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# Phytochemical Analysis And Determination Of Total Phenolics Content In Water Extracts Of Three Species Of Hedychium

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**Abstract:** Hedychium is a rhizomatous plant belonging to family Zingiberaceae. In this present study, three different species of genus Hedychium i.e. *H. spicatum*, *H. coronarium* and *H. rubrum* were analysed quantitatively for the presence of major phytochemical compounds. The total phenolic contents in extracts of the three different species were also analyzed. The dried and powdered rhizomes of each species were extracted with distilled water using Soxhlet extraction method. Phytochemical screening of the water extract of *H. spicatum* showed the presence of phenolic compounds, flavonoids, reducing sugar (carbohydrate), protein, steroids and triterpenoids, cardiac glycosides, tannin, saponin and oil. Phytochemical screening of the water extracts of both *H. coronarium* and *H. rubrum* also showed the presence of similar groups of compounds. *H. spicatum*, *H. coronarium* and *H. rubrum* tested negative for the presence of alkaloids. Both *H. spicatum* and *H. coronarium* showed negative test for phlobatannin but *H.rubrum* indicated the presence of phlobatannin. The total phenolic contents of the water extracts of each species were estimated by using UV Visible Spectrophotometer and expressed as gallic acid equivalents (GAE). The total phenolic contents of the water extracts of *H. spicatum*, *H. coronarium* and *H. rubrum* in terms of gallic acid equivalent were 29.39 ± 0.01, 34.93 ± 0.01 and 66.48 ± 0.01 mg /g of extract powder respectively.
**Keywords:** *Hedychium spicatum*, *Hedychium coronarium*, *Hedychium rubrum*, rhizome, phytochemical screening, Soxhlet extraction, water extract, total phenolic content, gallic acid equivalent (GAE).

## INTRODUCTION:

Medicinal plants have been an integral part of the development of modern civilization as it has been the oldest form of health care known to mankind. In developing countries, it is estimated that about 80% of the population rely on traditional medicine for their primary health care [1]. The medicinal value of these plants lies in bioactive phytochemical constituents that produce definite physiological action on the human body [2]. Some of the important phytochemicals are alkaloids, phenolic compounds, essential oils, flavonoids, tannins, terpenoids, saponins,etc [3]. They have been serving as potential sources of new compounds of therapeutics value and also as sources of lead compounds in the drug development.

In this present study, three different species of genus Hedychium i.e., *Hedychium spicatum*, *Hedychium coronarium* and *Hedychium rubrum* are chosen to study because of their immense ethnomedicinal and ornamental significances, and they also form an integral part of religious practices and sacred rituals. These

three different species have been analyzed to identify and compare their bioactive constituents present in them and their total phenolic contents have also been determined.

*Hedychium spicatum* is a perennial rhizomatous herb, commonly found in the Himalayas and sub-Himalayan region. It has thick straight stem with broadly lanceolate leaves. Its flowers are fragrant and white with an orange base. The fruit is globosely capsule; when ripe the three valves are reflexed exposing numerous small black seeds. Its rhizome is also traditionally used in treating dyspepsia, nausea, vomiting, liver complaints, diarrhoea and pains, etc [4]. The decoction of rhizome with Deodar sawdust is taken for the treatment of tuberculosis [5]. It has been a valued medicinal plant for possessing a variety of therapeutic properties like carminative, expectorant, tranquilizer, stomachic, antiseptic, vasodilator, analgesic, anti-inflammatory, antimicrobial, antifungal, antioxidant, pediculicidal, cytotoxic, anti-asthmatic, hypoglycemic, spasmolytic and hypotensive activities [6].

*Hedychium coronarium* is an erect rhizomatous herb. It is widely cultivated in tropical and subtropical regions of India. Its leaves are bright green and pubescent below. Flowers are white and sweetly scented. Its rhizomes are consumed by local people of Manipur. It is used as a febrifuge, tonic, excitant and anti- rheumatic in the Ayurvedic system of traditional Indian medicine [7]. It has been reported that its rhizomes are used for the treatment of diabetes, tonsillitis, infected nostrils, tumor and fever [8, 9].

