Antioxidant potentials of successive solvent extracts from the unexplored *Hedychium coronarium* rhizome

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Abstract *Hedychium coronarium* Koen. is considered as an endemic medicinal plant. In the current research antioxidant potential of six different extracts obtained by the successive solvent extraction method from the unexplored *H. coronarium* rhizome was evaluated. Various in vitro assays were performed to achieve the aim and variation in scavenging potential of the six extracts, in addition to quantification of phenolic and flavonoid compounds was done by HPLC. Among the six extracts, methanolic extract have showed highest Total phenolic content (TPC), metal chelation activity and free radical scavenging potential against DPPH, ABTS and superoxide anions as compared to other extracts. The highest scavenging potential against nitric oxide and hypochlorous acid was shown by acetone extract. The variation in anti-oxidant activity of the extracts may be due to the phyto-constituents present in different solvents. A significant correlation ($r^2 = 0.864$) was established between antioxidant and TPC of *H. coronarium* from principle component analysis. Thus the present study provides strong evidence that rhizome extract of *H. coronarium* is a potential source of bioactive

compounds and can be used as a remedy for diseases caused due to oxidative stress. Reported results could be helpful to develop novel drugs for the management of oxidative stress and associated diseases.

Keywords *Hedychium coronarium* · Methanolic extracts · Total phenol content · Free radicals · Antioxidant activity

Introduction

Free radicals are highly reactive atom or group of atoms having one or more unpaired electrons. The potentially reactive free radicals containing oxygen, known as reactive oxygen species (ROS) are continuously generated inside living organisms by different mechanisms (Liu and Huang 2015). ROS are produced internally as a normal part of metabolism within the mitochondria, whereas external factors such as smoking, environmental pollutants, radiation, drugs, pesticides, industrial solvents and ozone also stimulates the production of ROS as well as Reactive nitrogen species (RNS). In all organisms intracellular defense mechanisms are present to balance between the production and neutralization of free radicals and ROS. Imbalance between free radical production and antioxidant defenses leads to overproduction of ROS and the cells suffer the consequences of oxidative stress. The ROS-induced oxidative stress play a substantial role in the evolution of many ailments, including diabetes mellitus, cancer, hypertension, obesity, dyslipidemia etc. in addition to aging process (Hopps et al. 2010). To maintain normal physiological function of cells by overcoming oxidative stress, antioxidants are needed. An antioxidant is a substance which present at low concentrations, donates own electrons to ROS and neutralizes the adverse effects of the

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ROS (Pisoschi and Pop 2015). However, natural antioxidant mechanism can be inefficient, so dietary intake of antioxidants become important. In spite of the wide use of synthetic antioxidants in food processing, the natural antioxidants from plant resources have gained special interest. The antioxidant activity of plants is associated with the various phytochemicals present in the plant extracts such as phenols, flavonoids, carotenoids, anthocyanins, tannins and terpenes (Bharti et al. 2015) and the phenolic/flavonoid compounds attribute more to the biological activity of plant materials (Rice-Evans et al. 1996; Shahwar and Raza 2012).

The plant *Hedychium coronarium*, is a member of the Zingiberaceae family and commonly known as white ginger lily or Dolan champa, locally known as Gulbakawali. It is widely cultivated in Vietnam, India, and Southeast Asian countries. In Vietnam the rhizomes of *H. coronarium* have been used as a traditional medicine for the treatment of inflammation, skin diseases, headache, and sharp pain due to rheumatism (Kiem et al. 2011). Essential oil reported from rhizome of this species have been claimed to possess anti-inflammation, anti-microbial and mosquito larvicidal activity (Ho 2011). Recently, much attention has been paid to *Hedychium* species due to their diverse biological activities (Suresh et al. 2010) and ethno-pharmacological significance. Despite this, investigation related to the antioxidant activity is still not explored. In view of this background, the aim of the present study was to evaluate the antioxidant activity of *H. coronarium* rhizome extracts isolated from six different solvent extracts. Principle component analysis (PCA) was also done to find the significant correlation between phenolic content and potent free radical scavenging activity, which could combat effectively against oxidative stress and related diseases.

