WORLD JOURNAL OF PHARMACY AND PHARMACEUTICAL SCIENCES
SJIF Impact Factor 6.647
Volume 6, Issue 10, 874-887 Research Article ISSN 2278 – 4357

MICROSCOPIC AND PHYSICOCHEMICAL EVALUATION OF LEAVES OF CICHORIUM INTYBUS LINN

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Article Received on
02 August 2017,

Revised on 23 August 2017,
Accepted on 13 Sept. 2017,

DOI: 10.20959/wjpps201710-10217

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ABSTRACT

To study the pharmacognostic characters of a medicinally important plant *Cichorium intybus* Linn. In the present investigations an attempt has been made for the pharmacognostic standardization of leaves of *C. intybus* using various pharmacognostic parameters involved in organoleptic, microscopic, physicochemical constants, phytochemical screening, heavy metals analysis and TLC fingerprint profile. The plant is used to treat AIDS, cancer, diabetes, dysmenhorrhoea, impotence, insomnia, splentis and tachycardia. Result of the study indicate that besides the characteristic features like, leaf was dorsiventral, mesomorphic, hypostomatic and had prominent midrib and thin lamina, vessel elements were 250 µm long and 40 µm wide, large black irregular masses of bodies which possess unknown cell content and oil bodies of varying sizes etc., fluorescence analysis and thin layer chromatographic pattern of different extract can serve as finger prints of drug. The phytochemical screening revealed the presence of different phyto-constituents viz. alkaloids, glycosides, steroids, triterpenoids, sugar, tannins and flavanoids etc, were detected. Hence, the scientific 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: Physico-chemical values, Heavy metal analysis, HPTLC, Microscopy, Pharmacognostic evaluation, standardization.

INTRODUCTION

Cichorium intybus L. (Asteraceae), commonly known as chicory, is a perennial herb distributed in the temperate parts of the world and found wild in Punjab and Andhra Pradesh regions.[1] It is used in Indian system of medicine as a cardiotonic, anti-inflammatory, digestive, stomachic, liver tonic and diuretic drug.[2] The tuberous root of this plant contains number of medidicinally important compounds such as inulin, bitter sesquiterpenes lactone, coumarins, flavanoids and vitamins .[3] The main reported phytoconstituents of chicory roots are phenylacetic acid esters, cichoriosides,[4] sonchuside A,[5] ixerisoside, magnolialide[6] and endesmanolides.[7-8] The plant is useful in vitiated conditions of kapha and pitta, cephalalgia, hepatomegaly, inflammations, anorexia, dyspepsia and allergic conditions of skin [9]. In light of the importance of leaves of *C. intybus* 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. intybus* by using microscopic characters, physico-chemical values, heavy metal analysis and TLC fingerprint profile.

MATERIALS AND METHODS

Chemicals

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

Plant material

Leaves of *C. intybus* were collected from the Herbal garden of Jamia Hamdard, New Delhi and identified by taxonomist, Department of Botany, Faculty of Science, Jamia Hamdard, New Delhi. A voucher specimen (No. PRL/JH/05/28) was deposited in the herbarium of the Faculty of Pharmacy, Jamia Hamdard, New Delhi. After authentication, the leaves of *C. intybus* Linn. were dried at room temperature until they were free from moisture and subjected to botanical, physicochemical and histochemical studies.

Organoleptic and Microscopical Evaluation

The morphological studies including size, shape, apex, margin, surface, colour, odour, taste of the drug were carried out.[10] The important qualitative microscopic characters like stomatal index, vein termination, vein islet number and trichomes of both the surfaces was carried out by using standard procedures.[11] The microscopic studies carried out using the method described by O Brien et al.[12] Microphotography on different magnifications was carried out with nicon labtop 2 microscopic unit. The polarized light was used for the study of crystals, starch grains and lignified cell.[13]

Physicochemical Evaluation

The shade dried leaves were subjected to size reduction to get fine powder (# 40 mesh size) and then evaluated for different physico-chemical values such as ash value,[14] extractive values,[14] loss on drying,[10] total tannin,[10] total resin[10] and total fat[10] were determined.

