ISSN: 0975-8585

Research Journal of Pharmaceutical, Biological and Chemical Sciences

Total Flavonoid Content and Antioxidant Activity of Aqueous Rhizome Extract of Three Hedychium Species of Manipur Valley.

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ABSTRACT

Hedychium species is one of the important aromatic, ornamental medicinal plants of North east India. In the present study, total flavonoid content and antioxidant activity of aqueous rhizome extract of three Hedychium species namely *H. rubrum*, *H. coronarium* and *H. spicatum* were investigated. In antioxidant activity: free radical scavenging activity, reducing power and total antioxidant activity were determined and expressed in terms of standard used for the respective assay. Total flavonoid content of aqueous rhizome extract of *H. rubrum*, *H. coronarium* and *H. spicatum* in terms of quercetin equivalent (QE) was 3.22, 2.77 and 0.74 µg/100g of extract respectively. In DPPH assay *H. rubrum* (32.3%) showed the highest free radical scavenging activity followed by H. coronarium (21%) and lowest activity in *H. Spicatum* (5.76%). In reducing power method, both standard ascorbic acid and sample extracts were found to be increased in a dose dependent manner. Total antioxidant activity of aqueous rhizome extract of *H. rubrum*, *H. coronarium* and *H. spicatum* in terms of ascorbic acid equivalent (AAE) was 207.3, 157.5 and 102.6 µg/ml of extract. The present data indicates that aqueous 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. Among the three Hedychium species, *H. rubrum* was found to have the highest total flavonoid content and antioxidant activity.

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

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ISSN: 0975-8585

INTRODUCTION

Medicinal plants are the source of many modern drugs used for the treatment of various human diseases [1]. They are known for their potent antioxidant property as they contain bioactive compounds such as carotenoids, benzoic acid, cinnamic acids, folic acid, phenols and flavonoids [2-4]. During metabolism free radicals or reactive oxygen species (ROS) were generated resulting in oxidative stress. Oxidative stress is the root cause of several degenerative diseases such as cancer, diabetes, cardiovascular disorder, liver damage, neurodegenerative diseases, aging etc [5,6]. Antioxidants are the substances that will either delay or inhibit the oxidation of reactive oxygen species. Synthetic antioxidants such as butylated hydroxytoluene (BHT) and butylated hydroxyanisole (BHA) are reported to be toxic in human [7]. Plants are the potential source of natural antioxidants. Natural antioxidants have become the target of a great number of research studies in finding the sources of potentially safe, effective and cheap antioxidants [8]. Herbal drugs containing free radical scavengers are known for their therapeutic activity [9].

Flavonoids, the most common group of polyphenolic compounds are found ubiquitously in plants. Flavonoids are potent antioxidants. The capacity of flavonoids to act as antioxidants depends upon their molecular structure. The position of hydroxyl groups and other features in the chemical structure of flavonoids are important for their antioxidant and free radical scavenging activities [10,11].

Hedychium is a genus of flowering plants in the ginger family Zingiberaceae, native to lightly wooded habitats in Asia. The genus name Hedychium is derived from two ancient Greek words, hedys meaning "sweet" and chios meaning "snow". It is considered as highly valued ornamental garden plant due to its foliage, showy inflorescence having wide variety of colour. Recently, much research has been done on screening of phytochemical constituents and antioxidant activity of different Hedychium species. Hedychium species have wide range of medicinal values including vasodilator, hypoglycemic, hypotensive, stomachic, etc along with antimicrobial and anti-inflammatory properties [12-18]. In our previous paper the medicinal values of the selected Hedychium species namely *Hedychium rubrum*, *Hedychium coronarium* and *Hedychium spicatum* has already mentioned [19]. Considering the various uses and immense medicinal properties our work has been further carried out on the selected three Hedychium species. The present study was carried out with the objective to determine and compare the total flavonoid content and antioxidant activity in the aqueous rhizome extract of the selected Hedychium species.

