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Research Article

PHARMACOGNOSTIC STANDARDIZATION OF *CLEMATIS ERECTA* LINN

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ABSTRACT

The present investigation establishes histological characters, micrometric determinations and physicochemical parameters for *Clematis erecta* Linn. (Ranunculaceae). Transverse section of leaf showed presence of dorsiventral lamina comprising single layer of upper and lower epidermis, two layers of palisade cells below upper epidermis followed by 2-3 layers of spongy cells. The midrid portion of leaf showed are shaped vascular bundle in centre. Below the vascular bundle, rounded parencyma cells with intercellular spaces were observed. The powdered aerial parts of *C. erecta* showed presence of anomocytic stomata, unicellular covering trichome, pericyclic fibre, and lignified pitted vessel. Foreign organic matter content of air dried aerial parts of *C. erecta* was found to be 0.28%. Moisture content of air dried aerial parts of *C. erecta* was found to be 10.82%. The total ash was found to be about 6 times more than the acid insoluble ash in *C. erecta* whereas water soluble ash was about 2 times less than total ash. Water-soluble extractive value of *C. erecta* aerial parts was found to be about 11 and 2 times more, respectively, in comparison to petroleum ether and ethanol-soluble extractive value. Thin layer chromatography petroleum ether extract showed 2 spots using hexane: ethyl acetate (8:2) as the mobile phase, chloroform extract showed twelve spots for *C. erecta* and methanol extract where as TLC of methanol extract showed four spots using toluene: ethyl acetate: glacial acetic acid (85:15:1) as the mobile phase, employing 0.5% anisaldehyde as the visualizing agent. Phytochemical screening of various plant extracts showed presence of steroids, triterpenoids, tannins, polyphenols, coumarins and carbohydrates.

Keywords: Ash values, *Clematis erecta*, Coumarins, Extractive values, Triterpenoids.

INTRODUCTION

Clematis erecta Linn *(*Ranunculaceae), commonly known as Upright Virgin's Bower, has been traditionally used in treatment of various ailments such as insomnia, neuralgic and rheumatic headache, gonorrhoea, neuroses of men with pain in testicles and bladder, and reflex neuroses of women from ovarian or urinary irritation[1]. The plant is a homoeopathic remedy of much importance in disturbances of sleep, neuralgic pains in various parts, and has indications in fear of approaching misfortunes and impaired memory[2-3].

C. erecta has been used for the treatment of skin disease, pleurisy, eye diseases, and as antiseptic and antisyphilitic[4]. *C. erecta* is profoundly effective remedy related to all skin disease (like eczema, itching and formation of blisters and pimples). *C. erecta* is used in many diseases of men relating to the reproductive system, especially the inflammation of the right spermatic cord and inflammatory swelling of the right testis (orchitis). It is also effective in treating the inflammation of the bladder on the right side[5].

Phytochemically, *C. erecta* has been reported to contain quaternary isoquinoline alkaloids magnoflorine and corytuberine[6-7]. Saponin glycosides Clematoside A, Clematoside A` and Clematoside B[8].

The oil extracted from leaves of *C. erecta* exhibited strong antibiotic activity in wheat germination test[9]. Aqueous extract of the plant showed bactericidal and fungicidal effects[10].

As is the case with most of the traditional drugs, no work has ever been carried out for standardizing this potentially useful plant. Thus, the present investigations were planned with an objective to establish pharmacognostic standards for *C. erecta* thereby facilitating authentication of the correct plant material.

MATERIALS AND METHODS

Plant Material

Dried aerial parts of *C. erecta* were procured from K. R. Indo German American Trading Company, Kurukshetra (Haryana), India in the month of November 2008. Identity of the plant was confirmed through Dr. H.B. Singh, Scientist F, Head of Raw Material Herbarium and Museum (RHMD), National Institute of Science Communication and Information Resources (NISCAIR), New Delhi, India (Ref. No. NISCAIR/RHMD/Consult/-2008-09/1192/224 Dated 09-04-2009).