Preliminary Phytochemical Evaluation

Preliminary phytochemical screening of drug was carried out as per method described by Peach and Tracy.[15] The 10 g of dried and powdered leaf was extracted in a Soxhlet apparatus with petroleum ether, chloroform, acetone, methanol and water, successively. The extracts were dried and weighed. The presence or absence of different phyto-constituents viz. alkaloids, glycosides, steroids, triterpenoids, sugar, tannins and flavanoids etc, were detected. Fluorescence analysis study of powdered drug material with different reagents was carried out to observe the color reactions.[16] The bitterness value, sewelling index and foaming index were determined as per the WHO protocols[17] to determine the presence of bitters, mucilage, gum and saponins, respectively. The presence of heavy metals analysis was determined[17] to ensure the safety of drug, for its use in pharmaceutical formulations.

HPTLC Fingerprint Profile

HPTLC fingerprint profile of the drug was developed as per the TLC method described by Stahl.[18] Four extracts namely; petroleum ether, chloroform, acetone and ethanol; obtained from dried and coarse powdered of leaves of *C. intybus* were subjected to HPTLC analysis to find out the nature and approximate numbers of compound present. The extracts were applied (5µl each) on TLC plate (precoated silica gel G60 $F_{254,}$ aluminium sheets, 10cm × 10cm) in triplicate with band width of 8mm using CAMAG Linomat V applicating device on separate plates. The chromatogram were developed upto distance of 80mm at room temperature in different solvents systems via 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

Organoleptic Evaluation

C. intybus is a perennial herb, the leaves were simple, 7.5-15.0 cm long and 4-5 cm wide, radical broadly oblong, oblanceolate or lanceolate, crowded at the base forming a rosette arranged spirally on the stem, upper leaves were alternate, small, entire and lower leaves were large, pinnatifid lobes toothed and spreading thickly covered with hairs, Colour: dorsal surface-green, ventral surface-light green, Taste- bitter and Odour – characterstic. [Fig. 1]

Fig. 1: Leaves of *Cichorium intybus.*

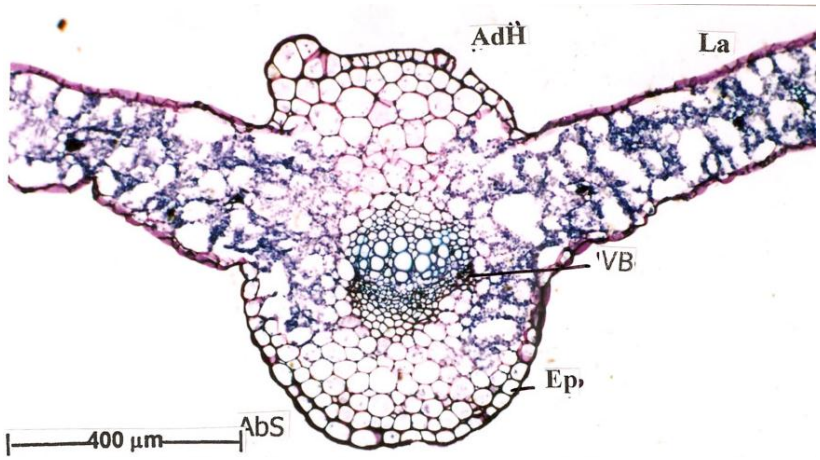


Fig. 2: Transverse section of *Cichorium intybus* leaf through midrib and lamina (10 x 2.5) showing:- AdH: adaxial hump; Abs: abaxial side; Ep: epidermis; La: lamina; VB: vascular bundle.

