

## Research Article



## Assessment of Total Flavonoid Content and Antioxidant Activity of Methanolic Rhizome Extract of Three Hedychium Species of Manipur Valley

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### ABSTRACT

In the present study methanolic rhizome extract of three Hedychium species namely *Hedychium spicatum*, *Hedychium coronarium* and *Hedychium rubrum* were evaluated for antioxidant activity using free radical scavenging activity, reducing power and total antioxidant activity. Total flavonoid content was also determined in each extract. Ascorbic acid and quercetin were used as reference standards. Total flavonoid content in methanolic rhizome extract of *H. spicatum*, *H. coronarium* and *H. rubrum* in terms of quercetin equivalent (QE) were 4.22, 2.47 and 21.25µg/100g of dried rhizome respectively. In DPPH assay *H. rubrum* (60.86%) showed the highest free radical scavenging activity followed by *H. Spicatum* (42.74%) and lowest activity in *H. coronarium* (32.42%). In reducing power method, standard ascorbic acid and sample extracts were found to increase with increase in concentration. Total antioxidant activity of methanolic rhizome extract of *H. spicatum*, *H. coronarium* and *H. rubrum* in terms of ascorbic acid equivalent (AAE) was 224.4, 205.6 and 279µg/ml of extract respectively. Results indicate that methanolic rhizome extract of all the three Hedychium species are potential source of antioxidant which may be due to the presence of flavonoid in the extracts. *H. rubrum* showed the highest total flavonoid content and antioxidant activity among the three Hedychium species.

**Keywords:** Hedychium, flavonoid, quercetin, ascorbic acid, DPPH, antioxidant

### INTRODUCTION

Plants and plant-derived products are part of the health-care system since ancient human civilization. The use of traditional medicine is wide spread and plants are still a large source of natural antioxidants that might serve as leads for the development of novel drugs.<sup>1</sup> Recent research with important bioactive compounds in many plant and food materials has received much attention. Medicinal plants are known for their potent antioxidant property as they contain bioactive compounds such as carotenoids, benzoic acid, cinnamic acid, folic acid, phenols and flavonoids.<sup>2-4</sup> Free radicals or reactive oxygen species (ROS) were generated during metabolism resulting in oxidative stress. Free radicals are associated with various physiological and pathological events such as inflammation, aging, mutagenicity and carcinogenicity. Most free radicals are extremely reactive. Free radicals, especially the oxygen radical, superoxide, when formed could lead to the formation of other radicals.<sup>5</sup> Antioxidants are the substances that will either delay or inhibit the oxidative power of reactive oxygen species. Studies have reported synthetic antioxidants such as butylated hydroxytoluene (BHT) and butylated hydroxyanisole (BHA) are toxic in human.<sup>6</sup> Natural antioxidants have become the target of a great number of research studies in finding the sources of potentially safe, effective and cheap antioxidants.<sup>7</sup> Epidemiological studies have indicated the relationship between the plant antioxidants and reduction of chronic diseases. These benefits area thought to result from the antioxidant

components of plant origin, vitamins, flavonoids and carotenoids. The studies in recent years have shown that polyphenols in plants scavenge active oxygen species and effectively prevent oxidative cell damage.<sup>8</sup>

Hedychium belongs to the Zingiberaceae family popularly called as ginger lily consists of about 80 perennial herbaceous species characterized by showy and scented flowers. They are generally grown as ornamental plants, but they are also used for their industrial (paper manufacture and perfumery) and medicinal properties.<sup>9-11</sup> Hedychium have been reported to possess antibacterial, antifungal, anti-inflammatory and insecticidal activities.<sup>12,13</sup> Certain species of Hedychium rhizome is often consumed as food in Manipur. Seeing its various uses, for our research we have selected three different Hedychium species namely *Hedychium spicatum*, *Hedychium coronarium* and *Hedychium rubrum*. *H. spicatum* is commonly known as spiked ginger lily and well distributed in the entire Himalayan region. Its rhizome is commercially available in Indian market as kapurkachari. Medicinal values of *H. spicatum* rhizome includes CNS depressant, analgesic, stomachic, carminative, bronchodilator stimulant and tonic, also used traditionally for treating nausea, vomiting, liver complaints, diarrhea, dyspepsia and pains, respiratory disorder etc.<sup>14-16</sup> *H. coronarium* is commonly known as white ginger lily. It is widely cultivated in tropical and subtropical regions of India. It has been used traditionally for the treatment of tonsillitis, infected nostrils, tumor and fever.<sup>17</sup> *H. rubrum* is a rare red species commonly known as red ginger lily which is endemic to the North