Materials and methods

Chemicals and reagents

Gallic acid, ascorbic acid, potassium ferricyanide (III), DPPH (2,2-diphenyl-1-picrylhydrazyl), ABTS (2,2-azino-bis(3-ethylbenzothiazoline)-6 sulphonic acid), TPTZ (2,4,6-tripyridyl-s-tiazine), catalase from bovine liver (966 U/mg solid) purchased from Sigma-Aldrich (USA). Hydrogen peroxide (30% v/v), TCA (Trichloroacetic acid), TBA (Thiobarbituric acid), NH_4Ac , $\text{Na}_2\text{HPO}_4 \cdot 2\text{H}_2\text{O}$, $\text{NaH}_2\text{PO}_4 \cdot 2\text{H}_2\text{O}$, Folin–Ciocalteu reagent, potassium per sulfate, sodium carbonate, deoxyribose, ferrozine, EDTA (ethylenediaminetetraacetic acid), sodium nitroprusside, sulphanilamide, NADH (Nicotinamide adenine dinucleotide) and all solvents were procured from Merck Co.

and Hi-Media Pvt. Ltd. All other reagents were of analytical grade.

Collection of plant material and sample preparation

Rhizomes of *H. coronarium* were collected from Indira Gandhi Krishi Vishwavidyalaya, Bilaspur, India. The plant was identified and authenticated by R.K.S Tiwary, Principal Scientist, Indira Gandhi Krishi Vishwavidyalaya, Bilaspur, C.G. The rhizomes of *H. coronarium* was cleaned under tap water followed by distilled water and dried in hot air oven at 40 °C. Dried samples were ground and stored at 4 °C until extraction. The extraction was carried out in a Soxhlet apparatus successively using solvents in increasing order of polarity hexane (HX), dichloro methane (DCM), ethyl acetate (EA), acetone (ACE), methanol (MET) and water (AQ). The extracts were concentrated under reduced pressure in a rotary evaporator and stored at 4 °C until analyzed. A stock solution of 10 mg/ml was used for the further experiments by dissolving dried extracts in DMSO.

Total phenolic content (TPC)

Total phenolic content was determined by the Folin–Ciocalteu method (Aadil et al. 2014) with slight modification. To 200 µl of crude sample, 10% Folin–Ciocalteu reagent (1.5 ml) was added. 5% Na_2CO_3 (1.5 ml) was added after 5 min and mixed well. Then the absorbance was taken at 750 nm after 2 h incubation in the dark. A standard curve with serial Gallic acid (ranging from 10 to 100 µg/ml) was used for calibration. The test was performed in triplicates and results were expressed as mg of gallic acid equivalent (GAE)/gram of extract.

Total flavonoid content (TFC)

The total flavonoid content in the plant extracts was determined using spectrophotometric method described by Siddhuraju and Becker (2003). To 0.5 ml of test samples 2 ml of distilled H_2O was added. Later 0.15 ml of AlCl_3 (10% w/v) was added and incubated for 6 min followed by the addition of 2 ml NaOH (4% w/v). After 15 min incubation absorbance was measured at 510 nm. A standard curve with serial quercetin (ranging from 10 to 100 µg/ml) was used for calibration. The test was performed in triplicates for each analysis and results were expressed as mg of quercetin equivalent (QE)/gram of extract.

Ferric reducing ability of plasma (FRAP)

The stock solutions, for FRAP assay included a 300 mM acetate buffer (3.1 g sodium acetate tri-hydrate in 16 ml glacial acetic acid, pH 3.6), 10 mM TPTZ (2,4,6-tripyridyl-

s-triazine) solution in 40 mM HCl and 20 mM anhydrous FeCl_3 solution (Aadil et al. 2014). The working solution was freshly prepared by mixing a 25 ml acetate buffer, 2.5 ml TPTZ solution, and 2.5 ml FeCl_3 solution followed by warming at 37 °C before use. 2.85 ml of the FRAP solution was allowed to react with samples (150 μl) for 30 min in the dark. Absorbance was taken at 593 nm taking ascorbic acid as standard.

Phosphomolybdate assay

The total antioxidant capacity of samples was quantitatively evaluated according to Aadil et al. (2014). An aliquot of 500 μl of plant sample (1 mg/ml) was mixed with 2 ml of test reagent (0.6 M sulfuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate) and incubated in a water bath at 70 °C for 90 min. Then the absorbance was measured against a blank at 695 nm after samples cooled down to ambient temperature.

