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Pharmacognostical Study of *Hedychium Spicatum* (Ham-Ex-Smith) Rhizome

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ABSTRACT

Objective: To explore a detailed pharmacognostic study of the rhizome of *Hedychium spicatum* Ham-ex-Smith (Zingiberaceae), a plant species which is commonly used in preparation of indigenous medicine. **Methods:** The macroscopy, microscopy, quantitative analysis, extractive values in ethanol and water, phytochemical screening, TLC and HPTLC of aqueous and ethanolic extract and DNA fingerprint of the rhizome were investigated. **Results:** Rhizome appeared to be 15-20 cm long, 20-25 mm in diameter, light-brown with 4-6 nodules. Transverse section of rhizome showed an outermost thick layer of suberised, dark brown cells in outer cork with 10 or more layers of irregular parenchymatous cells. Inner cork consisted of a few layered light brown rectangular radially arranged cells, followed by a wide zone of cortex having 30-40 cell layers. Rhizome powder was light brown in colour, bitter having camphorous odour, and fibrous texture. Alkaloids, carbohydrates, proteins, resins, saponins, steroid, tannin, starch and glycosides were present in both extracts while, flavonoids and triterpenoids were present only in ethanolic extract. TLC, HPTLC and DNA fingerprinting confirmed the chemical composition present in rhizome. **Conclusions:** The pharmacognostic profile of *Hedychium spicatum* rhizome is helpful in sample identification, quality and purity standards.

1. Introduction

Hedychium spicatum (Ham-ex-smith) belongs to family zingiberaceae, is a perennial rhizomatous herb. It grows throughout subtropical Himalaya in the Indian state of Assam, Arunachal Pradesh and Uttarakhand with in an altitudinal range of 1000 to 3000 m. It is tall stout herb with fleshy rhizomes, thick straight stem with broadly lanceolate leaves. *H. spicatum* rhizome is mentioned as shati in Ayurvedic classics and has been used in various dosage forms to treat cough, wound ulcer, fever, respiratory problems and hiccough. The rhizomes have a strong aromatic odour and bitter taste. The rhizome extract has been reported to contain essential oil, starch, resins, organic acids, glycosides, albumen and saccharides, which has been advocated for blood purification and treatments of bronchitis, indigestion, eye disease and inflammations. The rhizome has been reported to contain sitosterol and its glucosides, furanoid diterpene-hedychenone, 7- hydroxyhedychenone and Metoxycinnamate. Essential oil present in rhizome has cineole, terpinene, limonene, phellandrene, p-cymene, linalool and terpeneol as major constituents[1]. The rhizome has been reported to possess anti-inflammatory, anti-asthmatic, hypoglycaemic, vasodialator, spasmolytic, hypotensive, in vitro pediculicidal, cytotoxic and antimicrobial properties[2]. Authenticity, purity and assay are the three major attributes for standardization and quality control[3]. Hence, in the present work we made an attempt for the standardization of *H. Spicatum* rhizome by carrying out its pharmacognostic evaluation.

2. Materials and Methods

2.1. Plant material

The rhizome of H. spitactum Ham-ex-Smith was collected from Lansdown (Uttarakhand) in the month of October to November and confirmed with the sample preserved in the herbarium of the Department of Dravyaguna, Institute of Medical Sciences, Banaras Hindu University, Varanasi. A specimen of plant (DGM- 104) was preserved in the museum

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for further reference.

2.2. Macroscopic and microscopic analysis

Rhizome was studied macroscopically for important identification points, i.e. odour, taste and texture and for microscopical studies; a transverse section was prepared and stained[4]. Microscopy of power was investigated according to method of Kokate (2010)[5].

2.3. Physiochemical analysis

Physiochemical studies such as moisture content, total ash, foreign matter, acid insoluble ash, sulphated ash were determined according to WHO guidelines on quality control method for medicinal plants[6].

2.4. Phytochemical screening

Phytochemical screening was carried out by using the standard methods[5].