Eastern region of India. It has been used in folk medicine in Manipur but no scientific report is available for its medicinal uses.

Phytochemical screening and the total phenol content in the methanolic rhizome extract of the selected *Hedychium* species has been reported in our previous paper.<sup>18</sup> In continuation of our research work, total flavonoid content and antioxidant activity in the methanolic rhizome extract of the selected *Hedychium* species have been determined and compared in the present study.

## MATERIALS AND METHODS

### Collection of plant sample

Three *Hedychium* species i.e. *H. spicatum*, *H. coronarium* and *H. rubrum* were collected from the Imphal valley of Manipur, Northeast India. Plant material was identified by the Department of Botany, Imphal College, Imphal. Rhizomes of each species were removed and used for the study. They were washed with tap water and then rinsed in distilled water. The rhizomes were cut into pieces and shade dried. Dried rhizomes were ground into fine powder using mechanical grinder and kept in air tight container for further analysis.

### Preparation of sample extracts

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

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

40g of the dried powdered rhizome was extracted using 400ml of methanol by soxhletion for 2 days. Crude methanolic extract was obtained by evaporating the extract to dryness.

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

44g of the dried powdered rhizome was extracted using 440ml of methanol by soxhletion for 2 days. The extract was evaporated to dryness to obtain the crude extract.

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

41g of the dried powdered rhizome was extracted using 410ml of methanol by soxhletion for 2 days. Crude methanolic extract was obtained by evaporating the extract to dryness.

### Estimation of total flavonoid content

Total flavonoid content was estimated by Aluminium chloride colorimetric method

The principle involved in Aluminium chloride ( $AlCl_3$ ) colorimetric method is that  $AlCl_3$  forms acid stable complexes with the C-4 keto groups and either the C-3 or C-5 hydroxyl group of flavones and flavonols. In addition it also forms acid labile complexes with the ortho-dihydroxyl groups in the A- or B-ring of flavonoids (Figure 1).

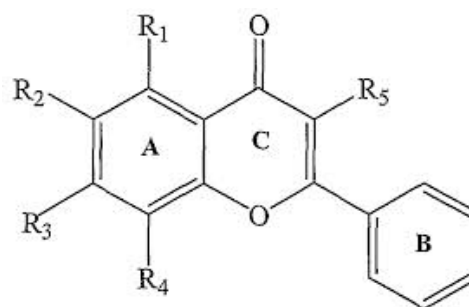


Figure 1: Basic structure of flavonoid

Studies have reported quercetin to be suitable reference for determination of total flavonoid content in plant sample extract. Therefore, quercetin solutions of various concentrations were used to make the standard calibration curve.

10mg of quercetin was dissolved in 100ml methanol and then diluted to 6.25, 12.5, 25, 50, 80, and 100 $\mu$ g/ml using methanol. Stock solution of plant extracts was prepared by dissolving 100 mg of the methanolic rhizome extract of each *Hedychium* species in 5ml methanol and transferred to 10ml volumetric flask and made up the volume with methanol. 10% aluminium chloride and 1M potassium acetate were prepared using distilled water.