MATERIALS AND METHODS

Collection of plant sample

Three different species of genus Hedychium i.e. *H. rubrum*, *H. coronarium* and *H. spicatum* were collected from the Imphal valley of Manipur, Northeast India. Identification of the plant material was done in the Department of Botany, Imphal College, Imphal. The rhizomes of each plant were washed in tap water and then rinsed in distilled water. The rhizomes were cut into pieces and dried under shade. The dried rhizomes of each plant were ground into fine powder using mechanical grinder.

Preparation of sample extracts

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

A. Aqueous extract of *Hedychium rubrum*:
46g of the dried powdered rhizome was extracted using 460ml of distilled water by soxhletion for 20 hrs. Crude aqueous extract was obtained by evaporating the extract to dryness.

B. Aqueous extract of *Hedychium coronarium*:
44g of the dried powdered rhizome was extracted using 440ml of distilled water by soxhletion for 20 hrs. The extract was evaporated to dryness to obtain the crude extract.

C. Aqueous extract of *Hedychium spicatum*:
44g of the dried powdered rhizome was extracted using 440ml of distilled water by soxhletion for 20 hrs. Crude aqueous extract was obtained by evaporating the extract to dryness

ISSN: 0975-8585

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).

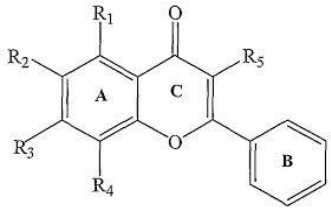

Figure 1: Basic structure of flavonoid

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

10 mg of quercetin was dissolved in methanol and then diluted to 6.25, 12.5, 25, 50, 80, and 100 µg/ml using methanol. Stock solution of extracts was prepared by dissolving 100 mg of the each extract in 5ml methanol and transferred to 10 ml 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 2.8 ml distilled water were added and mixed well. Sample blank for all the dilution of standard quercetin and all the three aqueous 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 if necessary before measuring their absorbance. Absorbance was taken at 415 nm against the suitable blank [20, 21].

Determination of free radical scavenging assay

The free radical scavenging capacity of the aqueous rhizome extracts was determined using DPPH assay [22]. DPPH solution (0.004% w/v) was prepared in methanol. Stock solution (1mg/ml) methanol extract of *H. spicatum*, *H. coronarium*, *H. rubrum* and standard ascorbic acid (0.5 mg/ml) were prepared using methanol. Various concentrations (10-50 µg/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 2 ml with methanol. After 30 minutes of incubation in dark at room temperature, the absorbance was read at 517 nm 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:

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

Estimation of reducing power

Various concentrations of the plant extracts (1mg/ml) in corresponding solvents were mixed with phosphate buffer (2.5 ml) and potassium ferricyanide (2.5 ml). This mixture was kept at 50ºC in water bath for 20 minutes. After cooling, 2.5 ml of 10% trichloro acetic acid was added and centrifuged at 3000 rpm for 10

min whenever necessary. The upper layer of solution (2.5 ml) was mixed with distilled water (2.5 ml) and a freshly prepared ferric chloride solution (0.5 ml). The absorbance was measured at 700 nm. Control was prepared in similar manner excluding samples. Ascorbic acid (1mg/ml) at various concentrations was used as standard. Increased absorbance of the reaction mixture indicates increase in reducing power [23].

Determination of total antioxidant activity

The phosphomolybdenum method was used to evaluate the total antioxidant activity of the extract [24]. 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 (1mg/ml) was mixed with 3.0 ml of the reagent solution separately. Mix well and measure the absorbance at 695nm. Reaction mixture was incubated at 95ºC for 90 min 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 micrograms per milligram of extract. Total antioxidant activity was calculated by using the formula

Total antioxidant = O.D. of test x concentration of standard in µg x made up volume of sample

RESULT AND DISCUSSION

The preliminary phytochemical screening and total phenol content of aqueous extract of rhizome of the three different Hedychium species was published in our previous paper. Our result showed that all the three species are rich source of phytochemicals. Total phenol content was found to be highest in *H. rubrum* followed by *H. coronarium* and least was noted in *H. spicatum* [19].

Flavonoids as one of the most diverse and widespread group of natural compounds are probably the most important natural phenols. These compounds possess a broad spectrum of chemical and biological activities including radical scavenging properties [25].

