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Pharmacopoeial Standardization of *Alectra chitrakutensis (M.A. Rau.) R. Prasad & R.D. Dixit* found in Chitrakoot Region.

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Abstract

Alectra chitrakutensis (M.A. Rau) R. Prasad & R.D. Dixit (Scrophulariaceae) is an endemic and critically endangered plant used in the treatment of various types of ailments and diseases like leprosy, tuberculosis, paralysis, piles, intestinal worms, constipation, leucorrhoea, fever, spermatorrhoea and as a blood purifier. Review of literature reveals that no pharmacognostical study has so far been carried out. The genuine whole plant sample of *Alectra chitrakutensis* was collected as a part of the study and subjected to macroscopic and microscopic investigation. The air-dried whole plant powder was extracted with different solvent systems such as hexane, acetone, chloroform, methanol, and ethanol and finally with sterile water and preliminary phytochemical analysis of the extracts including HPTLC assays were done and the R_f values were determined. Physico-chemical characters, fluorescence characters and extractive values of the whole plant powder in different solvent systems were also determined. The pharmacognostical parameters studied, may be used as a tool for the correct identification of the plant and also to test the adulterants if any. The present paper reports a systematic pharmacognostic study on *Alectra chitrakutensis* for the first time.

Key words : *Alectra chitrakutensis*, pharmacognosy, phytochemical analysis, HPTLC fingerprint.

1. Introduction

Medicinal plants have been used in virtually all cultures as a source of medicine, since times immemorial. Herbal Medicine is still the mainstay of health care in several developing countries. The widely used herbal remedies and health care preparations as described in ancient texts such as the Vedas and the Bible are obtained from commonly used traditional herbs and medicinal plants. The medicinal properties of these botanicals are being better understood and are

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attributable to the phytochemicals that specific plants contain. The efficacy and safety of herbal products therefore rely on the quality and proper identification of the raw material or the original plant source. One major obstacle that might impair the potential use of traditional medicine as medicine of choice is the lack of standardization.

Adulterations and substitutions are common in raw material trade of medicinal plants. Unintentional adulterations also exist in herbal raw material trade due to various reasons such as confusion in vernacular names between indigenous systems of medicine and local dialects, lack of knowledge about the authentic plant, non-availability of the authentic plant, similarity in morphology or aroma and careless collection. To avoid this accurate authentication is very important to prevent the adulteration of target plant with other plant species. The techniques used for the standardization of botanical preparations like HPLC, TLC, HPTLC, UV spectroscopy, mass spectroscopy, gas chromatography, infrared and NMR spectroscopy have limitations because the compositions and relative amount of chemicals in a species varies with growing conditions, harvesting periods, post-harvest processes and storage conditions. This can be misleading if the samples are deliberately adulterated with a marker compound. Also, it is difficult to distinguish closely related species due to similar chemical compounds. Identification of plants with botanical verifications is essential as contamination due to misidentification of plant species or parts is common. Therefore, it becomes necessary to develop more effective, accurate, reliable and sensitive methods for the authentication of herbs. In the present study an effort has been made to establish physicochemical, pharmacognostic and phytochemical parameters which could be helpful in identification of the authentic plant samples and differentiating it from adulterants.

The genus *Alectra* Thunb. of the family Scrophulariaceae is represented by more than 50 species of parasitic herbs which are distributed in tropical regions of Africa, South America and Asia. Three species are so far known from India viz. *Alectra sessiliflora* (Vahl) Kuntze, *A. thompsoni* Hook .*f*. and *A. chitrakutensis* (Rau) R. Prasad & R.D. Dixit.

Alectra chitrakutensis (Rau) R. Prasad & R. D. Dixit was first described by M. A. Rau in 1961 from Chitrakoot as a variety of *A. parasitica* A. Rich. The plant came to light after Prasad obtained encouraging results in the preliminary clinical trials of the rhizome in the treatment of leprosy. Then Rajgopalan and Seshadri (1964) worked out its chemical composition and Bedi (1967) published detailed information on its availability, collection and local uses. Saxena *et al* (1969) studied the soil properties of the habitat of the plant. Prasad and Dixit (1993) carried out a detail taxonomic study and raised the status of plant from varietal level to species level. Recently Khanna & Kumar (2007) studied the specimens of Alectra *chitrakutensis* (Rau) R. Prasad & R. D. Dixit, housed in the Herbarium of Botanical Survey of India, Central Circle, Allahabad (31799 & 38363) and reported that *Alectra chitrakutensis* (Rau) R. Prasad & R. D. Dixit is a subspecies of *Alectra parasitica* A. Rich. and given a new status as *Alectra parasitica* A. Rich. subsp. *chitrakutensis* K.K. Khanna & Anand Kumar.

