



In vitro* antioxidant and anthelmintic properties of rhizome extracts of *Hedychium spicatum

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Hedychium spicatum (Zingiberaceae) is an ayurvedic traditional medicinal plant known as Spiked Ginger Lily (English) (Anonymous 1994). It is a perennial rhizomatous herb, commonly found in subtropical Himalayan region. The plant is about 1 m tall with fleshy aromatic rhizomes and elongated stem with broadly lanceolate leaves and has different developmental stages of flowering and fruiting (Kirtikar and Basu 1999). The rhizome of the plant contains medicinal properties like antioxidant, antimicrobial, anthelmintic, antidiabetic, hepatoprotective, anti-inflammatory, antiemetic, antidiarrhoeal, analgesic, expectorant, antiasthmatic, emmenagogue, hypoglycaemic, hypotensive, insect repellent, antifungal, pediculicidal and cytotoxic activities (Asolkar *et al.* 1992).

Chemicals: DPPH, ABTS, rutin, gallic acid, ascorbic, trolox and pyrogallol were purchased from Himedia Chemicals and Loba Chemical Pvt. Ltd., Mumbai. The entire chemical and reagents used were of analytical grades.

Plant materials: Rhizome of *Hedychium spicatum* was collected and identified from Medicinal Plant Research Development Centre (MRDC), G. B. Pant University of Agriculture and Technology, Pantnagar. The rhizome were dried in shade and cut into small pieces and powdered and kept in close container for further uses.

Preparation of extracts: The coarsely powdered rhizome was subjected to extraction by using Soxhlet apparatus for extraction in different organic solvent, viz. methanol, ethanol, hydromethanol (1:1), hydroethanol (1:1) and aqueous extract. All the extracts were concentrated by using rotary vacuum evaporator and evaporated to dryness and kept at -20°C.

Determination of antioxidant property

DPPH radical scavenging assay: The 1, 1-diphenyl 1-

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2-picrylhydrazyl (α , α -diphenyl- β -picrylhydrazyl) (DPPH) free radical scavenging capacity of the extracts were determined as per Amarowicz *et al.* (2002) with minor modification.

$$\% \text{ inhibition} = \frac{\text{Absorbance}_{\text{control}} - \text{Absorbance}_{\text{sample}}}{\text{Absorbance}_{\text{control}}} \times 100$$

ABTS radical scavenging assay: ABTS, 2, 2'-azinobis (3-ethylbenzothiazoline-6-sulfonic acid) scavenging assay was performed by the method of Re *et al.* (1999).

$$\% \text{ of inhibition ABTS}^{\cdot-} \text{ of radical} = \frac{\text{Ab}_{\text{control}} - \text{Ab}_{\text{sample}}}{\text{Ab}_{\text{control}}} \times 100$$

Nitric oxide radical scavenging assay: Nitric oxide generated from sodium nitroprusside in aqueous solution at physiological pH interacts with oxygen to produce nitrite ions which are measured by Griess reaction (Green *et al.* 1982, Marcocci *et al.* 1994).

$$\% \text{ nitric oxide scavenging activity} = \frac{A_{546\text{nm}} \text{ sample}}{A_{546\text{nm}} \text{ control}} \times 100$$

Quantitative phytochemical analysis of extracts

Determination of total phenolic content: The concentration of phenolics in plant extracts was determined using spectrophotometric method (Singleton *et al.* 1999) with slight modification. The phenolics contents were expressed in terms of gallic acid equivalent (mg of GA/g of extract).

Determination of total flavonoids content: Total flavonoid content was estimated by Sakanaka *et al.* (2005) method with minor change. The mean of the absorbance were expressed as rutin equivalents in mg/g of extract.

Collection of *Hemonchus contortus* worms: *Hemonchus contortus* worms were procured from the abomasia of goat. The live and motile worms were selected and cleaned with normal saline solution. The live and cleaned worms were transferred in beaker containing lock's solution at 37°C (Bhatnagar *et al.* 1961). The authentication of the parasites was done at the Department of Veterinary Parasitology, College of Veterinary and Animal Sciences, G. B. Pant University of Agriculture and Technology, Pantnagar.