2.5. Thin layer chromatography (TLC)

Silica gel and distilled water were used to prepare a slurry coating materials and plates were coated by using the spreading device, with a layer about 0.30 mm thick coated plates were then dried and activated in oven for 30 min. a pencil line is drawn near the bottom and a small drop of ethanolic and aqueous extract solution were placed separately on it; and the spot were placed to become dry. Plates were placed in chromatographic chamber containing the Hexane: ethyl acetate, 4:1 and Rf value were recorded.

2.6. High performance thin layer chromatography (HPTLC)

HPTLC Analysis was performed on high performance silica gel 60 F254 TLC plates (Merka, KgaA, Germany). The plates were pre-cleaned by development to the top with methanol and dried in fume-hood before use. Sample solutions were applied to the plate by means of a Camag (USA) Linomat IV, automated spray on band applicator equipped with 100 μl syringe and operated with band length 10 mm, application rate 10 s μl^{-1}, table speed 10 mm s-1, and distance from bottom 10 mm. The volume applied for each analysis was 10 μl of the sample. Distance between tracks is 10 mm. The plates were developed 8 cm beyond the origin with solution of Toluene: Ethyl acetate: Acetic Acid (5.4 : 4.3 : 0.3) solvent system as mobile phase in vapor-equilibrated Camag (wilmingron Nc, USA) twin-trough chamber. After development the mobile phase was evaporated from the plate by drying in a fume-hood for 10min. Then the CAT-3 software controlling the CAMAG -TLC scanner used to scan the developed zones under Long and Shot UV and under white light after derivatization.

2.7. DNA finger print

2.7.1. DNA extraction

Rhizome tissue of plant (Hedychiutn spicatum Ham-ex-Smith) was used for extraction of total genomic DNA by following the protocol of Aromdee el al, 2011[7]. Five to thirty mg of rhizome tissue was frozen in liquid nitrogen and ground in a mircocetrifuge tube using a fitted pestle. The extraction of genomic DNA was perfume using the GeNeiTM Ultra pure plant Genomic DNA prep kit (Bangalore Genei, India), after that isolated genomic DNA were estimated by measuring absorbance at A260 nm with a VU Vis Spectrophotometer SL 159 (ELICO, India). Typically, 0.2- 0.8 mg of DNA were obtained per mg of fresh Rhizome. DNA samples were diluted to working solutions of 25ng. Finally, confirmed by agarose gel electrophoresis and stored at 4°C until use.

2.7.2. RAPD- PCR

RAPD analysis using the method of Olga et al., 2011[8]. Reaction were carried out in a final volume of 25 ml containing 20 mM tris-HCL pH 8.4, 50 mM KCl, 3 mM MgCl2, 0.2 mM dNTPs, 0.3mM primer, 10 mer polymerase (invitrogen). Amplifications were performed in a Peltier-Based Thermal Cycler Model P25+ (Cyberlab, USA). Programmed for 10mer primer having initial denaturation cycle (7 min at 94o C) followed by 10 cycles of denaturation (1 min at 94o C), annealing (2 min at 35o C) and extension (1 min at 72o C) with a final extension of 5 min at 72o C. The above reaction was performed at least twice to assess the consistency of the band profiles. Reaction products (10 ml) were resolved by electrophoresis in 1.2% agarose gels stained with ethidium bromide in 13 TAE buffer at 90 V for 140 min. all the reactions included negative controls in which DNA or primer was omitted in the amplification reaction mixture.

