RESEARCH ARTICLE

High-Performance Thin Layer Chromatography Method Development to Estimate Phytoconstituents in *Hedychium* species

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ABSTRACT

Objective: The *H. coronarium* (Zingiberaceae) is a perennial herbaceous plant. **C**hinese immigrants introduced the *H. coronarium* species as an ornamental plant in 1888. Its attractive flowers make it a desirable ornamental plant. The present study aims to evaluate the physiochemical characteristics and development of the (HPTLC) Fingerprint sequence profile of *Hedychium coronarium* leaves.

Method: Pharmacognostic characteristics and physiochemical parameters were performed according to the method prescribed. The mobile phase confirmation was completed using by performing (TLC). The development of the HPTLC fingerprint was performed with some modifications to get exact separation of phytochemicals using mobile phases such as Toluene: chloroform: ethanol (4:4:1).

Result: Pharmacognostic study reported the presence of unicellular trichomes, vessels, epidermal cells, oil globules, and vascular bundles. Pharmacognostic and physiochemical parameters like color, nature, odor, taste, foreign organic matter, loss on drying, ash value, extractive value, and the foaming index were found to be satisfied as documented in the results. The HPTLC fingerprinting reveals the presence of various chemical compounds expressed as Rf value.

Conclusion: From current scientific research work, *Hedychium coronarium* can be useful in modern medicine as various bioactive secondary metabolites were detected.

Keywords: *Hedychium coronarium*, Zingiberaceae**,** HPTLC fingerprint, Rf value.

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INTRODUCTION

Recent studies on bioactive chemicals found in plants and dietary sources have drawn a lot of attention. About 80% of the population in poor nations is thought to depend on a traditional system of medicine for their basic healthcare need. These plants show therapeutic potential in the human body due to their bioactive phytochemical components. Recent years have seen tremendous advancement in phytochemistry and pharmacological testing techniques, and as a result, new plant-based medications are entering the market as purified phytochemicals. Pharmacognosy is a field of research where, evolution of novel medicines is very important. It has been recognized that a number of crude compounds are used to make formulations and acts as a repository of therapeutically

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important chemicals that cannot be synthesized inexpensively by synthetic means. Moreover, the crude materials offer crucial intermediates for the final synthesis of active molecules. Alkaloids, tannins, saponins, phenolic compounds, essential oils, terpenoids, and flavonoids are some of the main phytochemicals which have potential sources of novel compounds with therapeutic value as well as serve as sources of lead compounds in drug development.[1]

A perennial medicinal plant called *H. coronarium Koen* is native to tropical Asia and is pretty popular on Indian subcontinent. It is widely disseminated in many other countries around the world, including Southern Africa, Eastern Australia, Southeast of the United States, and Central America.[2] It is found ubiquitous in tropical and subtropical areas, including India, Japan, South China, Brazil, and Southeast Asian countries. *H. coronarium* is a perennial maturing to 1.5 m by 1-m flowers from August to October. The traditional Chinese system of medicine mentioned that the rhizome and leaf of *H. coronarium* have been exploit to treat diabetes, contusion inflammation, headache, and sharp pain in rheumatism. Ayurvedic system of traditional medicine mentioned that plants act as excitant, febrifuge, tonic, and anti-rheumatic agents. Leaves are long, narrow at the apex, green in color, and have parallel venation. A leaf containing the essential oil is applied for carminative and anthelmintic purposes. The occurrence of the potentially beneficial labdane type diterpenes coronarins G, hedychicoronarin, peroxycoronarin D, 7- hydroxycalcaratarin A and (E) – 7-hydroxy-6-oxolabda-8, 12-diene-15, 16–dial was discovered through phytochemical analysis. Additionally, a diterpene by isocoronarin-D was identified from A Nepalese sample. Diterpenoids are also present, which are known to control blood sugar levels. Other significant hepatoprotective substances found in plant were benzoyl eugenol, ethoxy coronarin D, cytotoxic coronarin D, cytotoxic Diterpenoids and diarylheptanoid. Numerous chemotypic forms of essential Oils have been recorded in literature are as follows: α-pinene, β-pinene,1,8-cineole, β-caryophyllene, γ-elemene, β-trans ocimenone, linalool, caryophyllene oxide, β-caryophyllene, caryophylladienol I.[3-11] The *H. coronarium* has a variety of pharmacological properties, such as anti-bacterial, anti-angiogenic, anti-microbial, anti-oxidant, anti-inflammatory, anti-fungal, anti-urolithiasis, mosquito larvicidal, analgesic, cancer-chemopreventive, diuretic, anti-hypertensive, hypoglycemic and CNS depressive effects.[3-28] Hence, the present study was planned to study the plant's pharmacognostic characteristics, such as its morphology and phytochemical screening of plant *H. coronarium* in different solvents like ethanol, chloroform, and water. Fingerprinting of chloroform extract of leaves was performed to qualitative and quantitative analysis of secondary metabolites to ascertain whether it can be used for medicinal purposes.