Microscopic Studies of *C. erecta* Aerial Parts

Qualitative and quantitative studies on the plant were carried out using compound microscope (Rescholar, Ambala). Observations were made using ×10 eye piece and ×10, or ×40 objectives. Micrometric determinations viz., length and width of vessels, pericyclic fibres and diameter of calcium oxalate crystals were made using eye and stage micrometer (Erma, Japan). Leaf constants viz., stomatal number, stomatal index, vein-islet number and veinlet termination number were determined using camera lucida and stage micrometer following the procedure elaborated by Evans[11]. Photomicrographs were taken using binocular photomicroscopic apparatus (LEICA, Italy) attached with nikon digital camera.

Dried leaves of *C. erecta* were boiled with water until soft. Thin sections of leaves were cut by sharp blades, transferred on slides, cleared by warming with chloral hydrate (Reidel Research Laboratory Chemicals, Hapur) aqueous solution (250% w/v) and mounted in glycerine (Ranbaxy Laboratory Chemicals) aqueous solution (50% v/v). Similarly, powdered *C. erecta* aerial parts (# 60) were also cleared with chloral hydrate and mounted in glycerine. For micrometric determinations, aerial parts were disintegrated using Schulz's macerating fluid[12].

Table 1: Mean values of length, width and diameter of vessels, pericyclic fibres and calcium oxalate crystals of *C. erecta*.

Parameter	Mean[n] Length (μm)	Mean[n] Width (μm)
Vessels	280.6	40.5
Pericyclic fibres	786.0	14.6
	Mean[n] Diameter (μm)	
Calcium oxalate crystals	10.4	

n=50

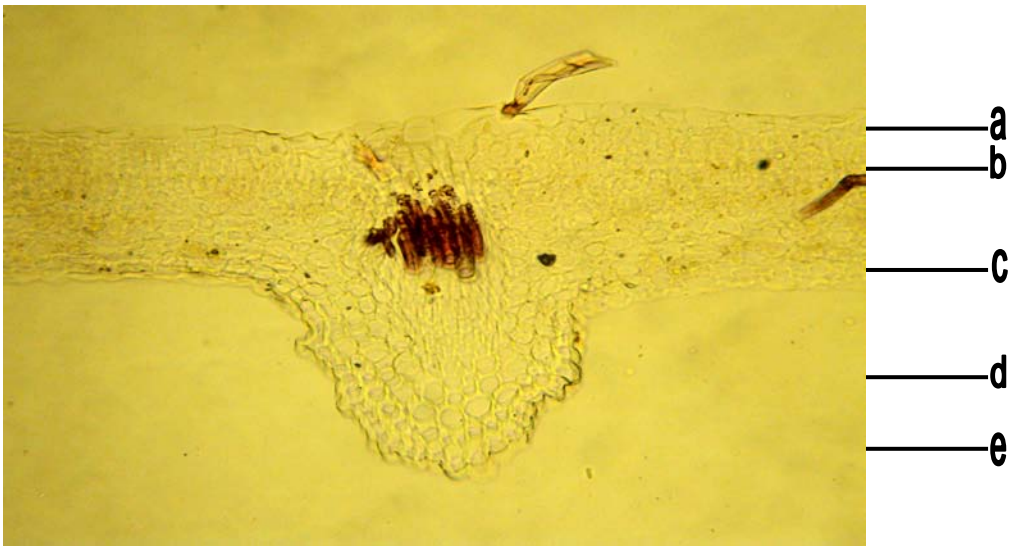

Fig. 1: Transverse section of leaf of *C. erecta* (100 X) (a) trichomes, (b) upper epidermis (c)palisade parenchyma (d) vascular bundle (e) spongy parenchyma (f) lower epidermis

Fig. 2 : Trichome in powdered aerial parts of *C. erecta* (100 X)

