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Antioxidant activity of *Hedychium spicatum* Buch.- Ham. rhizomes

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The different extracts of *Hedychium spicatum* Buch.-Ham. (Zingiberaceae) were evaluated for antioxidant activity by 1, 1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging activity and reducing power determination method. Total phenol and total flavonoid content in each extracts were also determined. Gallic acid and ascorbic acid were used as reference standards. They exhibited strong antioxidant DPPH radical scavenging activity with IC_{50} value of 0.43, 259.1, 386.7, 414.3, 77.01 and 51.54 µg/mL for gallic acid, petroleum ether, benzene, chloroform, methanol and aqueous extracts of rhizomes of *H. spicatum,* respectively. The absorbance for reducing power was found to be 0.540, 0.036, 0.042, 0.046, 0.050 and 0.072 for ascorbic acid, petroleum ether, benzene, chloroform, methanol and aqueous extracts of rhizomes of *H. spicatum,* respectively. Total phenol content was found to be 13, 33, 24, 32 and 35 mg equivalent to gallic acid per gram of petroleum ether, benzene, chloroform, methanol and aqueous extracts of rhizomes of *H. spicatum,* respectively. Total flavonoid content was found to be 4.6, 7, 7.5, 34.5 and 40 mg equivalent to rutin per gram of petroleum ether, benzene, chloroform, methanol and aqueous extracts of rhizomes of *H. spicatum,* respectively. The strongest antioxidant activity of the methanol and aqueous extracts could be due to the presence of flavonoids and phenols.

Keywords: *Hedychium spicatum* Buch.-Ham., Antioxidant, DPPH, Gallic acid, Ascorbic acid, Phenol, Flavonoid.

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Introduction

Hedychium spicatum Buch.-Ham. belonging to the family Zingiberaceae and commonly known as spiked ginger lily, is found in the entire Himalayan region[1]. It is a perennial rhizomatous herb and the rhizomes are an article of commerce which is called as *Kapurkachari* in Indian bazaar. Traditionally the rhizomes are used as febrifuge, tranquiliser, hypotensive, antispasmodic, CNS depressant, analgesic, anti-inflammatory, antimicrobial, antioxidant, antifungal, pediculicidal, cytotoxic, anthelmintic and in respiratory disorders[2,10]. The literature survey revealed that previous work has been done on essential oil compositions of the plant for various activities, viz. tranquiliser[11], pediculicidal[8], antimicrobial[5] and antioxidant activity[6].

Free radicals are reactive molecules involved in many physiological processes and human diseases such as cancer, ageing, arthritis, Parkinson syndrome, ischemia, toxin induced reactions, alcoholism, liver injury[12], etc. Research in finding a natural antioxidant from the plant source is therefore important as plants are potential source of immense chemicals for the treatment of number of aliments. Considering the above aspects, an attempt has been made to study the antioxidant activity of various extracts of rhizomes of this species. Different extracts of *H. spicatum* were evaluated for *in vitro* antioxidant DPPH free radical scavenging activity by reducing power method. Total phenol and total flavonoid content in each extracts were also determined.

Materials and Methods

Chemicals

DPPH (1, 1-diphenyl-2-picrylhydrazyl), gallic acid, rutin and ascorbic acid were purchased from Loba Chemie Pvt Ltd., Mumbai. All the chemicals and reagents used were of analytical grade.

Plant material

The rhizomes of *H. spicatum* were collected from Tirupati forest in the month of May 2010, identified and authentified by Dr. Shiddamallayya N, National Ayurveda and Dietetics Research Institute, Bengaluru, Karnataka (Ref.no. SMPU/NADRI/BNG/2010-11/307, date 3/8/2010). They were dried under shade then cut into small pieces; powdered and stored in a closed container at room temperature for future use. A

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voucher specimen was deposited in the Herbarium of Department of Pharmacognosy, The Oxford College of Pharmacy, Bengaluru.

Preparation of extracts

The dried rhizomes were coarsely powdered and subjected to successive extraction by soxhlation until the solvent in the side siphon tube is colorless. The extraction was done with different solvents in their increasing order of polarity such as petroleum ether (PE), benzene (BZ), chloroform (CH), methanol (ME) and distilled water (AQ). Each time the marc was dried and later extracted with other solvents. All the extracts were concentrated by rotary vacuum evaporator and evaporated to dryness. The yield was found to be 2.176, 0.831, 0.861, 6.06 and 5.41% w/w, respectively with reference to the air dried plant material.

