



Phytochemical investigation of extracts of rhizomes of *Hedychium Spicatum* Sm. in A. Rees of Himachal Pradesh, India

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ARTICLE INFO	ABSTRACT
<p>Received : 30 June 2021 Revised : 20 September 2021 Accepted : 04 October 2021</p> <p>Available online: 19 December 2021</p> <p>Key Words: <i>Hedychium Spicatum</i> Phytochemical screening Dextro rotatory Specific gravities Proteins Alkaloids Carotenoids</p>	<p><i>Hedychium Spicatum</i> is a rhizomatous perennial plant of various ethnomedicinal significance, which belongs to Zingiberaceae family. In the present study, <i>H. Spicatum</i> extracts were investigated for the presence of major phytochemical compounds. The dried and powdered rhizomes were extracted employing Soxhlet extraction with selective solvents of varying polarities viz water, ethanol, petroleum ether and diethyl ether. Qualitative phytochemical analysis of each of these extracts of <i>H. Spicatum</i> suggested the existence of flavonoids, phenolic compounds, carotenoids, alkaloids, reducing sugars (carbohydrate), proteins, steroids, saponins and oils. Greater extent of unsaturation was observed in diethyl ether and petroleum ether extracts. These extracts were also examined for their physico-chemical properties. All of the studied extracts were found to be optically active, specifically dextro rotatory. The phytochemicals present in the rhizomes suggest potential ethnomedicinal application of the species in the treatment, control and management of diseases and for new drug discovery.</p>

Introduction

The twenty first century has seen an unprecedented interest in traditional medicine accompanied by an enormous rise in the demand for drugs from plant sources. Herbal medicines have become increasingly quintessential part of the international pharmacopeia. Numerous studies were put forward in correlating phytochemical constituents of a plant with its pharmacological activity (Cseke *et al.*, 2016; Gupta, 1994; Vaidya *et al.*, 1994) research groups world over have correlated the botanical properties of plants with their pharmacological activity (Rawat *et al.*, 1997). An exhaustive multipronged research aimed at correlating botanical and phytochemical properties to specific pharmacological activities are expected in future (Bageeta *et al.*, 2016; Dahanukar *et al.*, 2000). Further studies can be carried out regarding extraction, isolation and identification of active principles and pharmacological studies of isolated compound based on these preliminary findings. Therefore, these scientific studies may be exploited to develop future drugs. While developing potent drugs from natural products various aspects need to be addressed, including the determination and selection of primary screening assays that are pivotal to ensure a selection of extract or molecule with appropriate pharmacological action worth pursuing. The plant material must primarily be exposed to an appropriate extraction process to assess the plant constituents for their biological activity. The term 'extraction' is a technique employed to separate and isolate target molecules using selective solvents through standard processes. An extensive range of technologies are available for the purpose. The crude extract is evaluated for its potential biological activity. The sequentially

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fractioned crude extracts are subjected to further suitable bio-assay tests, in order to evaluate them for potential biological activity (Bobzin *et al.*, 2000; Gaudêncio *et al.*, 2015).

Hedychium spicatum is a conventional herbal medicine, used as remedy of cough, wounds, ulcer (Badola, 2009; Dwivedi *et al.*, 2019; Giri *et al.*, 2010), fever (Sahu, 1979; Savitharamma *et al.*, 2007; Tavares *et al.*, 2020) breathing problems (Kumari *et al.*, 2011; Badoni *et al.*, 2010) and hiccup (Rawat *et al.*, 2021; Sahu, 1979; Savitharamma *et al.*, 2007). The plant *Hedychium spicatum* sm. in a. rees is a perennial rhizomatous herb (syn. *Gandasulium spicatum* (Sm.) Kuntze, *Hedychium acuminatum* Roscoe) belongs to zingiberaceae family. It is a native plant related to the genera *Hedychium*. It spreads all through the subtropical Himalaya at an altitudinal range of 1000–3000 m (Samant *et al.*, 1997; Thakur *et al.*, 1989). The rhizomes are usually recognised as Kapurkachari in local language or *Ban Haldi* (Samant *et al.*, 1997) in Hindi and *Shati* in Bangla and traditional name is *Gandhamulika* (Sahu, 1979; Chunekar, 1960). *Hedychium spicatum* grows up to at least one metre height with elongated stem. The rhizomes have a pungent odor and a bitter flavor. It closely resembles *Hedychium coronarium*. The white ascending flowers are borne in terminal spikes and the leaves are approximately 30 cm long glabrous beneath, earning it the name spiked ginger lily. The leaves vary in breadth, glabrous, oblong and lanceolate. Fruit is capsule type; glabrous and globose (Nadkarni, 1976; Kirtikar *et al.*, 1935). Rhizomes are externally yellowish-brown, which darken on storage. These are 15–20 cm long; 2.0–2.5 cm in diameter; with a rough reddish brown layer marked with numerous scars and circular rings; rudiments of rootlets are visible (Handa, 1997).