The assay was determined using 0.5ml of each extract stock solution and each dilution of standard quercetin taken separately in test tubes. To each test tube 1.5ml methanol, 0.1ml aluminium chloride solution, 0.1ml potassium acetate solution and 2.8ml distilled water were added and mixed well. Sample blank for all the dilution of standard quercetin and all the three methanolic rhizome extract were prepared in similar manner by replacing aluminium chloride solution with distilled water. All the prepared solutions were filtered through Whatmann filter paper before measuring their absorbance. Absorbance was taken at 415 nm against the suitable blank.<sup>19,20</sup>

### Determination of free radical scavenging assay

The free radical scavenging capacity of the methanolic rhizome extracts was determined using DPPH assay.<sup>21</sup> DPPH solution (0.004% w/v) was prepared in methanol. Stock solution (1mg/ml) of methanolic rhizome extract of *H. spicatum*, *H. coronarium*, *H. rubrum* and standard ascorbic acid (0.5mg/ml) were prepared using methanol. Various concentrations (10-50 $\mu$ g/ml) of the rhizome extract and ascorbic acid were taken in test tubes and 1ml of freshly prepared DPPH solution were added, the test tubes were protected from light by covering with aluminum foil. The final volume in each test tube was made to 2ml with methanol and incubated in dark for 30 minutes at room temperature. After incubation, the absorbance was read at 517nm using a spectrophotometer (UV-2700). Control sample was prepared containing the same volume of methanol and DPPH without any extract and reference ascorbic acid. Methanol was served as blank.

% scavenging activity of the DPPH free radical was calculated by using the following equation:

$$\% \text{ Scavenging Activity} = \frac{\text{Absorbance of the control} - \text{Absorbance of the test sample}}{\text{Absorbance of the control}} \times 100$$

### Estimation of reducing power

Various concentrations of the plant extracts (1mg/ml) in corresponding solvents were mixed with phosphate buffer (2.5ml) and potassium ferricyanide (2.5ml). This mixture was kept at 50°C in water bath for 20 minutes. After cooling, 2.5ml of 10% trichloro acetic acid was added and centrifuged at 3000 rpm for 10min whenever necessary. The upper layer of solution (2.5ml) was mixed with distilled water (2.5ml) and a freshly prepared ferric chloride solution (0.5ml). The absorbance was measured at 700 nm. Control was prepared in similar manner excluding samples. Ascorbic acid (0.5mg/ml) at various concentrations was used as standard. Increased absorbance of the reaction mixture indicates increase in reducing power.<sup>22</sup>

### Determination of total antioxidant activity

The phosphomolybdenum method was used to evaluate the total antioxidant activity of the extracts.<sup>23</sup> Antioxidants can reduce Mo (VI) to Mo (V) and the green phosphate / Mo (V) compounds at acidic pH, which have an absorption peak at 695 nm, were generated subsequently. 0.3 ml of the sample (1mg/ml) as well as ascorbic acid (0.5mg/ml) was mixed with 3.0ml of the reagent solution separately. Reaction mixture was incubated at 95°C for 90min under water bath. Absorbance of all the mixtures was measured at 695 nm

after cooling. Total antioxidant activity is expressed as the number of equivalents of ascorbic acid in microgram per milliliter of extract. Total antioxidant activity was calculated by using the formula:

$$\text{Total antioxidant} = \text{O.D. of test} \times \text{concentration of standard in } \mu\text{g} \times \text{made up volume of sample}$$

## RESULTS AND DISCUSSION

Flavonoids are plant secondary metabolites widely distributed in the plant kingdom. More than 6000 flavonoids have been identified in plants.<sup>24</sup> Figure 2 shows the standard calibration curve of quercetin (0.1mg/ml stock solution) for the determination of total flavonoid content in the methanolic rhizome extracts of *H. spicatum* and *H. coronarium*. Absorbance for *H. rubrum* was more than the absorbance shown by highest concentration of quercetin. Another standard calibration curve of quercetin with 0.2mg/ml stock solution was made following the same procedure and is indicated in Figure 3. From the second standard calibration curve, total flavonoid content in the methanolic rhizome extract of *H. rubrum* was determined. From the respective standard curves, concentration values of the three extracts were obtained and total flavonoid content (TFC) was calculated by using the following formula<sup>25</sup>:

$$\text{TFC} = \frac{R \times D.F \times V \times 100}{W}$$

Where R - Result obtained from the standard curve, D.F - Dilution factor, V - Volume of stock solution 100 - For 100 g dried plant and W - Weight of plant used in the experiment.