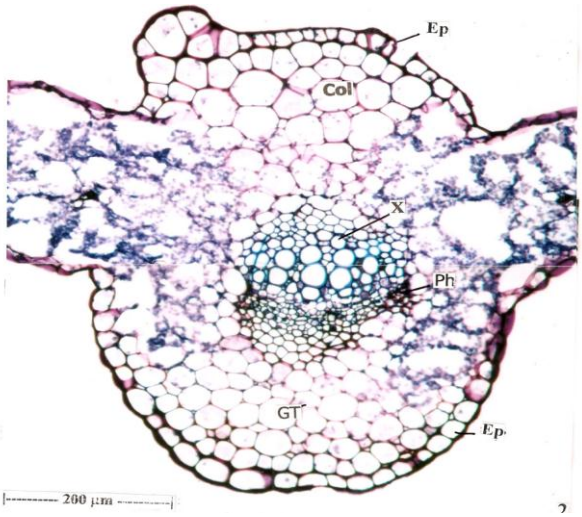


Fig. 3: Transverse section of *Cichorium intybus* leaf midrib enlarged (10 x 5) showing:- Col: collenchyma; Ep: epidermis; GT: ground tissue; Ph: phloem, X: xylem.

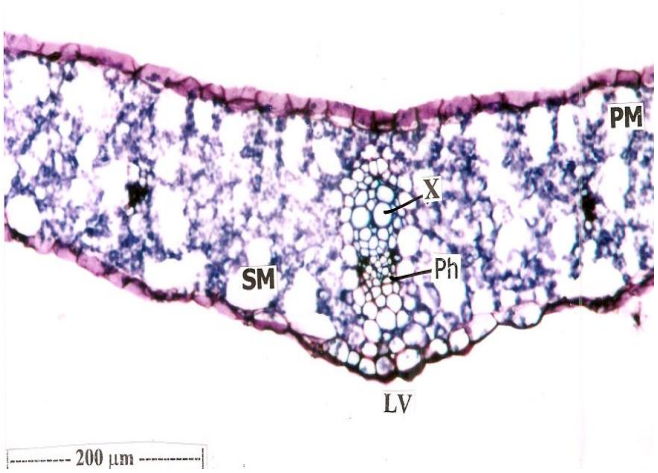


Fig. 4: Transverse section of *Cichorium intybus* leaf lamina (10 x 5) showing:-Ph: phloem; PM: palisade mesophyll; LV: lateral vein; SM: spongy mesophyll, X: xylem.

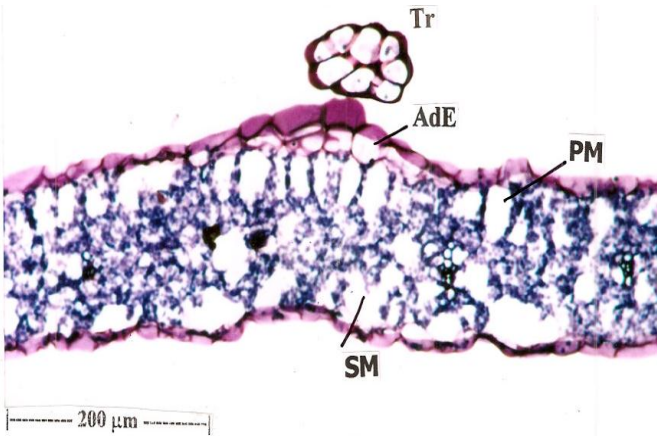


Fig. 5: Transverse section of *Cichorium intybus* leaf lamina (10 x 5) showing:-PM: palisade mesophyll; LV: lateral vein; SM: spongy mesophyll; X: xylem; Tr: trichome.

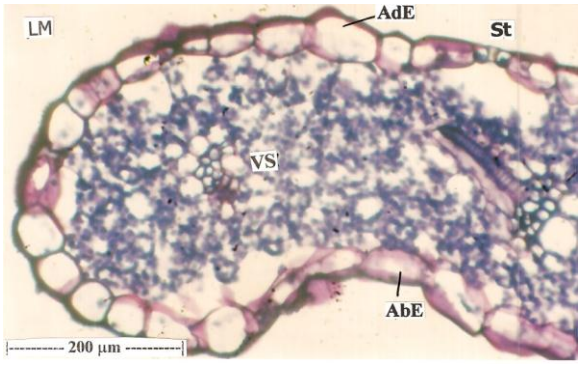


Fig. 6: Transverse section of *Cichorium intybus* leaf margin (10 x 5) showing:- AdE: adaxial epidermis; AbE: abaxial epidermis; LM: lamina; St: stomata; VS: vascular strand.

