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Original Article

Pharmacognostical-physico-chemical Evaluation and Development of HPTLC Finger print for *Cichorium intybus* L. fruits

Achintya Kumar Mandal, Shakila Ramachandran*, Kallingilkalathil Gopi Divya, Mattumal Rubeena, Koppala Narayana Sunil Kumar, Parameswaran Sathiyarajeswaran

ABSTRACT

Introduction: Many herbal medicines are lacking pharmacognostical, phytochemical, pharmacological and toxicological data even though used widely for medicinal purposes. *Cichorium intybus* L. (Asteraceae) – chicory is an ancient folklore medicine. Various parts of these plants are in use for a wide range of ailments including those affecting liver and kidney. The aim of the current study is to standardize the fruit of *C. intybus* for macroscopy, microscopy, physicochemical parameters, TLC photo documentation along with development of HPTLC fingerprint profiles. **Methods:** Following standard pharmacopoeial procedures, detailed macro-microscopic characterization along with preliminary phytochemical features of the drug has been recorded from the current study. **Results:** Macro-microscopic study has revealed the authenticity of this medicinal achene type fruit. Physico-chemical and HPTLC studies revealed constants for identification and authentication of fruits of *C. intybus*. **Conclusion:** The current study will serve as a reference tool for quality maintenance, authentication as well as scientific validation of chicory fruits.

Key words: *Chicory* fruits, Monograph, Quality control, Standardization.

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INTRODUCTION

Traditional system of medicines is the major backbone of primary health care systems throughout the whole world even in this century. The genus *Cichorium* comprises of six species majorly distributed in Asia and Europe.[1,2] *C. intybus* L. is an erect perennial herb with a fleshy taproot up to 75 cm.[2,3] It has a very pronounced history in folklore medicine as a very familiar plant in traditional system of medicine in Europe and Asia. Despite this fact, this plant is not described in European pharmacopoeia.[4] Chicory contains many medicinally important phytochemicals which belong to the categories, viz., carbohydrates, alkaloids, flavonoids, triterpenoids, tannins, fatty acids, volatile oils, etc.[5] The seed (botanically achene type of indehiscent fruit) contains triterpenoids, cichoridiol and intybusoloid along with 11 known compounds namely lupeol, fridelin, β-sitosterol, stigmasterol, betulinic acid, betunaldehyde, syringic acid, vanilic acid, betulin, 6,7-dihydroxycoumarin and methyl-α-D-galactopyranoside.[6] Chicory fruits are one of the main ingredients of jigerine, a commercial product of India which is used for the treatment of various diseases of liver.[7,8] Traditional uses, chemical constituents, pharmacological activities, toxicological studies, clinical trial reports, cultivation and sustainable uses of various anatomical parts of *C. intybus* has been reviewed by earlier researchers.[9] Alcoholic extract of seeds of *C. intybus* has been reported to exhibit a potent antihepatotoxic activity on CCl_4 induced liver damage in albino rats.[10] The histopathological study has shown the normalization of the fatty accumulation of the tissues and resolvation of necrosis.[7] Investigation of the hepatoprotective activity of aqueous methanolic extract of *C. intybus* seed resulted in a significant reduction in death rate, serum levels of alkaline phosphatase, glutamyl oxaloacetate transaminase and glutamyl pyruvate transaminase against acetaminophen and CCl_4 induced hepatic damage in mice.[11] The aqueous extract of *C. intibus* seed showed significant antimicrobial activity against *Staphylococcus aureus* but not against *Candida albicans*. Ethyl acetate extract showed activity against *Pseudomonas aeruginosa* and *Staphylococcus aureus*.[12] The present study is aimed at standardization the fruit of *C. intybus* and development of HPTLC finger profile for quality maintenance, authentication as well as scientific validation of this commercially important medicament.

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MATERIALS AND METHODS

Material

Cichorium intybus fruits were procured from local market of Mettur and was authenticated by Dr. M. Padma Sorna Subraminian, Research Officer (Botany), Siddha Medicinal Plants Garden, Mettur, Tamil Nadu.

