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Research Article

PHYSICO–PHYTOCHEMICAL INVESTIGATION AND ANALYTICAL STANDARDIZATION OF *Hedychium spicatum* Ham.ex Smith. (SATI)

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Abstract

Hedychium spicatum Ham.ex Smith (Sati) is an Ayurvedic herb useful for Respiratory, Dermatological problems, and in Tropical Pulmonary Eosinophilia etc and it is one of the ingredient of many an Ayurvedic formulations. The pharmacological activities like Anti-asthmatic, anti-inflammatory and anti-allergic activities of *Hedychium spicatum* were established. Here an attempt has been made to study the various analytical parameters like High Performance Thin Layer Chromatography finger printing, Physical analysis, pH, total ash, Acid insoluble ash, Bulk density, Trapped density, Heavy metals, Assay of marker compound by HPLC and these methods have a pivotal role in quality control and standardization. Analysis of the extract showed values within the Ayurveda Pharmacopeia and WHO guidelines and HPTLC graph showed peak value of the total height 954.8 and total area of 27632.3.

Key Words: Ayurveda; *Hedychium spicatum*; Analytical Techniques; HPTLC;

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INTRODUCTION

Plant and herbal materials are used widely in the developing and developed countries as a mainstream of medicine or as an alternative medicine. In recent times plant research has been increased all over the world and a large body of evidence has been accumulated to highlights the immense potential of the medicinal plants used in various traditional systems of medicine.[1][2] Standardization of herbal products is a big and important issue. Analytical techniques like High Performance Thin Layer Chromatography (HPTLC) finger printing, Physical analysis, pH, Total Ash, Acid insoluble Ash, Bulk density, Trapped density, Heavy metals, Assay of marker compounds by HPLC (High Performance Liquid Chromatography) Method has a pivotal role in quality control and standardization of herbal products. [6][7] Standardization and quality control of herbals as well as the Ayurvedic products are most essential for the acceptance globally. The World Health Assembly - in resolutions WHA31.33 (1978), WHA40.33 (1987) and WHA42.43 (1989) has emphasized the need to ensure the quality of medicinal plant products by using modern control techniques and applying suitable standards.[3][4]

Brief review of Sati

Hedychium spicatum belongs to the family Zingiberaceae, is grows in sub-tropical Himalayas and in Western Ghats of Kerala. It is commonly known as ginger lilies, *Hedichium*, Kapur Kachri and is a genus of herbs with thick, fleshy and branched rhizomes that grows to around 1 meter in length.[5]

Macroscopical characters

Rhizomes 15-20 cm long, 20-25 mm in diameter, externally yellowish-brown hut changed to dark brown on storage, drug available in pieces of 2.5 cm diameter, edge of each piece is covered by a rough reddish-brown layer marked with numerous scars and circular rings, rudiments of root-lets visible, odor- camphoraceous, taste- bitter.[5]

Microscopical characters

Transverse section of rhizome shows an outermost thick layer of submersed, dark brown cells of outer cork consisting of 10-15 or more layers of irregular parenchymatous cells, inner cork consisting 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 tissues, vascular bundles scattered irregularly throughout ground tissue, bundles closed and collateral possessing group of two or more xylem elements, ground tissue composed of large parenchymatous cells with abundant starch grains and oil.[5]

Phyto-chemistry

The dried rhizome of the plant contains essential oil, starch, resins, organic acids and a glycoside; albumen and saccharine. The essential oil has ethyl ester of p-methoxy cinnamic acid, d-sabirene cineole, sesquiterpenes and pentadecane methyl paracumarine acetate. It contain, ß-sitosterol and its ß-D-glycoside. [5]

Pharmacology

In preliminary pharmacological studies the drug is found to have a vasodilatory effect on coronary vessels, mild hypotensive property and a non-specific antispasmodic effect on smooth muscles. [5] Studies on the essential oil

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of the *Hedychium spicatum* rhizomes reveal that it possesses a mild tranquilizing effect of short duration. They depressed the conditioned avoidance response, rotarod performance and potentiated pento-barbitone hypnosis and morphine analgesia in rats. The crude ethanolic extract of rhizomes possesses anti-inflammatory and analgesic activity [5][18]. The anti-inflammatory activity was mainly localized in the hexane fraction from which 1% of pure active constituent was isolated. The root stalk is useful in local inflammations, nausea, asthma, bronchitis, hiccups and in pain. It has been described as useful, especially as an antiasthmatic agent. Clinical trials have been conducted in Tropical Eosiniphilia, with promising results. [5][8][9]