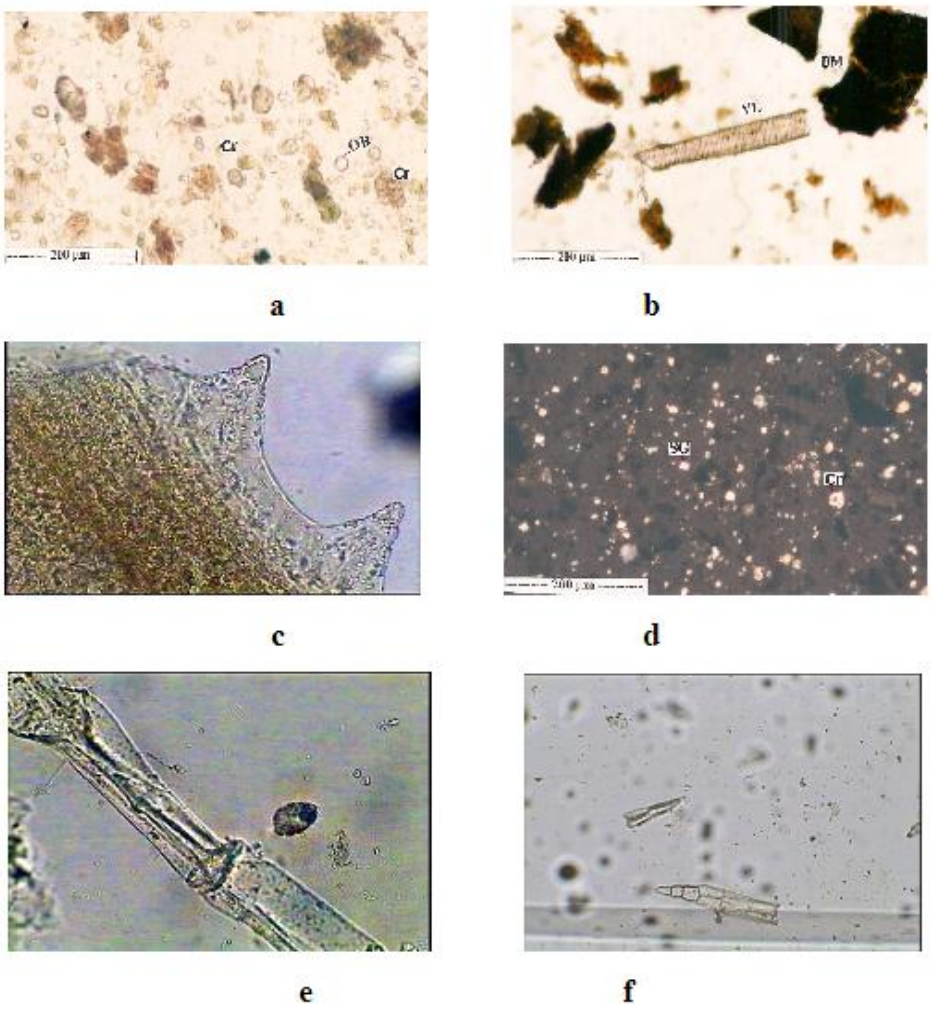


Fig. 7: Powder microscopy of *Cichorium intybus* leaf showing:-a. Calcium oxalate crystals and oil bodies (10 x 5). b. Vessel element and black masses (10 x 5); c. Calcium oxalate crystals and starch grains (10 x 5); d. multicellular multiserate trichome (40 x). e. collapsed trichome (40 x); f. multicellular uniserate trichome (40 x).

