ISSN (O): 2581-8740 | ISSN (P): 2581-8732
Kumar *et al.*, 2021
DOI: 10.21276/SSR-IIJLS.2021.7.6.1

Research Article

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Evaluation of Antioxidant Potential of *Hedychium spicatum* Rhizome Extracts from Bhowali Region, Uttarakhand, India

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Received: 16 Jun 2021/ Revised: 13 Aug 2021/ Accepted: 23 Oct 2021

ABSTRACT

Background: It is well-known that different extraction methods, including the technique, solvents, time and temperature extensively influence the antioxidant activity of plant secondary metabolites. In our study, *Hedychium spicatum* was used as a plant sample, collected from Bhowali region, Uttarakhand, India, using two extracting solvents (acetone and chloroform) to explore the antioxidant activity, total phenolic and flavonoid contents (TPC and TFC).

Methods: Initial phytochemical analysis was performed by evaluating the TPC and TFC content by Folin-Ciocalteu and $AlCl_3$ colorimetric assay. For the estimation of antioxidant activity of test samples, 2,2-diphenyl-1-picrylhydrazyl (DPPH) and ferric reducing antioxidant power (FRAP) methods were used for the determination of free radical scavenging activity, respectively.

Results: The FRAP results of acetone and chloroform samples was 335.782 and 254.116 µM $FeSO_4 \cdot 7H_2O$/g of dry extract, respectively. IC_{50} values for acetone and chloroform extract were calculated and used to interpret DPPH radical scavenging activity. Both tested extracts exhibited potent DPPH radical scavenging activity having IC_{50}, 113.11 and 294.23 µg/ml for acetone and chloroform extract, respectively. The result of TPC (12.82 mg equivalent to gallic acid) and TFC (13.998 mg equivalent to quercetin/gram) of dry extract respectively.

Conclusions: The overall results exhibit the high antioxidant potentiality of acetone extract as compared to chloroform extract, which could be due to its high phenolic and flavonoid content presence.

Key-words: Antioxidant potential, free radical scavenging activity, *Hedychium spicatum*, Medicinal plants, Plant extract, IC50

INTRODUCTION

In modern time, attention has increased to find natural antioxidants as an alternative option for medicines, cosmetic and foods items, as a substitute for synthetic antioxidant compounds to minimize the possibility of toxicity [1]. Medicinal plants have been investigated as potential sources of natural anti-oxidants secondary metabolites and other activities like anti-cancer, antibacterial, anti-mutagenic [2-4].

How to cite this article
Kumar M, Pratap V, et al. Evaluation of Antioxidant Potential of *Hedychium spicatum* Rhizome Extracts from Bhowali Region, Uttarakhand, India. SSR Inst. Int. J. Life Sci., 2021; 7(6): 2901-2907.

Access this article online
https://iijls.com/

Phytochemicals containing phenols are present in plants which help in protecting them from ultra-violet rays, grass-eating animals and other different forms of biotic/ abiotic factors [5]. The production of secondary metabolites by the plants are usually affected by different factors such as the strength of sunlight, altitude, elevated temperature, seasonal variation, different stress conditions including biotic and abiotic factors, rainfall, maturity at harvest [6].

H. spicatum is well-known as 'spiked ginger' Lilly or perfume ginger. It is associated with the family Zingiberaceae. *H. spicatum* is mainly found in Himalayan regions of India, and it's also natives to China, Myanmar, Thailand, and Ethiopia. It's a rhizomatous aromatic green herb with a hearty stew. The rhizome of *H. spicatum* is utilized in the prevention of several diseases in the

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mammalian health system, viz; rheumatoid arthritis/rheumatism, skin disease, inflammation, and vomiting. Additionally, rhizome extract of the *H. spicatum* is also used as antioxidants, anticancer, anthelmintic, antidiabetic, anti-inflammatory, analgesic, antiasthmatic, antifungal and other antimicrobial agents [4,7-15]. Some investigators also reported that extract of *H. spicatum* is used in traditional and modern medicine as well as cosmetic and perfumery industry.

The main objective of this study was to explore the antioxidant potential of *H. spicatum* rhizome extracts collected from Bhowali region, Uttarakhand, India. The extracts were prepared in two solvents, acetone and chloroform by maceration method to extract the possible phytochemicals or components in its more active form. The present study explored the strong antioxidant potential of the extracts to promote the traditional medicine.

