Arabian Journal of Chemistry (2016) 9, S1435–S1442

King Saud University

Arabian Journal of Chemistry

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ORIGINAL ARTICLE

Phytochemical studies, antioxidant activities and identification of active compounds using GC–MS of *Dryopteris cochleata* leaves

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Received 26 August 2011; accepted 17 March 2012
Available online 25 March 2012

KEYWORDS

Dryopteris cochleata;
In vitro;
DPPH;
Reducing power;
Quantitative screening;
Antioxidant

Abstract The increasing interest in powerful biological activity of plant phenolics and flavonoids outlined the necessity of determining their contents in *Dryopteris cochleata* leaves. The present investigation comprises, estimation of total phenolics, flavonoids, flavonols, tannins, carbohydrates, vitamin-C and protein content accompanied by an important *in vitro* antioxidant assays like DPPH-radical scavenging activity and reducing power for various leaf extracts. Quantitative screening provided a gateway to assess all the estimations, among which the significant role was played by the acetone extract. IC_{50} and EC_{50} of the *in vitro* assays of acetone depicted striking evidence in contrast to the other extracts. Supportively, GC–MS analysis revealed that many useful constituents indicating *D. cochleata* leaves could be useful for preparation of neutraceuticals as potent antioxidant to treat various human diseases and its complications.

1. Introduction

Use of plants as a source of medicine has been inherited and is an important component of the health care system. India is the largest producer of medicinal herbs and is appropriately called the botanical garden of the world (Ahmedull and Nayar, 1999). Approximately 20% of the plants found in the world have been submitted to pharmaceutical or biological tests (Suffredini et al., 2004). A special feature of higher angiospermic plants is their capacity to produce a large number of organic chemicals of high structural density, the so-called secondary metabolites. The accumulation of these phytochemicals in plants has generated knowledge for the production of desired phytoconstituents with the potential to act against multiresistant free radicals. Antioxidants are substances used by the body to protect itself from the damage caused by free radicals, whose overload has been linked to many diseases, few of which include heart diseases (Aviram, 2000), liver diseases and cancers (Owen et al., 2000). Hence, the research has been focussed on the use of antioxidants, with particular emphasis on naturally derived antioxidants, which may inhibit reactive oxygen species and may display protective effects. Plant phenolics, in particular phenolic acids, tannins and flavonoids are known to be potent antioxi-

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Peer review under responsibility of King Saud University.

Production and hosting by Elsevier

http://dx.doi.org/10.1016/j.arabjc.2012.03.018

dants and occur in vegetables, fruits, nuts, seeds, roots and barks (Pratt and Hudson, 1990). In the case of phenolic compounds, the ability of the phenolics to act as antioxidants depends on the redox properties of their phenolic hydroxyl groups that allow them to act as reducing agents, hydrogen-donating antioxidants and oxygen quenchers (Rice-Evans et al., 1996).

In addition to their antioxidant properties, these compounds display a vast variety of pharmacological activities such as anti-inflammatory, anticancer, anticarcinogenic, antibacterial, antioxidant, antifungal, antiviral activities etc. Phytochemicals with antioxidant activity: allyl sulfides (onions, leeks, garlic), carotenoids (fruits, carrots), flavonoids (fruits, vegetables) and polyphenols (tea, grapes). Many scientists have suggested that dietary antioxidants, such as ascorbate, α-tocopherol and carotenoids from fruits and vegetables could help to protect the cells from damage caused by oxidative stress and to fortify the defence system against degenerative diseases (Ames, 1983; Frei, 1991). With the onset of scientific research in phytochemistry, a systemic study of a crude drug emphasizes that secondary metabolites are derived as a result of plant metabolism.