Material and Methods

The authentic rhizomes were collected from CSIR-Institute of Himalayan Bioresource Technology, Palampur, Himachal Pradesh. The rhizomes were washed and then rinsed with distilled water. The rhizomes were chopped into pieces, shade dried and finally dried for 2–3 days at ambient temperature and then pulverized to obtain a coarse powder.

Preparation of rhizome extracts

(A) Aqueous extract: 500 ml double distilled water were added to 100 grams of the powdered rhizome and boiled for 1 h at 100°C. The extract was subjected to filtration and vacuum evaporation. A dark brown solid (15.4 g) was obtained from *Hedychium spicatum*.

(B) Ethanolic extract: In a Soxhlet extractor 100 g crushed material was placed and 500 ml of ethanol were added to it. The material was extracted for 72 hrs at 78°C. Dark brown mixture was obtained. 500 ml of ethanol were added again to the soxhlet and kept for another 72 hrs. The process was repeated till the extract became colourless. All the extracts were pooled and vacuum distilled. A dark brown solid (5.25 g) was obtained.

(C) Diethyl ether extract: In a Soxhlet extractor 100 g of the powdered rhizome was placed and 500 ml of diethyl ether were added. The material was extracted at 34°C for 72 hrs. The extract subjected to filtration and vacuum evaporation to yield yellowish brown viscous oil (3.56 g).

(D) Petroleum ether extract: In a Soxhlet extractor 100 g of the powdered rhizome was placed and 500 ml of petroleum ether were added. The extraction was carried out at 60–80°C for 72 hrs. The extract subjected to filtration and vacuum evaporation to yield yellowish light brown viscous oil (1.68 g).

Phytochemical Screening

Investigations for the presence of different class of natural product compounds, specific gravities, optical rotation, refractive index and pH were carried out by usual methods. The saponification values, acid values and iodine values were also determined for the essential oils and for the various extracted materials employing the established methods (Garratt, 1964; Guenther, 1963; Tyagi *et al.*, 2013) and in monographs of I.S.I. (1984).

Results and Discussion

Soxhlet extraction was employed and the yield has been found to be varying depending upon the polarity of the solvent used and the solubilities of the components present. The findings indicate that better extraction yields are obtained by using polar solvents (Iloki-Assanga *et al.*, 2015; Lapornik *et al.*, 2005; Nawaz, Nawaz *et al.*, 2020). The colours of the decoctions, too, were observed to be varying. Table 1 summarizes the yields and physico-

chemical properties of extracted materials, while the results of the phytochemical tests are described in Table 2. The extraction of the rhizomes through water provided a dark brown solid material with sharp odour and the yield was found to be maximum among all the solvents employed in the present work (Table 1). Presence of high percentage of water soluble substances in the rhizomes is suggested by highest yield in the case of the crude aqueous extract (Sravani *et al.*, 2012; Tchabo *et al.*, 2018). The existence of, reducing sugar (carbohydrate), protein, certain carboxylic acids, steroids and saponin as major phytochemical groups was indicated by the phytochemical screening of the aqueous extract of *H. spicatum* (Table 2). Low acid values and the pH values of higher than 5.0 indicate that not much free acids are present. The presence of comparatively high

molecular weight compounds and much less unsaturated compounds is suggested by relatively low saponification and iodine values of the aqueous extract. Extraction through ethanol gives dark brown solid with pungent odour. Most of the part of ethanol extract was insoluble in water but soluble in mixture of ethanol and water (80:20). The ethanol extract comprises of aldehydic and ketonic compounds, carbohydrates, steroids and the flavonoids but devoid of alkaloids. The yield obtained in ethanol extract was found to be lesser with respect to the yield obtained in the case of aqueous extract. The relatively high saponification value and lower iodine value of ethanolic extract is indicative of presence of relatively low molecular weight compounds with lesser degree of unsaturation.

