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Comparative Study Of Phytochemical Constituents And Total Phenolic Content In The Extracts Of Three Different Species Of Genus Hedychium

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Abstract: Hedychium is a rhizomatous perennial plant which belongs to family Zingiberaceae with various ethnomedicinal and ornamental significance. In this study, three different species of genus Hedychium i.e. *H. spicatum*, *H. coronarium* and *H. rubrum* were screened for the presence of major phytochemical compounds. The total phenolic contents of the three different species were also analyzed. The dried and powdered rhizomes of each species were extracted with methanol using Soxhlet extraction method. Phytochemical screening of methanolic extracts of each species was performed qualitatively. Phytochemical screening of the methanolic extract of *H. spicatum* showed the presence of phenolic compounds, flavonoids, alkaloids, reducing sugar (carbohydrate), protein, steroids and triterpenoids, cardiac glycosides, diterpene, tannin, saponin and oil. Phytochemical screening of the methanolic extracts of both *H. coronarium* and *H. rubrum* also showed the presence of similar groups of compounds but tested negative for the presence of alkaloids. Both *H. spicatum* and *H. rubrum* showed negative test for phlobatannin but *H. coronarium* indicated the presence of phlobatannin. The total phenolic contents of the methanolic extracts of each species were estimated by using UV Visible Spectrophotometer and expressed as gallic acid equivalents (GAE). The total phenolic contents of the methanolic extracts of *H. spicatum*, *H. coronarium* and *H. rubrum* in terms of gallic acid equivalent were 19.48±0.82, 26.22±1.17 and 28.16±0.45 mg /g of extract powder respectively.
Keywords: *H. spicatum*, *H. coronarium*, *H. rubrum*, rhizome, phytochemical screening, Soxhlet extraction, methanolic extract, total phenolic content, gallic acid equivalent (GAE).

Introduction:

Hedychium, popularly called ginger lily is a rhizomatous flowering plant belonging to family Zingiberaceae. The plant is native to tropical Asia and the Himalayas. India has rich diversity of ginger lily with 44 taxa, which includes 31 species and 13 varieties and is mainly distributed in Northeast India and South India [1]. 24 species of *Hedychium*, a genus of Zingiberaceae had been reported in Northeast India, out of the 65 valid taxon in the world which indicates its highest species concentration in this region [2]. It has high ethnomedicinal significance in India and even local people consume and use its rhizomes as an integral part of cuisines, cultural

practices and sacred rituals [3]. It is also highly valued as an ornamental garden plant due to its attractive foliage, showy inflorescence having a wide variety of colour. Taking into consideration the immense potential and significant uses of this plant genus, we have studied three different species i.e., *H. spicatum, H. coronarium* and *H. rubrum* and identified and compared the bioactive constituents present in them. Their total phenolic contents have also been analyzed which could prove beneficial in the development of new lead molecules for pharmaceutical uses.

Hedychium spicatum is commonly known as spiked ginger lily and is well distributed in the Himalayas and sub-Himalayan region. It is a tall perennial herb with fleshy aromatic rhizomes, thick straight stem with broadly lanceolate leaves. Its flowers are fragrant, white with an orange base. Its rhizome has been reported to be stomachic, carminative, bronchodilator stimulant and tonic; it is also traditionally used in treating dyspepsia, nausea, vomiting, liver complaints, diarrhoea and pains, etc [4]. Rhizome in the form of roasted powder is given for treatment of asthma and decoction of rhizome with Deodar sawdust is taken for tuberculosis [5]. The rhizome has been reported to possess anti-inflammatory, anti-asthmatic, hypoglycemic, vasodilator, spasmolytic, hypotensive, *in vitro* pediculicidal, cytotoxic and antimicrobial properties [6].