Dilution of extracts: All the extract, viz. methanolic, ethanolic, hydromethanolic, hydroethanolic and aqueous of *Hedychium spicatum* at different concentrations of each viz. 2% (20 mg/ml), 4% (40 mg/ml), 6% (60 mg/ml) and 8% (80 mg/ml) were prepared in lock's solution for evaluation of their anthelmintic activity. Thiabendazole (TBZ) was used as positive control. Stock solution A of thiabendazole was prepared by dissolving 100 mg of pure thiabendazole in 5 ml of dimethyl sulfoxide (DMSO) having 20 mg TBZ/ml (2%). Different concentrations were made from this stock solution having 2%, 4% and 6% TBZ in lock's solution.

Observation for estimation of anthelmintic efficacy: Adult *H. contortus* (10) were taken in each small petridishes having different dilutions of test extract in lock's solution, viz. 2% (20 mg/ml), 4% (40 mg/ml), 6% (60 mg/ml) and 8% (80 mg/ml). Total volume in each petridishes was kept at 15 ml. It was kept at 37°C±1°C for 12 h and number of live and dead adult worms were counted at 2, 4, 6 and 12 h interval. The minimum lethal time for all the ten worms in each extract was recorded. Each trial was conducted in triplicate. The viability of the worms was determined by pinch technique (the absence of motility for an observation period of 5–6 sec) as described by Neogi *et al.* (1961) and Egualé and Giday (2009). Corrected mortality was estimated as per the formula given by Sangwan and Sangwan (1998).

$$\% \text{ corrected mortality} = \frac{\text{Total mortality} - \text{Control mortality}}{\text{Total mortality}} \times 100$$

Extraction of plant extracts: The extraction percentage and physical properties of methanolic, ethanolic, hydromethanolic, hydroethanolic and aqueous rhizome extracts of *Hedychium spicatum* are given in Table 1.

Quantitative phytochemical analysis: Phytochemical constituents like total phenolic and total flavonoids contents in different extracts of the rhizome of *Hedychium spicatum* plants are presented in Table 2. The total phenolics and flavonoids contents were significantly higher (P<0.05) in methanolic extract of rhizome of *Hedychium spicatum* than the other extracts (Saravani and Paarakh 2012).

In vitro antioxidant potential of plant extracts: The ABTS, DPPH and nitric oxide free radical scavenging activities were determined to assess the *in-vitro* antioxidant

Table 1. Extraction percentage and physical properties of different extracts of powdered rhizome of *Hedychium spicatum*

Property	Rhizome extract of <i>Hedychium spicatum</i>				
	Methanolic	Ethanolic	Hydro-methanolic	Hydro-ethanolic	Aqueous
Extract ability (%)	4.94	4.60	8.28	6.54	2.34
Colour	Dark brown	Dark brown	Brown brown	Brown	Yellowish
Consistency	Semi-solid	Semi-solid	Semi-solid	Semi-solid	Semi-solid

Table 2. Quantitative phytochemical analysis of major phytochemical constituents present in different extracts of *Hedychium spicatum*

Parameter	Rhizome extract of <i>Hedychium spicatum</i>				
	Methanolic	Ethanolic	Hydro-methanolic	Hydro-ethanolic	Aqueous
Phenolic content (mg of GAE/g extract)	60.01±1.46	55.70±5.8	22.62±1.01	18.30±0.99	9.97±0.35
Total flavonoids contents (mg rutin/g extract)	538.70±36.63	532.74±18.74	120.51±13.56	93.10±33.10	20.46±3.83

Values are expressed as mean±SE of 3 replicates; GAE, Gallic acid equivalent.

potential of plant extracts. The median effective concentration (EC₅₀) of different extracts of rhizome of *Hedychium spicatum* in scavenging different types of oxidant radicals was determined by using straight line equation between different concentrations of extracts and per cent scavenging of free radicals generated in the medium (Table 3).