Evaluation of antioxidant activity

DPPH radical scavenging activity

The free radical scavenging activity of different extracts of rhizomes of *H. spicatum* were measured by 1, 1-diphenyl-2-picrylhydrazyl (DPPH)[13]. Briefly, 0.1 mM solution of DPPH in ethanol was prepared. The solution (1 mL) was added to 3 mL of different extracts in methanol at different concentration (100–1.56 µg/mL). The mixture was shaken vigorously and allowed to stand at room temperature for 30 min. Then the absorbance was measured at 517 nm by using a spectrophotometer (UV-VIS Shimadzu). Reference standard compound being used was gallic acid and experiment was done in triplicate. The IC_{50} value is the concentration of sample required to inhibit 50% of the DPPH free radical. The IC_{50} value for the sample was calculated using log-dose inhibition curve. Lower absorbance of the reaction mixture indicated higher free radical activity. The percent DPPH scavenging effect was calculated using the following equation:

$$\text{DPPH scavenging effect (\%)} = 100 \times A_1/A_0$$

Where A_0 was the absorbance of the control reaction and A_1 was the absorbance in presence of the standards or samples.

Reducing power determination

The reducing power of different extract was assayed by Oyaizu[14]. Different concentrations of the extracts (2.5 – 25 µg/mL) were mixed with 2.5 mL phosphate buffer (0.2 M phosphate buffer; *p*H 6.6) and 2.5 ml of 1% potassium ferricyanide solution and incubated at 50°C for 20 min. The above solution were cooled; mixed with 2.5 mL of 10 % trichloroacetic acid and the content were centrifuged at 1000 rpm for 10 min. 2.5 mL of supernatant was mixed with 2.5 ml of distilled water and 0.5 mL of 0.1% ferric chloride solution and allowed to stand for 10 min and absorbance was measured at 700 nm. Higher the absorbance, higher is the reducing power. Ascorbic acid was used as reference standard.

Estimation of total phenol (TP) content

The total phenol content was determined by Folin-Ciocalteu assay[15]. Different dilution of extracts (25–500 µg/mL) was made up to 3.5 mL, then 0.5 mL of Folin-Ciocalteu reagent followed by 2 mL of 7.5% sodium carbonate solution was added. The above solution is incubated at room temperature for 10 min and absorbance was measured at 650 nm. Total phenolic content is expressed as gallic acid equivalent (mg/g) of the dried weight.

Estimation of total flavonoid (TF) content

The total flavonoid content in the extracts was determined by method modified by Zhishen's method[16]. Different extracts of extract in methanol (3 mL; 25–500 µg/mL) was mixed with 0.1 mL of 10% aluminum chloride followed by 0.1 mL of 1M potassium acetate solution. Added 2.8 mL of water and kept for incubation at room temperature for 30 min. The absorbance was measured at 415 nm. The total flavonoid content is expressed as rutin equivalent (mg/g) of the dried weight.

Result and Discussion

The model for scavenging the stable DPPH radical is widely applied to evaluate antioxidant activities in a relatively short time as compared to other methods. The effect of antioxidants on DPPH radical scavenging was thought to be due to their hydrogen donating ability. DPPH is a stable free radical and accept an electron or hydrogen radical to become a stable diamagnetic molecule and therefore, inhibit the propagation phase of lipid peroxide[17,18].

The mechanism of action of DPPH is as follows:
$\rightarrow DPPH^{\cdot} + R\text{-}H \rightarrow DPPH\text{-}H + R^{\cdot}$

The odd electron in the DPPH free radical gives a strong absorption maximum at 517 nm and is purple in colour. The colour turns from purple to yellow when the odd electron of DPPH radical becomes paired with hydrogen from a free radical scavenging antioxidant to form the reduced DPPH-H. The resulting decolorization is stoichiometric with respect to number of electrons captured. The reduction capability

of DPPH radicals was determined by decrease in its absorbance at 517 nm induced by antioxidants. Hence, DPPH is usually used as a substrate to evaluate antioxidative activity of antioxidants.

Table 1 illustrates the percentage inhibition of DPPH radical by standard and different extracts. The IC_{50} value was found to be 0.43, 259.1, 386.7, 414.3, 77.01 and 51.54 µg/mL for gallic acid, petroleum ether, benzene, chloroform, methanol and aqueous extracts of rhizomes of *H. spicatum*, respectively.