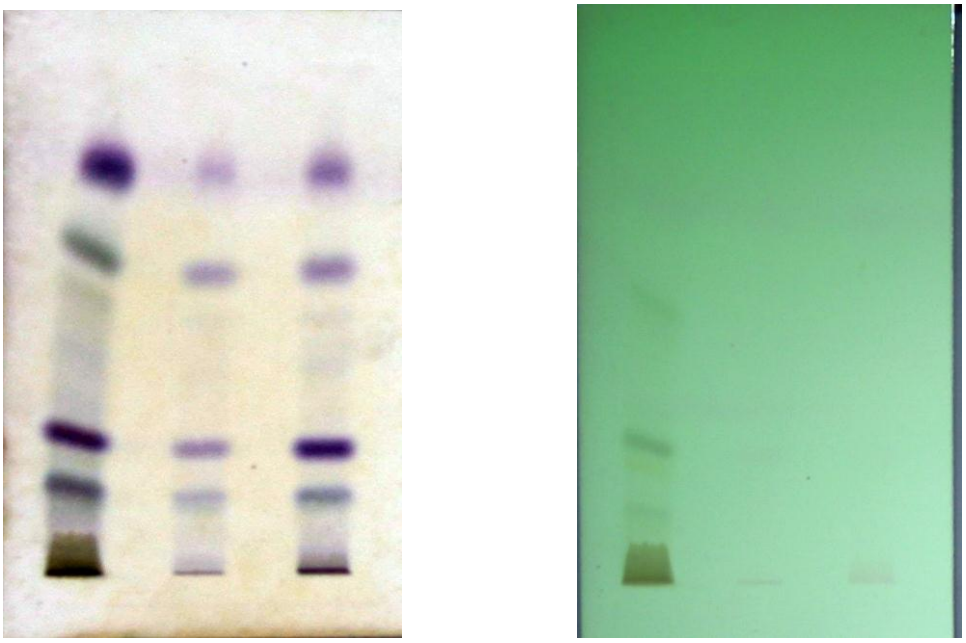
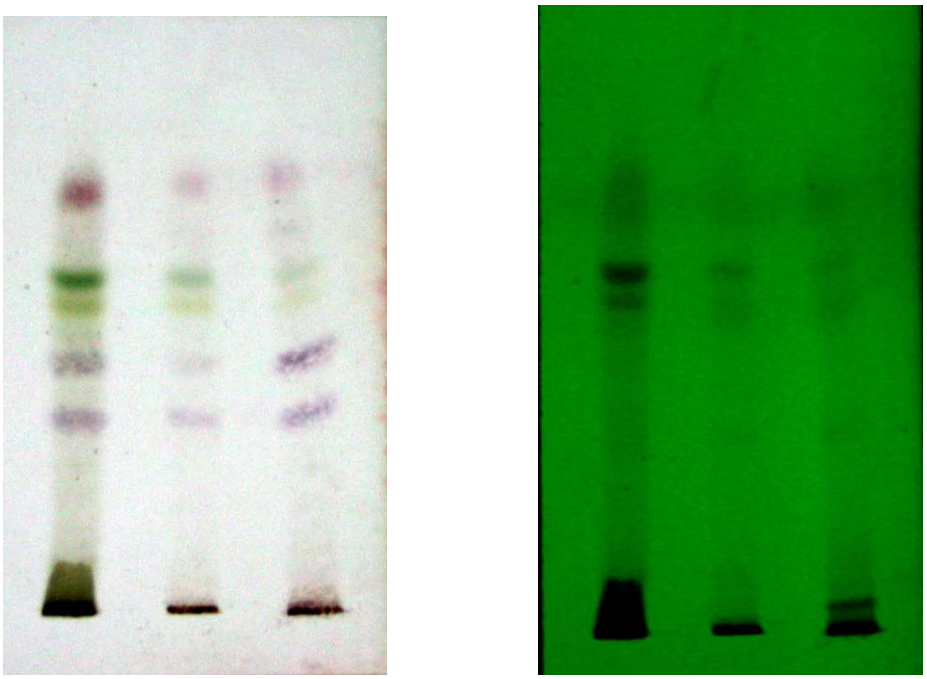


Fig. 8: HPTLC plates of petroleum ether extracts of *C. intybus* leaves upon derivatization with anisaldehyde in sulphuric acid at UV-254 and 366 nm.