Macro-microscopy and powder microscopy

The external features of the test sample were documented using Nikon COOLPIX 5400 digital camera followed by Zeiss Discovery.V8. For microscopy the sample was left in FAA (Formalin-5 ml + Acetic acid-5 ml + 70% Ethyl alcohol-90 ml) for more than 48 hr. The preserved specimens were hand cut into thin transverse section using 7 O'Clock platinum blades and were stained with safranine. Microsccopic features were photographed using Nikon ECLIPSE E200 trinocular microscope attached with Zeiss AxioCam ERc5s digital camera under bright field. The powdered sample was sieved through #80 mesh and a pinch of it was mounted on a microscopic slide with a drop of glycerin-water. Characters were observed using Nikon ECLIPSE E200 trinocular microscope attached with Zeiss AxioCam ERc5s digital camera under bright field. Diagnostic characters were captured, and photomicrographs were documented. Magnifications of the figures are indicated using pre-calibrated scale-bars.

Physico-chemical analysis

All the physico-chemical parameters were performed according to the method mentioned in standard books.[13]

Sample preparation for HPTLC

Exactly 2 g of coarse powder was taken in a Soxhlet apparatus and extracted with 100 ml each of *n*-hexane, chloroform and ethanol successively. These extracts were filtered, concentrated over water bath and made up to 10 ml with the corresponding solvents in standard flasks. The extracts were filled in separate sample vials of ATS 4 (CAMAG, Switzerland) for application.

Chemicals, solvents and materials

AR grade solvents *n*-hexane, chloroform, ethyl acetate, ethanol and formic acid were purchased from Merck. For visualizing purpose vanillin (1 g) sulphuric acid in ethanol (5%) solution (VSA) was used. For HPTLC, automatic sampler 4, twin trough chamber 10 × 10 cm, TLC visualizer, TLC scanner 4, TLC plate heater (all from CAMAG, Switzerland) were used.

15 and 20 μl of each extract were spotted using ATS 4 on silica gel $60F_{254}$ coated aluminium plates (6 × 10 cm) as 8 mm band width with 15 mm inter band distance. First application position was 15 mm and distance from the bottom of the plate was 10 mm. Plates were developed using solvent systems with *n*-hexane - ethyl acetate - formic acid (7.5:2.5:0.5, v/v/v), toluene - ethyl acetate - formic acid (6.5:4.0:0.5, v/v/v) and chloroform - methanol - formic acid (9:1: 0.8, v/v/v) for *n*-hexane, chloroform and ethanol extract respectively. The twin trough chamber was previously saturated for 15 min with the above mobile phases prior to development. The developed plates were dried, and photographs were taken by the visualizer under short UV and long UV. Scanning were performed by TLC Scanner 4 (Scanner_210441) under λ 254 and λ 366 nm in absorbance mode (D_2 lamp) and fluorescence mode (Hg lamp) respectively with a slit dimension 6×0.45 mm with scanning speed 20 mm/s. The scanned plates were dipped in VSA and heated over TLC plate heater at 105° C until the appearance of the coloured spots. Photographs were taken immediately at white light mode followed by scanning at λ 520 nm at absorption mode (W lamp).

1.1 Dried fruits **1.2** Fruits enlarged

Plate 1: Macroscopy *Cichorium intybus* L. fruit

RESULT AND DISCUSSION

Macroscopy

Macroscopically, the fruit of C. *intybus* is creamish - brown in colour, measures up to 3 to 3.5 mm in length and about 1.25 mm in breadth; oblong in shape pointed towards base and broader towards upper side; topped with scaly pappus derived from accrescent calyx; surface smooth and shining and comprises of about 8 ridges (Plate 1); odour and taste nil.

Microscopy

The pericarp of fruit consists of a single epidermal layer which bears randomly distributed groups of unicellular papillose trichomes; the outer mesocarp shows 3 to 4 layers of sclerenchymatous cells arranged beneath the epidermal cells while bulk of sclerenchymatous cells arranged under elevated areas giving the appearance of ridges and furrows; the inner mesocarp is made up of large compactly arranged columnar cells followed by thin layer compressed cells forming endocarp enclosing two cotyledons. Endocarp and testa are fused and cannot be differentiated as the fruit is an achene. The cotyledon has a thin walled parenchymatous epidermis enclosing parenchyma cells containing globoid aluerone grains (Plate 2).

Powder microscopy

Powder shows polygonal epicarp cells in surface and oblique views; pitted as well as normal parenchyma; parenchyma containing oil globules; cells of cotyledons containing aleurone grains; bundle of fibre and pitted vessels (Plate 3); odor and taste nil.

