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STANDARDIZATION OF *ERUCA SATIVA* LINN (SEEDS) USING PHARMACOPOEIAL AND HPTLC METHODS

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ABSTRACT

The seeds of *Eruca sativa* L. has the high therapeutic values such as teeth and gum tonic, emetic, spermatic tonic, diuretic, Emmenagogue and deobstruent. The seed contains gluconcasturtin, (4-Me-thio)-Bu-glucosinolate and erucic acid phyto-constituents which are responsible for therapeutic uses. Pharmacognostical studies revealed presence of mucilage, palisade like elongated parenchyma cells and cotyledonary parenchyma cells.TLC/HPTLC study revealed the presence of various types of phytoconstituents at different R_f values.

KEYWORDS: *Eruca sativa*, Microscopy, TLC/HPTLC.

INTRODUCTION

Eruca sativa L. is also called as *Eruca vesicaria*. Subsp. *Sativa* (Miller) Thell., *Brassica eruca* L., is an edible annual plant commonly known as salad rocket. It belongs to the family of Brassicaceae. The plant is an erect branched, glabrous, more or less hispid herb of 60 to 65 cm; it is a salad vegetable, native to the Mediterranean region from Morocco and Portugal in the west of Syria, Lebanon and Turkey in the East. It is also cultivated as field crop in many parts of India. *Eruca sativa* L. is traditionally used in the ailments of teeth, diuretics, aphrodisiac, Emmenagogue, deobstruent and in anemia.[11] In Unani system of medicine the seeds of *Eruca sativa* are used in the preparation of different types of formulations such as Jawarish-e-Atai, Safoof-e-Maleh, Sufoof-e-Moya, Habb-e-Jalinoos, Lubub-e- Sagheer, Majoon-e-Pambdana and Habb-e-Khabs-ul-Hadeed.[4] The microscopical features have not been reported yet as well as very few reports are available on physicochemical parameter studies. The present study is aimed to standardize the seeds of *Eruca sativa* using Pharmacopoeial and HPTLC methods.

MATERIAL AND METHODS

The seeds of *Eruca sativa* were collected from raw drug dealer R.N. Rajan & Co. Pvt., Limited, Chennai in the month of April. The seeds were identified and authenticated in the Department of Drug standardization Research Unit, Chennai by the Pharmacognosist, Dr Ms. S. Mageswari. Specimen of seeds was deposited in the Survey Unit of Medicinal Plants, Regional Research Institute of Unani Medicine, Chennai (Herbarium No. SMPU -3918).

(i) Processing of plant material for studies

The seeds of *Eruca sativa* L. were manually washed with the help of distilled water and the residual moisture evaporated at room temperature. The seeds were shade dried at room temperature, ground coarsely after drying and used for various analyses.[2,3]

(ii) Chemicals and Instruments

High Performance Thin Layer Chromatography, digital PH meter, digital flask shaker, Fluorescence microscope, camera Lucida, stage and eye piece micrometer, glass slides, cover slips, watch glass and other common glassware were the basic apparatus and instruments used for the study. Photographs in different magnification of all the necessary cells and tissue were taken with Nikon lab. Photo-2 microscopic unit. Some crystals, starch grains and lignified wall photographs were taken under polarized light microscope.

Solvents like chloroform, ethanol and reagents such as phloroglucinol, glycerin, HCl and sodium hydroxide procured were Merk grade. To take the fingerprint and chromatogram the instrument HPTLC Cammag make was used.

Pharmacognostical evaluation

(i) Exomorphology

The fresh seeds were collected and speared on a Petri dish and the different types of features were observed using magnifying glass and ruler.

(ii) Macro and microscopic studies

Macroscopic examination of the seed was carried out according to standard procedure.[5] Fresh seeds were collected for microscopial studies. Sections were taken through a microtome and free hand sections. Numerous temporary and permanent mount of the microscopial sections of seed specimen were made and examined using microscope.

Photomicrographs of the microscopial sections were captured with the help of Olympus fluorescence microscope.