UV (254 nm) **UV (366 nm)**

Fig. 9: HPTLC plates of chloroform extracts of *C. intybus* leaves upon derivatization with anisaldehyde in sulphuric acid at UV-254 and 366 nm.

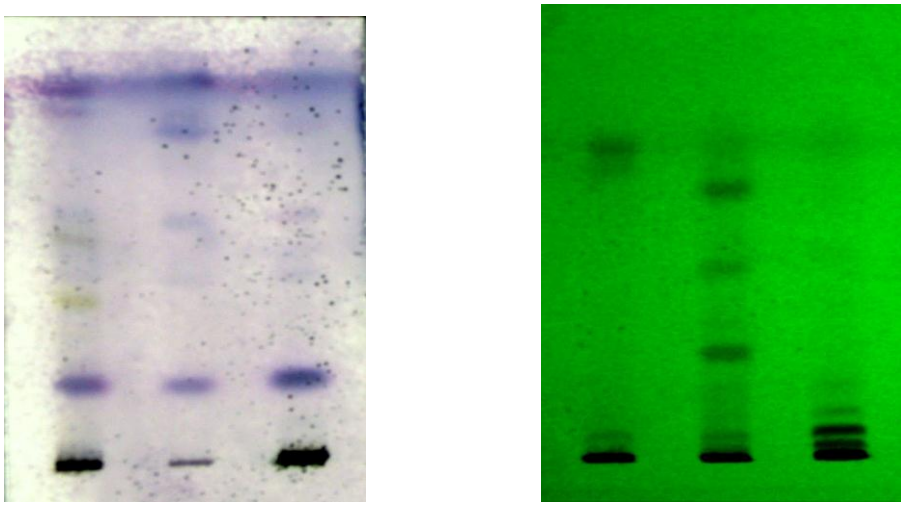


Fig. 10: HPTLC plate of ethanolic extracts of *C. intybus* leaves, stems and roots upon derivatization with anisaldehyde in sulphuric acid at UV-254 and 366 nm.

Table 1: Quantitative Microscopical Data of *C. intybus* Leaves.

S. No.	Leaf Constant Parameters	Range
1	Stomatal number in upper epidermis	10-14
2	Stomatal number in lower epidermis	27-32
3	Stomatal index in upper epidermis	15-20
4	Stomatal index in lower epidermis	21-29
5	Vein islet number	5-8
6	Vein termination number	10-13
7	Palisade ratio	2-4

Table 2: Phytochemical screening of leaf extracts of *C. intybus.*

S. No.	Constituents	Hexane ext	Pet.ether ext.	Chloroform ext.	Acetone ext.	thanol ext.	Aqueous ext.
1.	Alkaloids	+	-	+	-	+	+
2.	Carbohydrates	+	-	+	-	+	+
3.	Glycosides	-	-	-	+	+	+
4.	Phenolic compounds and Tannins	-	+	-	+	+	+
5.	Flavonoids	+	-	-	+	+	+
6.	Proteins and free amino acids	+	-	+	+	+	+
7.	Saponins	-	-	-	-	+	+
8.	Sterols	+	+	+	-	+	+
9.	Acidic compounds	-	-	-	-	-	-
10.	Mucilage	-	-	-	-	-	-
11.	Lipids/fats	+	+	-	-	-	-
12.	Resins	+	+	+	-	+	+
13.	Sesquiterpene	+	+	+	+	+	+

+ Present − Absent

The phytochemical screening of various extracts of leaf indicated the presence of carbohydrates, phenolic compounds and tannins, flavonoids, proteins and free amino acids, saponins, sterols, resins and sesquiterpene, etc.

Table 3: Percentage of loss on drying, ash and extractive value of *C. intybus* leaves.