Alectra chitrakutensis (Scrophulariaceae) known as 'Nirgundi' in Chitrakoot region is an important medicinal plant. This is an endemic and critically endangered plant grows as a parasite on the thread like roots of white flowered *Vitex negundo* L. It is a parasitic herb of 20-40 cm height, stem rhizomatous, well developed, orange-yellow, black on drying; leaves linear or lanceolate, up to 1.3 cm long,

flowers in terminal racemes, yellow; calyx 5 lobed, hairy, corolla 5 lobed, scarcely hairy, each lobe with 3 purple streaks; stamens 4, anthers dithecous, dorsifixed; ovary syncarpus, superior, bilocular; style long inflexed; stigma elongate, capsules globose, seeds minute, cuneiform, black. The plants grow from October to April mainly on sandy soils of Chitrakoot region of Madhya Pradesh and Uttar Pradesh.

2. Material and Method.

The genuine whole plant sample of *Alectra chitrakutensis* was collected from Sphaticshila, Sirsavan, along the banks of river Mandakni in sandy soils, parasite on the roots of generally old shrubs of *Vitex negundo*, which is gregarious in patches in the month of April 2009.The whole plant sample of *Alectra chitrakutensis* was dried under shade and powdered. Quantitative macroscopic and microscopic investigation was done and physicochemical studies like loss of weight on drying (Moisture contents), total ash, acid insoluble ash, water soluble ash, pH of 10 % w/v solution of aqueous extract, and successive extractive values were carried out by soxhlet extraction method as per the standard methods described in pharmacopoeia of India (2001).The fluorescence behavior of the powder drug in the visible light and ultraviolet light were carried out by soaking the powder in different reagent solutions and viewing under the light of required wavelength in a UV chamber. Preliminary Phytochemical analysis and high performance thin layer chromatography were also carried out as per standard methods.

3. Result and Discussion.

3.1 Macroscopic Investigation

Powdered drug is Dark brown in colour, Dusty with prominent odour & bitter taste. The powder completely passed on through sieve number 44 and less than 50 Percent pass on through sieve number 85.

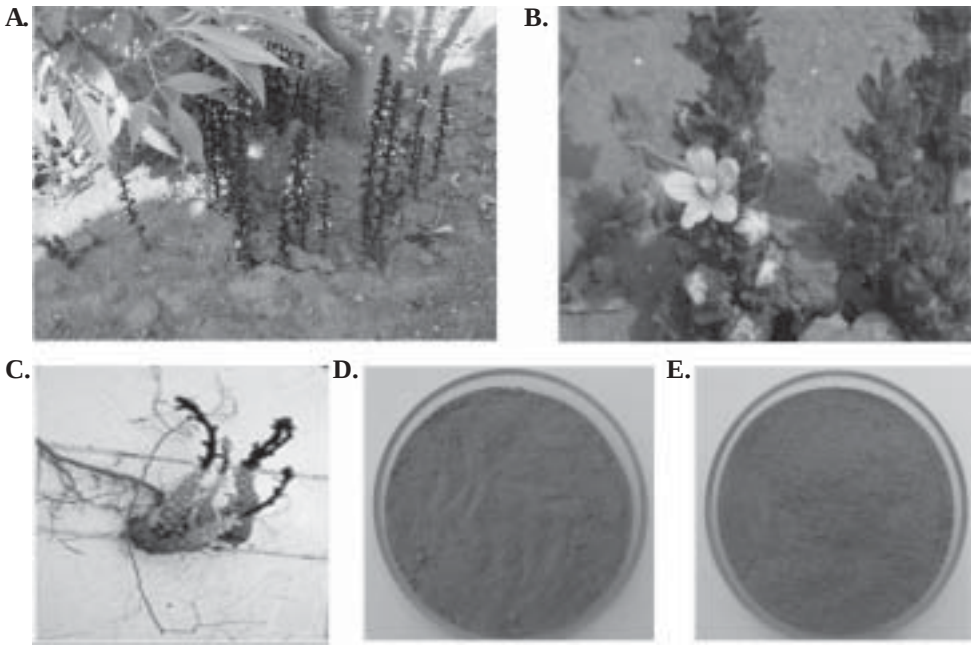

Figure 1: Photographs of Whole plant of *Alectra chitrakutensis* (A), Flowers(B), Rhizomes(C) & Two Crude drug samples after pulverization in which first sample (D) is collected and Manufactured by author and the second sample (E) is taken form Market (Chitrakoot Rasshala Pharmacy).

