

DRUG STANDARDISATION

Standardisation of Homoeopathic drug - *Aquilegia vulgaris* L.

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Abstract : In this present investigation, drug standardization studies are undertaken for quality assurance of a homoeopathic drug *Aquilegia vulgaris*. In Homoeopathy, it has been reported to have clinical indications for clonus hystericus and globus hystericus in women, sleeplessness and dysmenorrhoea. To establish the drug in the system, the standardization studies are mandatory and the useful parts of the plant drug are analyzed according to the standards of Homoeopathic Pharmacopoeia of India. The pharmacognostic and physico-chemical studies of the leaf and petiole are undertaken in present study. The diagnostic macro and microscopical characters of leaf and petiole, moisture content, ash and extractive values of crude drug are presented. The formulation of mother tincture is given. Thin layer chromatography (TLC) and Ultraviolet (UV) spectrometry are also undertaken for setting the pharmacopoeial standards of the drug.

Keywords : *Aquilegia vulgaris* L.; homoeopathy; standardization; pharmacognosy; physico-chemical.

Introduction

Aquilegia vulgaris L. (syn. *A. karelini* Baker) commonly known as 'European Columbine' belonging to family Ranunculaceae, is a perennial herb growing upto 60 cm. It is a native of Europe and also found growing in sub-alpine Himalayas. It is also planted in gardens. The herb is credited with diuretic, diaphoretic, antiscorbutic and tranquillizing properties.¹ In Homoeopathy, the drug was proved by Peters-Marshy: New Mat. Med. Sup. N.A.J. Hom., Feb., 1859 as referred from Bradford T.L. Index of drug proving 1901.² The drug is mentioned in Blackwood's Mat. Med.³, Clarke's Mat. Med.⁴ and Boericke's Mat. Med.⁵ This is a remedy for Clonus hystericus and Globus hystericus in women^{3,4}, sleeplessness, skin troubles, nervous trembling of the body, sensitivity to light and noise and dysmenorrhoea in young girls.⁵ Toxicologically, it is useful in sleep disorders with nervousness and oxidative stress. The flowering plant is poisonous to animals as it contains a cyanogenetic glycoside. An alkaloid, dihydroxydimethoxyaporphine (1.5%) has been isolated from its methanolic extract. The seeds

contain water-soluble proteins of low molecular weight and an alkaloid, aquilegine. The oil is reported to contain a new trienoic acid, ranunculeic acid (5-trans-9,12-cis,cis-octadecatrienoic acid).¹ The aerial parts of the plant are used in Homoeopathy. The drug is not included in Homoeopathic Pharmacopoeia of India but official in German Homoeopathic Pharmacopoeia.⁶ Literature survey reveals that there is no earlier work available pertaining to pharmacognostic and physico-chemical standards for the drug. Hence the present study has been carried out to determine pharmacopoeial standards of raw drug and mother tincture for quality assurance.

Material and methods

The authentic material of *A. vulgaris* was obtained from Survey of Medicinal Plants & Collection Unit, Emerald, Nilgiris District, Tamilnadu (India). The material was preserved in F.A.A. and used for anatomical studies following the method of Wallis.⁷ Epidermal peels have been taken by scraping with razor blade. Microtome sections were taken, stained and mounted as per schedule of Johansen.⁸ Photomicrographs were taken with the Olympus (SC-35) 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.⁹ Phytochemical screening

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and TLC profiling were also carried out by standard procedures.¹⁰⁻¹¹ For UV absorbance, the mother tincture was diluted with methanol and scanned from 200-900 nm on Perkin-Elmer UV spectrophotometer¹². The peak of maximum absorption (I_{max}) was recorded.

Observations

Macroscopy

A hardy perennial herb upto 60 cm in height. Leaves arise in tufts with a petiole which is 5–18 cm long, from a stout rhizome. The leaves are biternate. Leaflets are ovate to obovate and lobed. The fully developed stem leaves are reduced in size and form. Flowers nodding, blue varying to purple, white or pink, bisexual. Fruit, densely pubescent, 2.5 cm long.(Figure-1,2)



Fig.1-Flowering twig



Fig. 2-Complete flower

Microscopy

Leaflet: Epidermal cells are polygonal iso and anisodiametric, sides thin, wavy to sinuate and surface smooth on abaxial side while polygonal isodiametric, sides thick, curved to wavy and surface papillate on adaxial side; hypostomatic with anomocytic and tetracytic stomatal types. Stomata are sunken, subsidiaries 4 or 5 and indistinct; distributed all over, except on veins. Stomatal frequency is 280 per sq mm and Stomatal Index is 12.17 (ave.). The vein islet no. is 14.5 (ave.). The trichomes are unicellular conical, few and confined to lower surface.