ABTS radical scavenging assay: ABTS, 2, 2'-azinobis (3-ethylbenzothiazoline-6-sulfonic acid) scavenging assay of different extracts of *Hedychium spicatum* was determined. ABTS on reacting with potassium persulphate produces ABTS radical cation (ABTS⁺), a blue green chromogen. The effects of antioxidants on ABTS⁺ radical cation is due to its hydrogen donating ability which is visually observed by a change in colour of ABTS⁺ cation to colourless ABTS. The extent of decolourisation is an indicator of antioxidant activity present in the extracts. Per cent scavenging activity of different concentration of ascorbic acid and trolox (standard) and different plants extracts was calculated from per cent ABTS scavenging activity versus concentration. Methanolic extract was found to be more potent in scavenging the ABTS⁺ radical cation as compared to other extracts with least activity in aqueous extract. The calculated EC₅₀ values for different extracts are presented in Table 3. As per the ABTS scavenging capacity, the methanolic extract was most potent whereas aqueous extracts showed least antioxidant capacity (Sravani and Paarakh 2012).

DPPH radical scavenging assay: The molecule of 1, 1-diphenyl 1–2-picrylhydrazyl (a, a-diphenyl-b-picrylhydrazyl) (DPPH) is relatively stable nitrogen centered free radical in methanol induces purple colour in a solution with absorbance 517 nm wavelength. The effect of antioxidant on DPPH radical scavenging was due to donation of hydrogen atom that binds with the unpaired electron of DPPH radical and gives yellow colour (Blois 2001). The reducing capacity of DPPH radicals was determined by decrease in its absorbance at 517 nm induced

Table 3. Median effective concentration (EC₅₀) of different extracts of *Hedychium spicatum* in scavenging different type of radicals in the medium

Scavenging assay	Median effective concentration (EC ₅₀) (mg/ml)							
	Gallic Acid	Trolox (Std)	Ascorbic acid (Std)	Methanolic	Ethanollic	Hydromethanolic	Hydroethanollic	Aqueous
DPPH radicals	-	-	0.049	8.31	9.99	24.98	49.85	499.69
ABTS radicals	-	0.050	1.63	24.93	49.33	50.02	50.03	50.07
NO· radicals	0.015		0.10	3.57	12.5	12.5	25	-

Table 4. Anthelmintic activity of different extracts of rhizomes of *Hedychium spicatum*

Groups	Treatments	Concentration	Time taken for paralysis (min)	Time taken for death (min)	% corrected mortality
1.	Control	0	0	0	0
2.	Positive control (Thiabendazole)	2% (20mg/ml)	122.63±1.45	186.49±2.45	100
		4% (40mg/ml)	113.45±1.67	164.33±2.45	100
		6% (60mg/ml)	86.21±1.43	154.98±4.12	100
3.	Methanolic extract	2% (20mg/ml)	112.26±1.34	191.43±3.22	100
		4% (40mg/ml)	98.58±1.21	171.89±2.45	100
		6% (60mg/ml)	89.76±1.32	156.67±5.33	100
		8% (80mg/ml)	82.31±1.10	147.12±4.32	100
4.	Ethanollic extract	2% (20mg/ml)	123.22±1.21	205.64±4.67	100
		4% (40mg/ml)	111.43±2.12	194.67±3.61	100
		6% (60mg/ml)	96.35±2.09	181.47±4.11	100
		8% (80mg/ml)	87.83±1.11	166.77±3.31	100
5.	Hydromethanolic extract	2% (20mg/ml)	131.34±1.56	226.45±3.48	100
		4% (40mg/ml)	121.44±1.42	211.45±2.11	100
		6% (60mg/ml)	111.78±1.28	195.73±3.14	100
		8% (80mg/ml)	92.19±1.23	174.66±3.29	100
6.	Hydroethanollic extract	2% (20mg/ml)	134.67±1.31	235.74±3.23	100
		4% (40mg/ml)	123.78±1.35	221.29±4.31	100
		6% (60mg/ml)	118.34±1.61	198.79±3.34	100
		8% (80mg/ml)	96.56±1.73	183.91±2.23	100
7.	Aqueous extract	2% (20mg/ml)	0	0	0
		4% (40mg/ml)	0	0	0
		6% (60mg/ml)	201.94±3.54	253.33±5.62	50
		8% (80mg/ml)	195.45±4.32	263.87±4.32	70