3.2 Microscopic Investigation

Results of microscopic studies of whole plant powder shows small, rounded and compound starch grains, prismatic crystals of calcium oxalate, trichomes, thick walled -fibres, tannin cells, vessels with reticulate thickening, vessels with spiral thickening, long sclereids, thin walled. parenchymatous cells, vessels with simple pits and vessels with boarded pits.

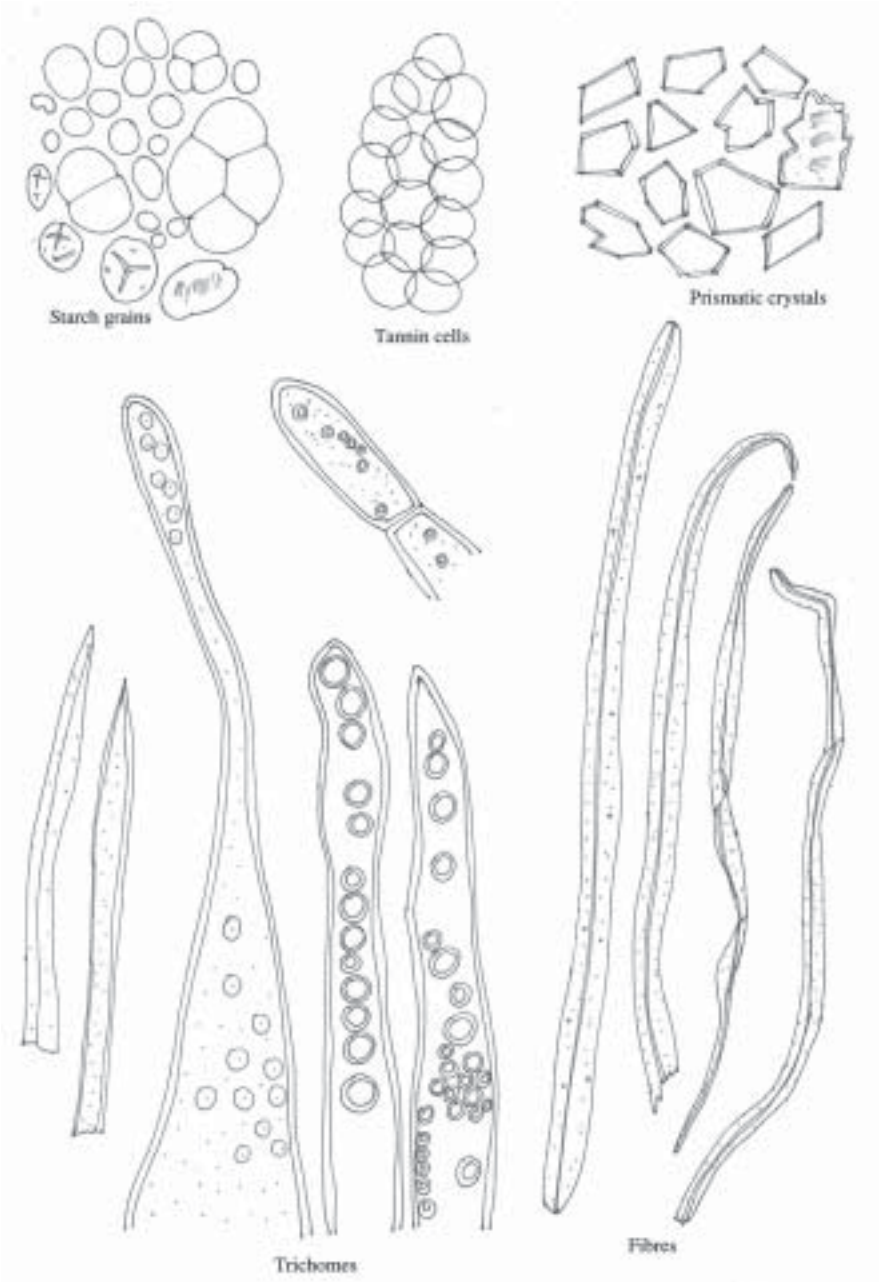

Figure 2: Camera Lucida Diagrams showing powder microscopy of *Alectra chitrakutensis.*