Figure 2 shows the standard calibration curve of quercetin for the determination of total flavonoid content in the sample extracts. From the standard curve, concentration values of all extracts were obtained and total flavonoid content (TFC) was calculated by using the following formula [26]

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

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.

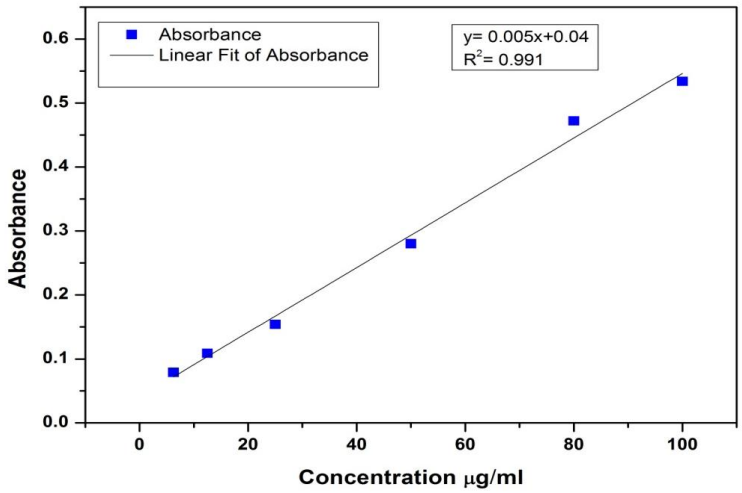

Figure 2: Standard calibration curve of quercetin

The total flavonoid content and total antioxidant activity of aqueous rhizome extract of the selected samples is shown in Table 1. Total flavonoid content in the aqueous rhizome extract of *H. rubrum*, *H. coronarium* and *H. spicatum* was found to be 3.22, 2.77 and 0.74 µg QE/100g of the extract respectively. This indicates that aqueous rhizome extract of *H. rubrum* has the highest total flavonoid content when compare to the other two Hedychium species. Similar result was noted in our previous study for the total phenol content of aqueous rhizome extract. Sravani and Paarakh [27] reported the total flavonoid content in aqueous rhizome extract of *H. spicatum* was 40 mg rutin equivalent per gram of extract which was much higher when compared to our result. This may be due the variation in the climatic condition and area from where the sample was obtained.

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

Sample	Total flavonoid content in µg/100g of extract (in QE)	Total antioxidant activity in µg/ml of extract (in AAE)
H. rubrum	3.22±0.02	207.28±0.26
H. coronarium	2.78±0.22	157.52±0.44
H. spicatum	0.76±0.02	102.6±0.45

Assays were performed in triplicates. Values are expressed as means±SD.
Total flavonoid content is expressed as quercetin equivalent (QE; µg quercetin /100g of extract)
Total antioxidant activity is expressed as ascorbic acid equivalent (AAE; µg ascorbic acid /ml of extract)

Similar trend was noted for the total antioxidant activity of aqueous rhizome extract of the three Hedychium species. The total antioxidant activity of *H. rubrum*, *H. coronarium* and *H. spicatum* in terms of ascorbic acid equivalent were 207.28, 157.52 and 102.6 µg/ml of extract respectively. The present data indicates that *H. rubrum* has the highest antioxidant activity when compared to *H. coronarium* and *H. spicatum* which may be due to higher phenol and flavonoid content in *H. rubrum*.

From the result, it was noted that DPPH scavenging activity increased with increase in concentration for both standard and aqueous rhizome extract of Hedychium species. Percentage scavenging activity at 50 µg/ml concentrations of standard ascorbic acid and aqueous rhizome extracts of *H. rubrum*, *H. coronarium* and *H. spicatum* were found to be 58.7%, 32.3%, 21% and 5.76% respectively. DPPH scavenging activity was highest in aqueous rhizome extract of *H. rubrum* among the three Hedychium species. DPPH assay is based on the ability of 2,2-diphenyl-1-picrylhydrazyl, a stable free radical to decolorize from purple to yellow color 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 decolorized [28]. The percentage scavenging activity of standard ascorbic acid and aqueous rhizome extract of the selected three Hedychium species is indicated in Figure 3.