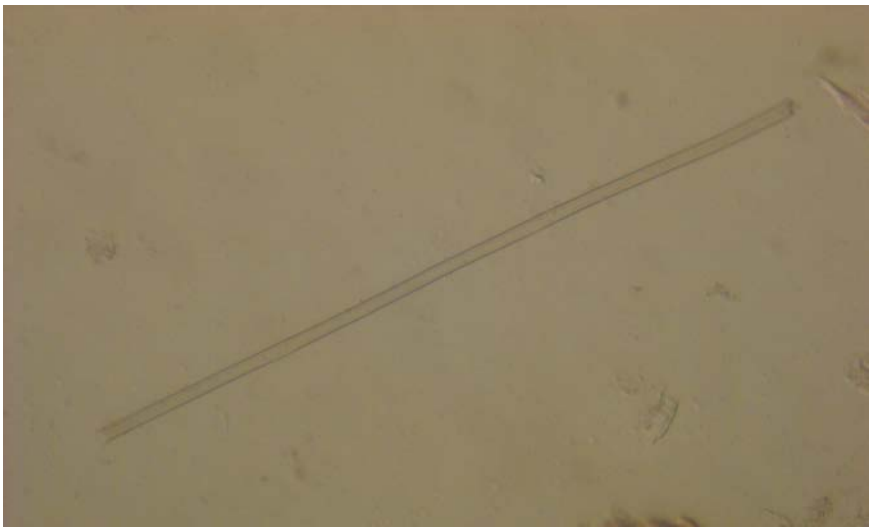

Fig. 3: Pericyclic Fibre in powdered aerial parts of *C. erecta* (100 X)

Fig. 4: Calcium oxalate crystals in powdered aerial parts of *C. erecta*. (X 400)

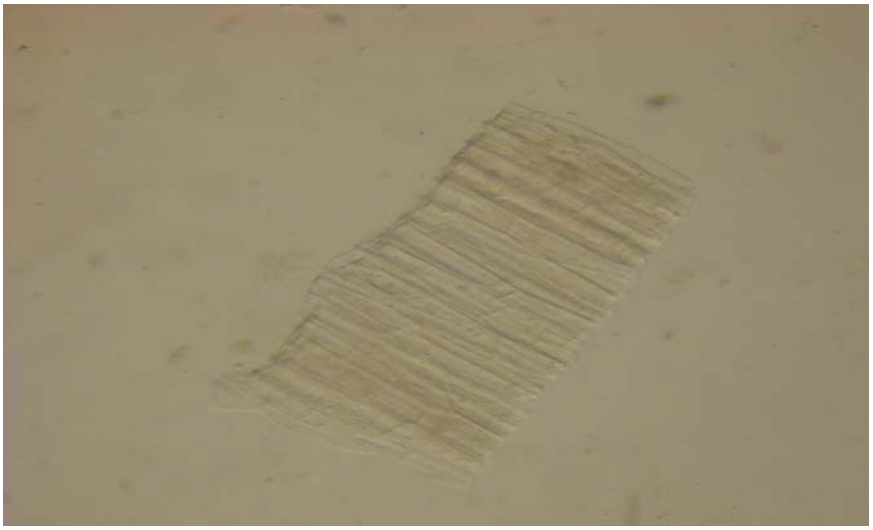

Fig. 5: Vascular bundle in powdered aerial parts of *C. erecta* (X 400)

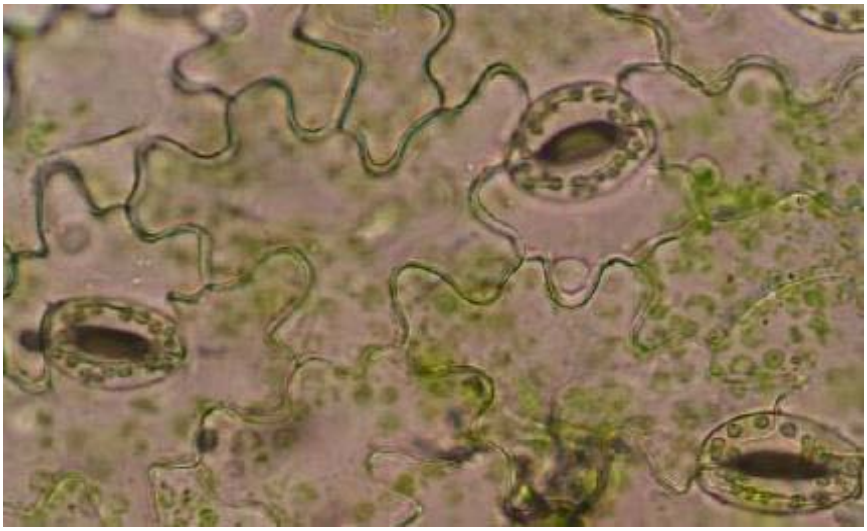

Fig. 6: Anomocyctic stomata in powdered aerial parts of *C. erecta* (X 400)