Physico-chemical analysis

Moisture plays animportant role in deterioration of a drug due to microbial load leading to decline in shelf life. The loss on drying value which indicates the presence of moisture content of air dried sample was found to be 3.43 %. Total ash which indicate the total inorganic content including physiological and non-physiological salts of the drug was estimated to be as 9.98 %. Water soluble ash which predicts the water soluble inorganic salts was calculated as 1.93 %. The acid insoluble ash, majorly composed of silica in a plant drug, was found to be 1.60 %. The water and ethanol soluble extractive value which indicate the solubility of the active ingredients of the plant in water and ethanol respectively were estimated to be 9.85 % and 11.25 %. The pH of the drug was determined as 5.86 which indicates the acidic nature of the plant (Table 1).

TLC Photo documentation

TLC finger print profile of *n*-hexane extract of *Cichorium intybus* L. fruits revealed 6 bands with R_f 0.18, 0.29, 0.37, 0.49, 0.58 and 0.75 (green) under short UV; 6 spots with R_f 0.23, 0.29, 0.36 (red), 0.42 (fluorescent blue), 0.55 and 0.63 (blue) under long UV; 8 spots with R_f 0.06, 0.14 (violet), 0.17 (ash), 0.32 (violet), 0.45, 0.53, 0.75 and 0.82 (purple) under white light (post derivatization) (Figure 1). Successive chloroform extract showed 6 bands with R_f 0.10, 0.21, 0.32, 0.51, 0.73 and 0.79 (green) under short UV; 7 bands with R_f 0.14, 0.21, 0.28 (blue), 0.32 (red), 0.53