Dryopteris cochleata that belongs to the family Dryopteridaceae is considered to possess great medicinal value. It has been reported to possess wide ethanomedical use, whose rhizome has antifungal property and is used as an antidote. It also has variety of applications against the disorders like epilepsy, leprosy, cuts, wounds, ulcers, swelling, pains and snake bites. The decoction of the dried rhizome, stem and stripe is used for blood purification (Shweta et al., 2005). The juice of fronds is used to treat muscular and rheumatic pain (Kunjani and Ananda, 2008). The leaf extract has been reported to have antibacterial activity (Pradeep et al., 2010). To the best of author's knowledge, there has been no works reported on the antioxidant activities of the leaf extracts. Hence, with a renewing interest, the goal of this piece of work will involve the possible screening of the active constituents in various crude extracts stepwise. The interactions of different active fractions are needed to explore the mechanism of the constituents present and their efficacies to be studied *in vitro* to assess their antioxidant properties.

2. Materials and methods

2.1. Chemicals

Folin–Ciocalteu's reagent, 1,1-diphenyl-2-picrylhydrazyl (DPPH), gallic acid, (±)-catechin, rutin, α-tocopherol, ascorbic acid, bovine serum albumin, glucose, tannic acid, solvents and other reagents used were of analytical grade purchased from Sigma Chemical Co. (St. Louis, MO, USA).

2.2. Plant collection and extraction

Healthy *D. cochleata* leaves were collected from Kolli Hills, Namakkal District, Tamil Nadu, identified and authenticated by Botanist, St. Xavier's College, Tirunelveli, Tamil Nadu, India.

The plant materials were washed with tap water, prior to distilled water, shade dried and powdered. The powdered plant materials were subjected to successive extraction with petroleum ether, chloroform, ethylacetate, acetone, methanol and water using Soxhlet extractor. The extracts were dried in vacuum pump at 40 °C. The dried crude extracts were stored in freezer at 0 °C for future use.

2.3. Phytochemical screening

The preliminary phytochemical screening tests were carried out to identify the useful constituents by standard methods (Onwukeame et al., 2007).

2.4. Determination of total phenolic contents

The total phenolics in the extracts were estimated by spectrophotometric assay (Barreira et al., 2008). One milliliter of sample (concentration 1 mg/mL) was mixed with 1 mL of Folin and Ciocalteu's phenol reagent. After 3 min, 1 mL of saturated sodium carbonate solution was added to the mixture and made upto 10 mL with distilled water. The reaction was kept in the dark for 90 min, after which the absorbance was read at 725 nm. Gallic acid was used for constructing the standard curve (20–100 μg/mL, $Y = 0.025x + 0.0378$, $R^2 = 0.997$) and the results were expressed as μg of gallic acid equivalents/mg of extract (GAEs).

2.5. Determination of total flavonoid contents

Flavonoid contents in the extracts were determined by spectrophotometric method (Barreira et al., 2008). The (250 μL) extract (concentration 1 mg/mL) was mixed with 1.25 mL of distilled water and 75 μL of a 5% $NaNO_2$ solution. After 5 min, 150 μL of 10% $AlCl_3$ solution was added. After 6 min, 500 μL of 1 M NaOH and 275 μL of distilled water were added to prepare the mixture. The solution was mixed well and the absorbance was read at 510 nm. (±)-catechin was used to calculate the standard curve (20–160 μg/mL, $Y = 0.0006x + 0.0603$, $R^2 = 0.9997$) and the results were expressed as μg of (±)-catechin equivalents (CEs) per mg of extract.

2.6. Estimation of total flavonol contents

One millilitre of leaf extract (concentration 1 mg/mL) was mixed with 1 mL aluminium trichloride (5 mg/mL) and 3 mL sodium acetate (25 mg/mL). The absorbance read at 440 nm was read after 2.5 h. The absorption of standard rutin solution (0.5 mg/mL) in methanol was measured under the same conditions (Grubesic et al., 2005). All determinations were carried out in duplicates. The amount of flavonols in plant extracts in rutin equivalents (RE) were calculated by the following formula. $X = (A.m_0)/(A_0.m)$, where X is the flavonol content, mg/mg plant extract in RE, A is the absorption of plant extract solution, A_0 is the absorption of standard rutin solution, m is the weight of plant extract (mg), and m_0 is the weight of rutin in the solution (mg).

