

DRUG STANDARDIZATION

Standardization studies on *Cuscuta reflexa* Roxb. – A new homoeopathic drug

H.C. Gupta¹, P. Subramanian², P. Padma Rao², D.S. Kushwaha¹ and C. Nayak^{1*}

¹Central Council for Research in Homoeopathy, New Delhi, India

²Drug Standardization Unit (H), Hyderabad (Andhra Pradesh), India

The Council has undertaken for the first time homoeopathic pathogenetic trials on *Cuscuta reflexa* Roxb. To establish the drug in the system, the standardization studies are mandatory. The diagnostic macro and microscopical characters of stem are presented. Moisture content, ash and extractive values of crude drug are determined. The preparation of the mother tincture and its physico-chemical characteristics are provided. Thin layer chromatography (TLC) and Ultraviolet-Visible (UV) spectrometry also are undertaken for setting the pharmacopoeial standards of the drug.

Keywords: *Cuscuta reflexa* Roxb.; homoeopathy; stem parasite; standardization

Introduction

Cuscuta reflexa Roxb. (Cuscutaceae) is a leafless twining stem parasite having pale white flowers with reflexed corolla lobes. The herb is found distributed throughout India as a dense yellow wiry mass on trees and shrubs during winter.¹ It is commonly known as Amarvela (Sanskrit), Dodder (English) and Akasbel (Hindi).^{1,2} The medicinal properties of the plant are attributed as purgative, used in flatulence and liver complaints and externally for itch.^{3,4} The parasite is reported to have been used in wash of sores, to be spasmolytic and anti-bacterial.^{5,6} Many chemical constituents have been isolated from *Cuscuta reflexa* such as cuscutin, amarbelin, beta-sterol, stigmasterol, kaempferol, dulcitol, myricetin, quercetin, coumarin and oleanolic acid.⁷

In Homoeopathy, small proving of the drug has been conducted first time by Central Council for Research in Homoeopathy on standard protocol involving double blind placebo controlled design.^{8,9} The symptoms observed during the proving indicate that the drug may be clinically useful in conjunctivitis, stye, cough, coryza, cervical spondylosis and urticarial eruptions. The standardization data on the drug is lacking in the literature. Hence the Council has undertaken

standardization studies to provide quality standards of the drug.

Material and Methods

The authentic material of *Cuscuta reflexa* Roxb. was obtained from Survey of Medicinal Plants & Collection Unit, Emerald, the Nilgiris District (T.N.). The work was carried out at Drug Standardisation Unit, Hyderabad. The material was preserved in F.A.A. and used for anatomical studies following the method given by Wallis.¹⁰ Epidermal peels were taken by scraping with razor blade. Microtome sections were taken, stained and mounted according to standard procedures.¹¹ The representative photomicrographs were taken with the Olympus (CH-2) trinocular microscope. Moisture content, ash value, extractive values of the raw drug, formulation of the mother tincture and its physico-chemical standards were determined as per procedures of Homoeopathic Pharmacopoeia of India.¹² Phytochemical screening and TLC profiling were also carried out by standard procedures.¹³ For UV absorbance, the mother tincture was diluted with methanol and scanned from 200-900 nm on Perkin-Elmer UV spectrophotometer.¹⁴ The peak of maximum absorption (λ_{max}) was recorded.

Observations

Macroscopy: Stem stout, twining, long, 1-3 mm thick, leafless, slender, yellow, glabrous, sometimes dotted with red spots, pungent, slightly bitter.

* Address for correspondence
Director General
Central Council for Research in Homoeopathy,
61-65, Institutional Area, D-Block, Janakpuri, New Delhi –110058.
E-mail: crrh@del3.vsnl.net.in



Fig. 1: Twining stem of *Cuscuta reflexa* on host plant

Microscopy: In surface, epidermis consists of polygonal linear cells; sides straight to curved with a few starch grains. Surface is striated; stomata a few, anomocytic and cyclocytic. Transection of the stem is rounded and slightly wavy in outline. Epidermis is one layered, covered by slightly thick cuticle; cells tabular, oval to oblong, few barrel shaped; walls thickened; contents dense with tannins and starch grains in a few cells. Cortex is 7-10 layered, cells polygonal to spherical, 15-38 μm (average 28) in diameter, walls

thin, contents slightly dense and a few with tannins, starch grains (8-15 μm in diameter) and rarely prismatic crystals. A layer of endodermis often discontinuous with tangentially long, oblong to polygonal cells, often densely filled with starch grains and crystals. The vascular bundles are 12-16 in number arranged in a ring, endarch, conjoint and collateral. The phloem is present towards outside and consists of phloem parenchyma, sieve cells and companion cells. Internal phloem is present in patches.