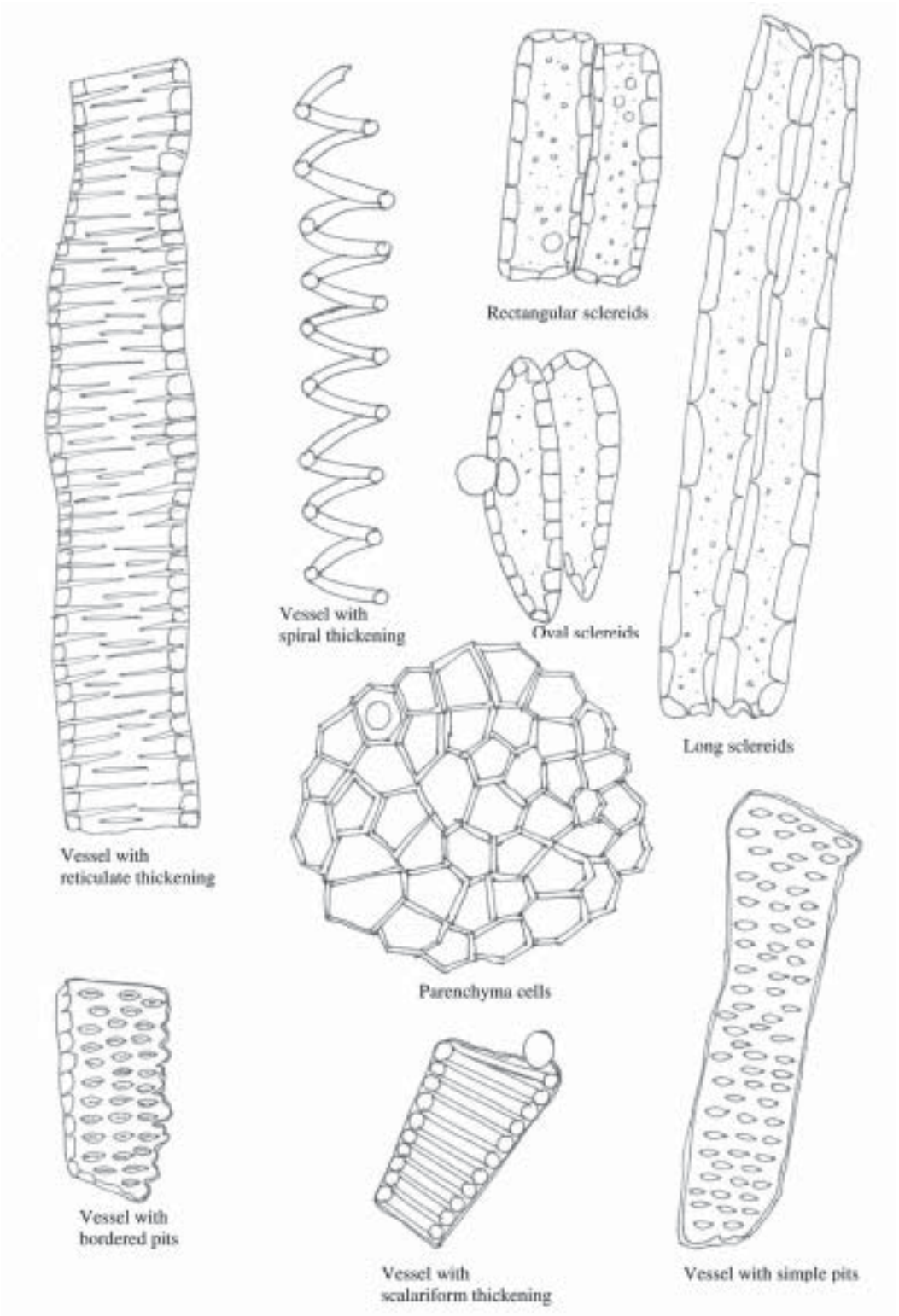

Figure 3: Camera Lucida Diagrams showing powder microscopy of *Alectra chitrakutensis.*

3.3 Physicochemical Parameters

The percent of loss of weight on drying, total ash, acid insoluble ash, water soluble ash, and pH of 10 % w/v solution of aqueous extract has been shown in Table 1. A known quantity of dried whole plant powder was extracted in a soxhlet apparatus with hexane, acetone, chloroform, methanol, ethanol and finally macerated with distilled water for 24 hours successively and the % of respective extractive values have been shown in Table 1.

3.4 Preliminary Phytochemical Screening

The successive extracts were tested for different constituents. The alcoholic and water extracts revealed presence of carbohydrates, resins and tannins (Table 2).

3.5 Fluorescence Analysis

Powdered drug under UV and visible light when treated with different reagents emitted various colour radiations which help in identifying the drug in powder form (Table 3).