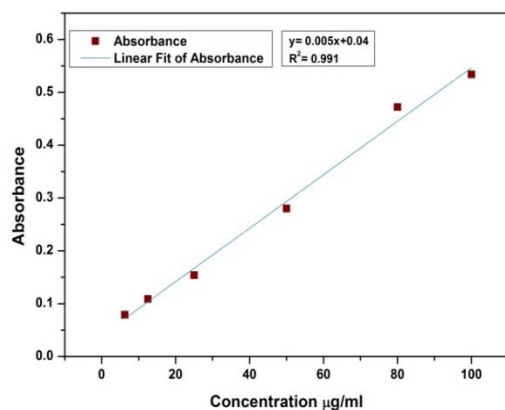


Figure 2: Standard curve of quercetin (0.1mg/ml stock solution)

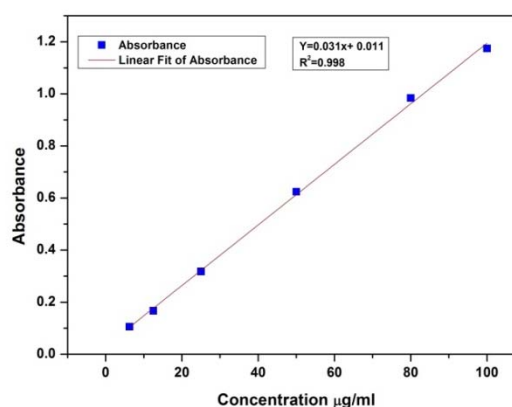


Figure 3: Standard curve of quercetin (0.2mg/ml stock solution)

Table 1: Total flavonoid content and total antioxidant activity of the studied samples

Sample	Total flavonoid content in µg/100g of dried rhizome (in QE)	Total antioxidant activity in µg/ml of extract (in AAE)
<i>H. spicatum</i>	4.22 ± 0.425	224.4 ± 0.7
<i>H. coronarium</i>	2.47 ± 0.079	205.6 ± 0.84
<i>H. rubrum</i>	21.25 ± 0.295	279 ± 0.08

The total flavonoid content and total antioxidant activity of methanolic rhizome extract of the three *Hedychium* species is shown in Table 1.

Assays were performed in triplicates. Values are expressed as means  $\pm$  SD.

Total flavonoid content is expressed as quercetin equivalent (QE;  $\mu$ g quercetin /100g of dried rhizome)

Total antioxidant activity is expressed as ascorbic acid equivalent (AAE;  $\mu$ g ascorbic acid /ml of extract)

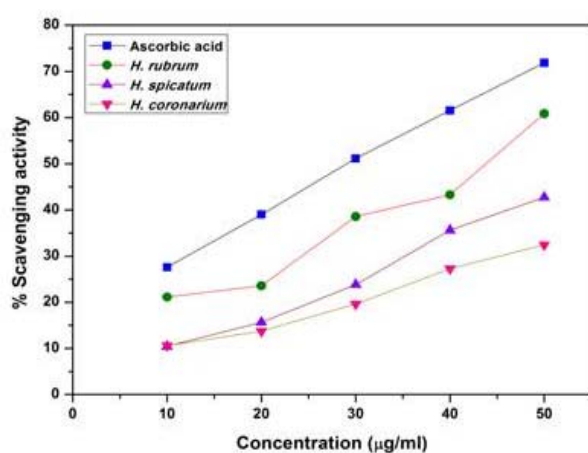
Total flavonoid content was determined from the respective standard curve of quercetin, results indicate that methanolic rhizome extract of *H. rubrum* has the highest total flavonoid content when compared to the other two *Hedychium* species. The absorbance of *H. rubrum* was found to be three times more than the absorbance shown by *H. spicatum* and *H. coronarium*. In our previous study, *H. rubrum* showed highest total phenol content among the three species.

Methanolic rhizome extract of *H. rubrum* showed the highest total antioxidant activity followed by *H. spicatum* and least activity was noted in *H. coronarium*.