2.7. Estimation of tannin contents

Tannin content of the extracts was measured by Folin–Denis method (Oyaizu, 1986). The various extracts (50 μL) were made up to 7.5 mL by adding double distilled water. Then 0.5 mL Folin–Denis reagent and 1 mL of Na_2CO_3 were mixed with it. Again the volume was made up to 10 mL by double distilled water. Absorption was recorded at 700 nm. Tannic

acid was used to calculate the standard curve (20–120 μg/mL, $Y = 0.069x + 0.0091$, $R^2 = 0.9985$) and the results were expressed as μg of tannic acid equivalents per mg of extract.

2.8. DPPH radical scavenging activity

Various concentrations of *D. cochleata* leaf extracts (0.3 mL) were mixed with 2.7 mL of methanol solution containing DPPH radicals (6×10^{-5} mol/L). The mixture was shaken vigorously and allowed to stand for 60 min in the dark. The reduction of the DPPH radical was determined by reading the absorbance at 517 nm (Barros et al., 2007). The radical-scavenging activity (RSA) was calculated as a percentage of DPPH discoloration, using the equation: $\%RSA = [(A_{DPPH} - A_S)/A_{DPPH}] \times 100$, where, A_S is the absorbance of the solution when the sample extract is added at a particular level and A_{DPPH} is the absorbance of the DPPH solution. The extract concentration providing 50% of radical-scavenging activity (IC_{50}) was calculated from the graph of RSA percentage against extract concentration. Ascorbic acid and α-tocopherol were used as standards.

2.9. Reducing power

The reducing power of *D. cochleata* leaf extracts was determined (Oyaizu, 1986). Various concentrations of different solvent extract (1 mL), phosphate buffer (1 mL, 0.2 M, pH 6.6) and potassium ferricyanide (1 mL, 10 mg/mL) were mixed together and incubated at 50 °C for 20 min. Trichloroacetic acid (1 mL, 100 mg/mL) was added to the mixture and centrifuged at 8000 rpm for 5 min. The supernatant (1 mL) was mixed with distilled water (1 mL) and ferric chloride (0.1 mL, 1 mg/mL) and then the absorbance was measured at 700 nm.

2.10. Estimation of carbohydrate content

Total carbohydrate contents were estimated by Anthrone method (Hedge et al., 1962). Glucose was used to calculate the standard curve (20–120 μg/mL, $Y = 20x$, $R^2 = 1$) and the results were expressed as μg of glucose equivalents per mg of extract.

2.11. Estimation of protein content

Total proteins were estimated by Lowry's method (Lowry et al., 1951). Bovine serum albumin was used to calculate the standard curve (20–160 μg/mL, $Y = 0.0004x + 0.032$, $R^2 = 0.9569$) and the results were expressed as μg of bovine serum albumin equivalents per mg of extract.

2.12. Estimation of ascorbic acid content

One mg of various extracts was treated with 4.0 mL of 10% trichloro acetic acid and centrifuged for 20 min at 3500 rpm and 0.5 mL of supernatant was then, mixed with 0.1 mL DTC reagent (2,4-dinitrophenylhydrazine-thiourea-copper sulfate reagent). The tubes were incubated at 37 °C for 3 h. Ice cold 65% H_2SO_4 (0.75 mL) was added and the tubes were allowed to stand at room temperature for an additional 30 min. The color developed was read at 520 nm (Omaye et al., 1962). Ascorbic acid was used to calculate the standard curve (20–160 μg/mL, $Y = 0.0043x + 0.03682$, $R^2 = 0.9652$) and the results were expressed as μg of ascorbic acid equivalents per mg of extract.