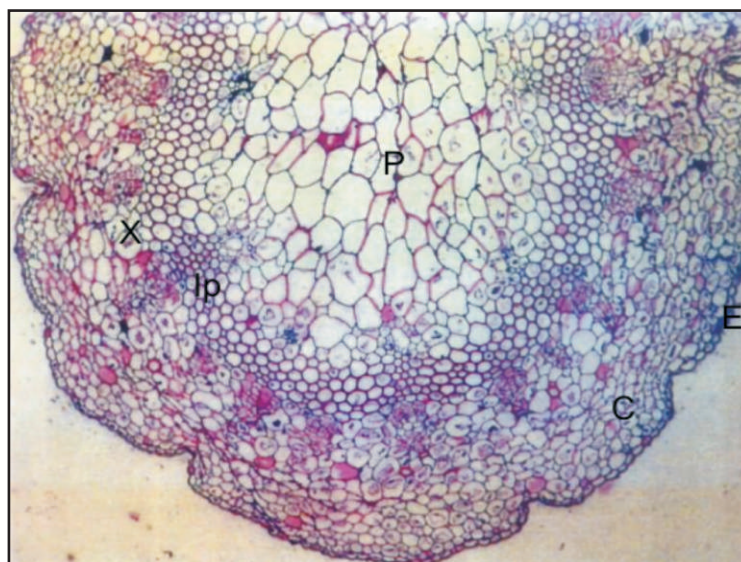


Fig. 2: Transection of Stem x 59

P – Pith; E – Epidermis; C – Cortex; X – Xylem; I – Internal phloem

Vascular cambium is present in some vascular bundles. The xylem is arranged in radial rows with helical, scalariform and annular secondary wall thickenings. Pith is extensive, and consists of large parenchymatous cells.

Powder studies: Greenish brown, slightly bitter without characteristic odour, powder shows fragments of parenchymatous cortical tissue; epidermal tissue

with a few anomocytic stomata; parenchyma with brownish masses, a few prismatic crystals, a few starch grains and pieces of vessels with helical, scalariform thickenings.

Physico-chemical studies

Phytochemical group tests: It indicates the presence of tannins, carbohydrates and alkaloids.

Table: 1 Raw drug standardization

S.No.	Parameters	Quantitative values
1.	Moisture content (at 105° C)	Not more than 17.12% w/w
2.	Total ash	Not more than 5.2% w/w
3.	Acid insoluble ash	Not more than 0.2% w/w
4.	Water soluble ash	Not more than 3.75% w/w
5. Extractive values in:		
a	Hexane	Not less than 0.5% w/w
b	Alcohol	Not less than 6.5% w/w
c	Chloroform	Not less than 1.5% w/w
d	Purified water	Not less than 18.5% w/w
e	Methanol	Not less than 13.3% w/w

Table: 2 Determination of Maximum Extractive Value in different ratio of alcohol

S.No.	% Alcohol	% Ex. value
1.	30	18.75
2.	40	19.75
3.	50	26.0
4.	65	35.6
5.	75	17.9
6.	85	13.5
7.	95	6.5

Preparation of the mother tincture

Mother tincture is prepared as per maximum extractive value in alcohol (65% v/v), by percolation technique (HPI 1971).

Table: 3 Formulation

Drug strength	1/10
<i>Cuscuta reflexa</i> in coarse powder	100 gm
Strong alcohol	683 ml
Purified water	350 ml
To make one thousand millilitres of the mother tincture.	

Table: 4 Standardization of the mother tincture

S.No.	Parameters	Observations
1	Organoleptic properties	
a	Appearance	Clear, non-viscous
b	Colour	Yellowish brown
c	Odour	Pleasant
2	Sediments	Absent
3	Weight / ml	Not more than 0.89 g
4	Total solids	Not less than 2.2 % w/v
5	Alcohol content	62-64 % v/v
6	pH (at R.T.)	4.0-4.5
7	Refractive index (at R.T.)	1.422
8	λ_{\max}	344 nm

Thin layer Chromatography (TLC)

TLC study of chloroform extract of the mother tincture of *Cuscuta reflexa* was carried out on silica gel 'G' plate

using toluene: ethyl acetate (93:7 v/v) and chloroform: methanol (9:1 v/v) as mobile phases. The number of spots obtained, colour and R_f are given as under.

Table: 5 Chromatographic Results

Solvent System	Detecting agent	R_f	Colour
Toluene: ethyl acetate (93:7v/v)	10% Methanolic KOH	0.07	Light brown
		0.15	-do-
		0.21	-do-
		0.38	-do-
Chloroform: methanol (9:1 v/v)	10% Ferric chloride	0.88	Grey
		0.97	-do-

Discussion and Conclusion

The raw drug consist of leafless, yellow, glabrous, twining long stem of 1-3 mm thicknes. Salient microscopical features of stem include epidermis with a few anomocytic and cyclocytic stomata; parenchymatous cortex with tannins, starch grains and rarely prismatic crystals of calcium oxalate; discontinuous endodermis; 12-16 endarch, conjoint and collateral vascular bundles in ring; internal phloem in patches and presence of extensive pith.

Various Physico-chemical constants of raw drug and mother tincture have been determined and presented in tables 1-4. λ -max after UV Spectrometry and R_f values (TLC studies) are also reported in Table 4 & 5.

Above morpho-anatomical characters of raw drug and physico-chemical constants are specific to drug *Cuscuta reflexa*. Thus the studies reported are referential for correct identification and can be taken as standards for raw drug and mother tincture of *Cuscuta reflexa* Roxb. Since the drug is not included in Homoeopathic Pharmacopoeia of India, the data are very useful as Pharmacopoeial standards of the drug in Homoeopathy.

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