Table 1: Physicochemical analysis of the extracts of *H. spicatum*

Properties	Extract of <i>H. spicatum</i>			
	Pet. Ether	Diethyl ether	Ethanol	Aqueous
Colour of Decoction	Yellow	Yellowish brown	Dark brown	Dark red
Colour of Extract	Yellowish light brown	Yellowish brown	Dark brown	Dark brown
State	Viscous oil	Viscous oil	Solid	Solid
Odour	Spicy	Sweet	Sweet	Sharp
Yield(% ,w/w)	3.67	4.86	6.21	17.41
pH	4.61	5.65	3.99	6.70
Refractive index (0.025% solution)	1.31	1.34	1.37	1.37
Specific gravity(30°/30°)	0.8662	0.8937	0.8646	--
Optical rotation(25°C) (0.025% solution)	3°20'	2°06'	4°01'	4°07'
Acid value	37.86	35.38	33.35	16.28
Saponification value	151.6	142.2	154.2	73.00
Iodine value	73.87	41.76	13.65	14.54

Table 2: Phytochemical Analysis of the extracts of *H. spicatum*

Chemical tests	Extract of <i>H. spicatum</i>			
	Pet. Ether	Diethyl ether	Ethanol	Aqueous
Proteins and amino acids				
Xanthoproteic test	-	+	-	-
Biuret test	-	-	-	+
Alkaloids				
Mayer's test	+	-	-	-
Carbohydrates				
Molisch test	-	-	+	+
Carotenoids				
Sulphuric acid test	-	-	-	-
Flavonoids				
Alkaline reagent test	+	+	+	-
Lead acetate test	+	+	+	-
Steroids				
Salkowski's test	-	+	+	+
Aldehydic				
Tollen's test	+	-	+	+
Fehling solution	+	-	+	+
Schiff's reagent	+	-	+	-
Ketonic				
Carboxylic	-	-	-	+
Phenolic				
Phenolic	+	-	-	-
Unsaturation				
Bromine water test	+	+	+	+
Alkaline KMnO ₄ test	+	+	+	+

Key:(-) = Absent, (+) = Present

Yield in diethyl ether is marginally lower than that in alcohol. Instead of solid material, it gave yellowish brown viscous oil. The obtained oil was almost neutral in character (pH 4.54) and lighter than water (Tables 1). It was found to be dextro rotatory with enough acid value (Tables 1) and high degree of unsaturation as indicated by its iodine value (Table 1). However, a relatively large saponification value suggests the existence of greater amount of low molecular weight compounds. The carbohydrates and were not found in the extracted mass but indicated presence of flavonoids, steroids and proteins. Because of the sweet, characteristic odor and its solubility in ether, the extracted oil can be used in perfumery.

Extraction through the non-polar solvent petroleum ether yielded reddish brown viscous oil. The yield of the substance obtained through petroleum ether extraction is the lowest of all the solvents, indicating lesser amount of fatty acids. Flavonoids and carbohydrates were absent but some aldehydes and phenolic compounds were found, as evident by

their positive tests in petroleum ether extract (Table 2). Petroleum ether extract of a plant material usually contains the fatty oils (Tse, 2005). A good saponification value (Table 1) indicated the presence of sufficient amount of saponifiable matter. A slight acidic pH of the extracted samples confirms their suitability in preparation of syrups, skin ointments and also as colorant for food and drug. All the extracts were dextro rotatory thus enhancing the probability of exhibiting biological activity as molecular chirality directs whole-cell chirality (Inaki, 2016; Morozov, 1979). Since phenolic compounds are present, it's possible that such extracts may show antioxidant effects. These extracts could be utilized to isolate and identify antioxidant molecules that could be studied further in the treatment of diseases caused by free radicals.

Conclusion

The study of *H. spicatum* extracts showed the presence of almost similar groups of compounds.

Carbohydrates, protein, steroids, and saponin are important phytochemical groups found in the aqueous extract of *H. spicatum*. These compounds in the form of water soluble extracts seem to be quite important as they find use in various herbal formulations and ethnomedicinal applications. Aldehydic and ketonic groups, carbohydrates, steroids, and flavonoids are found in the ethanol extract. The alcohol soluble compounds particularly flavonoids can act as antioxidants. The ether extracted oily mass rich in flavonoids and steroids with characteristic sweet odour can also be used in perfumery apart from pharmaceutical applications.

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The current investigation also discovered that the Pet. ether extract of *H. spicatum* has a considerable amount of phenolic compounds that may have antioxidant properties. As a result, this plant could be utilized to isolate and identify antioxidant chemicals that could be studied further in the treatment of diseases caused by free radicals.

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