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Pharmaocognostic evaluation of *Calendula officinalis* l.flowers

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ABSTRACT

Calendula officinalis is a most common medicinal and ornamental plant. The flowers of the plant has been used since long, in traditional system of medicine especially in Homeopathic system for the treatment of skin diseases as well as for its wound healing property. In the present investigations an attempt has been made for the pharmacognostic standardization of flowers of *C.officinalis* using macroroscopic and microscopic characters, physico-chemical constants, phytochemical screening, heavy metal analysis and TLC fingerprint profile. Results of the study indicates that besides the characteristic features like solitary inflorescence, median veins in ligulate florets, glandular trichomes, thick dark tannin filled cells and secretory canals in involucre etc., fluorescence analysis and thin layer chromatographic pattern of different extract can serve as finger prints of drug. Hence, the data generated will have potential utilization to determine correct identity, adulterants as well as for standardization and quality control of raw material used in the development of medicaments.

KEYWORDS

Calendula officinalis;
Pharmacognostic evaluation;
HPTLC;
Microscopy;
Physico-chemical values;
Heavy metal analysis.

INTRODUCTION

Calendula officinalis L. (Compositae) commonly known as Marigold, is an annual herb, cultivated for ornamental and medicinal properties in India, Europe and America. The plant has been employed for long time in folk therapy. Tincture and extracts of florets have been used topically to promote wound healing and to reduce inflammation; systemically, in the treatment of dysmenorrhea, angina, fever, gastritis, hypotension, jaundice and rheumatism[1-2]. The flowers have been used for the treatment of smallpox, measles, jaundice, wounds, ulcers and skin diseases[3] and are the important ingredient of several homeopathic formulations. The plant has been found to contain number of phytoconstituents like, triterpenoides[4-5], carotenoids[6], volatile oils[7] and flavonoides[8]. It has been reported to possess antiinflammtory[9], antitumour[10], immnuno stimulant[11], hypoglycemic[12] and antidiarrheal activities[13]. In light of the importance of flowers of *C.officinalis* in traditional and modern system of medicine, it was thought worthwhile to develop quality standard for the same. Hence, in the present investigation an attempt has been made to standardize *C.officinalis* by using microscopic characters, physico-chemical values, heavy metal analysis and TLC fingerprint profile.

MATERIALS AND METHODS

Chemicals and collection of plant material

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All the chemicals and reagents used were of analytical grade purchased from Sigma Chemical Co. (St Louis, MQ, USA) and Merck(Darmstadt, Germany).

Flowers of *C.officinalis* were collected from Herbal garden of Jamia Hamdard, New Delhi(February 2006), which was identified by Taxonomist, Department of Botany, Hamdard University. The voucher specimen(PC-015/2006) was deposited in phytochemistry research laboratory.

Morphological studies

The morphological study was carried out for shape, size, color, odour, taste and fracture of the drug[14].

Microscopic studies

The microscopic studies carried out using the method described by O Brien et al.[15]. Microphotography on different magnifications was carried out with nicon labphot 2 microscopic unit. The polarized light was used for the study of crystals, starch grains and lignified cell.

Physico-chemical studies

Different physico-chemical values such as ash value[16], extractive values[16], loss on drying[14], total tannin[17], total resin and total fat[14] were determined.

Preliminary phytochemical screening of drug was carried out as per method described by Peach and Tracy[18]. The 5g of dried and powdered leaf was extracted in a Soxhlet apparatus with hexane, chloroform, methanol and water, successively. The extracts were dried and weighed. The presence or absence of different phyto-constituents viz. triterpenoids, steroids, alkaloids, sugar, tannins, glycosides and flavanoids etc, were detected.

Fluorescence analysis study of powdered drug material with different reagents was carried out to observe the color reactions[19].

The bitterness value, swelling index and foaming index were determined as per the WHO protocols[20] to determine the presence of bitters, mucilage, gum and saponins, respectively.

The presence of heavy metals analysis was determined[20] to ensure the safety of drug, for its use in human being.

High performance thin layer chromatographic fingerprints

HPTLC fingerprint profile of the drug was developed as per the TLC method described by Stahl[21]. Three extracts namely; hexane, chloroform and methanol; obtained from dried and coarse powdered of flowers *C.officinalis* were subjected to HPTLC analysis to find out the nature and approximate numbers of compounds present.