Table 1: Physicochemical parameters of whole plant powder of *Alectra chitrakutensis*

Parameters	Average value (%)
Loss of weight on drying at 105^{0}C	4.00
Water soluble extractive value at room temperature (Cold extraction)	39.33
Alcohol soluble extractive value at room temperature (Cold extraction)	12.70
Total Ash value	8.87
Acid insoluble ash value	6.89
Water soluble ash value	5.54
pH(10% w/v aqueous extract)	4.8
Extractive value in percentage through soxhlet (Hot extraction)	
Hexane soluble extractive value	21.70
Acetone soluble extractive value	24.17
Chloroform soluble extractive value	3.50
Methanol soluble extractive value	42.17
Ethanol soluble extractive value	34.95
Distilled water soluble extractive value	41.63

Table 2: Preliminary phytochemical Screening of whole plant powder of *Alectra chitrakutensis.*

Test	Alcohol extract	Distilled water extract
Alkaloid	Negative	Negative
Flavonoid	Negative	Negative
Carbohydrate	Positive	Positive
Protein	Negative	Negative
Resin	Negative	Positive
Saponin	Negative	Negative
Tannin	Negative	Positive

Table 3: Ultra-violet analysis of whole plant powder of *Alectra chitrakutensis.*

Treatment	Visible Light	UV (254 nm)	UV (366 nm)
Crud Drug Powder	Dark Brown	Dark Brown	Blackish Brown
Methanol	Turmeric yellow	Greenish yellow	Dark Turmeric yellow
Ethanol	Yellow	Greenish yellow	Turmeric yellow
Chloroform	Light yellow	Light green	Turmeric yellow
Distilled. Water	Yellow	Yellow	Turmeric yellow

3.6 HPTLC Study

2 g of powdered plant material was extracted with 10 ml of methanol, under reflux on water bath for 5 minutes, filtered, concentrated to 5 ml & carried out the HPTLC. 2 ul of the extract was applied on silica gel plate & developed the plate to a distance of 10 cm using Toluene: Ethyl acetate (70:30) as mobile phase. After development the plate was allowed to dry at Room Temperature and examined the plate before & after derivatization under ultra violet Day Light, at 254 nm and at 366 nm. It showed major spots at under Day light, 254 nm, 366nm and major spots appears also at under Day light, 254 nm, and 366nm after derivatization with 5% Methanolic Sulphuric Acid.

Batch No. A – In - house (Manufactured by author in Ayurveda Sadan, Laboratory)

Batch No. B – Out-Side (Manufactured by Chitrakoot Rasshala Pharmacy)

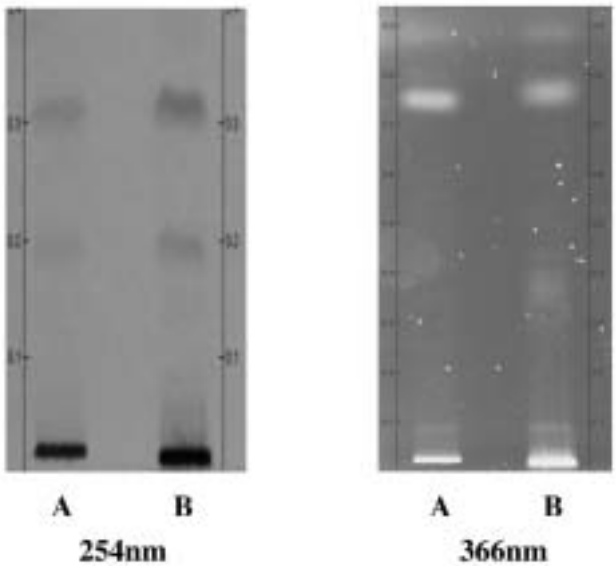

Figure 4: Methanolic extract chromatography of *Alectra chitrakutensis.*

Major spots seen before derivatization: Under 254 nm, at R_f 0.20 (Light black) and at R_f 0.31 (Light black).
Major spots seen after derivatization: Under 366 nm: at R_f 0.10 (Light Pink), at R_f 0.31 (Brown), at R_f 0.32 (Brown), at R_f 0.75 (Sky Blue), at R_f 0.77 (Sky Blue), at R_f 0.80 (Light Red) and at R_f 0.89 (Light Red).

4. Conclusion

This study presents a set of diagnostic characters of *Alectra chitrakutensis* that will help to identify the drug in fragmentary condition as well as in whole form. The results of parameters for preliminary phytochemical screening, UV analysis and HPTLC studies can act as biomarkers for identification and authentification of raw drug samples and play an important role in quality control and prevention of adulteration.

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