

Pharmacognostical studies on seeds of *Centrathium anthelminticum* Kuntze

Daksh Bhatia^{1*}, MK Gupta², Ankur Gupta³, Mamta Singh¹ and Gaurav Kaithwas¹

¹Department of Pharmaceutical Sciences, Faculty of Health and Medical Sciences
Allahabad Agricultural Institute (Deemed University), Naini, Allahabad-211 007, Uttar Pradesh, India

²Kota College of Pharmacy, Kota, Rajasthan, India

³Shreya Life Sciences Pvt. Ltd., Plot No. 13-15, Village Riapur, P.O. Bhagwanpur, Roorkee- 247 667, Uttarakhand, India

*Correspondent author, E-mail: bhatiadaksh@gmail.com; Phone: +91-9889909235 (Mob.)

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Abstract

The present communication deals with the pharmacognostical and preliminary phytochemical studies on the seeds of *Centrathium anthelminticum* Kuntze. Less reports are available on microscopical and phytochemical studies, hence, the present study was undertaken to investigate the same. All the parameters were studied regarding the WHO and pharmacopoeial guidelines. The study revealed the presence of patches of rounded to polygonal stone cells, trachieds showing pittings, thick walled cells, abundant covering and glandular trichomes, alleurone grains and brown tannin content. In fluorescence analysis no specific fluorescence was observed. HPTLC profile was also established for successive extracts of the seed using Camag HPTLC system.

Keywords: *Centrathium anthelminticum*, *Vernonia anthelmintica*, *Kaliziri*, *Somraj*
Pharmacognosy, Phytochemical, Fluorescence analysis.

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In the present study some pharmacognostical parameters such as foreign matter, moisture content, volatile matter, ash values, extractive values, microbial load on the drug, preliminary phytochemical screening and HPTLC profile using the seeds of this plant have been investigated.

Materials and Methods

The dried seeds of *C. anthelminticum* were collected from the local market of Jhansi. The seeds were stored in normal environmental conditions and they were authenticated at Raw Material, Herbarium and Museum NISCAIR, CSIR, New Delhi, India and sample was submitted in the museum (Ref. No. – NISCAIR/RHM/F-3/2004/Conslt/626/106).

About 500g of the seeds were soaked in a glass bottle (2 litre capacity) with sufficient light petroleum ether (40-60°C) for 24 hours and then the seeds were extracted using soxhlet apparatus with petroleum ether (40-60°C), chloroform and ethanol successively. Petroleum ether fraction was combined with the first fraction obtained after soaking. After that, these extracts were concentrated in rotary film evaporator under reduced pressure. For microscopical

Introduction

Herbal medicine is a triumph of popular therapeutic diversity. Almost in all the traditional medicine, the medicinal plants play a major role and constitute the backbone for the same. In order to make sure the safe use of these medicines, a necessary first step is the establishment of standards of quality, safety and efficacy. Keeping this fact in to consideration, the attempts were made to establish physicochemical standards of the plant *Centrathium anthelminticum* Kuntze syn. *Vernonia anthelmintica* Willd. (Hindi — *Kaliziri*, *Somraj*) belonging to family Asteraceae¹⁻³. In India, nine species of this genus are found, of which *C. anthelminticum* is of medicinal value. It is a tall, robust annual with

anthelmintic properties especially against threadworms. The active anthelmintic constituent is confined in the achenes (fruits) of the plant. The plant is widely distributed throughout India up to 1650 m altitude in the Himalyas and Khasi hills⁴. Seeds have a hot sharp taste. It is an important medicinal plant used in various Ayurvedic preparations and is also reported to be used in asthma, kidney troubles and cough. It is tonic, stomachic, and cure phlegmatic discharge from the nostrils. It also enters in to the prescription for leucoderma, psoriasis, and other skin infections⁵. The major classes of chemical constituent present in this plant are glycosides, carbohydrates⁴, phenolic compounds and tannins, flavanoids⁶, proteins, saponins, sterols⁷, lipids and fats⁸.

studies, free hand section of seeds were cut, cleared and stained with safranin according to the prescribed method⁹. A drop of HCl and phloroglucinol were used to detect the lignified cells in the cut sections and in the powdered drug, trichomes lengths were measured and photomicrographs were taken with micrometer (Erma, Tokyo) and photomicroscope (Olympus, New Delhi). Powder microscopy was performed as described earlier in the literature¹⁰. Physicochemical studies such as foreign matter, moisture content, ash values, extractive values and microbial load were performed according to official procedures^{11,12}. Dried seeds were used for the physicochemical studies. All the three extracts were used for primary phytochemical screening¹³. The fluorescence and general behaviour of the powdered drug in different solutions toward the visible and ultraviolet lights (254 and 365nm) was carried out¹⁴. TLC studies of the petroleum ether (40-60°C), chloroform and ethanol extracts were carried out in various solvents at 30°C using Silica gel G as adsorbant^{15, 16} and the same mobile phase were used for HPTLC profiles of the three successive extracts.