3. Result

3.1. Macroscopic characteristics

Rhizome 15-20 cm long, 20-25 mm in diameter, externally light- brown having nodes which vary from 4-8 in number. Rootlets are attached here and there in whole length .Numerous scars and circular rings, rudiments of root- lets are also visible. It has pleasant camphoraceous odour and bitter taste. (Figure 1)

Figure 1. Macroscopic characteristic of *H. spicatum* rhizome

3.2. Microscopic characteristics

Transverse section of rhizome shows an outermost thick layer of suberised, dark brown cells of outer cork consisting of 10–15 or more layers of irregular parenchymatous cells. Inner cork consists of a few layered light brown rectangular, radially arranged cells followed by a wide zone of cortex 30–40 cells thick, some cortical cells filled with flattened and oval oblong starch grains. Numerous oleo-resin cells also found in this region which have suberised walls containing green-yellow oil. A thin endodermal layer present beneath cortex. Central cylinder distinguished by presence of peripheral plexus of irregular congested vascular bundles with poorly developed mechanical tissue, Vascular bundles scattered irregularly throughout ground tissue. Bundle closed and colleteral possessing group of two or more xylem element. Ground tissue composed of large parenchymatous cells with abundant starch grains and oil. Transverse section showed the numerous cortical cells filled with yellowish green oils. After staining with iodine and phloroglucinal with concentrated HCl showed abundant starch grains in entire section and appearance of fibrovascular bundles (Figure 2 & 3).

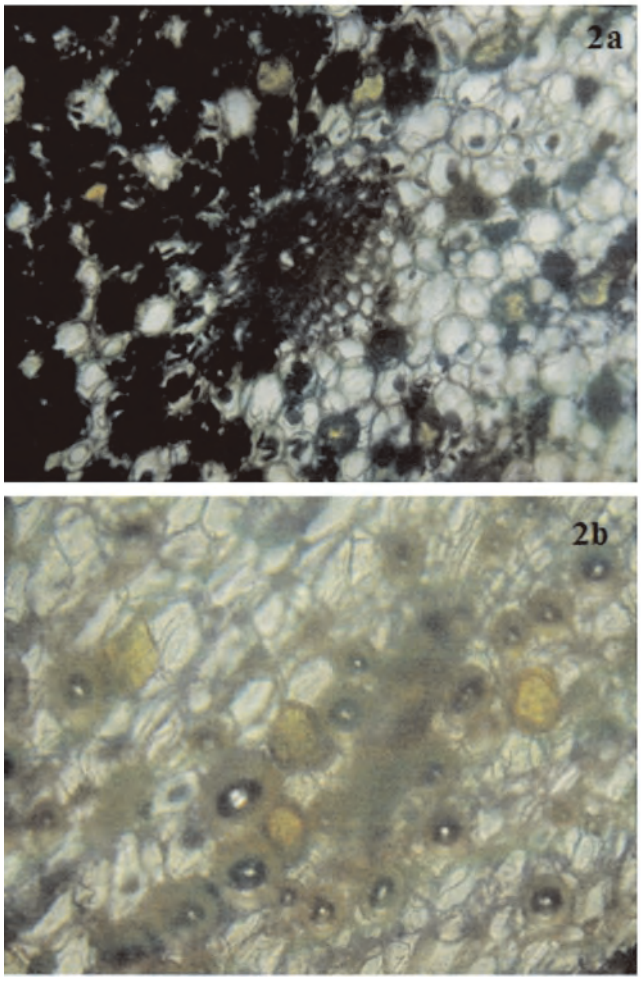

Figure 2. Microscopic characteristics of *H. spicatum*. 2a: T.S. of rhizome showing ground tissue of parenchymatous cell with abundant starch grain; 2b: T.S. of rhizome showing oil filled cortical cells.