Rhizomes of *Hedychium spicatum* Ham.ex Smith.[14][15][16] are stomachic, carminative, stimulant and tonic. They are used in dyspepsia.[10] The dried rhizomes of commerce on steam distillation yield 4% of an essential oil and its main constituent being ethyl-p-methoxy cinnamate. The oil may be used as perfume for soaps; hair oils and face powders etc.[11] The presence of alkaloids, saponins and flavonoids has been reported in the rhizomes.[12] The ethanolic extract of dried rhizomes showed antibacterial activity.[13]

MATERIAL AND METHODS

The physicochemical, organoleptic and spectrographic studies were conducted at Laila impex, R&D Division Vijayawada.

Collection and Authentication of Plant Material

Sati[14] (*Hedychium spicatum*) rhizomes were purchased from the local market. The identity of the plant was confirmed by Dr. Reddy KN, Botanist, Department of Taxonomy, Laila Impex , Vijayawada. A voucher specimen of the sample (No.22) Raw drug (Serial No: 3323) has been deposited in the institute.

The water extract of *Hedychium spicatum* kept in an airtight double foiled package in a cool temperature for further studies. (Batch Number: L10060616).

Physico-chemical studies

Physico-chemical parameter of the *Hedychium spicatum* was determined as per Guidelines of WHO.[4][5] Total Ash values, Loss on drying, Water soluble Ash, Acid insoluble Ash, Heavy metals, Alcohol soluble extractive and Water soluble extract values were determined at Laila impex, R & D Division, Vijayawada.

Microbial screening

Microbial screening was carried out at Laila impex, R & D Division, Vijayawada for the safe use of the individual plant extract and checked whether total aerobic count, total yeast and mould count is present or absent.

Preparation of Extracts

The Fresh Rhizome sample of *Hedychium spicatum* was air dried and powered. The dried powder of the rhizomes was treated for extraction by hot water for 6 hours. The process was repeated twice. The pooled extracted was concentrated and dried under vacuum, still it forms to Dry flakes. Dry flakes pulverized by Multi-mill / Micro-pulveriser and sieved on shifter and packed.

Spectroscopic screening

Thin layer Chromatography/HPTLC

Methonol reflexed *Hedychium spicatum* water extract at the ratio of 3x50 for one hour was subjected to high Performance Thin layer Chromatography using LINOMAR IV (CAMA G, Sonnemattstise,17, Switzerland) at Laila impex, R&D Division, Vijayawada.

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RESULTS

Six Samples in 3 batches *of Hedychium spicatum* water extract were studied for its characteristics and Physico-chemical standards. Physicochemical parameters of the water extract of the *Hedychium* like Total Ash, Water soluble Ash, Acid insoluble Ash, Water soluble extractive, Ethanol soluble extractive and Moisture content are shown in Table 1. Limits of Heavy metals in Water of *Hedychium spicatum* were shown in Table 2. Analysis reveals a minor presence of some of Heavy metals but the sample does not exceed the limits given according to Ayurveda pharmacopeia and WHO Guidelines. Microbial Screening of the Extract (Table 3) showed the Microbial count is within the WHO standards and safe for the formulation.

Assay by HPLC Method:

Value of Marker Compound-(p-Methoxy Cinnamic Acid Ethyl Ester): 0.014%

Table 1: Physico-chemical standards of Water extract of *Hedychium spicatum*

Sl.No.	Physico-chemical parameter	Value
1.	Particle size through 40 mesh	99.65%
2.	Loss on Drying (% w/w)	33.71%
3.	Water soluble Extractive (% w/w)	87.90%
4.	Alcohol soluble Extractive (% w/w)	40.50%
5.	pH	6.12
5.	Total ash (% w/w)	31.56%
6.	Acid insoluble Ash (% w/w)	2.12%
7.	Bulk Density (g/ml)	0.78