Table 2: Mean values of stomatal number, stomatal index, vein-islet number and veinlet termination number of *Clematis erecta*

Leaf constant	Range
Stomatal number	180-223
Stomatal index	17.1--19.7
Vein-islet number	19.5-22.5
Veinlet termination number	25.9-32.8

Foreign Organic Matter

Foreign organic matter in *C. erecta* aerial parts was determined by spreading 100 g aerial parts on clear smooth surface background by using a magnifying lens (10x)[12]. The experiment was done in triplicate.

Moisture Content

Moisture content of *C. erecta a*erial parts powder was determined by azeotropic distillation method following the procedure given in the Indian Pharmacopoeia[13]. The experiment was done in triplicate.

Ash and Extractive Values

Petroleum ether-, alcohol- and water-soluble extractive values, total ash, acid insoluble ash and water soluble ash of dried powdered aerial parts of the plant were determined following the procedures given in the Indian Pharmacopoeia[13]. Ash was prepared in a Muffle Furnace (Narang Scientific Works, New Delhi).

Table 3: Various physico-chemical parameters of *C. erecta* aerial parts

S. No.	Parameters	Observations[n] (% w/w)
1.	Foreign organic matter*	0.28
2.	Moisture content*	10.68
3.	Total ash**	10.82
4.	Acid insoluble ash**	1.74
5.	Water soluble ash**	6.04
6.	Pet. ether extractive value**	2.6
7.	Alcohol soluble extractive values**	13.6
8.	Water soluble extractive value**	27.60

n = 3; * Air dried weight basis; ** dry weight Basis

Thin Layer Chromatography (TLC) Fingerprint Profiles

Pre-coated aluminum based TLC sheets (Merck, Silica gel G, 0.2 mm) were used for Thin Layer Chromatography. Petroleum ether (60-80°C), chloroform (S.D. Fine Chemicals Pvt. Ltd.) and methanol (E. Merck, Mumbai), all of LR grade, distilled under normal atmospheric pressure were employed for extraction of the plant material. All solvents employed as mobile phase for thin layer chromatography were of LR grade.

Dried powder of *C. erecta* (2 g each) aerial parts was packed in filter paper sachet, placed inside 500 ml round bottom flasks, macerated (15 min) with petroleum ether (50 ml), and extracted under reflux (I h) on a boiling water bath. The chloroform and methanol extracts were prepared in a similar manner as explained above. Solvents from the respective extracts were recovered under reduced pressure using rotary vacuum evaporator (Gupta Scientific Store, Ambala). The dried petroleum ether, chloroform and methanol extracts were dissolved in 3 ml of respective solvents, and their volume was made up to 5 ml in volumetric flasks. Ten µl of the standard solution of each extract was loaded on TLC plates using 2 µl capillary tubes (CAMAG). The thin layer chromatograms were visualized by spraying with 0.5% anisaldehyde followed by heating at 105°C for 2 min.

Phytochemical Screening

Dried, coarsely powdered aerial parts of *C. erecta* (200 g) were successively extracted with petroleum ether, chloroform and methanol using a Soxhlet apparatus. The marc was air dried, and water extract was obtained by boiling with distilled water for 2 h, filtering, concentrating and drying in an oven at 40-50°C. All the four extracts were dissolved in respective solvents, and were screened for different classes of phytoconstituents[14].