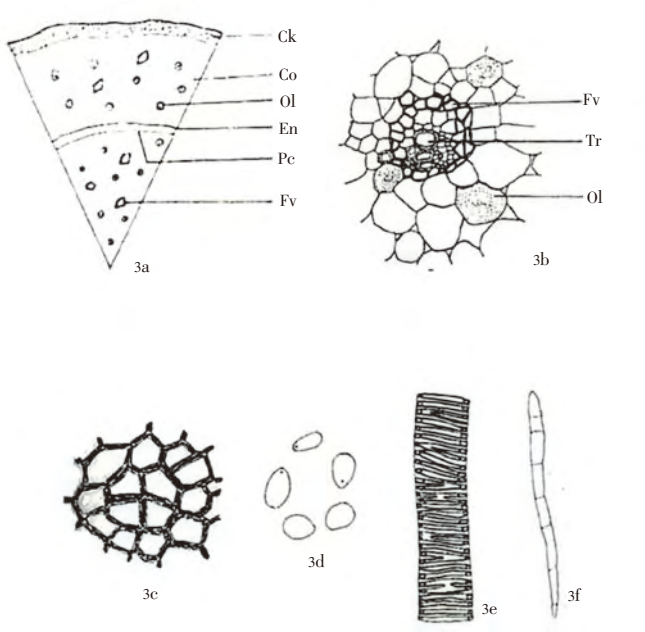

Figure 3. Schematic diagram of rhizome. 3a: T.S. of the rhizome; 3b: T.S. through fibrovascular bundle from the cortex x720; 3c: Cork cells x180; 3d: Starch grains x367; 3e: Vessels x367; 3f: Septate fiber x92. Ck: Cork; Co: Cortex; En: Endodermis; Fv: Fibrovascular bundle; Ol: Oil globule; Pc: Pericycle; Tr: Tracheid

3.3. Powder characteristics

Rhizome powder is light brown in colour, bitter having camphorous odour, and fibrous in texture. It shows abundant starch grains in entire section which will take purple colour. The shapes of starch grains were oval. With phloroglucinal and concentrated hydrochloric acid it gave the appearance of fibrovascualr bundles. Numerous cortical cells filled with yellowish green oil were also found (Figure 4).

3.4. Physicochemical analysis

Physiochemical studies such as moisture content, total ash, foreign matter, acid insoluble ash and sulphated ash were presented in table 1.

Table 1

Physicochemical characteristics of *H. spicatum* rhizome

Parameters	Value
Foreign matter	1.0%
Moisture content	2.8%
Total ash	7.6%
Acid insoluble ash	1.8%
Sulphated ash	8.3%
Alcohol soluble extractive	5.4%
Water soluble extractive	10.5%

3.5. Phytochemical Screening

The yield of ethanolic and aqueous extracts was 4% and 7%

respectively. The extracts revels the presence of alkaloids, carbohydrate, protein, resins, saponins, steroid, tannin, starch and glycosides in aqueous extract while flavonoids and tritepenoids were only present in ethanolic extract (Table 2).