The extracts were applied (5µl each) on TLC plate (precoated silica gel G60 F_{254} aluminium sheets, (10cm ×10cm) in triplicate with bandwidth of 8mm using CAMAG Linomat V applicating device on separate plates. The chromatograms were developed upto distance of 80mm at room temperature in different solvent systems as given in TABLE 4 using previously saturated twin trough chamber(CAMAG). The developed chromatograms were scanned at different wavelengths using CAMAG TLC Scanner III and deuterium and tunguston lamp in absorbance/fluorescence mode to determine best suitable wavelength showing maximum number of compounds.

RESULTS AND DISCUSSION

Macroscopic characters

The capitula (inflorescence) are solitary, heterogamous having ligulate florets and tubular florets. Ligulate florets were orange or orange-yellow in colour, 5-6mm in width, with 3-toothed apex and yellowish brown tube with projecting style and 2-lobed stigma while tubular florets about 7-8mm long, yellow or orange red 5-lobed corolla in colour and with orange-brown tube. The anthers are two lobed with thin connective tissue. The involucres 5-6mm in length, concave on the inner side, thick in middle and tapering towards margins. Morphological studies on the flowers were in accordance with previous data[1].

Microscopic characters

Outer epidermal layers of tubular floret composed with tangentially oblong dilated cells and inner epidermis with thin walled parenchyma cells. Small nest of vascular strands were seen in parenchyma zone. Glandular trichomes were mainly on inner side (Figure 1). Ligulate floret was folded twice and had three major

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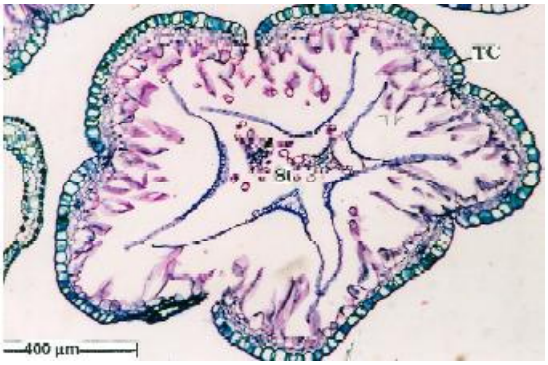

Figure 1: T.S. of tubular corolla
Abbreviations: TC, tubular corolla; St, stigma

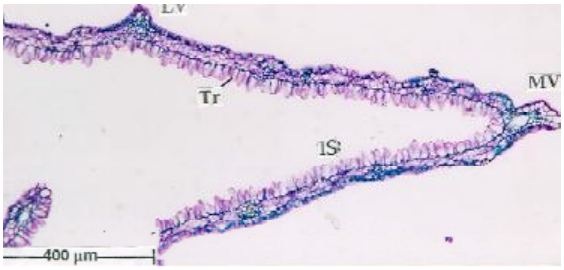

Figure 2: T.S. of ligulate corolla
Abbreviations: LV, lateral vein; MV, mid vein; Tr, trichome; IS, inner side

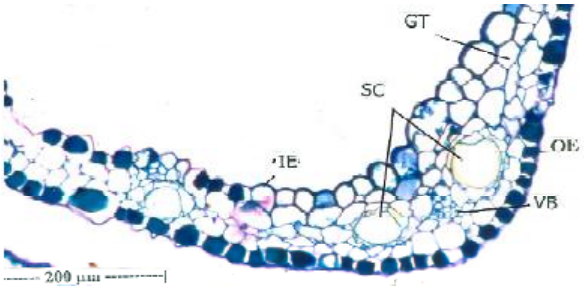

Figure 3: T.S. of involucre bract
Abbreviations: GT, ground tissue; IE, inner epidermis; SC, secretory canal; OE, outer epidermis; VB, vascular bundle

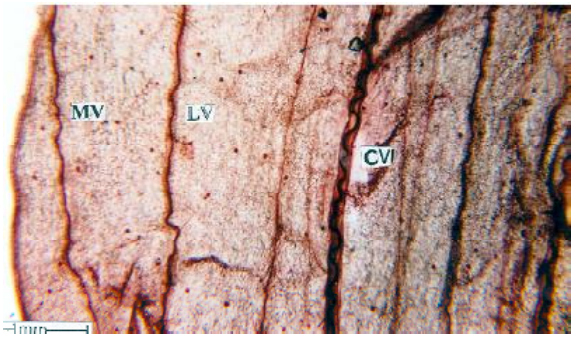

Figure 4: Venation pattern in the petal
Abbreviations: LV, lateral vein; MV, mid vein; CV, central vein

