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Original Article

COMPARATIVE PHYTOCHEMICAL EVALUATION OF ETHNOBOTANICALLY IMPORTANT MEDICINAL PARASITIC HERB: *ALECTRA CHITRAKUTENSIS*

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ABSTRACT

Objective: *Alectra chitrakutensis* (M. A. Rau) R. Prasad & R. D. Dixit is a critically endangered and ethnobotanically very important plant. Official drug i.e. dried rhizome of the plant has been reported to be used for treatment of leprosy, tuberculosis, paralysis, oedematous swelling, fevers, intestinal worms and constipation. Besides having high medicinal properties, detail studies on chemical constituents present in the rhizome of this particular species have not been done so far. Thus, in present study efforts were made for evaluation of phytochemical as well as physicochemical analysis of the rhizome collected from six different places of the Chitrakoot region of Madhya Pradesh (M. P.) and Uttar Pradesh (U. P.).

Methods: Phytochemical analysis of the rhizome was carried out as per standard protocol given in Ayurvedic Pharmacopoeia of India (API). Separation and qualitative phytochemical screening were done by using an advance technique of High-performance Thin Layer Chromatography (HPTLC).

Results: The **s**tudy revealed the presence of alkaloids, steroids, triterpenoids, flavonoids, glycosides, carbohydrates, starch, saponin, tannins, etc. in the rhizome of the plants, and the study confirmed the chemotaxonomic resemblance among all the collected plant materials.

Conclusion: Comparative study of six rhizome samples of *A. Chitrakutensis* provides authenticity for establishing Pharmacopoeial standardization of drug and evidence of the study proves the chemotaxonomic similarities of official drug.

Keywords: *Alectra chitrakutensis*, Endangered plant, Quality control, Physicochemical, Phytochemical analysis, HPTLC

INTRODUCTION

A. chitrakutensis, family Scrophulariaceae is a critically endangered annual root parasitic herb. It is locally known as 'Nirgundikand' found in confined localities in the Chitrakoot region of Madhya Pradesh and also in Uttar Pradesh, India [1]. The species grows as a parasite on the threadlike roots of white-flowered *Vitex negundo* L. Chitrakoot is very rich in ethnomedicobotanical diversity. The tribal people use locally available plant species for the treatment of human as well as livestock ailments [2]. *A. chitrakutensis* is one of them having high-value medicinal properties. Ethnobotanically, the whole plant has been reported to be used for treatment of diverse deadened diseases. However, the rhizome is used to cure leprosy, tuberculosis, paralysis, oedematous swelling, fevers, intestinal worms and constipation. The previous study has reported various ethnobotanical uses of rhizomes, for example, paste of the rhizome mixed with cow urine is given to leprosy patients [3]; similarly, dried and powdered rhizome is used to cure other skin diseases. For instant relief from constipation rhizome paste is taken with milk. Besides, rhizome paste is given once a day for 2-3 d to expel intestinal worms. Likewise, for the treatment of malaria, fresh rhizome paste with cow urine is given to a patient twice a day. Rhizome powder is also given to cure paralysis and piles. The plant and rhizome extract with almond, cucumber, watermelon, long cucumber, cardamom and rose petal is taken as an invigorating tonic. In case of Spermatorrhoea, rhizome powder is given with milk [2]. Preliminary phytochemical investigations carried out and reported the presence of steroids, glycosides, flavonoids, carbohydrates, alkaloids, saponins and tannins in the whole plant extracts [4]. A carotinoid, an orange-yellow color pigment azafrin, and mannitol were also found in the rhizome [5].

Since, *A. chitrakutensis* is a critically endangered and endemic species of high medicinal value. Until now, the importance of this species was recorded only in few folklore literatures; furthermore, no records were found to establish pharmacopoeial evaluation of the rhizome which is the plant part of medicinal interest. Owing to the significant medicinal properties of the rhizome, there is an urgent need to identify and characterize the various primary as well as secondary metabolites present in the rhizome so as to prepare pharmacopoeial standards and a novel drug of therapeutic use. Therefore, the present study deals with the physicochemical and phytochemical evaluation of the rhizome of the plant *A. chitrakutensis* collected from six different places.