RESULT AND DISCUSSION

Pharnacognostical studies

(i) Macroscopic

Seeds were observed in reddish orange colour, ellipsoidal minute about 3.5mm and 2mm wide, seed surface is smooth and mucilaginous, odour pungent and bitter in taste like mustard oil **(Fig. 1 & 2).**[6,7]

(ii) Microscopic

T. S. of seed shows outer seed coat consisting of single layer of thin walled cuticularised epidermis filled with mucilage **(Fig. 3 & 4)** (white coat on the surface of the soaked seed) followed by a single layer of thin walled hypodermal cells; palisade like cells consisting of single layer of thick walled elongated cells filled with reddish brown contents; **(Fig. 5 & 6)** inner seed coat consisting of single layer of less thickened cells with cell contents;[8] cotyledons large consisting of oval to polygonal moderately thick walled parenchyma cells filled with aleurone grains and oil. **(Fig. 7 & 8).**

(iii) Powder microscopy

The seeds were dried in shades and finally powdered and passed through sieves no. 125 and 180, separately to obtained fine and very fine powder respectively and then subjected for microscopic examination. The powdered drug was separately treated with phloroglucinol, hydrochloric acid solution, glycerin and iodine solution to determine the presence of lignified cells, calcium oxalate crystals and starch grains.[9,13]

(iv) Physio-chemical parameters

The Total ash, Acid insoluble ash and water soluble ash, loss on drying, foreign matter, were determined as per standard methods.[1] The different extractive values were determined to find out the amount of soluble components in suitable solvents such as water and alcohol.[10] **(Table 1)**

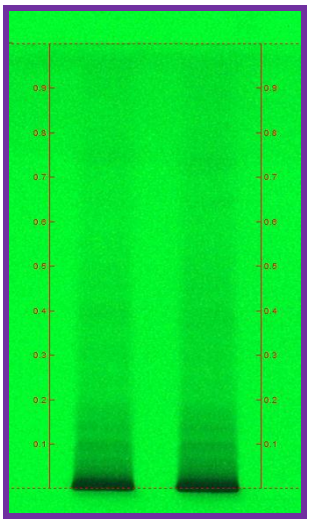
Table 1: Physio-chemical parameters.

S. No.	Parameters	Values	
		Chennai	New Delhi
1.	Foreign matter	Nil	Nil
2.	Loss on drying at 105°C	10.21 % 10.24 % 10.35 %	9.79% 9.85% 10.05%
3.	Total ash	5.03 % 5.21 % 5.32 %	4.96% 5.08% 5.10%
4.	Acid insoluble ash	1.52 % 1.75 % 1.89 %	1.72 % 1.78% 1.85%
5.	Alcohol soluble extractive	1.82 % 1.89 % 1.97 %	1.85% 1.88% 1.92%
6.	Water soluble extractive	9.08 % 9.16 % 9.28 %	9.05% 9.10% 9.19%

(v) HPTLC

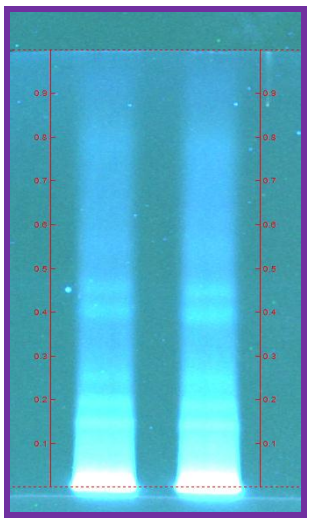
HPTLC profile of seeds of the extracts of chloroform and ethanol was carried out as per standard methods. High Performance Thin Layer Chromatography was performed on 10 cm × 10 cm TLC plates pre-coated with 0.25 µm thin layers of silica gel 60 F_{254} (E. Merck).[12] Both the extracts of chloroform and ethanol were applied on different plates as bands 10 mm wide by using of an applicator (Linomat V) fitted with a 100 µL syringe. The application positions X and Y were both 10 mm, to avoid edge effects. Linear ascending development to a distance of 80 mm with *Toluene: Ethyl acetate*: *9: 1 (v/v)* as mobile phase for chloroform extract was performed in a twin-trough glass chamber (20 cm × 10 cm) previously saturated with vapors of mobile phase for 20 min. The plates were dried in air and visualized under 254 nm and 366 nm for ultra violet detection and taken the fingerprints as evident in **Fig. 9–10**. Further, the same TLC plate was derivatized with vanillin-sulphuric acid reagent and visualized in white light obtained fingerprints were as evident in **Fig. 11**. The Chromatograph of chloroform extract of *Eruca sativa* at 254 nm is given in **Fig. 12**. The alcoholic extract of drug was performed similarly with the mobile phases of *Toluene: Ethyl acetate 1: 1 (v/v)* and then visualized in 254 nm, 366 nm and white light using **Fig. 13-15**. The Chromatograph of alcohol extract of *Eruca sativa* at 254 nm is given in **Fig. 16**. The Rf values of both Chloroform and Alcoholic extracts at 254nm, 366nm and white light are given in **Table 2.**