DPPH assay

The method of Aadil et al. (2014) was used for measuring the DPPH radical scavenging capacity of rhizome extracts. A stock solution of DPPH was prepared by dissolving 12 mg of DPPH in 50 ml of methanol. The working solution was prepared by diluting the stock solution to get an absorbance of 1.05 ± 0.02 . 2.8 ml of the working solution was added to 200 μl of the diluted plant sample (1 mg/ml). The reaction mixture was mixed properly and incubated in the dark for 15 min. Then the absorbance was taken at 517 nm using BHT as standard.

ABTS radical scavenging assay

ABTS radical scavenging activity was assessed by the method of Aadil et al. (2014). ABTS radical was produced by reacting 7.4 mM ABTS stock solution with 2.6 mM potassium persulfate and allowed to stand in the dark at room temperature for 12–16 h before use. Plant extracts (200 μl) were allowed to react with 2.8 ml of the ABTS solution for 2 h in the dark. The absorbance was taken at 734 nm using ascorbic acid as standard.

Hydroxyl radical scavenging assay

The scavenging activity of hydroxyl radicals was determined by the method of Barapatre et al. (2016). To 1 ml of test samples or reference, 1 ml phosphate buffer (20 mM, pH 7.4) containing ferric chloride (1 mM), EDTA (1 mM), deoxyribose (2.8 mM), 0.1 ml ascorbic acid (1 mM) and 0.5 ml hydrogen peroxide (20 mM) was added. After 1 h incubation at 37 °C, 1 ml of TBA (1%, w/v) and 1 ml of

TCA (2.8%, w/v) were added to the reaction mixture and further incubated at 100 °C for 20 min to develop the color. After cooling down to ambient temperature, the absorbance was taken at 532 nm using catechin as standard.

Superoxide scavenging activity

Superoxide radicals generated in 1 ml 20 mM Tris–HCl buffer pH 8.0 containing 0.05 mM nitroblue tetrazolium (NBT), 0.01 mM phenazine methosulphate (PMS), were pre incubated for 2 min with the test samples (Barapatre et al. 2016). The reaction was initiated by the addition of 0.078 mM NADH. Blue chromogen formed due to NBT reduction, was read at 560 nm using Quercetin as standard and results were expressed as percentage of inhibition of superoxide radicals.

Scavenging assay for nitric oxide radical

The scavenging activity of nitric oxide radical was assessed by the method of Barapatre et al. (2016). Nitrite ions were produced by incubating sodium nitroprusside (SNP) (5 mM, in PBS) with plant samples at 25 °C. After 120 min, 0.5 ml of the incubation solution was withdrawn and mixed with 0.5 ml of Griess reagent (1% sulfanilamide, 5% phosphoric acid and 0.1% NEDD in 1:1 ratio). The absorbance was measured at 550 nm against the blank and standard Curcumin.

Metal chelating activity

The ferrous ion chelating activity was determined according to the method of Barapatre et al. (2016). 1 ml of different samples of plant were mixed with 2.75 ml distilled water and 0.05 ml FeCl_2 (2 mM). The reaction was initiated by the addition of 0.2 ml ferrozine (5 mM) and the mixture was shaken vigorously. The absorbance was measured at 562 nm after leaving the mixtures at room temperature for 10 min, taking EDTA as reference.

Hypochlorous acid scavenging

The assay was done according to the method of Hazra et al. (2008) with little modification. Immediately before the experiment Hypochlorous acid (HOCl) was prepared by adjusting the pH of a 10% (v/v) solution of NaOCl to 6.2 with 0.6 M H_2SO_4 , and the concentration of HOCl was determined by measuring the absorbance at 235 nm using the molar extinction coefficient of $100 \text{ M}^{-1} \text{ cm}^{-1}$. The decrease in absorbance of catalase at 404 nm represented scavenging activity, with reference to BHT. The reaction mixture in a final volume of 2 ml, was contained 50 mM

phosphate buffer (pH 6.8), catalase (7.2 μ M), HOCl (8.4 mM) and plant extract. The assay mixture was incubated at 25 °C for 20 min and the absorbance was measured against an appropriate blank.