S.No.	Parameters	*C.intybus* (mean[a] S.D)
1	Loss on drying	4.09 ± 0.04
2	Total ash	8.67 ± 0.35
3	Water soluble ash	7.61 ± 0.39
4	Acid insoluble ash	0.69 ± 0.01
5	Water soluble extractive	11.94 ± 0.23
6	Alcohol soluble extractive	18.26 ± 0.12

Table 4: Fluorescence analysis study of *C. intybus* leaves powder.

S.No.	Chemical treatment	At 254nm	At 365 nm
1	Leaf powder as such	Light Green	Brownish Green
2	1N NaOH in methanol	Brownish green	Yellowish green
3	1N NaOH in water	Dull green	Brownish green
4	50% HCl	Blackish green	Blackish brown
5	50% HNO_3	Yellowish green	Brownish green
6	50% H_2SO_4	Blackish brown	Blackish green
7	Hexane	Green	Dull green
8	Petroleum ether	Dull green	Brown
9	Chloroform	Dull green	Green
10	Methanol	Yellowish green	Green
11	Glacial acetic acid	Light Green	Brownish Green
12	50% HCl	Blackish Green	Blackish Brown
13	50% HNO_3	Yellowish Green	Brownish Green
14	50% H_2SO_4	Blackish Brown	Blackish Green

Table 5: Total phenolic content in leaves of *C. intybus.*

S. No.	Part and extract used	Absorbance	Total phenolic content of GAE from calibration curve (mg/g)	%age of phenolic content
1	Leaf alcoholic extract	0.7155	0.0744	7.44
2	Leaf hydro-alcoholic extract	0.600	0.0624	6.24
3	Leaf aqueous extract	0.6205	0.0645	6.45

The total phenolic content in the alcoholic extract of leaf was found to be higher than the hydroalcoholic and aqueous extracts of leaves.

Table 6: Total flavonoid content in leaves *C. intybus*

S. No.	Part and extract used	Absorbance	Total flavonoid content of QE from calibration curve (mg/g)	% age of flavonoid content
1	Leaf alcoholic extract	0.600	15.00	1500
2	Leaf hydro-alcoholic extract	0.648	16.20	1620
3	Leaf aqueous extract	0.233	5.82	582

The total flavonoid content in the hydroalcoholic extract of leaves was found to be higher (16.20 mg/g) than the alcoholic and aqueous extracts.

Table 7: Heavy metal content in leaves of *C. intybus*

S.No.	Name of metal	Concentration of metal in ppm
1	Lead	3.8733
2	Cadmium	0.1092
3	Chromium	0.9604
4	Nickel	0.5052
5	Arsenic	0.4141
6	Mercury	0.1532

The heavy metal content in the different parts of *C. intybus* was found to be within the prescribed limits.

Table 8: TLC fingerprint profile of *C. intybus* leaves extracts

S. No.	Extract	Solvent system	No. of spots	R_f values	Visualizing agents
1	Leaf petroleum ether extract	Toluene: ethyl acetate: glacial acetic acid (9.5: 0.5: 0.2)	5	0.01, 0.20, 0.31, 0.72, 0.89	Anisaldehyde in sulphuric acid
2	Leaf chloroform extract	Toluene: ethyl acetate: chloroform: glacial acetic acid (5.0:2.0:3.0:0.2)	16	0.02, 0.07, 0.11, 0.17, 0.23, 0.30, 0.41, 0.52, 0.58, 0.61, 0.65, 0.69, 0.72, 0.75, 0.79, 0.88	Anisaldehyde in sulphuric acid
3	Leaf ethanol extract	Toluene: ethyl acetate: chloroform: glacial acetic acid (4.0:3.0:3.0:0.5)	17	0.07,0.15,0.21,0.27,0.34,0.39, 0.43,0.48,0.54,0.55,0.65,0.71, 0.79,0.81, 0.83,0.87,0.96	Anisaldehyde in sulphuric acid

Microscopical Evaluation

The leaf was dorsiventral, mesomorphic, hypostomatic and had prominent midrib and thin lamina [Fig.2]. Midrib had broad and low adaxial hump and prominently projecting, semicircular abaxial part, the epidermal layer was thick with dilated barrel-shaped cells and prominent cuticle. The epidermal layer was 30 µm thick. There were four layers of large, collenchyma cells in the adaxial part of the midrib. In the abaxial part two or three layers of similar large collenchyma cells occur in the outer portion of the midrib. The remaining ground tissue was parenchymatous and chlorenchymatous cells were found around the vascular bundles. The vascular strand was single, collateral and more or less circular and it was 200 µm in diameter. There were four or five short, parallel rows of wide, thin walled angular xylem elements and a thick area of phloem elements. The xylem elements were 30 µm wide [Fig. 3].