Reducing power assay measures the electron-donating capacity of an antioxidant. The reducing properties are generally associated with the presence of reductones, which have been shown to exhibit antioxidant action by breaking the chain reactions by donating a hydrogen atom. Reductones are also reported to react with certain precursors of peroxide, thus preventing peroxide formation[19]. Being good electron donors, phenolic compounds show the reducing power and have ability to convert the ferric ion Fe^{3+} to ferrous ion Fe^{2+} by donating an electron[20]. Increasing absorbance at 700 nm indicates an increase in reductive ability.

Table 2 illustrate the absorbance of standard and different extracts. The absorbance for reducing power at the concentration of 15µg/mL was found to be 0.540, 0.036, 0.042, 0.046, 0.050 and 0.072 for ascorbic acid, petroleum ether, benzene, chloroform, methanol and aqueous extracts of rhizomes of *H. spicatum*, respectively. The ability to reduce Fe (III) may be attributed to hydrogen donation from phenol compounds which are also related to the presence of reductant agent. Figure 1 shows the reducing potential of different extracts in the concentration range of 2.5–25 µg/mL.

The standard graph for gallic acid is represented in Figure 2. Total phenol content (Table 3) was found to be 13, 33, 24, 32 and 35 mg equivalent to gallic acid per gram of petroleum ether, benzene, chloroform, methanol and aqueous extracts of rhizomes of *H. spicatum*, respectively. The standard graph for rutin is shown in Figure 3. Total flavonoid content (Table 3) was found to be 4.6, 7, 7.5, 34.5 and 40 mg equivalent to rutin per gram of petroleum ether, benzene, chloroform, methanol and aqueous extracts of rhizomes of *H. spicatum*, respectively.

Significant correlations were observed between DPPH radical scavenging and reducing power, total phenol and total flavonoid. Many supportive reports

Table 1−DPPH free radical scavenging activity of standard and extracts of *H. spicatum*

Extract/standard	IC_{50} value (µg/mL) (mean ± SEM)
Gallic acid	0.43 ± 0.102[a]
Petroleum ether extract (PE)	259.1 ± 0.156
Benzene extract (BZ)	386.7 ± 0.212
Chloroform extract (CH)	414.3 ± 0.301
Methanol extract (ME)	77.01 ± 0.112[a]
Aqueous extract (AQ)	51.54 ± 0.175[a]

[a] $P<0.001$ when compared to control

Table 2−Absorbance of reducing power of standard and extracts of *H. spicatum*

Extract/standard (15 µg/mL)	Absorbance (mean ± SEM)
Ascorbic acid	0.540 ± 0.002
Petroleum ether extract (PE)	0.036 ± 0.013
Benzene extract (BZ)	0.042 ± 0.020
Chloroform extract (CH)	0.046 ± 0.031
Methanol extract (ME)	0.050 ± 0.012[a]
Aqueous extract (AQ)	0.072 ± 0.015[a]