Hedychium coronarium is a rhizomatous herb commonly known as white ginger lily or butterfly lily and is usually 1-2.5 m tall. The local people of Manipur consume its rhizomes as vegetable. It is widely cultivated in tropical and subtropical regions of India. Its leaves are simple, lanceolate with broad leaf base, where leaf base is shiny above and pubescent below. Flowers are white, broadly orbicular and sweetly scented. Its rhizome is used in the treatment of diabetes [7]. Traditionally it is used for the treatment of tonsillitis, infected nostrils, tumor and fever [8]. It is also used as antirheumatic, excitant, febrifuge and tonic [9]. It has been reported that the essential oil extracted from leaves, flowers and rhizome of the plant have molluscicidal activity, potent inhibitory action, antimicrobial activities, antifungal, anti-inflammatory, antibacterial and analgesic effects [8].

Hedychium rubrum is a rare red species which is endmic to the North Eastern region of India. It is a short plant reaching up to 4ft with reddish stem and green, lanceolate, glabrous leaves and bright red orbicular flowers. It has many flowers per stem. It lacks aroma and is a potential ornamental plant due to its flower having a long spike with red bracts, which alone makes it attractive [3]. Though the plant has ornamental characteristic, no scientific report is available to validate its folkloric medicinal uses.

Materials And Methods:

Collection and Processing of Plant Material:

Three different species of genus Hedychium i.e. *H. spicatum, H. coronarium* and *H. rubrum* were collected from the Imphal valley of Manipur, Northeast India. The plants were processed and analyzed. The rhizomes of each plant were washed in tap water and then rinsed in distilled water. The rhizomes were cut into pieces, shaded dried and finally dried in an oven at a temperature of 35-40^{0}C for 3 days. The dried rhizomes of each plant were pulverized by using grinder to obtain a powdered form.

Preparation of Extracts of Plant Material:

Plant extracts of each plant were prepared using methanol as extracting solvent:

A. Methanolic extract of *Hedychium spicatum*:

40g of the dried and powdered plant material (rhizome) was extracted with 400ml of methanol at 65^{0}C for 2 days using Soxhlet extraction method. After filtering and evaporating to dryness, the crude methanolic extract was obtained.

B. Methanolic extract of *Hedychium coronarium*:

44g of the powdered plant material (rhizome) was extracted with 440 ml of methanol at 65^{0}C for 2 days using Soxhlet extraction method. The extract was then filtered through filter paper and evaporated to dryness; finally the crude methanolic extract was obtained.

C. Methanolic extract of *Hedychium rubrum*:

41g of the dried and powdered plant material (rhizome) was extracted with 410ml of methanol at 65^{0}C for 2 days using Soxhlet extraction method. After filtering and evaporating to dryness, the crude methanolic extract was obtained.

Phytochemical Screening:

Chemical tests were carried out qualitatively on each extract using standard procedures to identify the phytochemical constituents [10, 11, 12, 13, 14].

1. Test for alkaloids:

Hager's test: Extracts were dissolved individually in dilute hydrochloric acid and filtered. Filtrates were treated with Hager's reagent (saturated picric acid solution). Presence of alkaloids confirmed by the formation of yellow coloured precipitate.

2. Tests for carbohydrates:

Benedict's test: Extracts were dissolved individually in distilled water and filtered. Filtrates were treated with Benedict's reagent and heated gently. Formation of orange red precipitate indicated the presence of reducing sugars.

Fehling's test: Filtrates were mixed with equal volume of Fehling's A and Fehling's B solutions and heated. Formation of brick red precipitate of cuprous oxide indicated the presence of reducing sugars.

3. Test for proteins:

Xanthoproteic test: The extracts were treated with a few drops of conc. nitric acid. Formation of yellow colour indicated the presence of proteins.

4. Test for flavonoids:

Alkaline reagent test: To the test solution, a few drops of sodium hydroxide solution were added. Formation of intense yellow colour which turns to colourless by addition of few drops of dilute acetic acid indicated the presence of flavonoids.

Lead acetate test: To the test solution, a few drops of lead acetate solution were added. Formation of yellow precipitate indicated the presence of flavonoids.

5. Test for phenolic compounds:

Lead acetate test: To the test solution, a few drops of 10% lead acetate solution were added. Formation of white precipitate indicated the presence of phenolic compounds.

Ferric chloride test: To the test solution, a few drops of ferric chloride solution were added. A dark green colour indicates the presence of phenolic compounds.