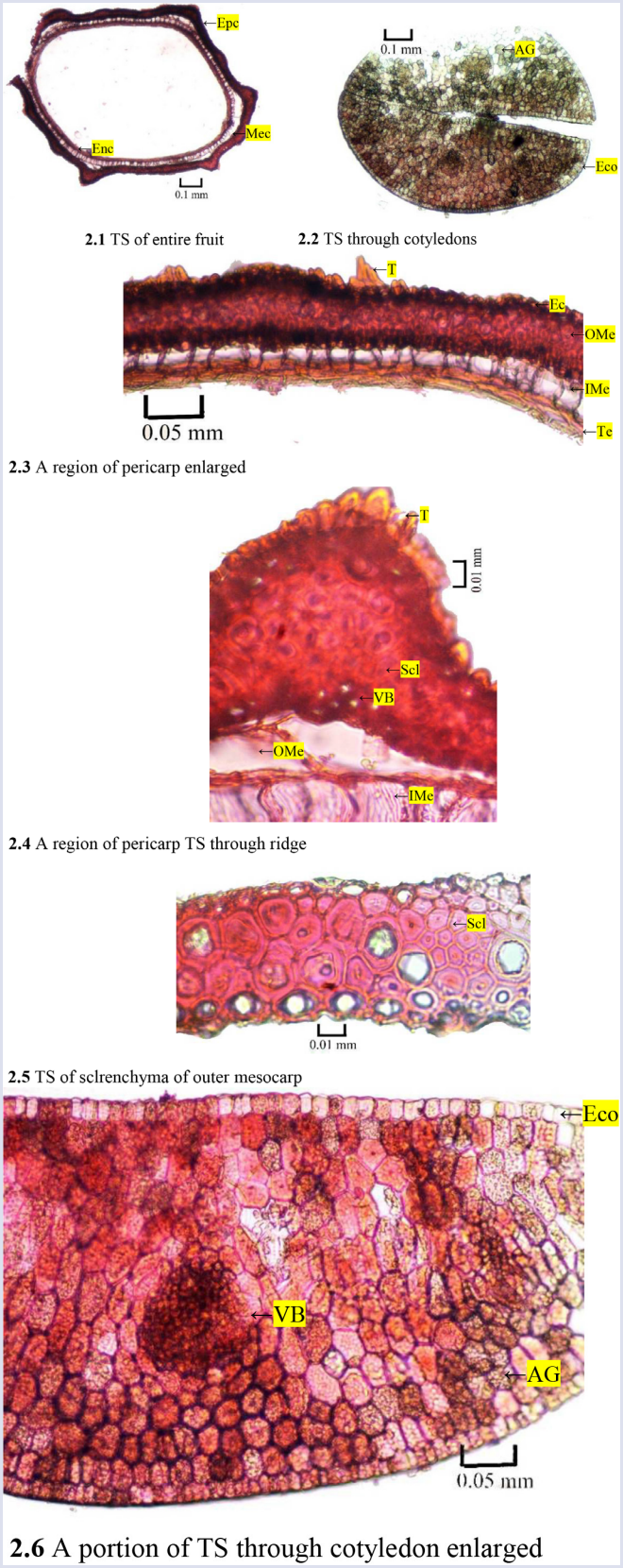


Plate 2: Microscopy *Cichorium intybus* fruit.
AG - Aleurone grains; Ec - Epicarp; Eco - Epidermis of cotyledon; Enc - Endocarp; IMe - Inner mesocarp; Mec - Mesocarp; OMe - Outer mesocarp; Scl - Sclerenchyma; Te - Testa; T - Trichome; VB - Vascular bundle.

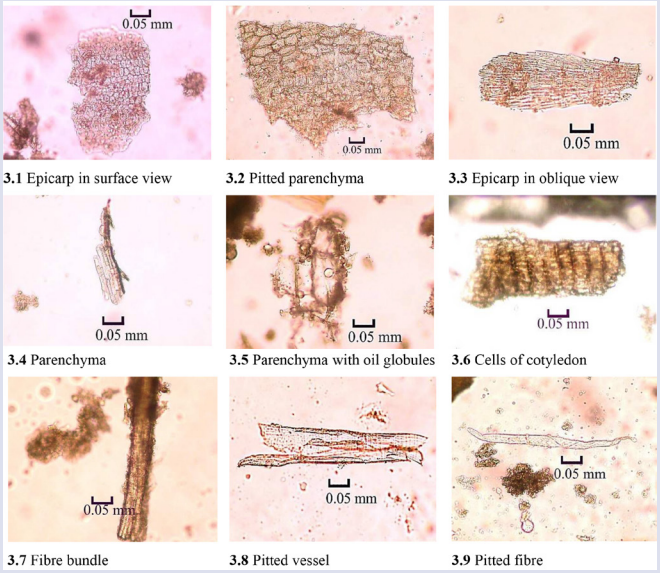


Plate 3: Microscopy of powder of *Cichorium intybus* fruit

Table 1: Physico-chemical constants of *Cichorium intybus* fruits.

Parameter	Result
Loss on drying at 105^0 C	3.43 %
Total ash	9.98 %
Water soluble ash	1.93 %
Acid insoluble ash	1.60 %
Water soluble extractive	9.85 %
Ethanol soluble extractive	11.25 %
pH	5.86
Successive Extraction	
n-Hexane	1.70 %
Chloroform	1.25 %
Ethanol	3.25 %

(violet), 0.64 and 0.73 (red) under long UV; 10 spots with R_f 0.07 , 0.09 (violet), 0.14 (grey), 0.16, 0.37 , 0.54 (purple), 0.61, 0.70, 0.75 and 0.81 (violet) under white light (post derivatization) (Figure 2). In case of successive ethanol extract of 6 bands with R_f 0.04, 0.11, 0.22, 0.37, 0.53, 0.85 (green) were found under short UV, 6 spots with R_f 0.11, 0.34 (blue), 0.40 (fluorescent blue), 0.58 (blue), 0.82 and 0.90 (red) were seen under long UV and 9 bands with R_f 0.18 (grey), 0.21, 0.36 (violet), 0.49 (brown), 0.53, 0.66, 0.74, 0.79 and 0.93 (violet) were found under white light (post derivatization) (Figure 3). The R_f values and corresponding colours of the bands are enlisted in for *n*-hexane, chloroform and ethanol extract respectively (Table 2).

HPTLC denistometric scan

The fingerprint profile of *n*-hexane extract under λ 254 nm revealed that the major peak is R_f 0.58 with an area of 18.52% followed by the peaks at R_f 0.29 (17.38 %), R_f 0.37 (9.39%) and 9 more minor peaks (Figure 4.1); under λ 366 nm, the major peak appeared at R_f 0.36 with an area 32.01%, second major peak at R_f 0.42 with an area 23.77% followed by R_f 0.29 with an area of 13.72% along with 8 more peaks (Figure 4.2); under white light after derivatization, the peak at R_f 0.75 with an area 25.22%

Table 2: R_f **values and color of spots of extracts of *Cichorium intybus* fruits.**