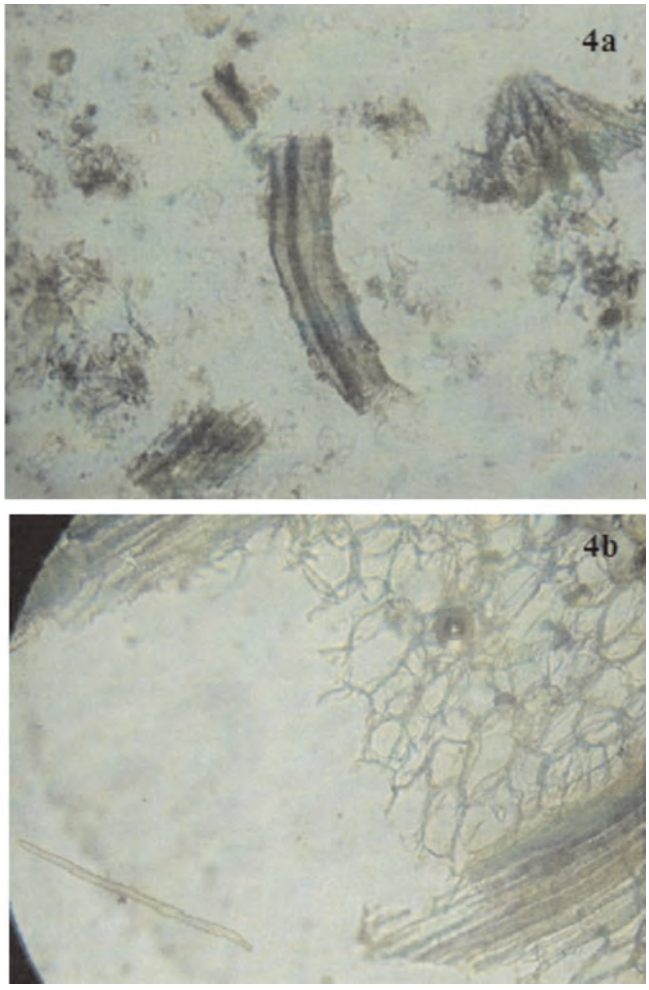

Figure 4. Powder characteristics of *H. spicatum*. 4a: Vessels; 4b: Fibers

Table 2

Phytochemical screening of *H. spicatum* rhizome

Chemical constituents	Chemical tests	Aqueous xtract	Alcoholic Extract
Alkaloids	Dragendroff Test	+	+
	Hager's test	−	+
	Wagner's test	−	+
	Mayer's test	+	+
Carbohydrate	Anthrone test	+	−
	Benedict's test	+	+
	Fehling's test	+	+
	Molisch's test	+	+
Flavonoids	Shinoda's test	−	+
Tritepenoids	Liebermann Burchard test	−	+
Proteins	Biuret's test test	+	+
	Moillon's test	+	+
Resins		+	+
Saponins	Foam test	+	+
Steroid	Liebermann Burchard test	+	+
Tannins	Ferric chloride test	+	+
Starch	Iodine test	+	+
Glycoside	Keller-kikkiani test	+	+

+: Present; − : Negative

3.6. TLC

Both the extracts contain the fluorescence component. Steroid was confirmed on TLC plate and produced purple color with vanilline sulphuric acid. After spray of ninhydrin an orange color appeared in aqueous extract due to presence of amino acid (Figure 5, table 3).

3.7. HPTLC

HPTLC plate was observed under UV 254 nm and UV 366 nm for four different sample of *H. spicatum* rhizome. The Rf values was ranging from 0.33 to 0.89 under UV 254 and 0.25 to 0.99 under UV 366 nm. After derivation with anisaldehyde sulphuric acid a purple colour appeared under white light to confirm the presence of steroids in the sample and Rf value ranging from 0.12 to 0.97. (Figure 6, table 4)

Table 3

Thin layer chromatography of ethanolic and aqueous extract of *H. spicatum*

Solvent system	Sample	Number of spot	Colour of spot			R.F Value
			UV light	With Ninhydrin	With Vanilline sulphuric acid	
Toluene: Ethyl acetate	Ethanolic extract	2	Light green	Purple	No colour change	0.384; 0.522
Toluene: Ethyl acetate	Aqueous extract	3	Light green	Purple	Orange colour	0.192, 0.384; 0.480

Table 4

HPTLC of ethanolic extract of *H. spicatum*

Observations	Rf Values			
	Varanasi	Genuine	Haridwar	Lucknow
Under UV 254 nm	0.33, 0.62	0.33, 0.48, 0.62, 0.89	0.33, 0.48, 0.68	0.33, 0.48
Under UV 366 nm	0.18, 0.22, 0.32, 0.86, 0.94	0.08, 0.18, 0.33, 0.71, 0.85, 0.91, 0.94	0.09, 0.25, 0.33, 0.56, 0. 86, 0.94, 0.99	0.33, 0.85, 0.94, 0.98
After derivation with Anisaldehyde sulphuric acid Under white light	0.12, 0.26, 0.32, 0.63, 0.67, 0.73	0.12, 0.16, 0.26, 0.32, 0.39, 0.47, 0.61, 0.73, 0.88	0.12, 0.17, 0.24, 0.32, 0.39, 0.46, 0.74, 0.97	0.33, 0.46, 0.52, 0.66, 0.71, 0.80, 0.97