Lipid peroxidation assay

For this assay, the perfused chicken liver was homogenized in 10% KCl using homogenizer at -4 °C. The homogenate was centrifuged at 800 g for 15 min, and clear cell-free supernatant used for the study of in vitro lipid peroxidation. 1 ml of 0.15 M KCl and 0.5 ml of rat liver homogenates were added to plant extract and peroxidation initiated by adding 100 μ l of 0.2 mM ferric chloride. The reaction was stopped after 30 min incubation at 37 °C by adding 2 ml of ice cold HCl (0.25 N) containing 15% TCA, 0.38% TBA, and 0.5% BHT (Singh et al. 2007). Then the reaction mixtures were heated at 80 °C for 60 min. followed by centrifugation. The absorbance of the supernatants after centrifugation was measured at 532 nm taking curcumin as standard.

Fourier transform infrared spectroscopy (FTIR) analysis

All extracts were characterized by FTIR to identify the functional groups. The FTIR spectrum of the samples properly grounded with KBr was observed in the range of 400–4000 cm^{-1} .

Quantification of phenolic and flavonoid compounds by HPLC

The separation and quantification of phenolic and flavonoid compounds were done by using HPLC (Shimadzu, Japan) system having the betasil C18 column (250 mm $\times$ 4.6 mm i.d, 5 μ m particle size. For phenolic compounds the detection was done at 288 nm and for elution the gradient mixture of aqueous phosphoric acid (0.026%, solvent A) and acetonitrile (solvent B) were used (Liu et al. 2006; Rababah et al. 2011). The gradient program was as follows: initial change from A–B (98:2) to A–B (77:23) for 0–20 min followed by a linear change to A–B (71.5:28.5) for the next 20–35 min.

Similarly for flavonoid compounds the detection was done at 370 nm using the mobile phase glacial acetic acid (solvent A) and acetonitrile (solvent B) in a step gradient manner as mentioned below (Mesbah et al. 2005): the ratio of A:B was 60:40 for 11 min, then 40:60 for 7 min and finally washed with 40:60 for next 10 min.

Statistical analysis

Principle component analysis (PCA) was performed by using Unscrambler 10.3 software package, to find the main

variance in the data set. The data were analyzed by one-way analysis of variance (ANOVA). All the results were expressed as mean $\pm$ SD for triplicate determinations and the significance of difference defined at the 99.9% ($p < 0.001^{***}$), 90% ($p < 0.01^{**}$) and 95% level ($p < 0.05^*$).

Results and discussion

Rhizome fractionation of *H. coronarium*

TPC and TFC of the six fractions [Hexane (HX), Dichloromethane (DCM), Ethyl acetate (EA), Acetone (ACE), Methanol (MET) and water (AQ)] were determined and represented in Table 1. The range of TPC varied between 7.27 ± 0.16 and 32.14 ± 0.03 mg of GAE/gram of dried sample. The significantly higher ($p < 0.05$) phenolic content was observed in the methanolic extract (32.14 ± 0.03 mg/g) followed by water (30.99 ± 0.37 mg/g) and ethyl acetate (21.52 ± 0.51 mg/g) extracts.

Total flavonoid contents were found to be significantly higher ($p < 0.05$) in acetone extracts than other extracts. The flavonoid content in *H. coronarium* rhizome extracts were in a range of 28.7 ± 0.44 to 143.06 ± 1.98 mg of quercetin/gram of dried sample as represented in Table 1. The order of TFC in the extracts was ACE > DCM > EA > HX > MET > AQ. The total phenolic and flavonoid content in the Ethyl acetate, acetone and methanol also quantified by HPLC (Table 2). The EA have the maximum amount of quercetin whereas the MET have maximum amount of Gallic acid and vallic acid i.e., phenolic compounds. The polarity of compounds present in raw material may attribute to the variation in phenolic and flavonoid content. Our results support the report that compounds containing $-\text{OH}$ and $-\text{COOH}$ functional groups in their structure such as phenolic acid and flavonoid are easily extracted by the polar solvents (Wong et al. 2014).