MATERIAL AND METHODS

Collection of Crude Drug

The leaves of *H. coronarium* were collected from the local area of Nigdi, Pune, Maharashtra, India. A herbarium specimen was submitted to the Botanical Survey of India, Pune, and the plant was confirmed.

Determination of Physicochemical Parameters

Loss on Drying

It is mass reduction reported as a percent of weight. This test identifies the amount of water and volatile materials in the raw material.

Precisely weighed 2 gm of the powdered drug was placed evenly scattered throughout the Petri dish. The sample was dried in Petri dish for three hours at a temperature of 60°C until a consistent weight was noted and the loss on drying was calculated.[29]

Examination of Foreign Organic Matter

Foreign substances in herbal medicines can either be ingested whole or mixed with the medicinal plant, such as soil, dust or stone. The prescribed amount of plant material was spread out on white tiles. Foreign objects were identified visually, collected, and a percentage was recorded.[29]

Total Ash Value

Ash values are useful for figuring out the purity and quality of the raw medication. When burned, crude material typically leaves ash that is composed of carbonates, sodium silicates, phosphates, sodium silicates, etc. One gram of powdered drug sample was precisely weighed in a silicon crucible, burned or four hours at A temperature not more than 450°C to remove all carbon, then cooled and weighed.[29]

Water Soluble Ash

The ash was reheated to a maximum of 450°C or 4 hours in a crucible after being boiled with 25 mL of water, filtered, and the insoluble material collected on an ash-free filter paper. The total weight of ash was deducted from the weight of the insoluble materials. The weight difference represents water soluble ash value.[29]

Acid Soluble Ash Value

Around 25 mL HCl (2M) was added to the ash and then heated for 5 minutes. It was filtered, insoluble material was collected on ash-free filter paper, rinsed with water, and burned for 4 hours at no higher than 450°C in a crucible coated with tar. Later, it was chilled in desiccators and weighed.[29]

Alcohol Soluble Extractive Value

A 100 mL solution of 90% alcohol was added to 5 gm of the precisely weighed coarse powdered drug, and the mixture was macerated for 24 hours. After being shaken repeatedly for the first six hours, it was filtered. A tarred dish was used to dry off alcoholic extract, which was then weighed.[29]

Water Soluble Extractive Value

A flask was used to macerate 5 gm of a precisely weighed coarsely powdered drug for 24 hours with 100 mL of chloroform water. In the following 6 hours, with frequent shaking, it was then filtered into a tarred dish to dry off 25 mL of chloroform extract, which was then measured.[29]

Table 1: Physicochemical parameters of *H. coronarium* Leaves

S. No.	*Organoleptic evaluation study*		*Observations*
1.	Parameters	Nature of powder sample	Coarse
		Color of leaf	Green
		Odour of leaf	Aromatic
		Taste of leaf	Bitter
2.	Physicochemical Evaluation	Loss on drying	4%
		foreign organic matter	1%
		Total ash Value	7%
		Water soluble ash value	3%
		Acid insoluble ash value	5%
		Alcohol soluble extractive value	8 %
		Water soluble extractive value	4.5%
		Foaming index	Less than 1

Table 2: Rf values of chloroform extract of *H. coronarium* leaves

Peak	*Start Rf*	*Start height*	*Max. Rf*	*Max height*	*Max %*	*End Rf*	*End height*	*Area*	*Area%*
1	0.11	0.0	0.14	46.8	7.39	0.15	20.0	883.8	5.13
2	0.15	20.1	0.17	36.6	6.21	0.20	1.4	755.3	4.39
3	0.20	1.7	0.22	30.1	5.10	0.24	12.4	604.0	3.51
4	0.24	13.1	0.27	62.3	10.57	0.29	0.0	1107.0	6.43
5	0.36	0.0	0.44	62.0	10.51	0.45	54.5	2380.0	13.82
6	0.46	54.8	0.48	73.4	12.44	0.49	67.0	1518.5	8.82
7	0.49	67.1	0.51	82.4	13.97	0.56	24.4	3273.9	19.03
8	0.56	25.2	0.60	123.0	20.86	0.65	38.7	5003.0	29.06
9	0.66	39.1	0.67	41.0	6.95	0.71	0.1	1010.0	5.87
10	0.71	0.1	0.74	32.3	5.47	0.77	0.5	678.4	3.94

Figure 1: *Hedychium coronarium* plant.