[a] $P<0.001$ when compared to control

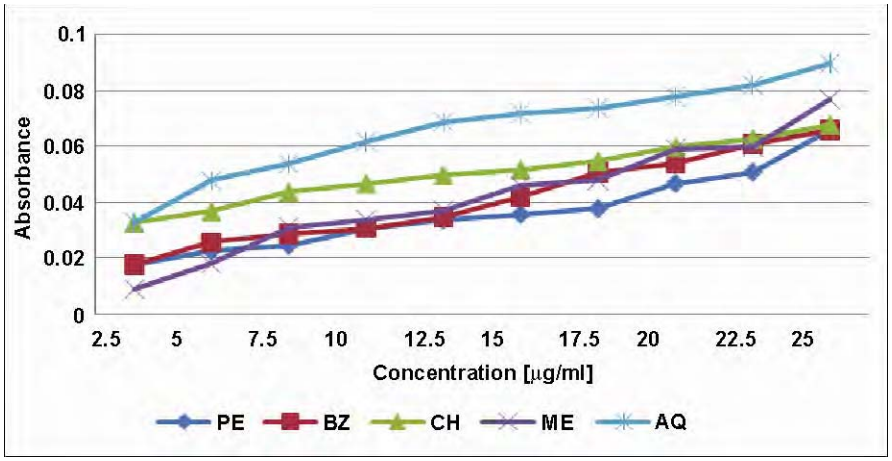

Fig. 1— Reducing potential of different extract of *H. spicatum*

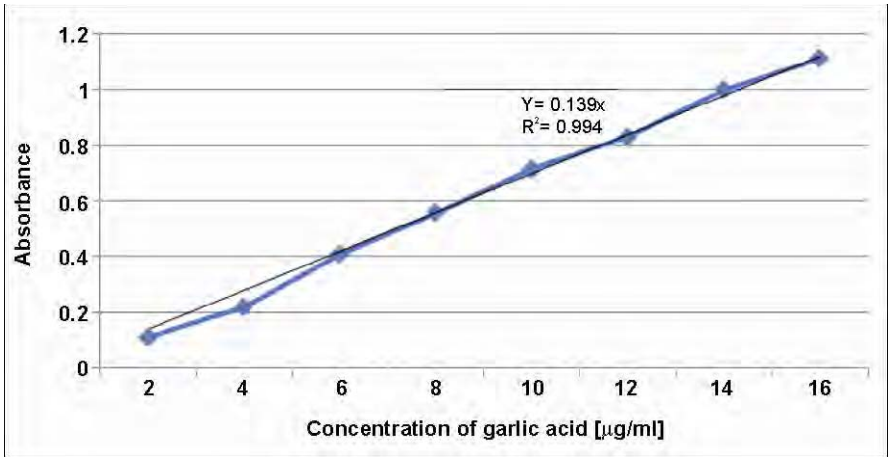

Fig. 2 — Standard graph of gallic acid

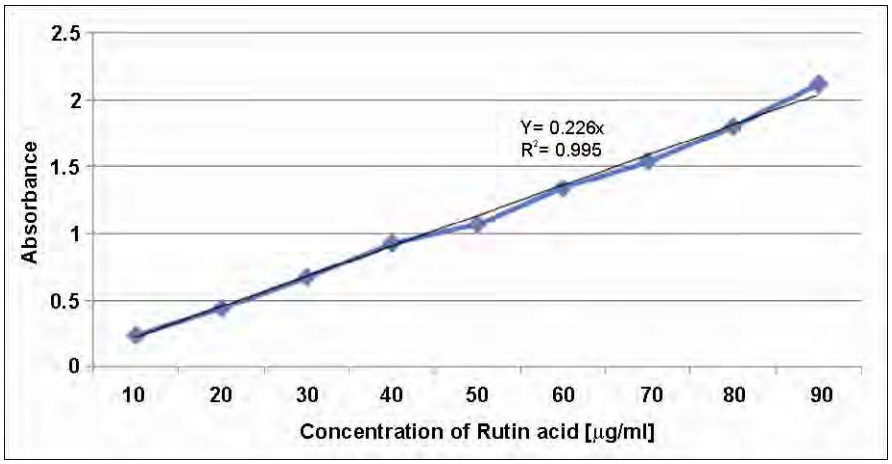

Fig. 3 — Standard graph for rutin

Table 3: Total phenol and flavonoid content of different extracts of *H. spicatum*

Extract	mg of gallic acid/g of extract (mean ± SEM)	mg of rutin/g of extract (mean) ± SEM
Petroleum ether extract (PE)	13 ± 0.010	04.6 ± 0.030
Benzene extract (BZ)	33 ± 0.015	07.0 ± 0.025
Chloroform extract (CH)	24 ± 0.019	07.5 ± 0.016
Methanol extract (ME)	32 ± 0.018	34.5 ± 0.023
Aqueous extract (AQ)	35 ± 0.301	40.0 ± 0.029

have emphasized the positive correlation between phenol content and antioxidant efficacy[21,22]. A positive correlation between antioxidant activity and polyphenol content was found, suggesting that the antioxidant capacity of the plant extracts is due the presence of their polyphenols[23,24].

Conclusion

The strongest antioxidant activity of the methanol and aqueous extracts could be due to the presence of flavonoids and phenols. The components responsible for the antioxidant activity of the extracts are unknown. Therefore, further research is needed for the isolation and identification of the antioxidant components in the extracts.

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References

1 Anonymous, The Wealth of India: A Dictionary of Indian Raw Materials and Industrial Products, Publications and Information Directorate, CSIR, New Delhi, Vol. V, 2004 (Reprint), 13-14.