MATERIALS AND METHODS

Chemicals and reagents- 2,2-diphenyl-1-picryl-hydrazyl (DPPH), $FeSO_4 \cdot 7H_2O$**,** sodium carbonate(Na_2CO_3), gallic acid, aluminium chloride ($AlCl_3$), potassium acetate, quercetin, 2,4,6- tripyridyl-s-triazine (TPTZ), Folin-Ciocalteu's reagent, HCl, ascorbic acid, Ferric chloride ($FeCl_3$), Sodium acetate (CH_3COONa), Glacial acetic acid, methanol, acetone, chloroform. The analytical grade chemicals were used.

Plant material collection- *H. spicatum* rhizomes were collected and confirmed from ICAR, NBPGR regional Station, Bhowali, Uttarakhand, India in the month of November 2017.

Plant extract preparation- The extraction process was done by following Singh *et al.* [16] method with some modifications. Briefly, the sample was washed twice with double distilled water and tween 80 (5%) to remove the impurities and was cut into small pieces, shade dried. Further, the dried sample was ground into coarsely powder and used for extraction with two solvents, acetone and chloroform (dried sample: solvent ratio was 1:10 w/v) using maceration method under shaking condition at 30°C and 120 rpm for 72 hour. The mouth of flasks were tightly plugged with non-absorbent cotton and tightly wrapped with aluminium foil to prevent evaporation. Finally, Whatman no.1 filter paper was used to filter both the extracts. The resultant extracts were used for different assays and remaining samples were kept at -20°C for further experiments.

Quantitative Phytochemical analysis

Assay of Total Phenolic Content (TPC)- Total phenolic content in *H. spicatum* extracts prepared in acetone and chloroform solvents was assayed by following Folin-Ciocalteu's colorimetric method with minor modifications [16]. Fifty micro liters of each *H. spicatum* extract was taken and 400 µl of double distilled water was added with 50 µl of Folin-Ciocalteu's reagent to each sample extract and incubated for 5 minutes at room temperature. Then, 500 µl of 7% (w/v) sodium carbonate was added to all sample extracts to neutralize the mixture and kept for 30 minutes at room temperature in dark conditions. After incubation, the blue colour appeared in the solution and the absorbance of each solution was measured at 765 nm using a UV-Visible spectrophotometer (Thermo Scientific-Evolution 201). TPC was quantified by using gallic acid standard curve and results were expressed in 'mg gallic acid equivalent (GAE) /g of dry extract'.

Assay of Total Flavonoid Content (TFC)- Total flavonoid content in both the extracts of *H. spicatum* was quantified following aluminium chloride ($AlCl_3$) colorimetric method with some modifications [17]. Briefly, 50 µl of each sample extract was taken and diluted with 250 µl of distilled water. Then, 250 µl of 10% (w/v) $AlCl_3$, 50 µl of 1 M potassium acetate and 650 µl of distilled water were sequentially added to each diluted extract. Further, solutions were incubated at room temperature for 30 minutes. Thereafter, the absorbance of each solution was taken at 415nm using UV-VIS spectrophotometer. Quercetin was used for standard curve for the estimation of TFC in each extract and results were expressed in terms of 'mg quercetin equivalent (QE)/g of dry extract'.

Assay of Antioxidant activity- Antioxidant potential of *H. spicatum* was evaluated by ferric reducing antioxidant power (FRAP) and 2,2-diphenyl-1-picryl-hydrazyl (DPPH) assays.

FRAP Antioxidant assay- The ferric reducing antioxidant power (FRAP) of each sample extract was determined by following method of Cai *et al*. [18] with some modifications. The reductive potential of acetone and chloroform extracts of *H. spicatum* was assessed based

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on reducing ferric to ferrous ions (Fe^{3+} to Fe^{2+}). In Brief, 30 µl of each extract was taken followed by the addition of 70 µl of double-distilled (DDW) and 900 µl of freshly prepared pre-warmed (37°C) FRAP reagent. Further, solutions were kept at 37°C for 10 minutes and the optical density was recorded at 593 nm. Ferrous sulphate ($FeSO_4$) was used as standard. For measurement of control, acetate buffer, solution of TPTZ and ferric chloride were used in the ratio of 10:1:1 without the addition of test extracts. Results of the FRAP were expressed in µM $FeSO_4 \cdot 7H_2O$/g of the dry weight of the test extract.