2.13. GC–MS analysis

For the GC–MS analysis a 30×0.25 mm $\times$ 0.25 μm df a 5% diphenyl/95% dimethyl poly siloxane column; was used in Clarus 500 Perkin–Elmer gas chromatograph with a Turbo mass gold-Perkin–Elmer detector. For GC–MS detection, an electron ionization system with ionization energy of 70 eV was used. Helium gas (99.999%) was used as the carrier gas at a constant flow rate of 1 mL/min. and an injection volume of 3 mL was employed (split ratio of 10:1). Injector temperature was 250 °C; ion-source temperature was 280 °C. The oven temperature was programed from 110 °C (isothermal for 2 min.), with an increase of 10 °C/min to 200 °C (no hold), then from 5 °C/min to 280 °C, ending with a 9 min. isothermal at 280 °C. Mass spectra were taken at 70 eV; a scan interval of 0.5 s and fragments from 45 to 450 Da. Total GC running time was 36 min. The relative percentage amount of each component was calculated by comparing its average peak area to the total areas. Software adopted to handle mass spectra and chromatograms was a Turbomass version 5.2.0.

2.14. Statistical analysis

Statistical analyses were conducted using SPPS software (16.0 version). Analysis of variance (ANOVA) in a completely randomized design and Turkey's multiple range tests were used to compare any significant differences between samples. Values were expressed as means ± standard deviations. All determinations were done at least in triplicate and all were averaged. The confident limits used in this study were based on 95% ($p < 0.05$).

3. Results

3.1. Phytochemical screening

The quantitative screening of the crude leaf extracts had revealed the presence of numerous phytoconstituents. The presence of them from copious, slightly to moderately present in all the test extracts has been listed in Table 1.

3.2. Total phenolic content

The acetone extract recorded the highest content of about 90.45 μg/mL. The next highest was observed in ethylacetate extract. Chloroform and methanol extracts closely ranged in their contents and the least observed extract was water of about 28.64 μg/mL.

3.3. Total flavonoid content

The acetone extract had recorded the highest content for flavonoid too, then stood the ethylacetate (145.78 μg/mL) and chloroform (122.56 μg/mL) extracts. The expected methanol extract recorded a low content of about 77.71 μg/mL. Least value was obtained for water and petroleum ether extract.

Table 1 Phytochemical screening of *Dryopteris cochleata* leaves extracts using various solvents.

Phytochemical constituents	Tests	Various solvent extracts					
		PE	CH	EA	AC	ME	WA
Alkaloids	Mayers test	–	+ +	+ +	+ + +	+ + +	+ +
	Wagners test	–	+ +	+	+ + +	+ +	+ +
	Hagers test	–	+ +	+ +	+ +	+ +	+ +
Phenolics/tannins	$FeCl_3$ test	+	+ + +	+ + +	+ + +	+ + +	+ + +
	Lead acetate test	+	+ +	+ + +	+ + +	+ + +	+ + +
	$K_2Cr_2O_7$ test	–	+ +	+ +	+ + +	+ + +	+ +
Flavonoids	Shinoda test	+	+ + +	+ + +	+ + +	+ + +	+ + +
Proteins/amino acids	Ninhydrin test	+	+	+ + +	+ + +	+ +	+ +
	Biuret test	+	+	+ +	+ + +	+ +	+ +
Carbohydrates	Molisch's test	+	+ +	+ +	+ + +	+ +	+ +
	Fehling's test	+	+ +	+ +	+ + +	+ + +	+ + +
	Barfoed's test	–	+ +	+ +	+ + +	+ +	+ + +
Fats/oils	Sudan IV test	+ + +	+	–	–	–	–
Steroids	Salkowski test	–	+ +	+	+	+	+ +
	Libermann's test	–	+	+	+	+	+ +
Saponins	Foam test	+ + +	+	–	+	–	–
Terpenoids	Knollar's test	–	+ +	+ +	+ +	+ +	+ +
Cardiac glycoside	Keller-Killiani test	–	+ +	+	+	+ +	+
Anthraquinones	Borntrager's test	–	–	+ +	+ +	+ +	+

PE = petroleum ether extract; CH = chloroform extract; EA = ethylacetate extract; AC = acetone extract; ME = methanol extract; WA = water extract.
+ + + = Copiously present; + + = moderately present; + = slightly present; – = absent.

3.4. Total flavonol content

Both acetone and ethylacetate extract recorded a significant flavonol content ranging around 47 μg/mL. Then thenext higher content of about 46.35 μg/mL was recorded by methanol extract. Unlike the other extracts chloroform recorded 26.40 μg/mL.