In the proposed HPTLC method the drug was spotted on to the precoated silica gel 60F-254 TLC plates (10×10cm with 200µm thickness, E. Merck, Germany). The chromatographic development was performed using a mixture of n-hexane: ethyl acetate (9:1) for petroleum ether extract, Ethyl acetate: dichloromethane (DCM): carbon tetrachloride (CCl₄): glacial acetic acid (GAA) (1:2:7:0.25) for chloroform extract and chloroform: methanol: GAA (8:1.5:0.5) for ethanol extract as mobile

phase under the following conditions; chamber saturation time, 30 min and temperature, 20° C. After development, the TLC plates were dried completely at room temperature. Plates were visualized using sulphuric acid-methanol (5%) solution as detecting agent. Quantification of chemical constituents were achieved by scanning with CAMAG TLC scanner 3 (slit dimension, 5 × 0.45mm; scanning speed 1 mm/s; wavelength of determination, 650 nm at absorbance mode) and the automated software produced the chromatogram by plotting absorbance against Rf values.

Results and Discussion

Macroscopically, the seed of *C. anthelminticum* is 4.5-6 mm long, dark brown in colour having a characteristic odour and intensely bitter taste. The surface of the seed is comprised of about 10 ridges. The ridges are covered with trichomes. The seed is oblong shaped, pointed from one side and hairy tapered from other end (Fig. 1.).

The outermost layer of the seed is single cell epidermal layer. In between the epidermal layer, there are unicellular bulb shape trichomes. Under this layer there are round structures of collenchymatous cell arranged in bundles giving an appearance of ridge from outside the seed. A sclerenchymatous layer is just above the seed coat giving a wave like appearance⁹. This serves for the mechanical support to the embryo, the

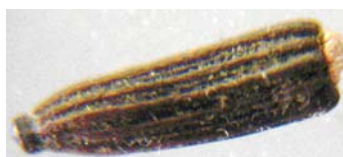


Fig. 1: *Centratherum anthelminticum* seed X 40

whole part comes under pericarp region. Beneath the pericarp seed coat is there. The outer integument of the seed coat is single cell layered. The cells are beaker shaped. Inner integument is thinner in comparison to outer integument. In the center embryonic (residual endosperm or perisperm) region is there (Fig. 2) which contains globoid aluerone grains (storage proteins) and lipid globules. For the Asteraceae family, seeds and fruits are considered as same.

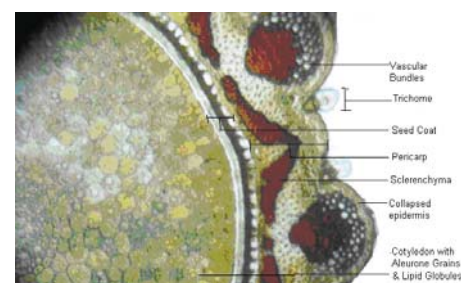


Fig. 2 : Transverse section of seed

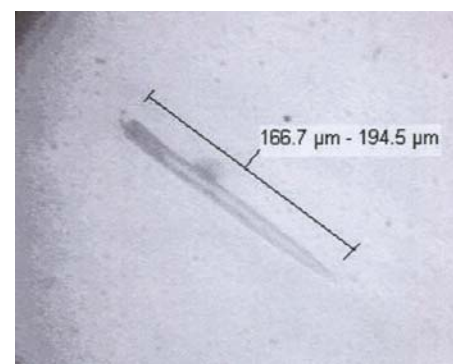


Fig. 3 : Trichome

The powder microscopy revealed the presence of abundant uniseriate trichomes (Fig. 3) which are 166.7-194.5 µm in length. Ground tissues are multilayered with thick walled, compactly arranged parenchymatous cells. Sclerenchymatous cells are also present. Thin walled fibres and trachieds from hilum furrow showing pittings are also found. Stone cells are elongated and rectangular with broad and narrow lumen.

The results of physico-chemical analysis and extractive values are given in Table 1. Preliminary qualitative chemical tests were performed which shows that plant is credited with flavone glycosides, saponins, steroids, carbohydrate, protein and fats. No specific fluorescence was observed in fluorescence analysis. The values of other physical constants like foreign matter, moisture content, ash values and microbial load are believed to be lying with in the permissible limits and these values can be used for further investigations. All the three successive extracts were developed on chromatographic plates with many ratios of different solvents and the best eluent mixture was used further for HPTLC profile to minimize errors in TLC pattern (Table 2 and Figs 4-6).