DPPH Antioxidant assay- DPPH scavenging assay of both the extracts was performed using the procedure with minor modifications following Chrzczanowicz *et al.* [19]. Each extract was dissolved in its extracting solvents (acetone and chloroform). Briefly, for DPPH assay of each extract, the reaction mixture contained 30 µl of each test sample, 825 µl of methanol and 45 µl of 1 mM DPPH solution (prepared in methanol). Further, the mixture was kept in dark condition for 30 minutes of incubation. After that, the absorbance of solutions was recorded at 517 nm using spectrophotometer with respect to blank. For the positive control, ascorbic acid was used as standard. The DPPH scavenging activity of extracts was expressed in terms of percentage inhibition, which was calculated by using the following formula [16]:

% Inhibition= [(Absorbance of Control sample– Absorbance of Test sample)/Absorbance of Control] x 100

IC_{50} results of sample extracts and standard were calculated from graph (% inhibition) plotted against different concentrations by probit analysis using SPSS (version 22 for window).

Statistical Analysis- The overall experiments of antioxidant activity such as FRAP, DPPH scavenging activity as well as the evaluation of phytochemicals like total flavonoid and phenol contents were performed in triplicates. Results have been expressed as mean±standard error for each extract. Correlation coefficients (r) among TPC, TFC, DPPH scavenging activity and FRAP antioxidant activity of test extracts were determined by SPSS (version 22 for window).

RESULTS

Total flavonoid Content (TFC)- Total Flavonoid Content of acetone and chloroform extracts prepared from *H. spicatum* sample were expressed as quercetin equivalent (QE), which varied from 13.998±0.990 and 9.794±0.568 mg QE/g extract, respectively as represented in Table 1. TFC results emphasize the strong potential of acetone extract (mixture of polar and less polar solvent) to pull out the flavonoid as well as polyphenols from the cell wall.

Total Phenolic Content (TPC)- Total phenolic content of acetone and chloroform extract of *H. spicatum* was solvent dependent and represented in terms of mg GAE/g of dry extract. A significant difference was found in TPC values of tested acetone and chloroform extracts, 12.82±0.225, 9.262±0.9 mg gallic acid equivalents (mg GAE/gram of dry extract) respectively (Table 1). It is interpreted from the result of TPC, that acetone is the suitable solvent system for the extraction process of *H. spicatum* compared to solvent chloroform.

FRAP Antioxidant assay- The results of reducing potential of acetone and chloroform extracts of *H. spicatum* in terms of FRAP are mentioned in Table 1. FRAP value was found more for acetone extract (335.782±2.716 µM $FeSO_4.7H_2O$/g of dry extract) as compared to chloroform extract (254.116±1.367 µM $FeSO_4.7H_2O$/g of dry extract). Although, the reason for antioxidant activity may be due to several other processes such as peroxides decomposition, radical scavenging, reductive capacity, chain initiation prevention etc.

DPPH scavenging assay- The colour change of DPPH (violet to yellow) caused by scavenging activity of free DPPH radicals during the reaction is the basis of this assay. The results of this study (Table 1) showed the dose dependent pattern of test extracts (acetone and chloroform). The IC_{50} result of acetone was 113.11±0.409 µg/ml and chloroform was 294.23±0.841 µg/ml, respectively. The acetone extract showed more DPPH scavenging activity as compared to chloroform extract.

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Table 1: Total phenolic, flavonoid contents and antioxidant activity of acetone and chloroform extracts of *H. spicatum*

H. spicatum Extract	Total Phenolic (mg GAE/g of dry extract)	Total flavonoid (mg QE/g of dry extract)	FRAP activity (µM $FeSO4·7H_2O$/g of dry extract)	DPPH % scavenging activity (IC_{50}, µg/ml)
Acetone	12.82±0.225	13.998±0.990	335.782±2.716	69.043±0.41 (113.11±0.409)
Chloroform	9.262±0.9	9.794±0.568	254.116±1.367	62.28±0.84 (294.23±0.841)

Each values in the table represents the mean± SE (n=3)

Correlation Coefficient- The results of present investigation was also analyzed statistically in which a significant correlation was found between antioxidant activity and polyphenolic content (Table 2). It should also be noted that in the present study FRAP result was highly correlated with flavonoid of both extracts (acetone and chloroform) shows in Table 2.