3.5. DPPH radical scavenging activity

Fig. 1 illustrates a significant decrease in the concentration of DPPH free radical with various concentrations of the plant extracts, due to their scavenging ability against the standard ascorbic acid and α-tocopherol. IC_{50} values have been calculated that represents the concentration of an inhibitor that is required for 50% inhibition of its target (i.e.) DPPH radicals. The scavenging effects of the extracts were in the order: ascorbic acid > acetone > ethylacetate > α-tocopherol > chloroform > methanol > water > petroleum ether.

3.6. Reducing power

Reducing power was seen to increase with increase in the concentration of the extracts as shown in the Fig. 2. EC_{50} was calculated from the graph to determine the concentration of extracts that provided 0.5 absorbance at 700 nm. The reducing ability of the extracts was in the order: ascorbic acid > acetone > ethylacetate > α-tocopherol > chloroform > methanol > water > petroleum ether, unlike the order seen for DPPH scavenging activity.

3.7. Estimation of carbohydrates

In the case of carbohydrates, acetone and ethylacetate extracts exhibited higher contents while a moderate content for water and chloroform extracts were observed. A lesser content (38.10 μg/mL) was observed for methanol extract.

3.8. Estimation of tannins

The highest value of about 102.02 μg/mL was recorded by the acetone extract. About one-half of the content was only recorded by the ethylacetate extract and then the methanol extract. Chloroform and water extracts showed lesser contents.

3.9. Estimation of protein

The order obtained in the protein content of extracts were acetone > ethylacetate > methanol > chloroform > water > petroleum ether. Protein content of about 174.02 μg/mL was recorded for the acetone extract.

3.10. Estimation of vitamin C

The acetone and ethylacetate extracts exhibited higher vitamin content of about 157.37 and 156.04 μg/mL, respectively followed by methanol (152.19 μg/mL) extract. While the remaining extracts showed insignificant amounts of vitamin-C.

3.11. GC–MS analysis

Interpretation on mass spectrum GC–MS was conducted using the database of National Institute Standard and Technology (NIST) having more than 62,000 patterns. The spectrum of the unknown component was compared with the spectrum of the known components stored in the NIST library. Six compounds were identified in *D. cochleata* leaf extract by GC–MS analysis was shown in Fig. 3. The active principles with their retention time (RT), molecular formula, molecular weight (MW) and concentration (%) were taken into account.

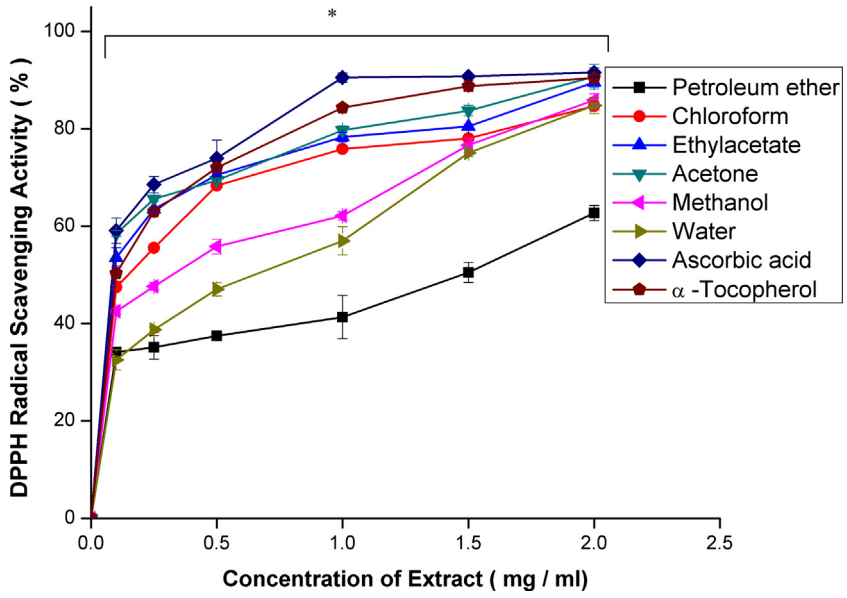

Figure 1 DPPH radical scavenging activities of *Dryopteris cochleata* leaves extracts in different concentrations. Each value represents a mean ± S.D. Symbols represent statistical significance from control ($^{*}p < 0.05$).