Table 4: Results of thin layer chromatography of petroleum ether and chloroform extracts of *C. erecta* aerial parts

Extract	Mobile phase	Number of spots (R_f values)
Petroleum ether	Hexane : Chloroform(8 : 2)	Two spots (0.86, 0.93)
Chloroform	Toluene : Ethyl acetate : Glacial acetic acid(8.5: 1.5:0.1)	Twelve spots (0.18, 0.25, 0.31, 0.40, 0.42, 0.45, 0.50, 0.62, 0.68, 0.75, 0.81, 0.87)
Methanol	Toluene : Ethyl acetate : Glacial acetic acid(8.5: 1.5:0.1)	Four spots (0.53, 0.73, 0.80, 0.88)

* Spots were visualized by spraying with 0.5% anisaldehyde followed by heating at 110°C for 2 min in hot air oven.

Estimation of Aflatoxins, Heavy Metals, Arsenic, Pesticides and Microbial Content in C. erecta Aerial Parts

Quantitative determinations of aflatoxins, heavy metals, arsenic, pesticides and microbial content in *C. erecta* aerial parts were done at analytical laboratory of OSCAR Analytical Pvt. Ltd. Baddi, Solan (Certificate No. 2009/10/10280 dated 14-10-2009).

Table 5: Estimation of aflatoxins, heavy metals, arsenic and pesticides in *C. erecta* aerial parts

Parameters	Observation (As prescribed by WHO)
Aflatoxins B1	Not detected
B2	Not detected
M1	Not detected
M2	Not detected
Total	Not detected
Heavy metals	Complies
Arsenic	Complies
Pesticides:	
Heptane, Lindane, Heptachlor, Di Aldrin, HCH Isomer, Endrin, DDT	Not detected

Table 6: Microbial content in *C. erecta* aerial parts

Microbes	Observation	Limit (As prescribed by WHO)
Total bacterial count	156 cfu/10gm	NMT 1000 cfu/gm
Total fungal count	Nil cfu/10gm	NMT 100 cfu/gm
Pathogen		
Salmonella	Absent	Should be absent
E.coli	Absent	Should be absent
Pseudomonas auroginosa	Absent	Should be absent
Staphylococcus aureus	Absent	Should be absent

RESULTS AND DISCUSSION

Figure 1 shows transverse section of *C. erecta* leaf. Representative photomicrographs of trichome, fibre, calcium oxalate, vascular bundle and stomata respectively are shown in figures 2-6.

Table 1 shows the mean values of length and width of vessels, pericyclic fibres, and diameter of calcium oxalate crystals of *C. erecta* aerial parts. Table 2 Shows mean values of stomatal number, stomatal index, vein-islet number and veinlet termination number of *Clematis erecta*. Table 3 shows mean values for various physico-chemical parameters of *C. aerial* aerial parts. Results of thin layer chromatography of various extracts of *C. erecta* are shown in Table

4. Tables 5-6 show the microbial content, aflatoxins, heavy metals, arsenic, and pesticides residue limits in *C. erecta* aerial parts.

Authentication of plant material is an indispensable prerequisite before using it as research material or as medicine. Therefore, it was planned to establish pharmacognostic standards for *C. erecta* so as to have reliable parameters to authenticate the plant.

In India, *C. erecta* has not been reported from wild sources. It is cultivated to meet the demands of the pharmaceutical industry, especially those manufacturing homoeopathic drugs. *C. erecta* aerial parts were procured from a cultivated source and its identity was further confirmed through NISCAIR, New Delhi. The positively identified plant material was used to generate pharmacognostic standards.

C. erecta was subjected to qualitative and quantitative microscopic studies. Transverse sections of leaf as well as the powdered aerial parts of the plant were studied for microscopic characters. Transverse section of leaf showed presence of dorsiventral lamina comprising single layer of upper and lower epidermis, two layers of palisade cells below upper epidermis followed by 2-3 layers of spongy cells. The midrid portion of leaf showed are shaped vascular bundle in centre. Below the vascular bundle, rounded parencyma cells with intercellular spaces were observed. The powdered aerial parts of *C. erecta* showed presence of anomocytic stomata, unicellular covering trichome, pericyclic fibre, lignified pitted vessel and cluster crystals of calcium oxalate.

