (length-3-6 cm, Width-1.5-2 cm), smell- pungent, taste- bitter and odor- characteristic of leaves. Microscopic studies were done by preparing a thin section of midrib and lamina region of *Calendula officinalis*. The section was cleared with chloral hydrate solution and then stained with phloroglucinol and hydrochloric acid, mounted in glycerin. A separate section was prepared and stained with iodine solution for the

identification of starch grains. Powder (#60) of the dried leaves was used for the observation of powder microscopical characters. The powder of the dried leaves was used for the observation of microscopical characters. The powder drug was separately treated with phloroglucinol–HCl solution, glycerin and iodine solution to determine the presence of lignified cells, calcium oxalate crystals and starch grains 4. As a part of quantitative microscopy, stomatal number, stomatal index, vein islet terminations were determined by using fresh leaves of the plant and the results are shown in respective tables.

Table No. 1: Anatomical detail of the plant part

Parameter	<i>Calendula officinalis</i>
Epidermal cell	5-9 μ m
• Thickness	
Stellar region	Conjoint, collateral and open type
Phloem	Reticulate

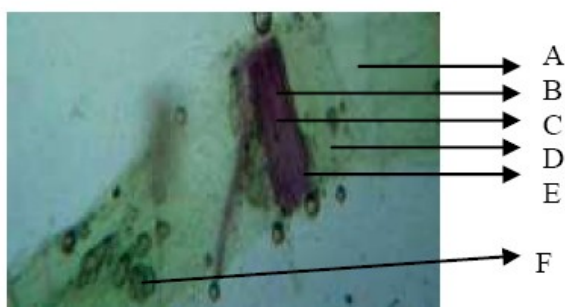


Fig 1: Traverse section

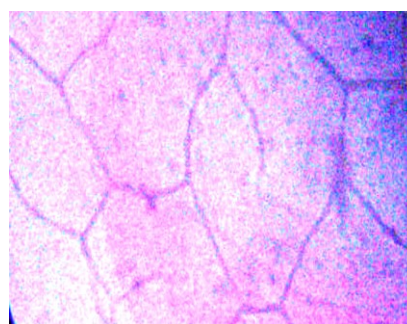


Fig2: Epidermal cell

(A) Epidermal cell, (B) Phloem, (C) Cambium, (D) Parenchyma cell (E) Xylem (F) Stomata

Table No. 2: Quantitative Microscopy of Leaves of *Calendula officinalis*

Particulars	<i>Calendula officinalis</i>
Vein islet number	5-7
Vein termination number	6-8.5
Stomatal number	
• Upper surface	2-2.5
• Lower surface	4-4.5
Stomatal index	
• Upper surface	22-25
• Lower surface	28-30

Determination of physicochemical parameters

Varies physicochemical parameters were evaluated for leaves part of *Calendula officinalis* as per guidelines described by W.H.O., results are given in table 3

Table No. 3: Physiochemical parameter of *Calendula officinalis*

Parameter	Percentage
LOD	18%
Crude fibre	32%
Ash values	
• Total ash values	14%
• Acid in soluble	10%
• Water soluble	6%
Extractive values	
• Pet. ether	1.6%
• Alcoholic	2.4%
• Aqueous/ water	21.6%

Preliminary phytochemical screening

Preliminary phytochemical investigation of different extracts of the leaves of *Calendula officinalis* was observed:

- Petroleum ether extract shows the presence of carotenoids, steroids, and saponin, tannin.
- Chloroform extract shows the presence of steroids and tannin, triterpenes.
- Ethanolic extract shows the presence alkaloids, flavonoids, and saponin.
- Aqueous extract shows the presence of flavonoids and saponin.

Optimization of TLC solvent system for the extract

Different solvent systems were tried for developing a TLC system for identification of constituents in the extract based on the literature survey and the mobile phase showing maximum separation was selected for the study

For pet. ether- Toluene: ethyl acetate: glacial acetic acid (5:7:1drop)

For chloroform- Toluene: ethyl acetate: glacial acetic acid (6:4:1drop)

For ethanol- Chloroform: methanol: glacial acetic acid (8:2:1drop)