MATERIALS AND METHODS

Collection and identification of plant material

Authentic plant materials were collected from six different places in the Chitrakoot region of M. P. and U. P. in between the month of February-March 2012 under the supervision and guidance of R. L. S. Sikarwar; speciemen herbarium was prepared and submitted vide herbarium number 319 in the repository at "Arogyadham" (JRD Tata Foundation for Research in Ayurveda and Yoga Science), Deendayal Research Institute, Chitrakoot, (M. P.). The distinctive places from where the material was collected are Herbal Garden, Deendayal Research Institute, Chitrakoot (M. P.), Narayanpur Hills, Chitrakoot (U. P.), Bank of Mandakini river, Satna, Chitrakoot (M. P.), Bank of Bagey river, Badausa (U. P.), Bank of Bagey river, Banda (U. P.) and Lodwara Hill, Chitrakoot (U. P.).

Processing of plant material

Rhizomes were separated from aerial parts of the plant, thoroughly washed with tap water and dried in the hot air oven at 50C and powdered by using a grinder. After grinding all the samples were passed through sieve of 40-60 mesh size and kept in a container for further use.

Powder analysis

The fluorescence study of powdered drugs was performed using different chemicals under the UV light (254 nm and 366 nm) and in visible light according to the standard methods [6-7].

Physicochemical evaluation

Physico-chemical analysis such as moisture content, ash value, and extractive values were performed using standard protocols given in Ayurvedic Pharmacopoeia of India [8].

Table 1: Fluorescence study of rhizome powder of '*A. chitrakutensis*' collected from six different places

Reagents/Samples	Herbal Garden, Chitrakoot (M. P.)			Bank of Bagey river, Banda (U. P).			Lodwara Hill, Chitrakoot (U. P.)			Narayanpur Hills, Chitrakoot (U. P.)			Bank of Bagey river, Badausa (U. P.)			Bank of Mandakini river, Satna, Chitrakoot (M. P.)		
	Day Light	UV 254 nm	UV 366 nm	Day Light	UV 254 nm	UV 366 nm	Day Light	UV 254 nm	UV 366 nm	Day Light	UV 254 nm	UV 366 nm	Day Light	UV 254 nm	UV 366 nm	Day Light	UV 254 nm	UV 366 nm
Powder (P) as such	Brown	Dark Brown	Black	Brown	Dark Brown	Black	Brown	Dark Brown	Black	Brown	Dark Brown	Black	Brown	Dark Brown	Black	Brown	Dark Brown	Black
P+Nitrocellulose in amyl acetate	Blackish Brown with Greenish Tinge	Fluorescence Greenish brown	Black with Yellow tinge	Blackish Brown with Greenish Tinge	Fluorescence Greenish brown	Black with Yellow tinge	Blackish Brown with Greenish Tinge	Fluorescence Greenish brown	Black with Yellow tinge	Blackish Brown with Greenish Tinge	Fluorescence Greenish brown	Black	Blackish Brown with Greenish Tinge	Fluorescence Greenish brown	Black	Dark Brown with Greenish Tinge	Fluorescence Greenish brown	Black
P+1N NaOH	Chocolate Brown	Dark Brown	Black	Chocolate Brown	Dark Brown	Black	Chocolate Brown	Dark Brown	Black	Chocolate Brown	Dark Brown	Black	Chocolate Brown	Dark Brown	Black	Chocolate Brown	Dark Brown	Black
P+1N NaOH+Amyl acetate	Dark Brown	Dark Brownish Black	Black	Dark Brown	Dark Brownish Black	Black	Dark Brown	Dark Brownish Black	Black	Blackish Brown	Black	Black	Blackish Brown	Black	Black	Dark Brown	Black	Black
P+1N HCl+Nitrocellulose in amyl acetate	Brown with Greenish Tinge	Dark Brown with fluorescence Green tinge	Black	Brown with Greenish Tinge	Dark Brown with fluorescence Green tinge	Black	Brown with Greenish Tinge	Dark Brown with fluorescence Green tinge	Black	Dark Brown	Blackish Brown with fluorescence Green tinge	Black	Dark Brown	Blackish Brown with fluorescence Green tinge	Black	Blackish Brown	Brown	Black
P+1N NaOH in methanol	Dark Green	Dark Green	Green	Dark Green	Dark Green	Green	Dark Green	Dark Green	Green	Dark Green	Blackish Brown	Green	Dark Green	Blackish Brown	Green	Dark Green	Dark Brown	Green
P+50% KOH	Chocolate Brown	Black	Black	Chocolate Brown	Black	Black	Chocolate Brown	Black	Black	Chocolate Brown	Black	Black	Chocolate Brown	Black	Black	Blackish Brown	Fluorescence Green tinge	Black
P+1N HCl	Dark Brown	Dark Brown	Black	Dark Brown	Dark Brown	Black	Dark Brown	Dark Brown	Black	Brown	Black	Black	Brown	Black	Black	Brown	Black	Black
P+50% H_2SO_4	Black	Black	Black	Black	Black	Black	Black	Black	Black	Black	Black	Black	Black	Black	Black	Blackish Brown	Black	Black
P+50% HNO_3	Rusty Yellow	Black with Green tinge	Black with Green tinge	Rusty Yellow	Black with Green tinge	Black with Green tinge	Rusty Yellow	Black with Green tinge	Black with Green tinge	Almond Brown	Dark Brown	Black	Almond Brown	Dark Brown	Black	Chocolate Brown	Black	Black
P+Conc. HNO_3	Rusty Yellow	Black with Yellow tinge	Black with Green tinge	Rusty Yellow	Black with Yellow tinge	Black with Green tinge	Rusty Yellow	Black with Yellow tinge	Black with Green tinge	Turmeric dark/pale Yellow	Rusty Yellow	Black	Turmeric dark/pale Yellow	Rusty Yellow	Black	Pale Yellow	Brown	Black
P+Acetic acid	Dark Brown	Black	Black	Dark Brown	Black	Black	Dark Brown	Black	Black	Blackish Brown	Brown with fluorescence Green tinge	Black	Blackish Brown	Brown with fluorescence Green tinge	Black	Dark Brown	Rusty Yellow	Black
P+Conc. H_2SO_4	Black	Black	Black	Black	Black	Black	Black	Black	Black	Black	Black	Black	Black	Black	Black	Black	Dark Brown	Black
P+Iodine water	Brown	Dark Brown	Black	Brown	Dark Brown	Black	Brown	Dark Brown	Black	Dark Green	Dark Brown	Black	Dark Green	Dark Brown	Black	Dark Green	Black	Black