Medicinal plant materials should be entirely free from visible signs of contamination by moulds or insects, and other animal contamination, including animal excreta. No abnormal odour, discoloration, slime or signs of deterioration should be detected. It is seldom possible to obtain marketed plant materials that are entirely free from some form of innocuous foreign matter. However, no poisonous, dangerous or otherwise harmful foreign matter or residue should be allowed. During storage, products should be kept in a clean and hygienic place, so that no contamination occurs. Special care should be taken to avoid formation of moulds, since they may produce aflatoxins. Macroscopic examination can conveniently be employed for determining the presence of foreign matter in whole or cut plant materials. However, microscopy is indispensable for powdered materials. Any soil, stones, sand, dust and other foreign inorganic matter must be removed before medicinal plant materials are cut or ground for testing. Foreign organic matter content of air dried aerial parts of *C. erecta* was found to be 0.28%.

Presence of excess moisture in the plant acts as an adulterant and can cause decomposition in the plant material as it promotes microbial growth. Thus, it should be determined and controlled. Moisture content of air dried aerial parts of *C. erecta* was found to be 10.68%. Moisture content of the aerial parts was accounted for calculating values of other physicochemical parameters on dry weight basis.

Determination of ash is useful for detecting adulteration with spurious, exhausted drugs, and excess of sandy and earthy matter. Most drugs contain calcium oxalate crystals, sometimes in large and variable amounts. The acid insoluble ash is determined to remove all the variable constituents of the ash using dilute hydrochloric acid. The water soluble ash is used to detect the presence of material exhausted with water. The total ash was about 6 times more than the acid insoluble ash in *C. erecta*, indicating the presence of large number of calcium oxalate crystals or other acid soluble inorganic matter in *C. erecta*. The water soluble ash was about 2 times less than total ash in *C. erecta*.

Petroleum ether, ethanol and water were used to evaluate the extractable constituents in the aerial parts of *C. erecta* in terms of extractive value. Water-soluble extractive value of *C. erecta* was found to be about 11 and 2 times, respectively, in comparison to petroleum ether- and ethanol-soluble extractive value.

Amongst various chromatographic techniques, thin layer chromatography is a handy technique for studying separation pattern of various extracts of plant material. TLC fingerprint profiles are useful for the identification/authentication of plant material.

In order to prepare qualitative TLC fingerprint profiles of petroleum ether, chloroform and methanol extracts of aerial parts of *C. erecta,* the plant materials were subjected to a standardized extraction procedure wherein petroleum ether, chloroform extracts and methanol were obtained by direct extraction with petroleum ether, chloroform and methanol. Standard solutions of the extracts were prepared and loaded quantitatively on silica gel TLC plates. Petroleum ether extract showed two spots using hexane: ethyl acetate (8:2) as the mobile phase, chloroform extract, showed twelve spots using toluene: ethyl acetate: glacial acetic acid (85:15:1) as the mobile phase and four spots were observed in TLC of methanol extract of *C. erecta* using toluene: ethyl acetate: glacial acetic acid (85:15:1) as the mobile phase , when visualized with 0.5 % anisaldehyde.

Phytochemical screening of various extracts of *C. erecta* viz., petroleum ether, chloroform, methanol and water was carried out using standard procedures. Petroleum ether extract of *C. erecta* showed presence of steroids and triterpenoids, whereas chloroform extract showed presence of steroids and triterpenoids, methanol extract tested positive for steroids, triterpenoids, tannins, coumarins and polyphenols, and water extract indicated the presence of carbohydrates and proteins. Various toxic residues such as pesticides, arsenic, heavy metals, aflatoxins, and microbes in *C. erecta* complies the limits as prescribed by World Health Organization.

CONCLUSION

Lack of standardization is the major stumble block in exploiting the potential of traditionally used herbal medicines. The present investigation could successfully evolve important standardization parameters for *C. erecta* viz., qualitative and quantitative microscopic characters, ash values, extractive values, qualitative TLC fingerprint and phytochemical profiles of petroleum ether, chloroform and methanol extracts of the plant. These standardized parameters would be of immense help in authenticating *C. erecta*.

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