Table 2: Correlation coefficients (r) between total phenolic, flavonoid contents and antioxidant activity of different extracts of *H. spicatum*

Parameter	TPC	TFC	FRAP	DPPH
		Acetone		
TPC	1			
TFC	0.989	1		
FRAP	-0.982	-.999*	1	
DPPH	0.056	-0.095	0.133	1
		Chloroform		
TPC	1			
TFC	0.986	1		
FRAP	-0.911	-0.967	1	
DPPH	-0.902	-0.816	0.644	1

***Correlation is significant at the 0.05 level (2-tailed)**

DISCUSSION

A large number of secondary metabolites specially polyphenolic compounds are produced by plants to cope up the various types of physical stress such as ultraviolet radiation as well as biotic stresses like pathogens [20-22]. Natural compounds, such as polyphenolic compounds have been received a great deal for many health benefits, including anti-inflammatory, antimicrobial and antioxidant activities in recent years [23-27]. Therefore, the present investigation aimed to explore the antioxidant potential of *H. spicatum* rhizome. The results of our study in terms of flavonoid and phenolic content of *H. spicatum* in total were found to be based on extracting solvents used (Table 1). It is well evident from earlier studies that the polyphenolic content of plants reflects the antioxidant potential [28,29].

The study from some researchers on different bioactive components relates the antioxidant potential in terms of ferric reducing antioxidant power (FRAP) and assayed by measuring the conversion of Fe^{3+} into Fe^{2+} by sample extracts [30]. Some studies also reported the antioxidant potential of rhizomes of *H. spicatum* methanol extracts and were related to the high content of phenolic compounds. The results of different studies on extracts/oil are variable for the *H. Spicatum* depending on sample collection of geographical location [10,11,31,32]. In our study, high phenolic content was found in acetone extract of *H. spicatum* sample and also showed remarkable ferric reducing ability. The reducing ability of a bioactive compound may indicate its potential to have the antioxidant capability. In the present study, the tested extracts of *H. Spicatum* showed powerful antioxidant DPPH radical scavenging activity having IC_{50} value of 113.11±0.409 and 294.23±0.841 µg/ml for

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acetone and chloroform extract, respectively, which was comparatively effective than IC_{50} (414.3 µg/ml) value for chloroform extract of *H. spicatum* by Sravani and Paarakh [33]. Tian *et al.*[34] results on *H. flavum* reported the high TPC and TFC (50.08–57.42 mg GAEs/g extract; 12.45–21.83 mg REs/g extract), respectively. Total phenolic content was 12.82±0.225 and 9.262±0.9 mg equivalent to gallic acid per gram of acetone and chloroform extract respectively. The results of this study explored the potential of *H. spicatum* extracts prepared in solvents acetone and chloroform having high antioxidant activity might be due to the high content of phenolic antioxidant compounds present in it.

CONCLUSIONS

In present study, the influence of different solvents (acetone and chloroform) on antioxidant activity and polyphenolic compounds of *H. spicatum* extracts was reported. Plant extracts showed significant content of phenolic and flavonoidal compounds. Moreover, our findings demonstrated that the tested extracts exhibited strong activity against DPPH and Fe+3 radicals. The present study represented a significant correlation between polyphenolic compounds and antioxidant activity, on the other hand, it is also examined that acetone extract has more antioxidant activity in both analysed assays as well as also has more contents of polyphenolic compounds.

It is now evident that this plant species has tremendous medicinal properties and is effective in the treatment of various health issues. *H. spicatum* could be a potential herbal source of antioxidants used for therapeutic purposes. However, further in vitro as well as in-vivo studies are needed to establish its antioxidant potential for human health care and wellbeing. As our results also showed high antioxidant activity, which can be used as a better antioxidant option in the management of oxidative stress and for the discovery of new lead compounds from it in the discovery of drugs in future prospects.

ACKNOWLEDGMENTS

Manish Kumar and Vinay Pratap are grateful to University Grant Commission, New Delhi for their research fellowship to pursue Ph.D course.

CONTRIBUTION OF AUTHORS

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Data analysis and interpretation- Dr. Manoj Kumar Singh, Manish Kumar
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