Chloroform extract



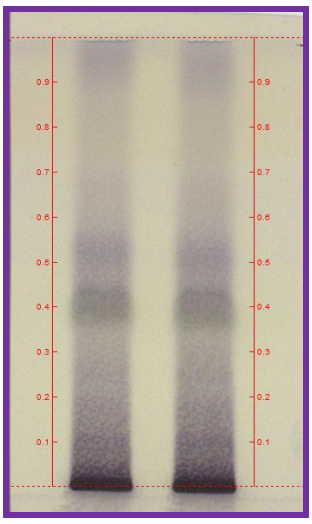
A B

Figure 9



A B

Figure 10

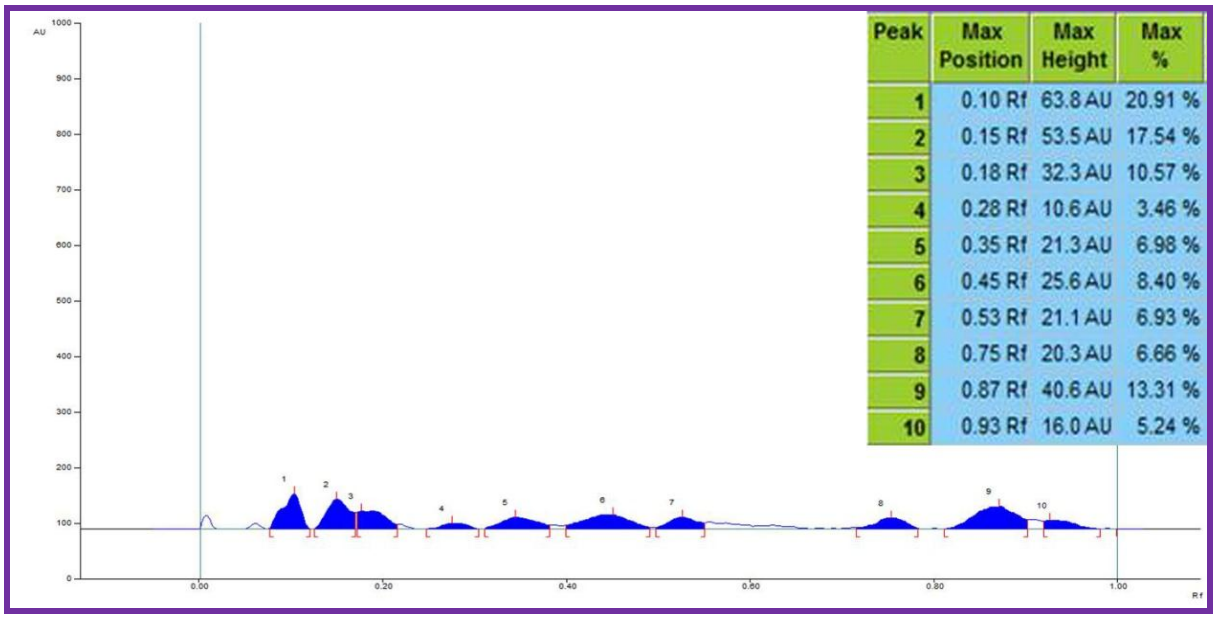


A B

Figure 11

UV - 254 nm **UV - 366 nm** **V - S Reagent**

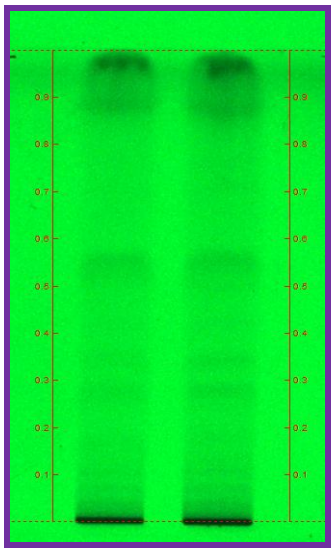
Solvent System : Toluene :Ethyl acetate (9 : 1)



Peak	Max Position	Max Height	Max %
1	0.10 Rf	63.8 AU	20.91 %
2	0.15 Rf	53.5 AU	17.54 %
3	0.18 Rf	32.3 AU	10.57 %
4	0.28 Rf	10.6 AU	3.46 %
5	0.35 Rf	21.3 AU	6.98 %
6	0.45 Rf	25.6 AU	8.40 %
7	0.53 Rf	21.1 AU	6.93 %
8	0.75 Rf	20.3 AU	6.66 %
9	0.87 Rf	40.6 AU	13.31 %
10	0.93 Rf	16.0 AU	5.24 %

Figure 12: HPTLC finger print profile of chloroform extract of *Eruca sativa* at 254 nm.