Phytochemical constituents of *H. coronarium*

FTIR spectra of different extracts showed characteristic peaks and variations corresponding to different functional groups. The variation in band spectra reveals that the nature of the functional groups may depend on the solvent system used. The FTIR spectra of all extracts were represented in Fig. 1. Bands nearby 2927 cm^{-1} , assigned to the C–H groups of alkane (Starlin et al. 2012) were invariably observed in all extracts. The extracts in ACE, MET and AQ have shown higher intensity at $\sim 3389 \text{ cm}^{-1}$ compared to other fractions, corresponds to O–H absorption. Bands at $\sim 1640 \text{ cm}^{-1}$ associated with C=O of amides were

Table 1 Total phenolic (TPC), flavonoid content (TFC), FRAP and phospho-molybdate assay (PMA) of plant extracts

Plant extract	TPC ^a	TFC ^b	FRAP ^c	PMA ^d
HX	7.27 ± 0.32	45.56 ± 0.12 ^{**}	49.82 ± 0.11 [*]	72.11 ± 0.9 ^{***}
DCM	15.63 ± 0.07 ^{**}	141.65 ± 5.19 ^{***}	50.28 ± 0.34 [*]	39.61 ± 2.5
EA	21.52 ± 0.51 ^{***}	117.10 ± 0.64 ^{***}	152.64 ± 0.28 ^{***}	172.34 ± 3.4 ^{***}
ACE	18.38 ± 1.81 ^{**}	143.06 ± 1.98 ^{***}	47.64 ± 0.22	65.52 ± 1.13 ^{***}
MET	32.14 ± 0.03 ^{***}	29.92 ± 0.51 ^{NS}	165.22 ± 0.80 ^{***}	117.56 ± 0.9 ^{***}
AQ	30.99 ± 0.37 ^{***}	28.7 ± 0.44	173.85 ± 0.34 ^{***}	81.88 ± 1.36 ^{***}

Values represent mean ± standard deviation (SD) of three independent experiments (n = 3). According to one way ANOVA values within in a Column are significantly different, NS non significant, *** $p < 0.001$, ** $p < 0.01$ and * $p < 0.05$

^a TPC expressed in mg of gallic acid equivalent/gram of extract

^b TFC expressed in mg of quercetin equivalent/gram of extract

^c FRAP value expressed as equivalents of gallic acid (mmol)

^d Phosphomolybdate assay of test compounds expressed as equivalents of ascorbic acid (mmol)

HX in TPC, AQ in TFC, ACE in FRAP and DCM in PMA analysis was taken as a reference for one way ANOVA analysis

Table 2 Phenolic/Flavonoid contents in extracted solutions from plant extract

Phenolic/flavonoid compounds (mg per 100 g extract)			
Extract	Gallic acid	Vallic acid	Quercetin
EA	6.35	7.57	0.4
Ace	7.8	9.31	0.09
Met	8.7	10.39	0.02

observed in methanol and aqueous extracts. The DCM and ACE extracts have shown peak at $\sim 1679\text{ cm}^{-1}$ corresponding to the C = O group of an aldehyde. The band at $\sim 1022\text{ cm}^{-1}$ was assigned to aliphatic amines shown in HX, DCM and EA extracts. Except AQ and HX all extracts have peak $\sim 630\text{ cm}^{-1}$ indicating the presence of a C–H group of alkyne (Zhao et al. 2015). The characteristic peaks found in the FTIR spectra of ACE extract at ~ 1510 and $\sim 1030\text{ cm}^{-1}$ corresponds to C=O absorption and C–OH stretching vibration respectively (Rohaeti et al. 2015).

Antioxidant activity of *H. coronarium* rhizomes

FRAP and phospho-molybdate activity of rhizomes

As the antioxidant activity of plant extracts is influenced by many factors such as a test system and composition of extracts, so single assay could not evaluate the accurate result. Therefore more than one type of antioxidant activity is carried out to explore various mechanisms of antioxidant assay (Gan et al. 2010). FRAP assay depends on the reduction of ferric ion into ferrous ion. The FRAP activity of six extracts varied from 47.64 ± 0.22 to

173.85 ± 0.34 mmol. The FRAP value of water extract was significantly higher followed by MET extract. As reported earlier, the essential oils from flowers of *H. coronarium* had shown FRAP value $0.22\text{ }\mu\text{mol/mg}$ (Lu et al. 2009) So, according to the recent study the rhizomes could be a better source of antioxidants.