Name of the extract	UV 254 nm		UV 366 nm		White light after derivatization	
	R_f	Colour	R_f	Colour	R_f	Colour
n-Hexane	Green	0.18	Blue	0.06	Violet	0.06
	Green	0.27	Blue	0.09	Violet	0.11
	Green	0.31	Red	0.22	Violet	0.13
	Green	0.35	Red	0.28	Grey	0.17
	Green	0.48	Red	0.35	Violet	0.30
	Green	0.60	F Blue	0.41	Purple	0.44
	Green	0.73	Blue	0.53	Purple	0.54
			Blue	0.62	Purple	0.74
					Purple	0.83
Chloroform	Green	0.10	Blue	0.13	Violet	0.06
	Green	0.21	Blue	0.21	Violet	0.09
	Green	0.32	Blue	0.28	Grey	0.14
	Green	0.51	Red	0.32	Grey	0.16
	Green	0.73	Violet	0.52	Purple	0.37
	Green	0.80	Red	0.64	Purple	0.53
			Red	0.74	Violet	0.62
					Violet	0.69
					Violet	0.75
					Violet	0.82
Ethanol	Green	0.05	Blue	0.11	Grey	0.19
	Green	0.12	Blue	0.34	Violet	0.22
	Green	0.20	F Blue	0.42	Violet	0.36
	Green	0.36	Blue	0.60	Brown	0.50
	Green	0.55	Red	0.68	Violet	0.53
	Green	0.86	Red	0.85	Violet	0.66
	Green	0.93	Red	0.92	Violet	0.74
					Violet	0.80
					Violet	0.94

F - Fluorescent

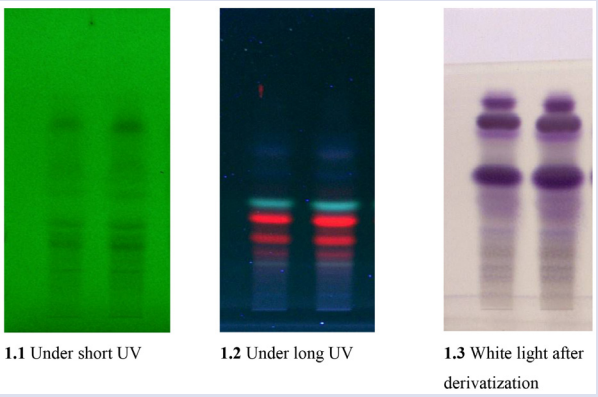


Figure 1: Photo documentation of n-hexane extract of *Cichorium intybus* fruit.

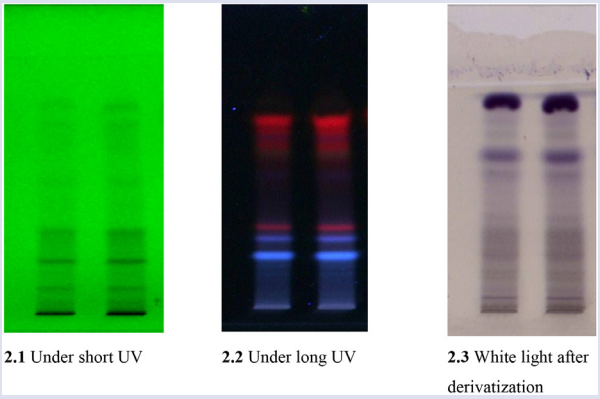
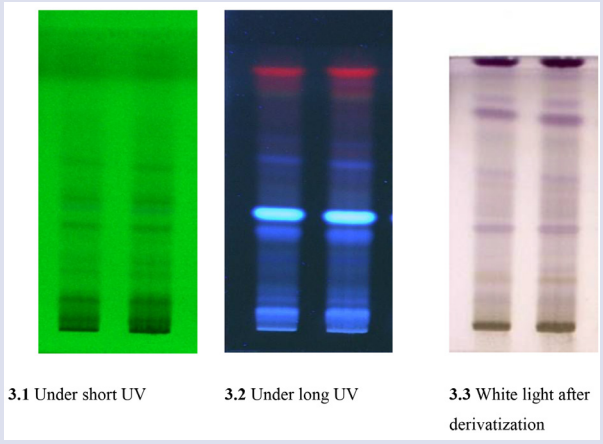
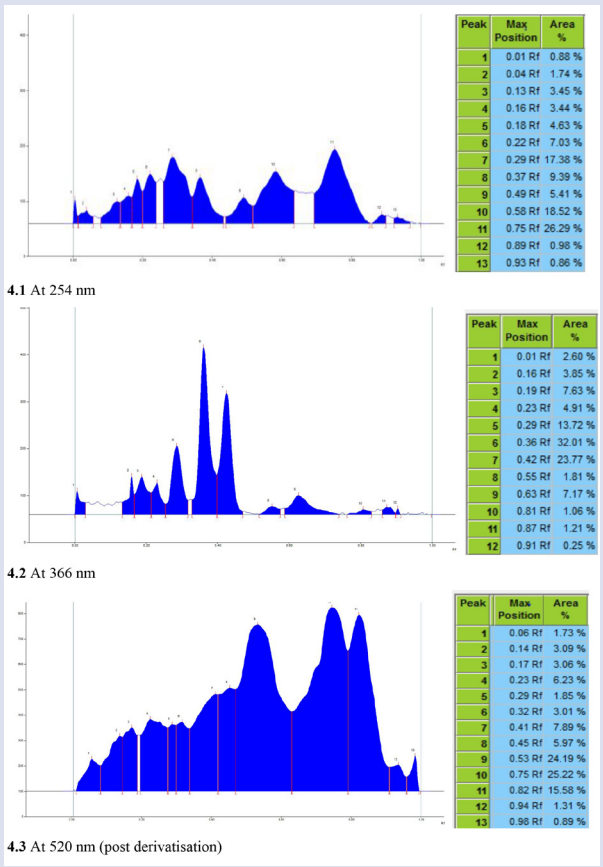