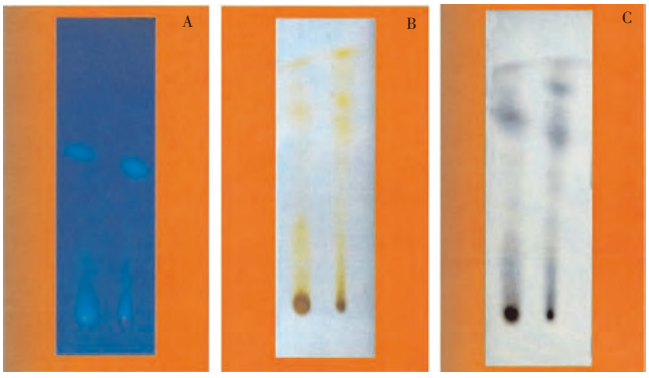

Figure 5. TLC Plates of aqueous and ethanolic extract of *H. spicatum* using Hexane:Ethyl acetate 4:1 as solvent. 5A: Under UV light; 5B: After treatment with iodine vapours; 5C: After treatment with vanilline sulphuric acid.

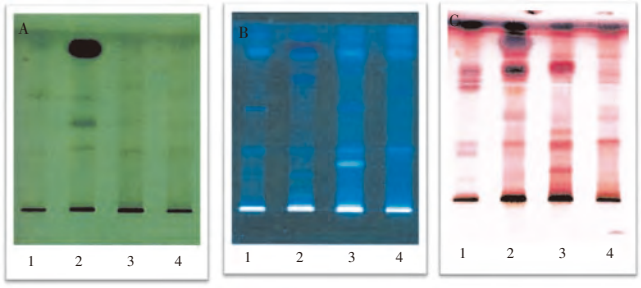

Figure 6. HPTLC of ethanolic extract of *H. spicatum*. 6A: Under UV 254nm; 6B: Under UV 366nm; 6C: After derivation with anisaldehyde sulphuric acid

3.8. DNA finger print

Bands of the sample and marker compound were observed. Three prominent bands were observed in DNA finger printing of *H. spicatum* with primer TGGTCCTGGC (Figure 7).

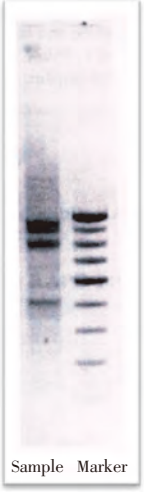

Figure 7. PCR amplification electrophoretic patterns obtained with amplified DNA (RAPD) of *H. spicatum* rhizome

4. Discussion

World Health Organization (WHO) recommend and promotes herbal remedies in national health care programmes because these drugs are easily available at low cost and safe but numerous concerns regarding the purity; safety and quality of herbal medicines have been observed so there is a need to ensure quality control of medicinal plant products by using modern techniques and applying suitable standards. According to WHO, standardization is an essential measure of quality, purity and authenticity[9]. Microscopic method is simple and reliable method for authentification and standardization of the plant materials. The preliminary phytochemical analysis will reveal the chemical nature of the drug and explore the source of pharmacologically active chemical compounds[10]. Thin layer chromatography study of aqueous and ethanolic extract confirms presence of different constituents i.e. steroid, alkaloids and amino acids. Chromatographic technique and DNA fingerprinting can be highly useful method for identification of the plant material and its chemical components. HPTLC of ethanolic extract from different sample of *H. spicatum* was done. The HPTLC analysis of different sample indicates the variation in quantitative and qualitative characters. RAPD technique includes rapidity, simplicity and no need of prior genetic information of the plant. DNA fingerprinting has given the genomic marker of genuine *H. spicatum* which is expressed by the bands observed after gel electrophoresis of extracted DNA from the rhizome[11].

The present work was undertaken with a view to lay down standard of *H. spicatum* rhizome which could be useful for authenticity of the rhizome. The pharmacognostic parameters could be useful in the identification and standardization of a crude drug.

Conflict of interest statement

We declare that we have no conflict of interest.

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