2 Dixit VK and Varma KC, Effect of essential oils of rhizomes of *Hedychium spicatum* on central nervous system, *Indian J Pharmacol*, 1979, **11** (2), 147-149.
3 Rastogi RP and Mehrotra BN, Compendium of Indian Medicinal Plants, (Central Drug Research Institute, Lucknow and National Institute of Science Communication, New Delhi), 1980, 369-370.
4 Tandon SK, Chandra S, Gupta S and Lal J, Analgesic and anti-inflammatory effects of *Hedychium spicatum*, *Indian J Pharma Sci*, 1997, **59** (3), 148-150.
5 Bishit GS, Awasthi AK and Dhole TN, Antimicrobial activity of *Hedychium spicatum*, *Fitoterpia*, 2006, **77** (33), 240-242.
6 Joshi S, Chanotiva CS, Agarwal G and Prakash O, Terpenoid compositions and antioxidant and antimicrobial properties of the rhizome essential oils of *Hedychium spicatum*, *Chem Biodivers*, 2008, **5** (2), 299-309.
7 Aqil F and Ahmad I, Broad spectrum antibacterial and antifungal properties of certain traditionally used Indian medicinal plants, *World I Micro and Biotech*, 2003, **19**(6), 653-657.
8 Jadhav V, Kore A and Kadam VJ, *In vitro* Pediculicidal activity of *Hedychium spicatum* essential oil, *Fitoterapia*, 2007, **78** (7-8), 470-473.
9 Reddy PP, Rao RR, Shashidhar J and Sastry BS, Phytochemical investigation of labdane diterpenes from the rhizomes of *Hedychium spicatum* and their cytotoxic activity, *Bio Org and Med Chem Let*, 2009, **19** (21), 6078-81.
10 Sravani T and Paarakh PM, Evaluation of anthelmintic activity of *Hedychium spicatum* Buch., *Int J Res Pharm Sci*, 2011, **2**(1), 66-68.
11 Sravani T and Paarakh PM, *Hedychium spicatum* Buch. Ham.- an overview, *Pharmacologyonline Newsletter*, 2011, **2**, 633-642.
12 Halliwell B, Free radicals, antioxidants and human disease: curiosity, cause or consequence, *Lancet*, 1994, **344**, 721-724.
13 Shimoda K, Fujikawa K, Yahara K and Nakamura T, Antioxidative properties of xanthan on the autooxidation of soybean oil in cyclodexin emulsion, *J Agric Food Chem*, 1992, **40**, 945-948.
14 Oyaizu M, Studies on products of browning reactions: antioxidant activities of products of browning reaction prepared from glucose amine, *Japan J Nutr*, 1986, **44**, 307-315.
15 Singleton VL, Orthofer R and Lamuela RM, Analysis of total phenol and oxidation substrates and antioxidants by means of Folin- ciocalteau reagent, *Methods Enzymol*, 1999, **299**, 152-77.
16 Zhishen J, Mengcheng T and Jianming W, The determination of flavonoid contents in mulberry and their scanvenging effect on superoxide radicals, *Food Chem*, 1999, **64**, 555-559.
17 Havsteen B, Flavonoids, a class of natural products of high pharmacological potency, *Biochem Pharmacol*, 1983, **32**, 1141-1148.
18 Soares JR, Dins TCP, Cunha AP and Almeida LM, Antioxidant activities of some extract of *Thymus zygis*, *Free Radical Res*, 1997, **26**, 469-478.
19 Matsushige K, Basnet P, Kadota S and Namba T, Potent free radical scavenging activity of dicaffeoyl quinic acid derivatives from propolis, *Trad Med*, 1996, **13** (13), 217-228.
20 Shon MY, Choi SD, Kahng GG, Nam SH and Sung NJ, Antimutagenic, antioxidant and free radical scavenging activity of ethyl acetate extracts from white, yellow and red onions, *Food Chem Toxic*, 2004, **42**, 659-666.
21 Kukic J, Petrovic S and Niketic M, Antioxidant activity of four endemic *Stachys taxa*, *Biol Pharmac Bull*, 2006, **29** (4), 725-729.
22 Canadanovic J, Cetkovic G, Djilas S, Tumbas V, Bogdanovic G, Mandic A, Markov S, Cvetkovic S and Canadanovic V, Radical scavenging, antibacterial and anti-proliferative activities of *Melissa officinalis* L. extracts, *J Med Food*, 2008, **11** (1), 133-143.
23 Ng TB, Liu F and Wang ZT, Antioxidative activity of natural products from plants, *Life Sci*, 2000, **66**, 709-723.
24 Kiselova Y, Ivanova D, Chervenkov T, Gerova D, Galunska B and Yankova T, Correlation between *in vitro* antioxidant activity and polyphenol content of aqueous extracts from Bulgarian herbs, *Phythother Res*, 2006, **11**, 961-965.