*Hedychium rubrum* is an endemic species of the North Eastern region of India. Its stem is reddish and leaves are green lanceolate and glabrous. Flowers are red, orbicular and lack fragrance. There has been no report for its ethnomedicinal uses. Though it is less hardy, it has high ornamental potential due to its flower having a long spike with red bracts [10].

## 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, dried under shade and finally dried in an oven at a temperature of 35-40$^0$C for 1 day. The dried rhizomes of each plant were pulverized by using mechanical grinder to obtain in powdered form.

### *Preparation of Extracts of Plant Material*:

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

#### A. Water extract of *Hedychium spicatum*:

44g of the dried and powdered plant material (rhizome) was extracted with 440ml of distilled water for 20 hrs using Soxhlet extraction method. After filtering and evaporating to dryness, the crude water extract was obtained.

#### B. Water extract of *Hedychium coronarium*:

44g of the powdered plant material (rhizome) was extracted with 440 ml of distilled water for 20 hrs using Soxhlet extraction method. The extract was then filtered through filter paper and evaporated to dryness; finally the crude water extract was obtained.

#### C. Water extract of *Hedychium rubrum*:

46g of the dried and powdered plant material (rhizome) was extracted with 460ml of distilled water for 20 hrs using Soxhlet extraction method. After filtering and evaporating to dryness, the crude water extract was obtained.

***Phytochemical Screening*:**

Chemical tests of water extracts of each plant were carried out qualitatively using standard procedures to identify the major phytochemical constituents [11, 12, 13, 14, 15].

**1. Test for alkaloids:**

**Hager's test:** Extracts were dissolved individually in dilute hydrochloric acid and filtered. Filtrates were treated with Hager's reagent. Formation of yellow coloured precipitate indiated the presence of alkaloids.

**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.

**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.

**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.

**Ferric chloride test:** To the test solution, a few drops of ferric chloride solution were added. An intense green, purple, blue or black colour 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.

***Determination of Total Phenolic Content*:**

The amount of phenol content in the water extracts of three different species of *Hedychium* i.e. *H. spicatum*, *H. coronarium* and *H. rubrum* were determined with Folin-Ciocalteu reagent [16, 17]. 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 $45^0$C for 15 min. The absorbance of each sample was measured at 760 nm using UV Visible Spectrophotometer. Gallic acid (5-30 µg/ml) was used as a standard compound. The gallic acid standard calibration curve was established by plotting concentration (µg/ml) versus absorbance (nm) ($y= 0.011x + 0.062$; $R^2= 0.993$), where y is absorbance at 760 nm and x is concentration (**Figure-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 [18]:

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

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 water extract of the plant, g.

**Table-1: Comparative analysis of phytochemical constituents of three different species of Genus *Hedychium*:**

| **Phytochemical constituents** | **Chemical tests** | **Water extract of *H. spicatum*** | **Water extract of *H. coronarium*** | **Water extract of *H. rubrum*** |
|---|---|---|---|---|
| Alkaloids | Hager's test | - | - | - |
| Carbohydrates (reducing sugar) | Benedict's test<br>Fehling's test | -<br>+ | -<br>+ | +<br>+ |
| Proteins | Xanthoproteic test | + | + | + |
| Flavonoids | Alkaline reagent test | + | + | + |
| Phenolic compounds | Lead acetate test | + | + | + |
| Tannins | Lead acetate test<br>Ferric chloride test | +<br>- | +<br>+ | +<br>+ |
| Steroids and Terpenoids | Salkowski's test | + | + | + |
| Saponin | Froth test | + | + | + |
| Cardiac glycosides | Keller-killiani test | + | + | + |
| Oil | | + | + | + |
| Phlobatannin | | - | - | + |