by the antioxidant. Per cent scavenging activity of different concentration of ascorbic acid (standard) and plants extracts was calculated from per cent DPPH scavenging activity versus concentration curve. The calculated EC₅₀ values of ascorbic acids and different extracts are depicted in Table 3. Methanolic extract showed least EC₅₀ value indicating its potent antioxidant capacity in comparison to other extracts (Sravani and Paarakh 2012, Rawat *et al.* 2011). Lower the EC₅₀ value, higher will be the antioxidant potential which increases after increasing the dose (Blois, 2001).

Nitric oxide scavenging activity: The NO· radicals have many essential physiological roles but their excessive production produces harmful effects. It is a diffusible free radical that plays important role in biological systems including neuronal messenger, vasodilatation, antimicrobial and antitumour activities (Hagerman *et al.* 1998). Thus, the

regulation of NO radical production level in biological tissue is important for avoiding harmful effect produced by excessive NO radicals (Beasley *et al.* 1991). Thus, the NO· radicals scavenging activity of the extracts of *Hedychium spicatum* was observed in this study which was concentration dependent. The extracts of *Hedychium spicatum* thus might have the property to counteract the formation of NO radical and in turn be of considerable interest in preventing the harmful effect of excessive NO generation in the body (Sravani and Paarakh 2012, Rawat *et al.* 2011).

Finally, a significant direct correlation was observed between the DPPH, ABTS, NO radicals scavenging activity and polyphenols contents of the extract i.e. the higher is the polyphenols contents, higher will be the antioxidant capacity. Thus, the methanolic extract of rhizome of *Hedychium spicatum* had maximum polyphenols like total

phenols and total flavonoid contents; therefore, it showed maximum antioxidant capacity (Kiselova *et al.* 2006).

Anthelmintic efficacy: Methanolic extracts of *Hedychium spicatum* were as effective as the positive control treated with thiabendazole at 2%, 4% and 6% concentrations (Table 4). The methanolic extracts showed that the time of paralysis and time of death were similar to the positive control at 2%, 4%, 6% and 8% concentrations. Hydroethanolic and hydromethanolic extracts took more time for paralysis and death. Aqueous extract at 2% and 4% did not show any mortality but higher concentrations of 6% and 8% showed 50% and 70% corrected mortality, respectively (Sravani and Paarakh 2011, Goswami *et al.* 2011). Although, organic solvents have sufficient amount of diterpenes, flavonoids, polyphenols and other phytoconstituent which might be responsible for the anthelmintic property (Sravani and Paarakh 2014).

SUMMARY

In the present study, different extracts of *Hedychium spicatum* were evaluated for *in vitro* antioxidant property by ABTS, DPPH and nitric oxide free radical scavenging assay. The methanolic extracts showed IC₅₀ minimum as compared to other extracts. Total flavonoid and phenolic contents were determined and methanolic extracts showed the maximum contents of flavonoids (38.70±36.63 mg of rutin equivalent/g of extract) and phenolic (60.01±1.46 mg of gallic acid equivalent/g of extracts), respectively. The *in vitro* anthelmintic efficacy of methanolic extract @ 2%, 4%, 6% and 8% concentrations against the *Hemonchus contortus* was found maximum as compared to other extracts and standard drug thiabendazole. The time of paralysis and time of death of the parasite were recorded and was found minimum for methanolic extract against *Hemonchus contortus* than the other extracts. Thus, the extracts of the *Hedychium spicatum* may be used for therapeutic purposes in animal and human health.

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