Foaming Index

The foaming index is mainly due to the saponin content in the drug. One gram powder sample was transferred in 100 mL water and then boiled for 30 minutes and filtered. This decoction was poured in test tubes in consecutive portions of 1–10 mL and adjusted volume equal to 10 mL with water. The test tubes were shaken for 15 seconds and stood for 15 minutes; then, foam height was measured.[30]

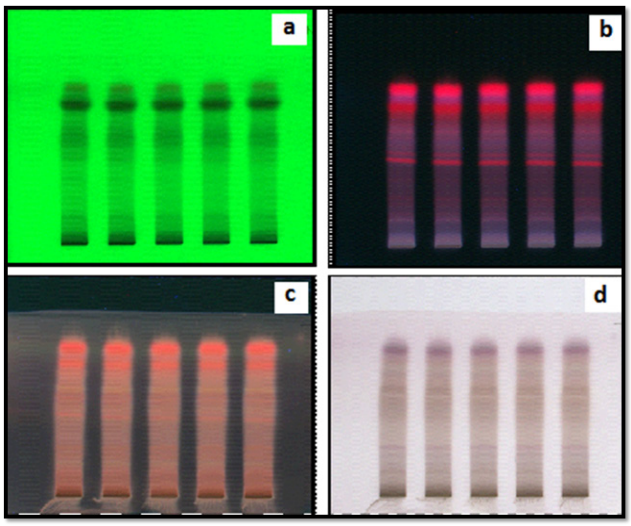

Figure 2: a: HPTLC fingerprinting at 254 nm, b: HPTLC plate observation at 366 nm, c: HPTLC analysis at 366 nm (contrast), d) HPTLC Plate observation at white light.

Qualitative TLC Analysis

Extract Preparation

The leaves of *Hedychium coronarium* were washed and dried. It was later ground into a fine powder and then extracted

with chloroform as solvent by maceration method, and phytochemical analysis was done.[29-31]

Preparation of TLC Plate

A slurry of Silica Gel GF 254 was prepared and later applied to plates of thickness 0.4 mm. The size of the plates was 10x20 cm. The plates were later activated at 110ºC for 1-hour.

Solvent System

The solvent system was prepared from Toluene: chloroform: n-butanol: ethanol in a ratio (4:4:1:0.5). The spots were detected under UV at a wavelength corresponding to 254 nm.

HPTLC Fingerprinting

HPTLC examination was performed using the CAMAG semi-automatic HPTLC system, which included digital TLC Sampler 4, development chamber, chromatogram immersion machine, TLC plate warmer III, and Reprostar 3 report generated appliance. The sample was added in solvent to get 10 mg/mL concentrations, and 2 µL of the sample was sprayed onto silica gel plates 60 F-254 (Merck, Germany) as 8 mm bands, 5 mm from the lower border. Investigating multiple systems with various polarities led to the discovery of the optimal mobile phase. The tank saturation was done with a 25 mL optimized mobile phase for 20 minutes at 25°C and 47–47% relative humidity, and the glass twin-trough was used for development. The mobile phase was composed of chloroform, Toluene, and ethanol. The results were examined and recorded under 254 nm and 366nm UV light, derivatized with reagent, and then observed under white light after 5 minutes of development and drying. The mobile phase, *i.e.,* Chloroform: Toluene: Ethanol (4: 4: 1), provides good Rf values results. This method is appropriate for a quick screening of chemical constituents.

RESULT AND DISCUSSION

The *H. coronarium* plant given in Figure 1 is reported for the presence of many phytoconstituents and pharmacological activities. The powder sample taken for the study was coarse as fine powder may get blocked during filtration. The chloroform extract showed the existence of Steroids, Glycosides, Alkaloids, Flavonoids, and Saponins. Color, odor, and taste were found to be green, aromatic, and bitter, respectively. The physiological evaluation parameters, like percent loss on drying, were found to be 4%. For the confirming purity of the crude drug, an evaluation of foreign organic matter was performed, and it was found to be 1%. The ash values, *i.e.,* %total, water-soluble, and acid-insoluble ash, were 7, 3, and 5%, respectively. The extractive values were found to be 8% (alcohol soluble) and 4.5 % (water soluble), respectively. The foaming index was performed to know the presence of saponin content. It was found to be less than (Figure 1). The physicochemical parameters were studied, and observations were documented in Table 1. The chloroform extract was tested for the presence of phytoconstituents by using the TLC analysis method. HPTLC is an instrument useful for the identification of phytoconstituents. It gives chromatographic fingerprints which can be visible and compiled as electronic images. In the present study, the Rf values were documented in Table 2, and HPTLC fingerprinting in Figure 2.

CONCLUSION

Traditional medicine is the collection of information, abilities, and procedures based on previously conducted theories and tests on how to employ herbs to treat physical and mental illnesses as well as to maintain health.[31] The *H. coronarium* can be useful as a medicinal plant as the identification of various bioactive secondary metabolites was detected by using the HPTLC method.

CONFLICT OF INTEREST

All authors declares no conflict of interest.

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