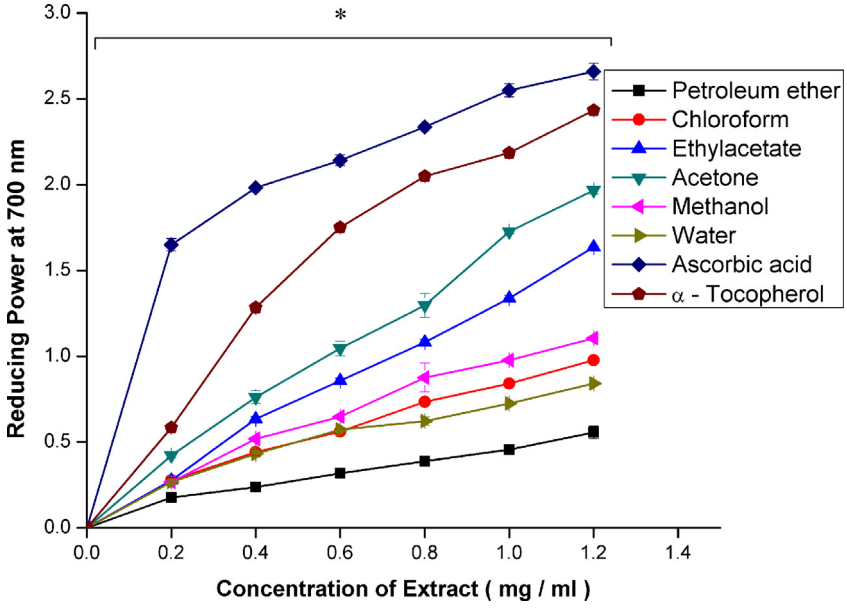

Figure 2 Reducing power activity of *Dryopteris cochleata* leaves extracts in different concentrations. Each value represents a mean ± S.D. Symbols represent statistical significance from control ($^{*}p < 0.05$).

For almost all the contents and activities thereby, the petroleum ether extract exhibited lower effects, which may be due its less polarity. All the contents and the estimations are neatly portrayed in Table 2 for the readers to get the fruit of this labor.

4. Discussion

The antioxidant activities of plants are mainly contributed by the active compounds present in them. In this study, the antioxidant activity and the content of active compounds has been determined stepwise for various extracts. Numerous important chemical constituents have shown their strong presence that has paved the way to estimate their contents and activities.

The results obtained proved the viability of the used methods in determining the phenolic and flavonoid contents in all the test extracts. The data clearly outline the richest source of phenolics and flavonoids in acetone extracts. Equally ethylacetate and chloroform extracts had showed their significant contents compared to the phenolic and flavonoid compounds ubiquitously that constitute the main class of natural antioxidants present in plants. It has presented a good content in acetone extract, thereby allowing it to express an excellent antioxidant activity. Thus, the natural flavonoids with pheno-