Phytochemical screening

The preliminary phytochemical screenings for the chemical constituents like steroids, alkaloids, saponins, glycosides, flavonoids, starch, tannins, resins, etc. were determined by the standard methods [9].

HPTLC fingerprint analysis

HPTLC (CAMAG, Switzerland), an advance technique was used for qualitative screening of different phytoconstituents [10]. For this, 1 g powdered material of each sample was extracted using 10 ml methanol in water bath for 5 min. The extracts were filtered through Whatman No. 1 filter paper separately for each sample. Similarly, stock solution (1 mg/ml) of standard azafrin; a pure compound isolated from plant material by using standard protocol [11] was also prepared. Filtrated extracts and standard solution of azafrin (2 µl) were applied on 20x20 cm TLC plates of silica gel G F254 (Merck, Germany) with the help of Linomat 5 CAMAG TLC Applicator.

The plate was developed up to 18.5 cm by using the solvent system Toluene: Ethyl acetate: Glacial acetic acid (4:5:0.1 v/v/v) in a CAMAG Twin-through chamber previously saturated with mobile phase vapor for 30 min. at room temperature. After development, plates were air dried at room temperature and documented by using CAMAG visualizer documentation system. Anisaldehyde sulfuric acid reagent was used as derivetizing agent. After derivetization, plates were dried at 100 °C on a hot plate for 5 min and visualize under 254 nm, 366 nm and visible light.

RESULTS

Powder analysis

Fluorescence study of all six samples of rhizome powder under UV light, with or without chemical, treatment was done. The color developed by the powder with different chemical reagents under UV, and visible lights are summarized (table 1). Plant materials collected from different places showed somewhat different color characterization of rhizome powder. Similarities in results were obtained among the rhizome samples collected from Herbal garden, Arogyadham, Dindayal Research Institute, Chitrakoot (M. P.), Bank of Bagey river, Banda (U. P.) and Lodwara Hill, Chitrakoot (U. P.). While, Narayanpur Hills, Chitrakoot (U. P.) and Bank of Begey river, Badausa (U. P.) also produced similar results, whereas material from Bank of Mandakini river, Satna, Chitrkoot (M. P.) showed unlike color than others.