The lateral veins do not project much beyond the leaf surface. It consisted of ventrically oblong narrow xylem and phloem strands unsheathed by thin parenchymatous cells [Fig. 4]. The lamina was 150 µm thick, it had thick epidermal layer of dilated circular or barrel shaped cells with thick cutical [Fig. 5]. The mesophyll tissue consists of short, wide palisade cells and 6-8 layers of small lobed and loosely arranged spongy parenchyma cells. The marginal part of the lamina was semicircular with undifferentiated mesophyll tissue [Fig. 6]. Quantitative microscopical evaluation was done for histological features such as stomatal index, stomatal number, vein islet number, vein termination number, palisade ratio [Table 1].

Powdered Analysis

Leaf powder green in colour powder; exhibits narrow, long, cylindrical vessel elements with scalariform lateral wall thickening. The vessel elements were 250 µm long and 40µm wide.There were large black irregular masses of bodies which possess unknown cell content. Oil bodies of varying sizes were frequently shown in the powder. Calcium oxalate crystals were fairly abundant up to 30 µm wide. Starch grains were also shown along with the crystals, grains were circular and concentric type and starch grains were 8-12 µm in diameter. The following anatomical features were found helpful to diagnose the crude drug: calcium oxalate crystals, starch grains, oil bodies, trichomes and stomata were present in the leaf. [Fig.7].

Preliminary Phytochemical Screening

Preliminary phytochemical screening revealed the presence alkaloids, glycosides, steroids, triterpenoids, sugar, tannins and flavanoids etc were mentioned in the table [Table 2].

Physico-chemical values

The content of total ash, acid insoluble ash and water soluble ash was calculated in mg/g of air dried leaf material, loss on drying and extractives values have been evaluated [Table 3]. Fluorescence analysis study was done by using different solvents [Table 4]. Quantitative estimation of total phenolic content [Table 5], total flavonoid content [Table 6] and the percentage of heavy metals were quantified [Table 7]. Moisture content of *C. intybus* leaves was found to be 4.07 % w/w The bitterness values, swelling factor and foaming index of leaf was found to be zero, 2.8 ml and less than 100 respectively. The total resin content in the leaves was found to be higher (2.53 mg/g).

HPTLC Fingerprints Analysis

Petroleum ether, chloroform and ethanol successive extacts were analysed by HPTLC for development of fingerprints. The chromatograms obtained after development in different solvent system followed by scanning at 254nm in absorbance mode depicted presence of number of substances in the extracts [Fig. 8, Fig. 9 and Fig.10]. Petroleum ether, chloroform and ethanol showed presence of 5,16,17 spots respectively, with different R_f values [Table 8]

DISCUSSION

Standardization of the crude drugs is an essential tool for evaluating the purity, quality and identity of a drug. Morphological evaluation is helpful in the authentification og crude drug by evaluating the external apperence such as colour, shape, size, odour, taste and so on. Microscopica analysis of a crude drug is necessary for the quantitative identification of closely allied and adulterants or substituents present in crude drug which can be distinguished by using optical microscopy. Even identifying the powder characterstics of crude drugs is useful in authentification of drug and identification of adulterents. The physicochemical parameters such as ash value, extractive values, loss on drying, swelling index, foaming index, fluorescence analysis, total phenolic, total resin and total flavonoids contents were helpful in the identification and authentification of the plant material. The preliminary phytochemical screening will reveals the presence of chemical constituents in the crude drug.

CONCLUSION

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

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