Figure 2: Photo documentation of chloroform extract of *Cichorium intybus* L. fruit.



3.1 Under short UV **3.2** Under long UV **3.3** White light after derivatization

Figure 3: Photo documentation of ethanol extract of *Cichorium intybus* L. fruit.



Peak	Max Position	Area %
1	0.01 Rf	0.88 %
2	0.04 Rf	1.74 %
3	0.13 Rf	3.45 %
4	0.16 Rf	3.44 %
5	0.18 Rf	4.63 %
6	0.22 Rf	7.03 %
7	0.29 Rf	17.38 %
8	0.37 Rf	9.39 %
9	0.49 Rf	5.41 %
10	0.58 Rf	18.52 %
11	0.75 Rf	26.29 %
12	0.89 Rf	0.98 %
13	0.93 Rf	0.86 %

4.1 At 254 nm

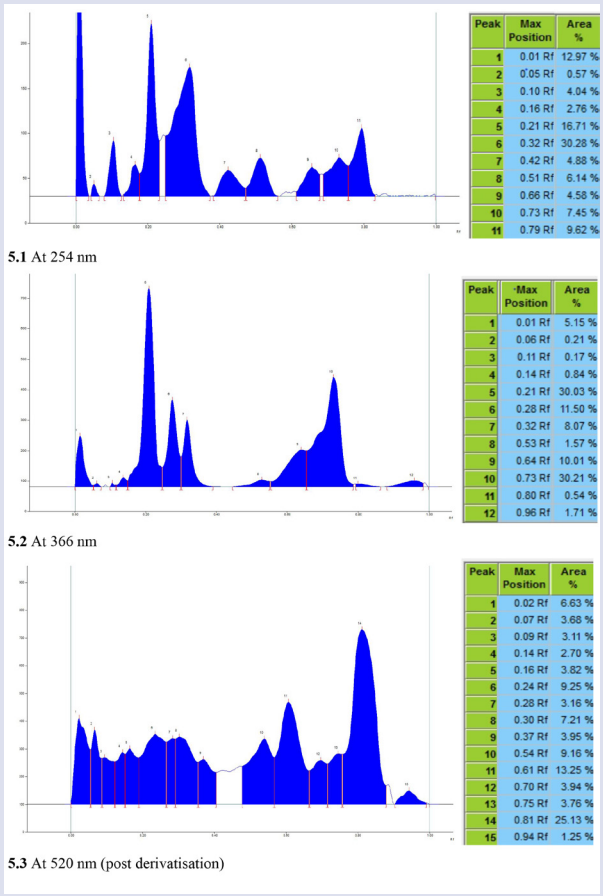
Peak	Max Position	Area %
1	0.01 Rf	2.60 %
2	0.16 Rf	3.85 %
3	0.19 Rf	7.63 %
4	0.23 Rf	4.91 %
5	0.29 Rf	13.72 %
6	0.36 Rf	32.01 %
7	0.42 Rf	23.77 %
8	0.55 Rf	1.81 %
9	0.63 Rf	7.17 %
10	0.81 Rf	1.06 %
11	0.87 Rf	1.21 %
12	0.91 Rf	0.25 %