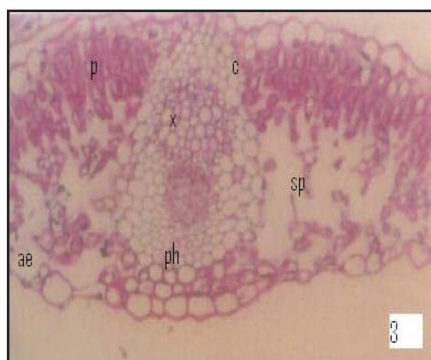


Fig.3 -V.S. of leaf at midvein



Fig.4-V.S. of leaf at margin

Abbreviations

ae	:	aerenchyma
c	:	collenchyma
p	:	palisade
ph	:	phloem
sp	:	spongy
x	:	xylem
m	:	margin
pa	:	papillate epidermis

In V.S. the leaflet at midvein is flat adaxially and ribbed towards abaxial side, 281-302 μ m (291) thick and lamina wings 129-162 μ m (144) thick. The lamina is dorsiventral with rounded margins (Fig. 3 & 4). The epidermis 1-layered with cells on the adaxial side larger, papillate besides, a few flat, barrel shaped, oval to spherical while those on abaxial side smaller. Mesophyll consists of 2-3 layered palisade occupying half of the area of lamina, cells of various shapes, cylindrical, columnar, dumbbell and clavate, 16-30 μ m (23) long and 5.5-14 μ m (9) wide, walls thin, contents dense with dark tannins and chloroplasts. Palisade ratio ranges from 13.3 to 15.3. The spongy parenchyma is loose, 4-6 layered, with large intercellular spaces (Fig. 3 & 4).

The ground tissue of midvein consists of 2-layered collenchyma on abaxial side with angular and lamellar cells often filled with chloroplasts and tannins. Parenchyma is 5-6 layered, cells polygonal, thin walled with dense contents (Fig.3).

The vascular tissue at midvein consists of a single ovate bundle which is endarch, conjoint, collateral and apericyclic. The xylem elements are 40-50 in number, arranged in clusters or radial rows. The secondary walls of xylem elements show helical, scalariform and rarely annular thickenings. Phloem is on abaxial side as a spherical mass. The secondary and tertiary veins also exhibit similar arrangement as in midvein.

Petiole: Transverse section circular in outline; epidermis is single layered and interrupted by stomata occurring more on abaxial side. A few unicellular conical hairs appear over the surface. The hypodermis consists of 1-2 layered collenchymas consisting of oval to spherical cells which are lamellar or angular, often containing chloroplasts. The cortex consists of a narrow zone of 4-5 layers of loosely arranged chlorenchymatous tissue. The vascular bundles are arranged in a circle and capped by sclerenchyma. The vascular bundles are endarch, conjoint and collateral of various sizes. The Ground tissue is parenchymatous with a hollow cavity at the center (Fig.5).

Powder Microscopy

Pieces of abaxial epidermal cells with sinuous sides and anomocytic stomata. Adaxial epidermal cells with wavy sides and papillae. Pieces of tracheary tissue of midvein. Conical hairs and fragments of chlorenchyma tissue.

Physico-chemical studies: Standardization of raw drug, formulation of mother tincture and its standardization are summarized in Table 1-3.

Table 1: Standardisation of Raw Drug

S.No	Parameters	Quantitative values
1	Moisture content (Loss on drying at 105° C)	Not more than 4.4 % w/w
2	Total ash value	Not more than 10 % w/w
3	Acid insoluble ash value	Not more than 2.4 % w/w
4	Water soluble ash value	Not more than 4.6 % w/w
5	Alcohol soluble extractive value	Not less than 2.6 % w/w
6	Water soluble extractive value	Not less than 8.3 % w/w
7	Extractive values in	
	a. Hexane	Not less than 0.4 % w/w
	b. Chloroform	Not less than 0.8 % w/w
	c. Ethyl Acetate	Not less than 1.0 % w/w
	d. 40% Ethanol	Not less than 3.0 % w/w
	e. 50% Ethanol	Not less than 3.1 % w/w
	f. 60% Ethanol	Not less than 2.7 % w/w
	g. 70% Ethanol	Not less than 2.6 % w/w
	h. 80% Ethanol	Not less than 1.70 % w/w

Organoleptic Characters

- Colour : light dull green
- Touch : smooth
- Taste : slightly bitter
- Odour : no characteristic

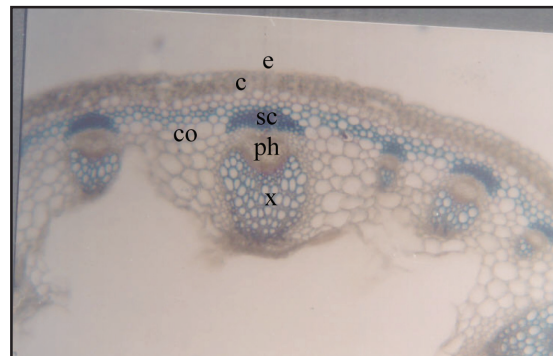


Fig. 5- T.S. of petiole- A portion enlarged

Abbreviations

- c : collenchyma
- ph : phloem
- x : xylem
- e : epidermis
- sc : sclerenchyma
- co : cortex

Table 2: Formulation of mother tincture (Percolation technique was used)⁹

Alcohol	50% v/v
Drug strength	1/10
Preparation :	
<i>Aquilegia visnaga</i> fruits in coarse powder	100 g
Strong alcohol	525 ml
Purified water	500 ml
To make one thousand milliliters of the mother tincture	

Mother tincture was prepared in 50% alcohol on the basis of Maximum Extractive Value (MEV).