Quantitative physico-chemical analysis

The results of physicochemical analysis *viz.*, foreign matter, moisture content, total ash, acid insoluble ash, alcohol, water extractive values and successive Soxhlet extractive values of different extractives like hexane, chloroform, methanol and water of the rhizome powder are presented in table 2 and 3. Plant material collected from different places throughout the Chitrakoot region showed not much significant difference. Thus, the values obtained from a physicochemical study are useful to establish the identity and strength on the rhizome part for further study.

Qualitative phytochemical screening of successive soxhlet extractives

The phytochemical analysis using different reagents such as n-hexane, chloroform, methanol and water extracts showed the presence of starch, steroids, terpenoids, alkaloids, tannins, flavonoids, glycosides, proteins, and amino acids in different successive extracts of all the collected samples have been presented in table 4. Glycoside was the major constituent found in chloroform, methanol and water extracts.

HPTLC study

In densitometry scan of Thin Layer Chromatography (TLC) profiles of the methanolic extracts of all six rhizome samples of *'A. chitrakutensis'* showed an almost resembling band pattern which indicated that the six samples are having similar phytochemicals. Only the quantity of particular phytoconstituents may vary (fig. 1). For TLC, solvent system was developed to get high-quality resolution and separation of bands. Even if there were some variations in the profiles which might be due to some environmental factors. Rf values were calculated under 366 nm and are summarized in table 5.

Table 2: Comparative physicochemical values of the rhizome of *'A. chitrakutensis'* collected from six different places

S. No.	Physico-chemical parameters	Herbal garden, chitrakoot (M. P.)	Narayanpur hills, chitrakoot (U. P.)	Bank of mandakini river, satna, chitrakoot (M. P.)	Bank of bagey river, badausa (U. P.)	Bank of bagey river, banda (U. P.)	Lodwara hill, chitrakoot (M. P.)
1.	Foreign matter	2.12	2.00	2.00	2.2	2.12	2.1
2.	Moisture content (%)	7.06 ±0.13	5.27 ±0.04	6.16 ±0.02	7.96 ±0.03	7.01 ±0.03	5.99 ±0.01
3.	Total Ash (%)	5.98 ±0.07	5.83 ±0.15	5.91 ±0.02	5.88 ±0.06	5.89 ±0.08	5.79 ±0.09
4.	Acid Insoluble Ash (%)	1.34 ±0.03	1.32 ±0.04	1.29 ±0.01	1.29 ±0.05	1.30 ±0.00	1.29 ±0.01
5.	Alcohol soluble extractive value (%)	7.66 ±0.74	7.81 ±0.44	7.52 ±0.24	8.26 ±0.56	8.02 ±0.15	7.55 ±0.25
6.	Water soluble extractive value (%)	27.93 ±1.43	27.92 ±0.06	27.00 ±0.05	27.91 ±0.09	27.77 ±0.05	27.75 ±0.06

Table 3: Comparative soxhlet extractive values of rhizome of *'A. chitrakutensis'* collected from six different places

Solvent	Herbal garden, chitrakoot (M. P.)	Narayanpur hills, chitrakoot (U. P.)	Bank of mandakini river, satna, chitrakoot (M. P.)	Bank of bagey river, badausa (U. P.)	Bank of bagey river, banda (U. P.)	Lodwara hill, chitrakoot (M. P.)
Hexane	1.83±0.03	1.64±0.02	1.74±0.03	1.81±0.02	1.59±0.02	1.41±0.02
Chloroform	5.29±0.05	5.24±0.02	5.28±0.02	5.34±0.03	5.32±0.02	5.13±0.01
Methanol	32.99±0.45	31.24±0.23	30.18±0.42	33.26±0.43	29.83±0.31	32.23±0.26
Water	20.73±0.12	20.71±0.09	20.74±0.07	20.79±0.07	20.79±0.11	20.72±0.13

Table 4: Qualitative phytochemical screening of successive soxhlet extractives of rhizome samples of '*A. chitrakutensis*' collected from six different places

Phytoconstituent (s)	Hexane	Chloroform	Methanol	Water
Starch	-	-	-	+
Steroids	+	+	-	-
Triterpenes	+	-	-	-
Flavonoids	-	-	+	-
Resins	+	+	-	-
Alkaloids	-	-	+	-
Reducing sugars	-	-	+	+
Glycosides	-	+	+	+
Tannins	-	-	+	+
Saponins	-	-	+	+
Carbohydrates	-	-	-	+
Lipids	+	-	-	-
Amino acids	-	-	-	+
Oils	+	-	-	-