The present data indicates that *H. rubrum* has the highest antioxidant activity when compared to *H. spicatum* and *H. coronarium* which may be due to higher phenol and flavonoid content in *H. rubrum*.

In free radical scavenging assay, it was noted that DPPH scavenging activity increased with increase in concentration for both standard and methanolic rhizome extract of *Hedychium* species. Percentage scavenging activity at 50 $\mu$ g/ml concentrations of standard ascorbic acid and methanolic rhizome extracts of *H. spicatum*, *H. coronarium* and *H. rubrum* were found to be 71.86%, 42.74%, 32.42% and 60.86% respectively.

The percentage scavenging activity of standard ascorbic acid and methanolic rhizome extract of the selected three *Hedychium* species is indicated in Figure 4.



**Figure 4:** DPPH scavenging activity of standard and methanolic rhizome extract of selected *Hedychium* species

Methanolic rhizome extract of *H. rubrum* showed highest DPPH scavenging activity among the three *Hedychium* species.

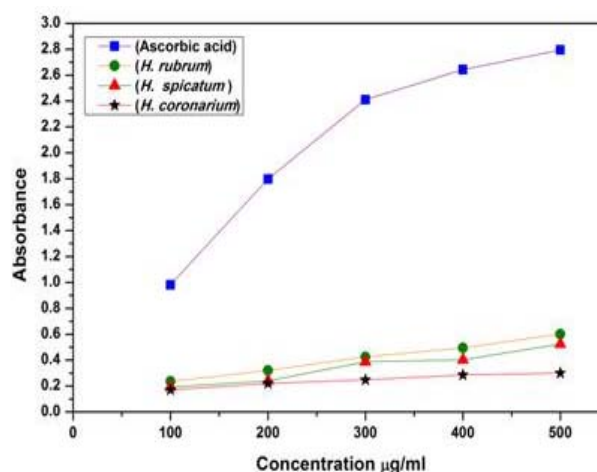
DPPH assay is based on the ability of 2,2-diphenyl-1-picrylhydrazyl, a stable free radical to decolourize from purple to yellow colour in presence of antioxidants. The DPPH contains an odd electron, which is responsible for the absorbance at 517nm and also for a visible deep purple colour. When DPPH accepts an electron donated by an antioxidant compound, the DPPH is decolourized.

The reduction capability of DPPH radicals was determined by decrease in its absorbance at 517nm induced by antioxidants. Hence, DPPH is usually used as a substrate to evaluate antioxidant activity of antioxidants.<sup>26</sup>

From the reducing power assay as shown in Figure 5, absorbance increased with increase in concentration for both standard and sample extract. Methanolic rhizome extract of *H. rubrum* showed the highest reducing power among the three *Hedychium* species.

When compared with the standard, reducing power of all the three species was found to be less. Reducing power may serve as a significant reflection of the antioxidant activity as it is associated with antioxidant activity.<sup>16</sup>

Compounds with reducing power indicate that they are electron donors and can reduce the oxidized intermediates of lipid peroxidation processes, so that they can act as primary and secondary antioxidants.<sup>27</sup>



**Figure 5:** Reducing potential of standard and methanolic rhizome extract of three *Hedychium* species

Essential oil obtained from *H. coronarium* flower showed high DPPH scavenging activity and reducing power as Lu in their studies.<sup>28</sup>

Antioxidant activity of methanolic rhizome extract of *H. coronarium* has also been reported by Ho<sup>29</sup>.

Sravani and Parakh have reported that there are significant correlations between radical scavenging and reducing power, total phenol and total flavonoids in *H. spicatum* as per their studies.<sup>16</sup>



## CONCLUSION

From the results it was observed that methanolic rhizome extract of all the three *Hedychium* species possess antioxidant activity. *H. rubrum* showed the highest total antioxidant activity, DPPH scavenging activity and reducing power followed by *H. spicatum* and least activity was observed in *H. coronarium*. The highest antioxidant activity of *H. rubrum* among the species may be due to higher total flavonoid content. Further investigations need to be carried out for the isolation and characterization of bioactive compound present in the rhizome of *H. rubrum* which is responsible for higher antioxidant activity in *H. rubrum*.

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