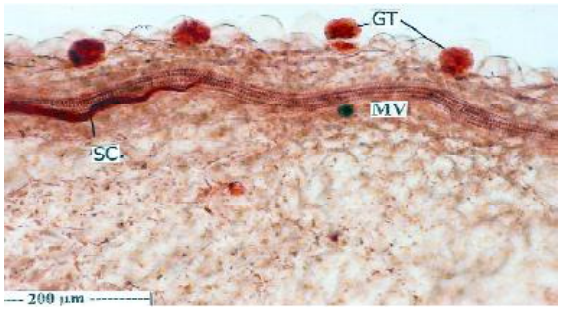

Figure 5: Marginal vein with secretory canal and glandular trichomes
Abbreviations: MV, mid vein; GT, ground tissue; SC, secretory canal

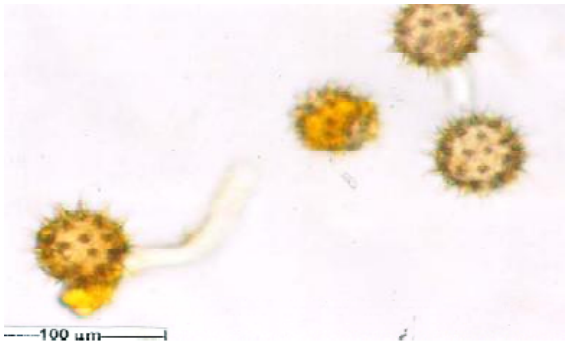

Figure 6: Pollen grains with exine

median veins and small veinlets. It was folded along the three major veins. The veins were prominent and projected as hemispherical lump on the outer part with small vascular bundles. The outer epidermis of ligulate floret consist of three or four layers of parenchyma zones and dense outgrowth of trichomes on inner side(Figure 2). The outer epidermis of involucre composed of rectangular or sqaurish cells with thick dark tannin fitted cells and inner epidermis with hemispherical cells. All along the involucre, there were wide, circular secretory canals(Figure 3).

Powder microscopy

The powdered material of the flower showed fragments of petals and pollen grains. The petal showed prominent central vein, thin lateral veins and marginal veins (Figure 4). The veins were invariably associated with the wide, undulate secretory canals. Glandular trichomes were frequently seen in the powder(Figure 5). Abundant pollen grains, spherical in shape with dense spiny exine were found in drug powder (Figure 6).

Physico-chemical values

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TABLE 1: Percentage of loss on drying, ash and extractive value of *C.officinalis* flower

Parameters	*C.officinalis* (mean[a]±S.D)
Loss on drying	5.17±0.19
Total ash	9.12±0.02
Water soluble ash	3.67±0.59
Acid insoluble ash	1.35±0.09
Water soluble extractive	11.08±0.26
Alcohol soluble extractive	18.15±0.18

[a]mean value of six readings

TABLE 2 : Preliminary phytochemical screening of *C. officinalis* flower extracts

Extract	Chemical consituetns	*C.officinalis*
Hexane	Triterpenoids	++
	Resins	-
Chloroform	Steroids	+++
	Triterpenoids	+
	Alkaloids	-
Alcohol	Flavonoids	++
	Alkaloids	-
	Reducing sugar	-
	Glycoside	+++
	Tannin	+
Water	Alkaloids	-
	Reducing sugar	-
	Saponin	++
	Tannin	-

TABLE 3: Fluorescence analysis study of *C.officinalis* flower powder

Solvent Used	UV light (254nm)	UV light (366nm)
Drug powder as such	Brown	Green
NaOH in methanol	Light Green	Green
NaOH in water	Cherry Red	Light Red
Benzene	Yellowish Brown	Reddish Brown
Acetone	Yellowish Brown	Light Brown
Ethyl acetate	Red	Green
Chloroform	Red	Light Green
Dil. H_2SO_4	Light Green	Light Green
Con. HCl	Reddish Brown	Green
Distilled water	Brown	Dark Green
50% HCl	Light Brown	Green
Dil. HNO_3	Reddish Brown	Green
Conc. H_2SO_4	Brown	Green