Table 5: TLC analysis of six rhizome samples of '*Alectra chitrakutensis*' collected from six different places at 366 nm

Rf values	Azafrin	Herbal Garden, Chitrakoot (M. P.)	Narayanpur Hills, Chitrakoot (U. P.)	Bank of Mandakini river, Satna, Chitrakoot (M. P.)	Bank of Bagey river, Badusa (U. P.)	Bank of Bagey river, Banda (U. P.)	Lodwara Hill, Chitrakoot (U. P.)
0.02	-	+	+	+	+	+	+
0.04	-	+	+	-	-	-	-
0.05	-	-	+	-	+	-	-
0.24	-	-	-	+	+	-	-
0.32	-	+	+	+	+	+	+
0.41	-	+	+	+	+	+	+
0.54	-	+	+	+	+	+	+
0.59	+	+	+	+	+	+	+
0.70	-	+	+	+	+	+	+
0.75	-	+	+	+	+	+	+

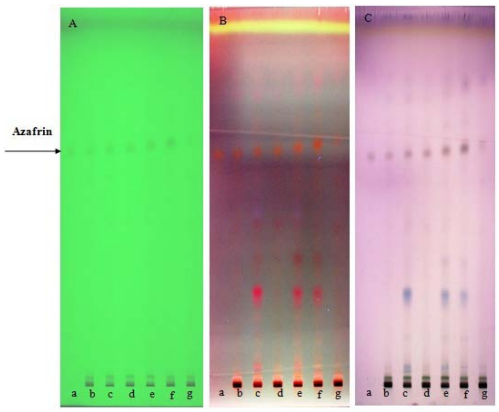

Fig. 1: HPTLC Fingerprint profile of methanolic extracts after derivetization. a. Azafrin standard b. Narayanpur Hills, Chitrakoot (U. P.), c. Bank of Mandakini river, Satna, Chitrakoot (M. P.), d. Bank of Bagey river, Badausa, (U. P.), e. Bank of Bagey river, Banda (U. P.), f. Herbal Garden, Deendayal Research Foundation, Chitrakoot (M. P.) g. Lodwara Hill, (U. P.) at A.254 nm, B.366 nm and C.422 nm respectively

DISCUSSION

The presence of medicinal phytochemicals *viz*. alkaloids, steroids, flavonoids, glycosides, terpenoids, etc. provides authenticity to the high curative value of the plant. Only very few records are noted in the physicochemical or pharmacognostical studies which were done on whole plants and not confined to rhizome, which is the plant part of medicinal interest [4, 12]. Similarly, preliminary phytochemicals analysis of another species *Alectra parasitica* A. Rich collected from Akola district (Maharashtra) has been conducted by using whole plant powder in various extracts, and they found the analogous phytochemicals present on the plant [13]. On the other hand, as also indicated above no records were found in the pharmacognostical standardization/phytochemical analysis by using only rhizomatous part of the plant. Therefore, it is the first attempt to study pharmacognostical evaluation and screening of phytochemicals of plant *A. chitrakutensis* by using an advanced and sophisticated instrument HPTLC. The results obtained from the different tests performed for physicochemical, and phytochemical analysis was given in a comparable manner for all six rhizome samples to lay down quality standards using a wide range of data. Thus, the study indicates that there is no significant chemotaxonomic variation found among the plants collected from different places of Chitrakoot region.

CONCLUSION

The present manuscript will be proven as an important document for pharmacognostical standardization and quality control because a wide range of rhizome samples was compared on their physicochemical as well as the phytochemical basis. The study also revealed that the plants are found to be similar chemotaxonomically as well as in their phytochemical, compounds present under the form of primary as well as secondary metabolites, in particular, species of *A. chitrakutensis* and was confirmed by HPTLC finger print profile, which showed similar bands in all the samples. The present study will definitely open up the new avenues for further researches on isolation, identification and characterization of some new secondary metabolites of promising medicinal uses. Consequently, the present data will certainly provide the platform to make new drugs for mankind to cure threatened diseases.

CONFLICT OF INTERESTS

Declared none

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