The total antioxidant activity of the rhizome extracts was also evaluated by phosphomolybdenum method. The antioxidants can reduce Mo (VI) to Mo (V), and the green phosphate/Mo (V) compounds, give an absorption peak at 695 nm (Abdille et al. 2005). The total antioxidant activity of *H. coronarium* extracts was measured by monitoring the absorbance of the reaction mixture at 695 nm and represented in Table 1. Reaction mixture showing higher absorbance indicates stronger total antioxidant capacity. Our results showed that the ethyl acetate extract have shown the maximum antioxidant power (172.34 ± 3.4 mmol) followed by methanol extract (117.56 ± 0.9 mmol). The varying response of the extracts to the different antioxidant tests may be due to the different structure of antioxidants as well as due to the different redox potential during the transfer of electrons/hydrogen from antioxidants (Loo et al. 2008).

Activity of *H. coronarium* rhizome extracts against DPPH and ABTS radicals

Free radicals play a key role in biological damage as well as in the aging process. DPPH, purple colored stable free radical which turns into a stable yellow compound on reacting with an antioxidant. The reduction capacity is determined by a decrease in its absorbance at 517 nm. The extent of reaction depends on the hydrogen donating ability of antioxidants, so this assay is used commonly to

Fig. 1 FTIR spectra of six different solvent extracts extracted from *H. coronarium* rhizome

evaluate free radical scavenging potential of plant extracts. The IC_{50} value of different extracts ranged between 119.79 ± 0.05 and 371.28 ± 0.96 μg as represented in Table 3. The methanol extract showed maximum inhibition (83.47%) having IC_{50} values of 119.79 ± 0.1 μg against the free radicals, nearer to standard Ascorbic acid (125.63 μg). This result supports the hypothesis that the phenolic composition of sample influences free radical scavenging capacity. As reported earlier, the essential oils from flowers of *H. coronarium* had shown IC_{50} value 1091.00 $\mu\text{g/ml}$ (Lu et al. 2009). Hence this report proves that the rhizome extracts were potential scavenger of DPPH free radicals.

ABTS assay depends on the ability of antioxidant compounds to scavenge ABTS radical. It helps to measure the antioxidant capacity of lipophilic and hydrophilic compounds in the sample (Iqbal et al. 2015). A significant variation was observed in the percentage inhibition of six samples. Similar to DPPH assay the highest scavenging activity (95.00%) was shown in methanol extract. The IC_{50} value of methanol was 105.51 ± 0.13 μg , lower than that of control (133.08 μg). The ABTS scavenging potential of the extracts were in the order $\text{MET} > \text{EA} > \text{AQ} > \text{DCM} > \text{ACE} > \text{HX}$. The FTIR study of the samples suggests that the higher free radical scavenging activity of methanol might be due to an O–H group of alcohol, which appears near about ~ 3389 cm^{-1} in FTIR spectrum (Li et al. 2010).

Hydroxyl scavenging activity of *H. coronarium*

The high reactivity of OH^- enables it to react with a wide range of molecules, such as sugars, amino acids, lipids, and nucleotides (Wang et al. 2008). So for the protection of living systems, removal of OH^- is important. The reaction between Fe^{2+} and H_2O_2 generates hydroxyl radical, which attacks deoxyribose and degrades it into fragments that react with thiobarbituric acid on heating at low pH to form a pink color. The inhibition of hydroxyl radicals by the plant extracts were determined by monitoring the reaction between deoxyribose degradation products and TBA (Gonc et al. 2013). The results are shown in the Table 3 and the highest hydroxyl radical inhibition (75.33%) is shown by ethyl acetate extract with IC_{50} values of 672.61 ± 8.94 μg . The highest hydroxyl radical scavenging activity of EA extract may be due to reactive chemical bond or functional group formed by the oxygen atom of ethyl acetate (Li 2013).

High superoxide scavenging of *H. coronarium*

The less reactive superoxide radicals can give rise to toxic hydroxyl radicals by damaging cellular macromolecules and involved in many pathological processes (Elmastaş et al. 2006). Thus, scavenging of superoxide radicals would be a promising remedy for these diseases. In this experiment superoxide radicals generated from dissolved oxygen by PMS-NADH coupling, which can be estimated by their ability to reduce NBT. The ability of the extracts, as well as standard to quench radicals in the reaction mixture indicated by a decrease in absorbance at 560 nm. As shown in Table 3, the highest superoxide radical scavenging activity was observed by methanol with a 64.19% reduction in NBT production at 1 mg/ml concentration, whereas the control Quercetin have 51.39% inhibition at the same concentration. The lowest % inhibition was shown by AQ extract (19.16%).