6. Test for tannins:

Lead acetate test: To the test solution, a few drops of 10% lead acetate solution were added. Precipitate formation indicated the presence of tannin.

7. Test for steroids and triterpenoids:

Salkowski's test: Extracts were treated with chloroform and filtered. The filtrates were treated with few drops of conc. sulphuric acid, shaken well and allowed to stand. Appearance of red colour in the lower layer indicated the presence of steroids. Formation of reddish brown colour of interface after addition of conc. sulphuric acid to the side carefully (without shaking) indicated the presence of terpenoids.

8. Test for saponins:

Froth test: Extract was added to 2-3 ml of distilled water. The mixture was shaken vigorously. Formation of foam indicated the presence of saponin.

9. Test for cardiac glycosides:

Keller Killiani test: To the test solution, 2ml of glacial acetic acid containing a few drops of $FeCl_3$ solution was added. 1ml of conc. H_2SO_4 was added along the side of the test tube carefully. A brown ring at the interface indicated the presence of deoxysugar of cardenoloides. A violet ring may appear beneath the brown ring, while in the acetic acid layer, a greenish ring may also form just gradually throughout the layer.

10. Test for oil:

A small quantity of the extract was pressed between the two filter papers. Oil stain on the filter papers indicated the presence of oil.

11. Test for phlobatannin:

Extract was boiled with 2 ml of 1% hydrochloric acid. Formation of red precipitate indicated the presence of phlobatannin.

12. Test for diterpenes:

Copper acetate test: Extracts were dissolved in water and treated with 3-4 drops of copper acetate solution. Formation of emerald green colour indicated the presence of diterpene.

Determination of Total Phenolic Content:

The amount of phenol in the methanolic extracts of three different species of *Hedychium* i.e. *H. spicatum*, *H. coronarium* and *H. rubrum* were determined with Folin-Ciocalteu reagent [15, 16]. 2.5 ml of 10% Folin-Ciocalteu reagent and 2 ml of Na_2CO_3 (2% w/v) were added to 0.5 ml of the sample (3 replicates) of each plant extract solution (1mg/ml). The resulting mixture was incubated at 45^0C for 15 min. The absorbance of each sample was measured at 760 nm using UV Visible Spectrophotometer. Gallic acid (0-3 µg/ml) was used as a standard compound. The gallic acid standard calibration curve was established by plotting concentration (µg/ml) versus absorbance (nm) ($y= 0.024x + 0.032$, $R^2= 0.968$), where y is absorbance at 760 nm and x is concentration (**Graph-1**). Total phenolic content in the plant extract was expressed as gallic acid equivalent (mg of gallic acid equivalent/ g of sample) and was calculated by the formula [17]:

$T = (C \times V)/M$

Where, T = total content of phenolic compounds, mg/g plant extract, in GAE; C = concentration of gallic acid established from the calibration curve, µg/ml; V = volume of extract, ml; M = weight of methanolic plant extract, g.

Table-1: Comparative Analysis of Phytochemical Constituents in the three different species of *Hedychium*