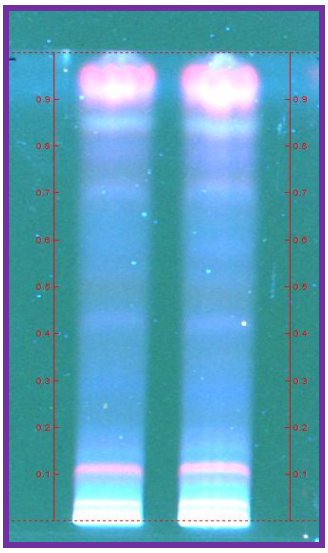
Alcohol Extract



A B

Figure 13

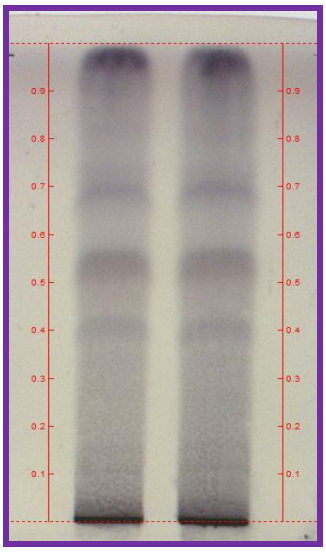
UV - 254 nm



A B

Figure 14

UV - 366 nm



A B

Figure 15

V - S Reagent

Solvent System : Toluene :Ethyl acetate (1 : 1)

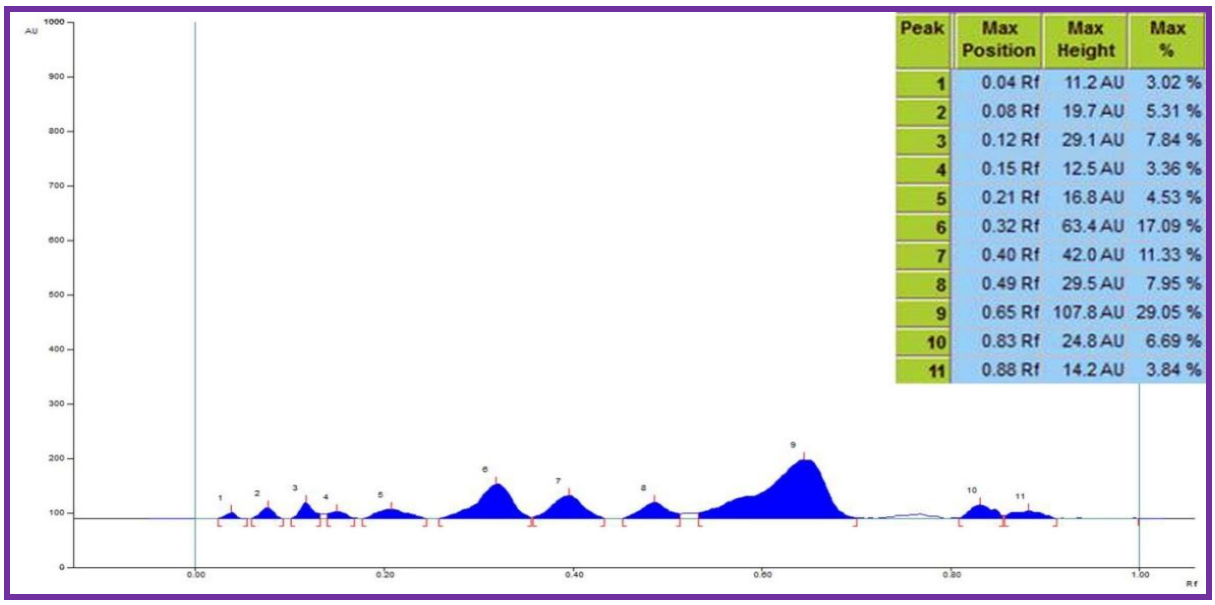


Figure 16: HPTLC finger print profile of alcohol extract of *Eruca sativa* at 254 nm.

Table 2: Rf value of phyto-chemicals present in chloroform and ethanol extract of *Eruca sativa* at different wave-lengths.

Wave-length(nm)	Chloroform extract	Ethanol extract
254	0.86, 0.78, 0.68, 0.52, 0.49, 0.37, 0.34, 0.25 and 0.15 (Green)	0.88, 0.70, 0.55, 0.35, 0.27, 0.17 and 0.10 (Green)
366	0.87, 0.75 (Blue), 0.69 (Pink), 0.57 (Blue), 0.55, 0.49 (Pink), 0.44, 0.40 (Blue), 0.35 (Pink),	0.91 (Pink), 0.84, 0.70, 0.42 (Blue) and 0.11 (Pink)

	0.25 (Blue), 0.22 (Pink) and 0.13 (Blue)	
Visible light after derivatization in vanillin-sulphuric acid reagent	0.82 (Grey), 0.72 (Pink), 0.64, 0.56, 0.42, 0.36 (Grey), 0.14 (Blue) and 0.11 (Grey)	0.83 (Grey), 0.70, 0.55, 0.42 (Violet), 0.15 and 0.11(Grey).