Table 3 Antioxidant and radical scavenging activity of plant extracts

Extract	DPPH ^a	ABTS ^b	Hydroxyl ^c	Superoxide ^d	NO ^e	Fe ²⁺ chelation ^f	Hypo ^g	LPO ^h
HX	256.55 ± 17.96***	228.09 ± 7.23**	889.96 ± 11.53***	36.06 ± 4.7*	653.87 ± 11.32 ^{NS}	45.12 ± 2.42***	359.59 ± 14.33***	54.06 ± 0.67 ^{NS}
DCM	163.69 ± 8.45 ^{NS}	131.85 ± 1.19 ^{NS}	1108.4 ± 26.19***	19.16 ± 1.39***	368.3 ± 1.79***	73.76 ± 0.33**	370.02 ± 7.59***	24.44 ± 0.46***
EA	129.43 ± 2.47 ^{NS}	108.97 ± 3.02 ^{NS}	672.61 ± 8.94***	42.07 ± 1.13 ^{NS}	423.02 ± 4.73***	65.26 ± 0.30 ^{NS}	352.61 ± 0.2***	44.33 ± 0.8***
ACE	371.28 ± 0.96***	164.92 ± 9.01 ^{NS}	865.77 ± 3.11***	48.6 ± 1.39 ^{NS}	304.74 ± 0.3***	73.54 ± 0.38**	318.31 ± 6.44***	83.74 ± 0.18***
MET	119.79 ± 0.1 ^{NS}	105.51 ± 0.13 ^{NS}	1067.82 ± 7.11***	64.19 ± 0.26*	361.29 ± 4.32***	76.42 ± 0.17***	358.72 ± 1.78***	73.95 ± 0.03***
AQ	127.37 ± 3.07 ^{NS}	119.95 ± 3.43 ^{NS}	1105.54 ± 18.43***	19.94 ± 0.95***	433.46 ± 18.63***	72.28 ± 0.012*	884.41 ± 29.36**	73.09 ± 0.46***
Control	125.63 ± 10.55 ^B	133.08 ± 30.29 ^{AA}	151.37 ± 15.24 ^{CC}	51.39 ± 2.61 ^Q	694.76 ± 38.24 ^C	66.66 ± 1.04 ^E	1151.13 ± 68.98 ^B	52.01 ± 0.11 ^C

Values represent mean ± standard deviation (SD) of three independent experiments (n = 3)

According to one way ANOVA values within in a Column are significantly different, NS non significant, *** $p < 0.001$, ** $p < 0.01$ and * $p < 0.05$

^a Concentration of test compound required to scavenge 50% inhibition of DPPH radical (in µg)

^b Concentration of compound required to scavenge 50% inhibition of ABTS radical (in µg)

^c Concentration of compound required to scavenge 50% inhibition of hydroxyl radical (in µg)

^d Percentage inhibition of superoxide radicals by the test compound

^e Concentration of test compound required to scavenge 50% inhibition of nitric oxide radical (in µg)

^f Metal Chelating ability expressed in % scavenging

^g Concentration of test compound required to scavenge 50% inhibition of Hypochlorous ions (µg)

^h Percentage inhibition of lipid peroxidation by the test compound

AA Ascorbic acid, B BHT, C Curcumin, CC Catechin, E EDTA, Q Quercetin

H. coronarium scavenges nitric oxide

Nitric oxide, a free radical generated from sodium nitroprusside which regulates various physiological processes in mammalian cells. It reacts with oxygen and forms nitrite but its excess production is toxic to tissues, leading to various diseases (Bera et al. 2015). The scavenging activity of the plant products may help to inhibit formation of nitric oxide and arrest chain of reactions initiated by NO, which is detrimental to human health. Among the six extracts, acetone extract showed the highest nitric oxide inhibition ability (82.03%) compared to other extracts and standards. The IC_{50} value of acetone extract was found to be 304.74 ± 0.15 μ g, lower than that of control (694.76 μ g). There was no significant correlation found between NO radical scavenging activity and TPC. As the ACE extract had maximum TFC, so the flavonoids may be responsible for NO scavenging activity.