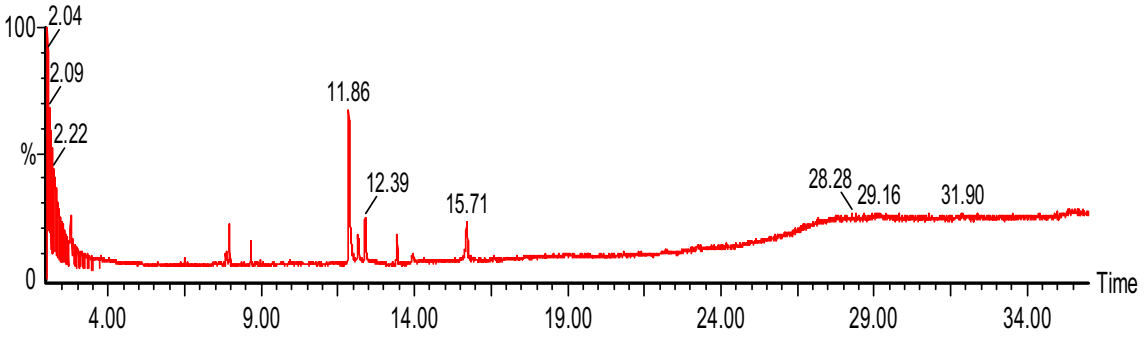

Figure 3 Chromatogram obtained from the GC–MS with the extract of *Dryopteris cochleata* leaves.

Table 2 Estimations of various contents of *Dryopteris cochleata* leaves by various solvents.

Phytochemical constituents (μg/mg extract)	Petroleum ether	Chloroform	Ethylacetate	Acetone	Methanol	Water
Total phenolic content (GAEs)*	17.71 ± 0.18	32.98 ± 1.20	43.48 ± 0.37	90.45 ± 1.26	30.86 ± 0.18	28.64 ± 0.37
Total flavonoid content (CEs)*	9.16 ± 1.41	122.56 ± 4.75	145.78 ± 5.17	146.99 ± 6.01	77.71 ± 5.04	25.74 ± 4.87
Total flavonol content (RE)*	10.37 ± 0.40	26.40 ± 0.24	47.30 ± 0.32	47.62 ± 0.32	46.35 ± 0.42	44.39 ± 0.24
Total Tannin content*	22.31 ± 1.58	30.20 ± 1.58	56.82 ± 4.34	102.02 ± 4.34	41.64 ± 3.33	35.87 ± 3.33
Total ascorbic acid content*	38.03 ± 0.37	79.03 ± 3.02	156.04 ± 0.25	157.37 ± 1.71	152.19 ± 1.38	101.05 ± 2.93
Total protein content*	33.56 ± 2.45	73.84 ± 4.91	114.85 ± 3.81	174.02 ± 5.51	78.09 ± 4.91	66.75 ± 4.25
Total carbohydrate content *	16.34 ± 0.33	43.96 ± 0.86	89.76 ± 1.54	93.96 ± 0.38	38.10 ± 3.24	79.98 ± 0.87

* The values are means of three replicates with standard deviations (mean ± S.D.; $n = 3$), $p < 0.05$.

lic groups in acetone extracts have exhibited potent scavenging activity. The flavonoid content of this extract was striking evidence for its activity. The flavonol contents have also recorded higher contents in acetone extracts. Though they are present in plants in lesser quantities, they exhibit stronger antioxidant activity in the acetone extract. Among the various extracts, the second highest was the ethylacetate in all the above explained contents. It has given a good competence with the acetone extract, especially in the flavonol content. As given in the results, the acetone extract had fetched high tannin content. In no other estimation there has been a wide variation of contents between the extracts recorded. These tannins are the polyphenolic compounds that have direct antioxidant activity, especially the scavenging properties. Hence, the accumulation of this content is considered to be the main criteria for the antioxidant activity of *D. cochleata* leaves.

Antioxidant and anti-free radical activities of this species would suggest the adaptive strategies for its potential importance to get a beneficial outcome. Thus, DPPH radical scavenging activity and reducing power activity are highly considered reliable methods to quantify the antioxidant activities. Highest scavenging activity was observed with the acetone extract, whose IC_{50} value was 83.4 μg as opposed to the IC_{50} value of ascorbic acid, 82.36 μg and α-tocopherol 94.9 μg, the well known antioxidants. It is noteworthy that the scavenging activity of the plant extract is ahead the standard, which as a whole can prove its substantial protective effect against human disorders with sufficient *in vivo* studies. Furthermore, the other extracts have recorded essential IC_{50} values, inparticular the ethylacetate extract (93.86 μg). The highest IC_{50} values were given by water and petroleum ether extracts indicating their least efficiencies. Scavenging of DPPH radical that was found to rise with increasing concentration of the plant extracts is shown in Fig. 1.