***Eruca sativa* L.(Seeds)**

Seed – Surface view

Figure-1

Single seed enlarged

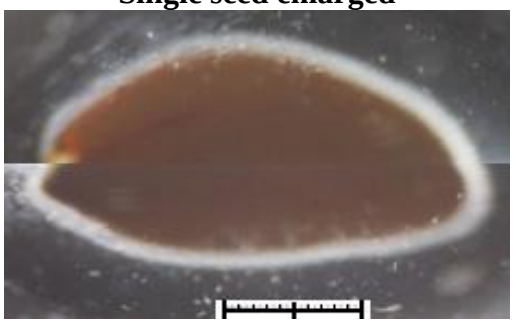


Figure-2

T. S. of Seed

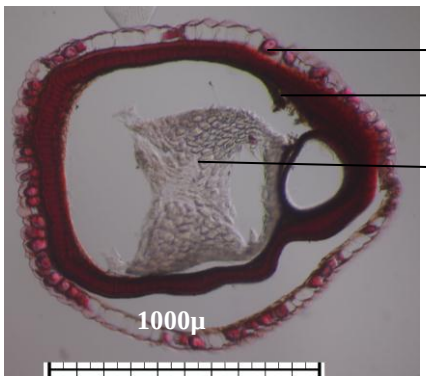


Figure-3

T. S. of Seed Coat

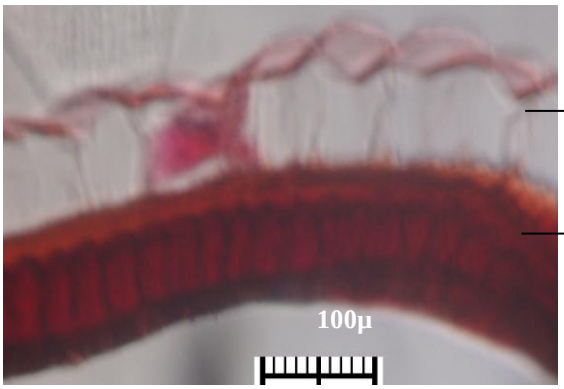


Figure-4

***Eruca sativa* L. (Powder Microscopy)**

Palisade like thick walled parenchyma cells

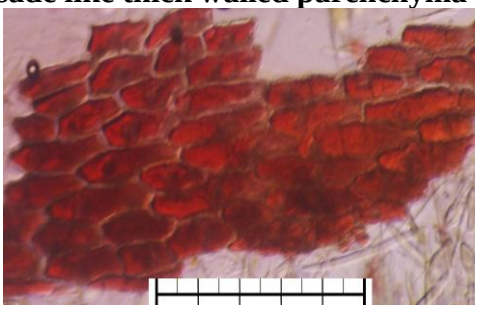


Figure-5

Cotyledonary parenchyma

Figure-6

Mucilaginous epidermal cells

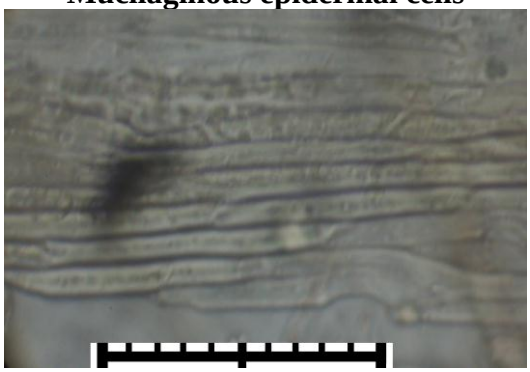


Figure-7

Thin walled parenchyma cells

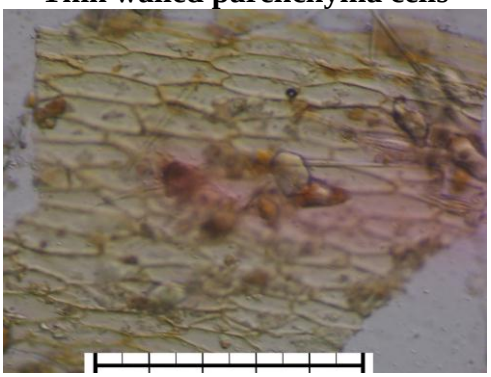


Figure-8

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