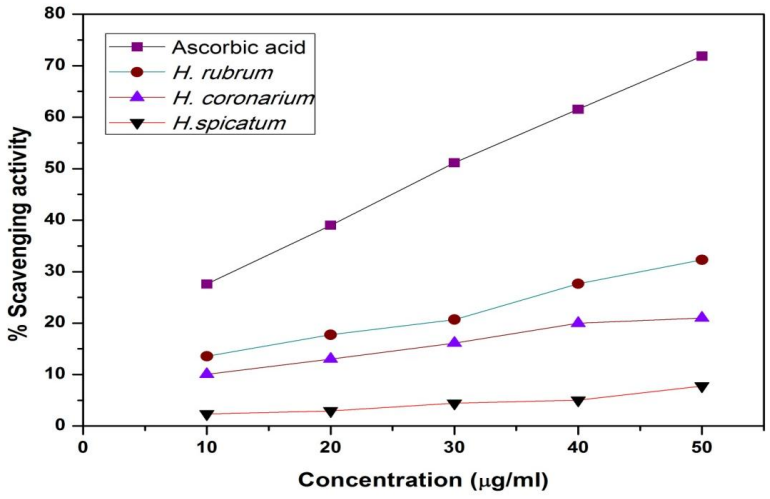

Figure 3: DPPH radical scavenging activity of the studied samples

ISSN: 0975-8585

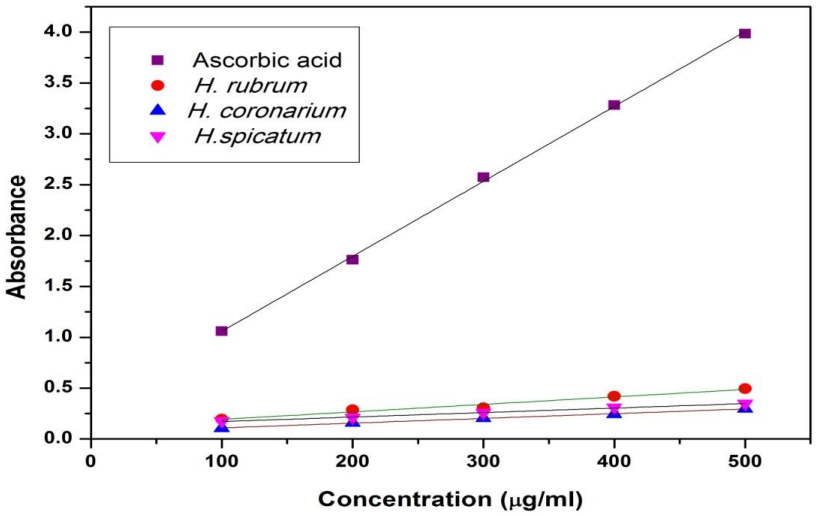

Figure 4: Reducing power of the studied samples

From the reducing power assay as shown in Figure 4, absorbance increased with increase in concentration for both standard and sample extract. *H. rubrum* showed the highest reducing power among the three Hedychium species. Reducing power was found to be less for all the three species when compare with the standard. Reducing power is associated with antioxidant activity and may serve as a significant reflection of the antioxidant activity [29]. 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 [30]. Essential oil obtained from *H. coronarium* flower was found to have high DPPH scavenging activity and reducing power as Lu *et al*. (2009) in their studies [31].

CONCLUSION

From the present study, it has been found that all the aqueous rhizome extract of three Hedychium species showed antioxidant activity. Among them, *H. rubrum* have the highest total antioxidant activity, DPPH scavenging activity and reducing power followed by *H. coronarium* and least activity was noted in *H spicatum*. The highest antioxidant activity of *H. rubrum* among the species may be due to higher total flavonoid content. Further research can be carried out for the isolation and characterization of bioactive compound responsible for higher antioxidant activity in *H. rubrum*.

ACKNOWLEDGEMENT

Authors are thankful to the Department of Biotechnology, Govt. of India for financial support.

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