Table 3: Standardisation of Mother Tincture

S.No.	Parameters	Observations
1.	Organoleptic profile a. appearance b. colour c. odour	clear, non-viscous Husky green Non characteristic
2.	Sediments	absent
3.	Weight per ml	Not more than 0.92 g
4.	Total solids	Not less than 3.0% w/v
5.	Alcohol content	45- 48% v/v
6.	pH	5.5 – 6.5
7.	λ max	631, 663 nm

Qualitative phytochemical tests

Preliminary phytochemical study indicates the presence of alkaloids, steroids, saponins, flavonoids, proteins, starch and tannins.

Identification (TLC)

Evaporate 25 ml mother tincture on water bath to remove alcohol. Extract the residue with 3x25 ml chloroform. Concentrate the chloroform extract to 2 ml and carry out TLC on Silica gel 'G' plates, using chloroform: ethyl acetate (9 : 1 v/v) as mobile phase. On spraying with 10% ethanolic sulphuric acid, eight spots appear at R_f 0.10, 0.16, 0.28, 0.44, 0.56, 0.74, 0.84, 0.98 (all brownish black) shown in Fig.6.

Discussion and conclusion

Aquilegia vulgaris L., commonly known as 'European Columbine' is a perennial herb belonging to the family Ranunculaceae. The aerial parts of the plant, predominantly the leaves are used as medicine in Homoeopathy. The leaves are biternate with ovate to obovate leaflets. Petiole is 5-18 cm long. The epidermal cells are polygonal, iso-anisodiametric with

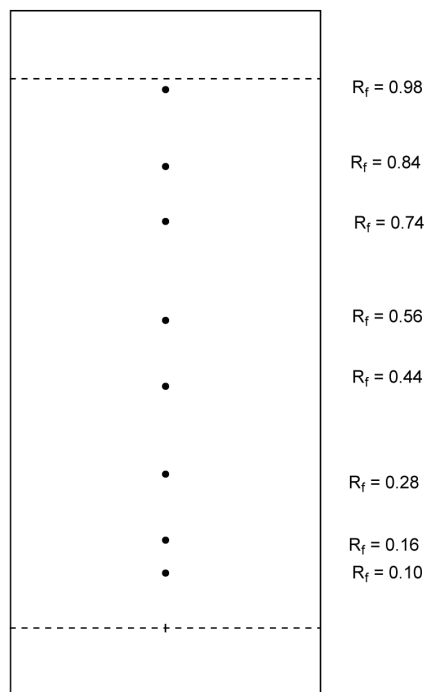


Figure.6. TLC retention behavior of *Aquilegia vulgaris* L.

sides wavy to sinuate on abaxial side and curved to wavy and papillate on adaxial side. The leaf is hypostomatic with anomocytic and tetracytic stomata. The stomatal frequency is 280 per sq mm; Stomatal index is 12.17 and Vein islet number is 14.5. The palisade ratio is 13.3 to 15.3. A few unicellular conical hairs are found on the abaxial side.

In V.S. the midvein is flat on adaxial side and ribbed towards abaxial side. The lamina is dorsiventral with rounded (obtuse) margins (Fig.4). Mesophyll possesses 2-3 layered palisade and loose 4-6 layered aerenchymatous spongy tissue.

The ground tissue at midvein is made up of 2-layered collenchyma on abaxial side followed by 5-6 layered parenchyma.

There is a single ovate vascular bundle, which is endarch, conjoint, collateral and aperiocyclic. The xylem elements have helical, scalariform and annular thickenings. The phloem is on abaxial side as a spherical mass.

The petiole in T.S. is circular. Epidermis is 1-layered and interrupted by stomata. A few unicellular conical hair also occur on the surface. Collenchyma is 1-2 layered with cells angular or lamellar often containing chloroplasts. The cortex is scanty, 4-5 layered and chlorenchymatous. The vascular bundles are endarch, conjoint and collateral, arranged in a ring and capped by sclerenchyma (absent in young petiole). A hollow central obliterated pith is present.

The powder microscopic and organoleptic characters are provided that are already described in the text.

The values of physico-chemical studies viz., total ash, water soluble ash, acid insoluble ash, extractability in alcohol, water and different solvents fall in the acceptable range. Phytochemical screening and chemical characters are often more precise, easily definable and rapidly detectable for deciding the abundance or absence of a particular constituent. TLC studies reveal eight distinct spots and UV study exhibits two prominent peaks which will be taken as characteristic standards of the drug.

The above morpho-anatomical characters of raw drug and physico-chemical constants are very specific to drug *A. vulgaris*. The studies provide referential information for correct identification and standardization of raw drug and Mother tincture. Since this drug is not yet included in Homoeopathic Pharmacopoeia of India, the data shall be very useful as Pharmacopoeial standards.

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