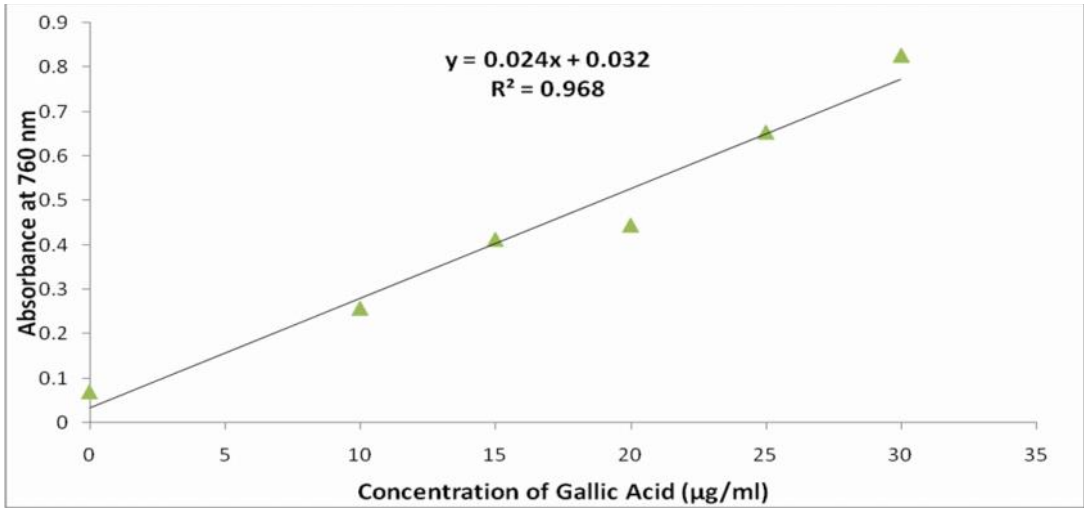
Chemical constituents	**Chemical tests**	**Methanolic extract of *H. spicatum***	**Methanolic extract of *H. coronarium***	**Methanolic extract of *H. rubrum***
Alkaloids	Hager's test	+	-	-
Carbohydrates (reducing sugar)	Benedict's test Fehling's test	+ +	+ +	+ +
Proteins	Xanthoproteic test	+	+	+
Flavonoids	Alkaline reagent test Lead acetate test	+ -	+ -	+ +
Phenolic compounds	Lead acetate test Ferric Chloride test	+ -	+ +	- +
Tannins	Lead acetate test	+	+	+
Steroids and Terpenoids	Salkowski's test	+	+	+
Saponin	Froth test	+	+	+
Cardiac glycosides	Keller-killiani test	+	+	+
Oil		+	+	+
Phlobatanin		-	+	-
Diterpene	Copper acetate test	+	+	+

Key: + = Present and – = Absent

Table-2: Total phenolic content in the methanolic extracts of *H. spicatum*, *H. coronarium* and *H. rubrum*

Sample	**Concentration (mg/ml)**	**mg of gallic acid/g of extract (Mean ± Standard Deviation)**
Methanolic extracts of *H. spicatum*	1	19.48±0.82
Methanolic extracts of *H. coronarium*	1	26.22±1.17
Methanolic extracts of *H. rubrum*	1	28.16±0.45

Graph-1: Standard curve of Gallic acid



Results And Discussion:

The phytochemical analysis conducted on the methanolic extract of *H. spicatum* revealed the presence of phenolic compounds, flavonoids, alkaloids, reducing sugar (carbohydrate), protein, steroids and triterpenoids, cardiac glycosides, diterpene, tannin, saponin and oil as major phytochemical groups. Phytochemical screening of the methanolic extracts of both *H. coronarium* and *H. rubrum* also showed the presence of phenolic compounds, flavonoids, protein, steroids and triterpenoids, cardiac glycosides, diterpene, tannin, saponin and oil but tested negative for the presence of alkaloids. Phlobatannin was present in the methanolic extract of *H. coronarium* but *H. spicatum* and *H. rubrum* showed negative for phlobatannin (Table-1).

The amount of total phenolic content was determined with the Folin-Ciocalteu reagent. Gallic acid was used as standard compound. The standard graph for gallic acid is represented in Graph-1 ($y= 0.024x + 0.032$, $R^2= 0.968$), where y is absorbance at 760 nm and x is concentration. Phenolic compounds are a group of antioxidant agents which act as free radical scavengers [18]. The total phenolic contents of the methanolic extracts of *H. spicatum*, *H. coronarium* and *H. rubrum* in terms of gallic acid equivalent were 19.48±0.82, 26.22±1.17 and 28.16±0.45 mg/g of extract powder respectively (Table-2). *H. rubrum* showed the maximum phenolic content as compared to *H. spicatum* and *H. coronarium*.

Conclusion:

The comparative study of the phytochemical compounds of the methanolic extracts of *H. spicatum*, *H. coronarium* and *H. rubrum* show the presence of almost similar groups of compounds. The present study also reveals that the methanolic extracts of three different species of *Hedychium* contain a moderate quantity of phenolic compounds which might exhibit antioxidant activity. Thus, these plants may be used for the isolation and identification of antioxidant compounds which can be studied further in the treatment of free radicals associated diseases.

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