The total ash, acid insoluble ash, water soluble ash and mean values of different solvent extractives have been tabulated in TABLE 1. Presence and absence of different phyto-constituents were detected (TABLE 2). Result of fluorescence analysis study was tabulated in TABLE 3. The percentage of successive extractives were calculated and depicted in histogram (Figure 7). Quantitative estimation of total tannin, resin, fat, bitterness value and swelling index were also determined and

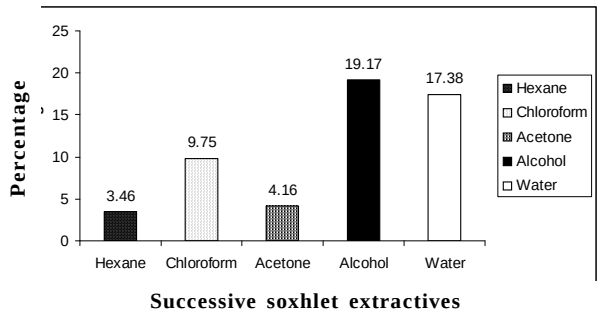

Figure 7: Successive extractive values of *C.officinalis* flower

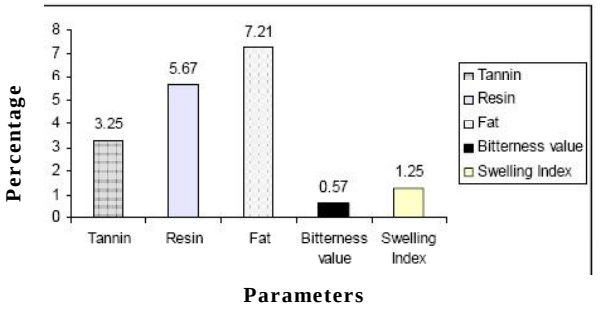

Figure 8 : Physico-chemical studies of *C .officinalis* flower

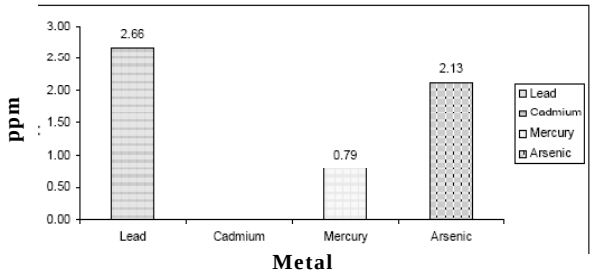

Figure 9 : Heavy metal analysis study of *C.officinalis* flowers

TABLE 4: TLC fingerprint profiles of *C.officinalis* flower extracts

Extract	Solvent system	Wavelength(nm)	No. of spots (R_f values)
Hexane	Toluene:chloroform: ethylacetate (4:4:1.0)	366	19 (0.02, 0.08, 0.15, 0.18, 0.23, 0.28, 0.33, 0.34, 0.38, 0.48, 0.64, 0.67, 0.70, 0.78, 0.80, 0.85, 0.89, 0.91, 0.93)
Chloroform	n-butanol: glacial acetic acid: water (5.6: 1.0: 1.6)	366	6 (0.02, 0.08, 0.66, 0.70, 0.76, 0.96
Methanol	Toluene: ethyl acetate (6: 1.0)	366	17 (0.02, 0.04, 0.09, 0.13, 0.22, 0.25, 0.31, 0.40, 0.45, 0.48, 0.61, 0.65, 0.67, 0.72, 0.80, 0.90, 0.96)

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results are presented in histogram (Figure 8). The foaming index was found to be less than 100. The percentage of heavy metals were quantified and depicted in histogram (Figure 9).

HPTLC fingerprints of flower extracts

Hexane, chloroform and methanol successive extracts were analysed by HPTLC for development of fingerprints. The chromatograms obtained after development in different solvent system followed by scanning at 366nm in absorbance mode depicted presence of number of substances in the extracts. Hexane, chloroform and methanol showed presence of 19, 6, and 17 spots respectively, with different R_f values as given in TABLE 4.

CONCLUSION

The results of present investigation reveals that the data generated can be used for determining correct identity of flowers and detection of adulterants as well. The quality pharmacognostic standards developed could be helpful for standardization of raw materials used for development of single drug and compound formulations as well as for their quality control.

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