H. coronarium extracts promotes Fe^{2+} chelation

Being an essential element, Iron is required for oxygen transport, respiration and for enzyme activity. It causes oxidative damage to cellular components due to high reactivity (Yen et al. 2005). Hence the ability of different extracts to chelate metal ion were tested by a method based on reaction between ferrozine and Fe^{2+} , which produces a violet complex. The presence of chelating agent decreases violet color by interrupting the complex formation. In the present investigation, the highest % inhibition (76%) was shown by methanol extract and % inhibition of different extracts was in the order MET > DCM > ACE > A-Q > EA > HX. The percentage inhibition shown by the standard EDTA is 66.66%. The results suggest that the *H. coronarium* plant extract is a potent chelating agent than the standard.

H. coronarium facilitates hypochlorous scavenging activity

HOCl is produced by oxidation of Cl^- ions in the presence of H_2O_2 and induce cell lysis, causes protein oxidation and inactivates antioxidant enzymes like catalase (Visioli et al. 1998). The results indicated that the acetone extract (IC_{50} value -318.31 ± 6.44 μ g) scavenged hypochlorous acid more efficiently than standard (IC_{50} value -1151.13 ± 68.98 μ g) and other extracts. The % inhibition was ranged from 78.57 to 21.79% and the order was ACE > EA > MET > HX > DCM > AQ.

H. coronarium inhibits lipid peroxidation

Lipid peroxidation is an oxidative deterioration of polyunsaturated fatty acids which leads to formation of free

radicals and ultimately leading to membrane damage (Wang et al. 2008). Therefore, inhibition of lipid peroxidation is considered as an important index for measuring antioxidant potential. The efficacy of lipid peroxidation (LPO) scavenging activity of solvent extracts was shown in Table 3. In the present study, the acetone extract showed highest LPO scavenging activity (83.74%) at 1 mg/ml concentration. There was no significant relation found between phenolic content and the lipid peroxidation.

Correlation among *H. coronarium* TPC and different antioxidant activity

Principle Component analysis (PCA) was carried out to get the information about the similarities and differences among the six rhizome extracts and to explore the relationship between different antioxidant assays. The first two principal components exhibited 93 and 4% of the total variance in the data set respectively. PC1 showed correlation with TPC, FRAP, DPPH and ABTS scavenging activity (Figure S1). PC2 mainly elucidated variation between samples with regard to TFC. Accordingly, Methanol extract having maximum phenolic content as well as maximum % inhibition against DPPH and ABTS radicals was situated to the right of PC1 (Figure S2). On the other hand, Hexane with low levels of TPC and weak scavenging activity was located on the opposite side of PC1. In the positive part of the PC2 the DCM and acetone extracts appeared due to their relatively high flavonoid content. TFC loaded mainly on the second component, while TPC has a low loading which explains that there is no significant correlation between TPC and TFC. Pearson correlation analysis ($r^2 = 0.795$) was used to demonstrate significant correlation between TPC and DPPH radical scavenging activity. Similarly, significant correlation was obtained between TPC and ABTS assay ($r^2 = 0.881$) as well as between TPC and FRAP ($r^2 = 0.864$).

Conclusion

The rhizome extracts of *H. coronarium* exhibited high antioxidant activity. The FTIR data support the presence of hydroxyl, carboxyl groups, may responsible for the antioxidant property. In addition to this the phenol and flavonoid compounds quantified by HPLC supported the role of these compounds for the antioxidant property. This study indicates a positive correlation between bioactive compounds of *H. coronarium* and antioxidant potential. Considering the obtained results performed experiments, our study provide strong scientific evidence for considering the *H. coronarium* rhizome extracts as natural antioxidants targeting various diseases caused due to oxidative stress.

Hence we conclude that *H. coronarium* rhizome could be used as a natural anti-oxidant in oxidative stress that may present in pathological conditions. But further investigation with the pure compounds is needed to understand the mechanism behind its significant medicinal importance in preventing various diseases induced by oxidative stress.

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Compliance with ethical standards

Conflict of interest The authors declare no conflict of interest.

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