4.2 At 366 nm

Peak	Max Position	Area %
1	0.06 Rf	1.73 %
2	0.14 Rf	3.09 %
3	0.17 Rf	3.06 %
4	0.23 Rf	6.23 %
5	0.29 Rf	1.85 %
6	0.32 Rf	3.01 %
7	0.41 Rf	7.89 %
8	0.45 Rf	5.97 %
9	0.53 Rf	24.19 %
10	0.75 Rf	25.22 %
11	0.82 Rf	15.58 %
12	0.94 Rf	1.31 %
13	0.98 Rf	0.89 %

4.3 At 520 nm (post derivatisation)

Figure 4: Densitometric scan of n-hexane extract of *Cichorium intybus* fruit.



Peak	Max Position	Area %
1	0.01 Rf	12.97 %
2	0.05 Rf	0.57 %
3	0.10 Rf	4.04 %
4	0.16 Rf	2.76 %
5	0.21 Rf	16.71 %
6	0.32 Rf	30.28 %
7	0.42 Rf	4.88 %
8	0.51 Rf	6.14 %
9	0.66 Rf	4.58 %
10	0.73 Rf	7.45 %
11	0.79 Rf	9.62 %

5.1 At 254 nm

Peak	Max Position	Area %
1	0.01 Rf	5.15 %
2	0.06 Rf	0.21 %
3	0.11 Rf	0.17 %
4	0.14 Rf	0.84 %
5	0.21 Rf	30.03 %
6	0.28 Rf	11.50 %
7	0.32 Rf	8.07 %
8	0.53 Rf	1.57 %
9	0.64 Rf	10.01 %
10	0.73 Rf	30.21 %
11	0.80 Rf	0.54 %
12	0.96 Rf	1.71 %

5.2 At 366 nm

Peak	Max Position	Area %
1	0.02 Rf	6.63 %
2	0.07 Rf	3.68 %
3	0.09 Rf	3.11 %
4	0.14 Rf	2.70 %
5	0.16 Rf	3.82 %
6	0.24 Rf	9.25 %
7	0.28 Rf	3.16 %
8	0.30 Rf	7.21 %
9	0.37 Rf	3.95 %
10	0.54 Rf	9.16 %
11	0.61 Rf	13.25 %
12	0.70 Rf	3.94 %
13	0.75 Rf	3.76 %
14	0.81 Rf	25.13 %
15	0.94 Rf	1.25 %

5.3 At 520 nm (post derivatisation)

Figure 5: Densitometric scan of chloroform extract of *Cichorium intybus* fruit.

came out as the major followed by the peak at R_f 0.53 (24.19%) and R_f 0.82 15.58 (15.58%) along with 9 more minor peaks (Figure 4.3). The finger print profile of successive chloroform extract showed the major peak at R_f 0.32 with an area of 30.28% followed by R_f 0.21 (16.71%), R_f 0.79 (9.62%) along with 7 more minor peaks (Figure 5.1) under λ 254 nm; under λ 366 nm the major peak appeared at R_f 0.73 with an area 30.21% followed by the peak at R_f 0.21 with an area 30.03% along with 9 more peaks (Figure 5.2); under white light the fingerprint profile showed the major peak at R_f 0.81 with an area 25.13%, whereas the peak having R_f 0.61 with an area 13.25% was second major and the peak having R_f 0.24 with an area 9.25% was the third major peak. It also showed 11 more minor peaks (Figure 5.3). The finger print profile of successive ethanol extract under λ 254 nm showed the major peak at R_f 0.85 with an area of 15.67% followed by R_f 0.90 (12.67%), R_f 0.42 (12.03%) along with 10 more minor peaks (Figure 6.1); under λ 366 nm, the major peak appeared at R_f 0.40 with an area 53.75% followed by the peak at R_f 0.34 with an area 15.19% along with 7 more peaks (Figure 6.2); under white light, the fingerprint profile showed the major peak at R_f 0.93 with an area 26.87%, whereas the peak having R_f 0.74 with an area 15.00 % was second major and the peak having R_f 0.89 with an area 11.52 % was the third major peak; it showed 12 more minor peaks (Figure 6.3).