Table 2: Limits of Heavy Metals in Water extract of *Hedychium spicatum*

Sl.No.	Heavy metal	Values
1	Arsenic	< 1ppm
2.	Lead	< 5ppm
3	Cadmium	< 1ppm

Table 3: Microbial Screening in Water extract of *Hedychium spicatum*

Sl.No.	Microbial type	Values
1.	Total plate count	5000 CFu/g
2.	Yeast Moulds	< 10 CFu/g
3.	*Escheria coli*	Absent
4.	*Salmonellae*	Absent
5.	*S. aureus*	Absent
6.	*Staphylococcus aureus*	Absent
7.	*Pseudomonas aeruginosa*	Absent

High performance Thin Layer Chromatography

High performance Thin Layer Chromatography (HPTLC) of *Hedychium spicatum* (DPB No: L10060516) under Spectrum M: TEF3_3 shown as figure 1. And peak values of High performance Thin Layer Chromatography (HPTLC) of *Hedychium spicatum* was mentioned in the Table 4. (Figure 2) Total height of High performance Thin Layer chromatographic of *Hedychium spicatum* is 954.8 and total area is 27632.3.

DISCUSSION

The water extract of *Hedychium spicatum* (Sati), was studied for organoleptic characters and subjected to physicochemical analysis to standardize by HPTLC method for quality control and further studies and utility. Simple and available techniques like High Performance Thin Layer Chromatography finger printing, Physical analysis, pH, Total Ash, Acid insoluble Ash, Bulk density, Trapped density, Heavy metals, and Assay of marker compound by HPLC techniques were selected for standardization of *Hedychium spicatum.* The Physicochemical Analysis, Microbial screening test results of *Hedychium Spicatum* water extract was within the prescribed limits of Ayurveda Pharmacopoeia and is of standard quality. [5]

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Figure 1.Rhizomes of *Hedychium spicatum*

Figure 2.HPTLC of *Hedychium spicatum*

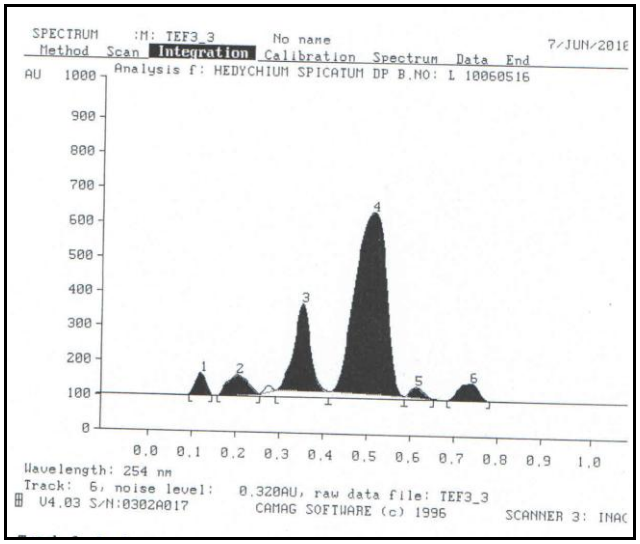

Table 4: High performance Thin Layer Chromatography

Peak	Start		Max			End		Area	
#	Rf	H	Rf	H	%	Rf	H	F	%
1	0.10	2.5	0.12	62.1	6.51	0.15	0.0	781.5	2.83
2	0.16	0.0	0.20	56.8	5.95	0.25	0.0	1383.4	5.01
3	0.29	0.0	0.35	245.7	25.73	0.41	0.0	4815.1	1.43
4	0.41	0.0	0.51	514.9	53.93	0.58	0.0	19087.7	69.08
5	0.58	0.0	0.61	27.7	2.90	0.65	0.0	426.6	1.54
6	0.68	0.0	0.73	47.6	4.93	0.78	0.0	1137.9	4.12

Even though a number of species of *Hedychium* are available in the market genuinety of *Hedychium spicatum* can be standardized by using the marker compound identification. The HPTLC finger print of the drug is also useful to verify the quality and determine the same drug in compound formulations.[6] Microbial and Heavy metals values are within the limits of Ayurveda Pharmacopeia and WHO Guidelines.

CONCLUSION

Organoleptic, physicochemical and Spectrometric values of the present study are useful in identification and authentication of the water extract of *Hedychium spicatum* - Sati rhizome.

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