Table 1 : Physicochemical analysis of seeds

Physical Constants	
Foreign Matter (%)	2.68
Moisture (%)	10.8
Ash (%)	4.837
Acid insoluble ash (%)	0.4823
Water soluble ash (%)	4.92
Sulphated ash (%)	11.8117
Microbial load	6.2 X 10 ⁶
Extractive values (g/50g of Drug)	
Petroleum ether	6.189
Chloroform	5.900
Ethanol	5.686

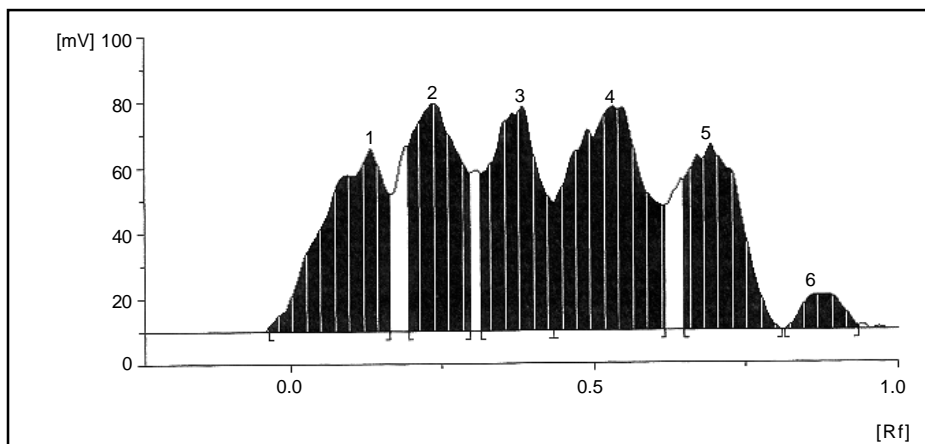


Fig. 4 : HPTLC chromatogram of petroleum ether extract

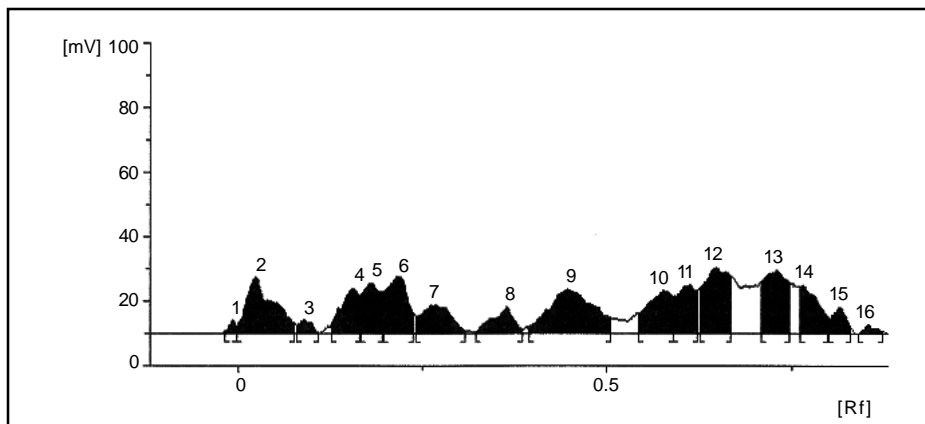


Fig. 5 : HPTLC chromatogram of chloroform extract

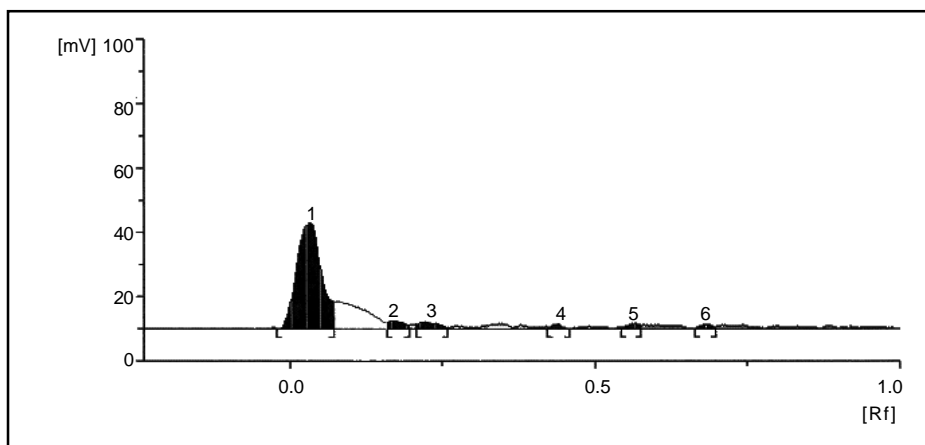


Fig. 6 : HPTLC chromatogram of ethanol extract

Table 2 : Chromatographic studies of successive seed extracts

S. No.	Extracts	Solvent Systems	Developing Reagents
1.	Petroleum ether	<i>n</i> -Hexane: Ethyl acetate (9:1)	5% Sulphuric acid in MeOH
2.	Chloroform	Ethyl acetate: Dichloromethane: Carbon tetrachloride: Glacial acetic acid (1:2:7:0.25)	-do-
3.	Ethanol	Chloroform: Methanol: Glacial acetic acid (8:1.5:0.5)	-do-

Conclusion

Standardization of herbal drugs is a topic of great concern. They are subject to variability as derived from heterogeneous sources. This variability can have both an advantageous and a disadvantageous effect. The main disadvantages are that the activity of the material may vary and that inferior material may be produced. *Kaliziri* is an Ayurvedic herb known for its anthelmintic activity and various medicinal properties. So the efforts were made to provide the scientific data to standardize the plant material for further studies. Microscopic, macroscopic and other physical values and parameters will help to identify the correct species of the plant since no such scientific data is available for the same.

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