Table No. 4: Phytochemical screening of *Calendula officinalis*

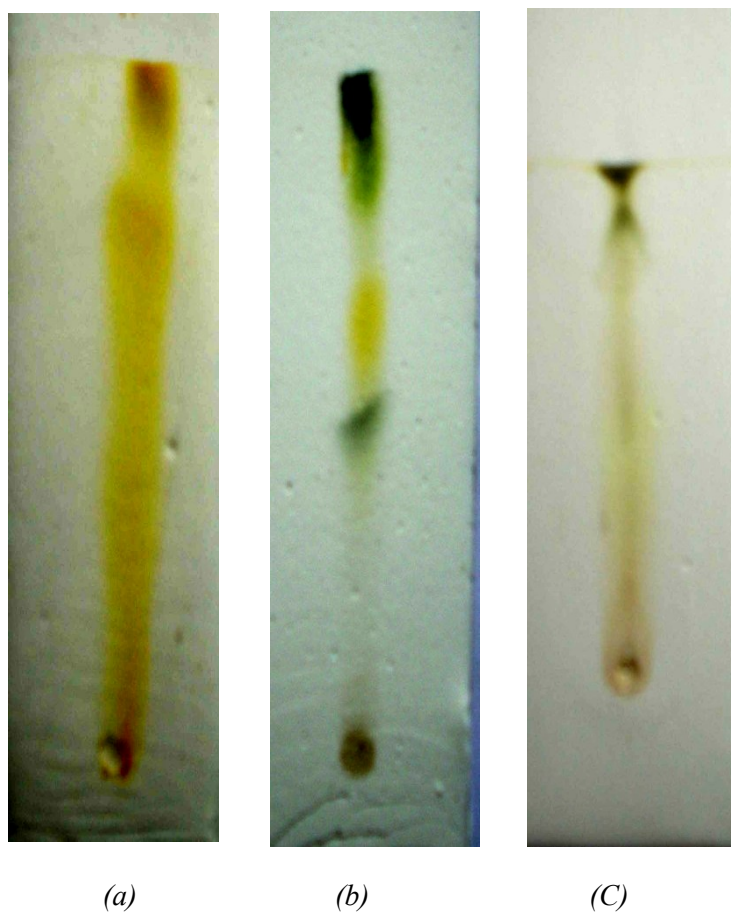
Chemical constituent	Tests	Pet. ether. Ext.	Chloroform. Ext.	EtOH. Ext.	Aq. Ext.
Alkaloids	• Dragendroff's test	--	--	++	--
	• Mayer's test	--	--	++	--
	• Wagner's test	--	--	++	--
Carbohydrates	• Benedicts test	--	--	--	--
	• Fehling's test	--	--	--	--
	• Iodine test	--	--	--	--
	• Glucose test	--	--	--	--
	• Molish,s test	--	--	--	--
Carotenoids	• Ext. + conc. HCl + PhOH.	++	--	--	--
	• Ext. + 85% Sulphuric acid	++	--	--	--
Flavonoids	• Lead acetate test	--	--	++	++
	• Sodium hydroxide test	--	--	++	++
	• Shinoda test	--	--	++	++
Saponin	• Foam test	++	--	++	++
Steroid	• Libermann's butchered test	++	++	--	--
	• Cholesterol test	++	++	--	--
Tannin	• Ferric chloride test	++	++	--	--
Tritrpenes	• Salkowski test	++	++	--	--

In the calculated Rf values and 2 spots in Petroleum ether extract, 4 spots in chloroform extract, 2 spots in ethanolic extract were found and shows to good separation.

The results are compiled in *table5 and figure 3*

Table No. 5: TLC analysis of *Calendula officinalis*

Extracts	Spots	Colors	Rf values
Pet. ether	1	Yellow	0.7
	2	Reddish yellow	0.9
Chloroform	1	Green	0.4
	2	Yellow	0.6
	3	Yellowish green	0.8
	4	Dark green	0.9
Ethanollic	1	Light pale green	0.6
	2	Dark pale green	0.7
Aqueous	-	-	-

**Fig3: TLC plates of (a)Pet. ether, (b) Chloroform, (c) Ethanollic****CONCLUSION**

The plant *Calendula officinalis* is a perennial plant belongs to family Asteracea, which is used in ayurveda. In study of plant *Calendula officinalis* pharmacognostic, preliminary phytochemical screening, physicochemical properties have been evaluated.

For the proper identification of plant physicochemical parameter (ash value, crude fibre, extractive values, moisture content) provide useful information. In morpho-anatomical studies the transverse section has been

examined. Form the present investigation it is evident that certain characters such as color of leaves, stoma in epidermal layer, xylem, phloem, cambium can provide useful parameter.

Preliminary phytochemical investigation different extracts of leaves *Calendula officinalis* shows the presence of alkaloids, carotenoids, flavonoids, saponin steroid, tannin and tritpenes.

TLC profile analysis was found to be a useful tool to provide the in plant *Calendula officinalis*

REFERENCES

1. Wealth of India- Raw Materials (1992) Vol. III Publication and Information Directorate, C.S.I.R., New Delhi, 55.
2. Chopra, Nayar and Chopra (1956) Glossary of Indian Medicinal Plants, Publication and Information Directorate, C.S.I.R., New Delhi, 1956, 45.
3. The Useful Plants of India (1992) Publications and Information Directorate, C.S.I.R., New Delhi, 96.
4. Pietta P, Bruno A M P& Rava A J.1992, *J chromatogra* 593, 165- 170
5. Kokate C K (2005) Practical Pharmacognosy, 4th Edn. Vallabh Prakashan, Delhi, 110-111.
6. Indian Pharmacopoeia 1985 Vol II, 3rd Edn., Ministry of Health and Family Welfare, Govt. of India, Controller of Publication, New Delhi, 310.
7. Mohammed Ali. (1994) Text book of Pharmacognosy, 1st Edn., CBS Publishers and Distributers, Delhi, 81, 116, 372, 447.
8. www.Botanical.com