**Key: + = Present and – = Absent**

## RESULTS AND DISCUSSION:

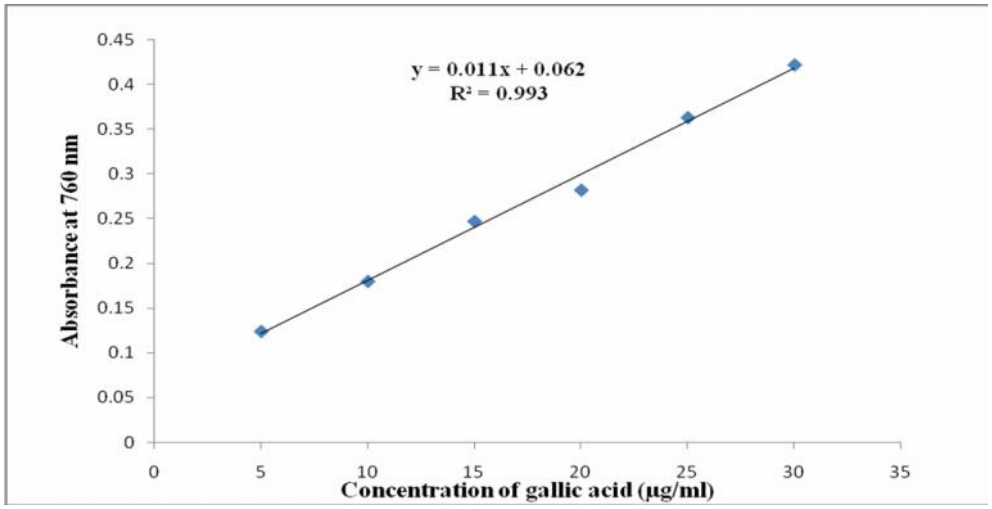
The phytochemical analysis conducted on the water extract of *H. spicatum* revealed the presence of phenolic compounds, flavonoids, reducing sugar (carbohydrate), protein, steroids and triterpenoids, cardiac glycosides, tannin, saponin and oil as major phytochemical groups. Phytochemical screening of both *H. coronarium* and H. rubrum also showed the presence of phenolic compounds, flavonoids, protein, steroids and triterpenoids, cardiac glycosides, tannin, saponin and oil. These phytochemical compounds are the major compounds which impart medicinal value of the plant. Water extracts of the three species of *Hedychium* tested negative for the presence of alkaloids. Phlobatannin was present only in water extract of *H. rubrum* but *H. spicatum* and *H. coronarium* showed negative test for phlobatannin (Table-1).

Phenolics are a major group of compounds that act as primary antioxidants or free radical scavengers. The amount of total phenolic content was determined with the Folin-Ciocalteu reagent. Gallic acid was used as standard compound. The calibration curve for gallic acid is represented in Figure-1 (y= 0.011x + 0.062; $R^2$= 0.993), where y is absorbance at 760 nm and x is concentration. The total phenolic contents of the water extracts of *H. spicatum*, *H. coronarium* and *H. rubrum* in terms of gallic acid equivalent were 29.39 ± 0.01, 34.93 ± 0.01 and 66.48 ± 0.01 mg/g of extract powder respectively (Table-2). *H. rubrum* showed the maximum phenolic content as compared to *H. spicatum* and *H. coronarium.*

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

| Sample | Concentration (mg/ml) | mg of gallic acid/g of extract (Mean ± Standard Deviation) |
|---|---|---|
| Water extract of *H. spicatum* | 1 | 29.39 ± 0.01 |
| Water extract of *H. coronarium* | 1 | 34.93 ± 0.01 |
| Water extract of *H. rubrum* | 1 | 66.48 ± 0.01 |

**Figure-1: Calibration curve of Gallic acid**




## CONCLUSION:

The phytochemical analysis of the water extracts of *H. spicatum*, *H. coronarium* and *H. rubrum* shows the presence of almost similar groups of compounds. This present study can help in choosing superior race of the plant. It has also been found that the water extracts of three different species of *Hedychium* contain a good quantity of phenolic compounds. These plants can be studied further to know their biological effects which could be a beneficial in the treatment and controlling of various diseases.

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