CONCLUSION

Cichorium intybus fruit (chicory seed) is an important ingredient of several Ayurveda, Siddha, Unani and other traditional formulations for the treatment of various ailments. The current attempt of preparation of monograph including macro-microscopy, powder microscopy, physico-chemical standardisation, TLC finger print profiles will help to check the quality of the drug prior to its use in traditional medicine.

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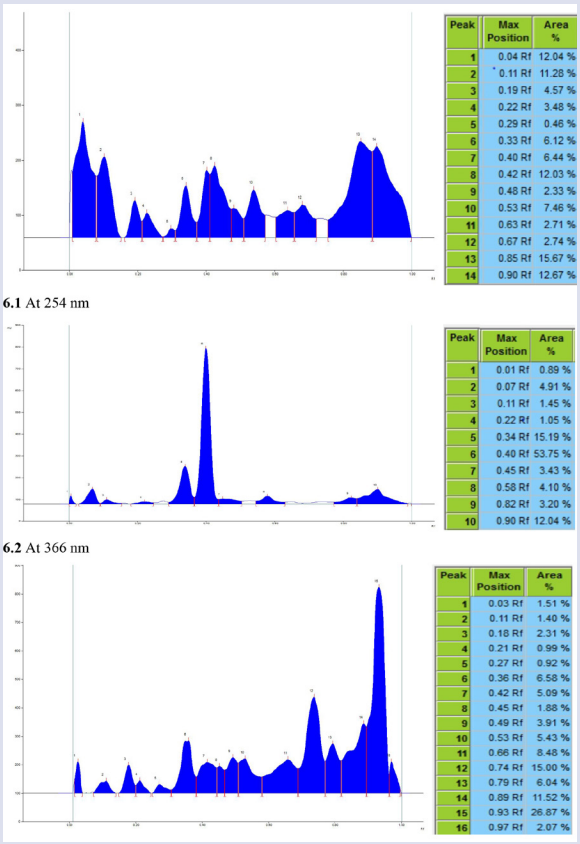

Figure 6: Densitometric scan of ethanol extract of *Cichorium intybus* fruit.

CONFLICT OF INTEREST

We declare no conflict interest with any.

ABBREVIATIONS

AG: Aleurone grains; **Ec:** Epicarp; **Eco:** Epidermis of cotyledon; **Enc:** Endocarp; **IMe:** Inner mesocarp; **Mec:** Mesocarp; **OMe:** Outer mesocarp; **Scl:** Sclerenchyma; **Te:** Testa; **T:** Trichome; **VB:** Vascular bundle.

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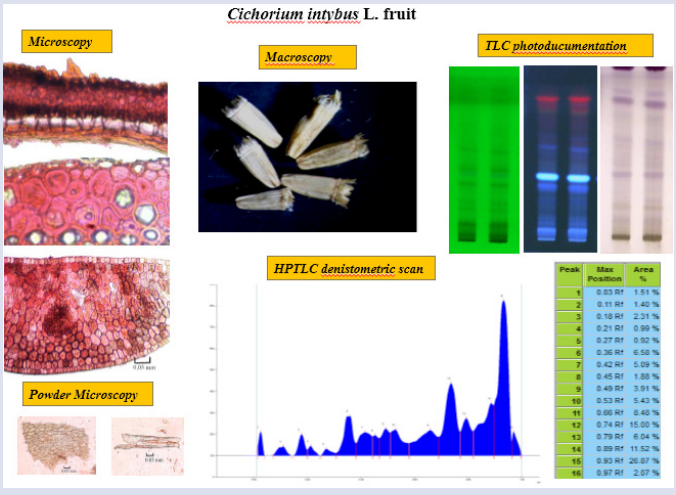
GRAPHICAL ABSTRACT



SUMMARY

- *Cichorium intybus* fruit (chicory seed) is an important ingredient of several Ayurveda, Siddha, Unani and other traditional formulations for the treatment of various ailments.
- The current attempt of preparation of monograph including macro-microscopy, powder microscopy, physico-chemical standardisation, TLC finger print profiles will help to check the quality of the drug prior to its use in traditional medicine.

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