Additionally, it has been determined that the antioxidant activity of the phenolics and flavonoids are mainly due to their redox properties, which can play an important role in adsorbing and neutralizing free radicals, quenching singlet and triplet oxygen or decomposing peroxides (Hasan et al., 2008). It was found that the reducing powers of all extracts increased with increase in their concentration (Fig. 2.). The various solvent fractions of *D. cochleata* that exhibited a good reducing power of EC_{50} are 243, 327, 378, 494 μg for acetone, ethylacetate, methanol, chloroform and water extracts respectively, indicating the ability of samples to reduce Fe(III) to Fe(II) (i.e., reducing power of sample). These data suggested that the acetone extract had served as a significant indicator to react against free radicals to convert them into more stable non-reactive species and to terminate radical chain reaction.

The nutrient phytochemicals are increasingly being recognized as potential health promoters in reducing the risk of diseases. Hence, the contents of carbohydrate, protein and vitamin-C were also estimated. These major constituents have also shown their copious presence in the screening test too and hence there prevailed an interest. With a deeper investigation among the extracts, acetone and ethylacetate extracts have presented considerable contents for all these estimations. These nutrient compounds are in agreement with the phenolic, flavonoid, flavonol contents and the antioxidant activities thereby. Protein content of the acetone extract has revealed a vast variation among the other extracts. Whereas, the carbohydrate and vitamin-C contents showed roughly closer values, chloroform and methanol extracts have given a satisfactory content.

The compounds identified using GC–MS analysis have been interpreted as given in Table 3. The phenolic compound ($C_{17}H_{27}NO_2$), whose molecular weight given as 277 may be highly responsible for their antioxidant activity. Terpene compound also has some useful properties. Phytol ($C_{20}H_{40}O$)

Table 3 Biologically active phyto-compounds identified of *Dryopteris cochleata* leaves by GC–MS analysis.

Retention time	Name of the compound	Molecular formula	Molecular weight	Peak area %
7.87	1*H*-3a,7-Methanoazulene, octahydro-1,4,9,9-tetramethyl-	$C_{15}H_{26}$	206	2.61
7.96	Phenol, 2,6-bis(1,1-dimethylethyl)-4-methyl-, methylcarbamate	$C_{17}H_{27}NO_2$	277	7.39
8.66	1,6,10-Dodecatrien-3-ol, 3,7,11-trimethyl-, [*S*-(*Z*)]-	$C_{15}H_{26}O$	222	3.91
11.86	Phytol	$C_{20}H_{40}O$	296	56.09
13.44	1,2-Benzenedicarboxylic acid, butyl octyl ester	$C_{20}H_{30}O_4$	334	8.70
15.71	Oxirane, tetradecyl-	$C_{16}H_{32}O$	240	21.30

a common compound reported to posses diuretic, antimicrobial, anti-inflammatory and anticancer activity (Jananie et al., 2011). Thus this analysis has provided a support for the isolation process.

It is interesting that plant extracts employed in the present study posses antioxidant activity accompanied with a very important criteria (i.e.) scavenging property. Various solvents employed here is just to get the maximum extraction of the contents, in which acetone has marked its capacity of being highly advantageous. Very essential phytochemical constituents responsible for the antioxidant activity have served a basis for the target of this paper. It is likely that these compounds would give a complete success of free radical termination. Though the acetone extract was focussed to be efficient all through the discussion, it can be noted that the ethylacetate extracts were also closely active. The latter estimations for nutrition like protein, carbohydrate and vitamin have added a massive support through which the properties of *D. cochleata* could be scaled. Thorough chemical analyses of these active constituents is underway and hope to reveal some interesting applications of the chemicals isolated as well as their bioactivities. The work presented in this paper encourages a systematic *in vivo* study for its further exploitation to develop extensive knowledge about this rare species. The leaf extracts of *D. cochleata* can also be employed therapeutically as the rhizome of this species does.

5. Conclusions

The results obtained in this work are noteworthy, not only with respect to the antioxidant activities of the acetone extracts, but also with respect to its content of various phytochemical compounds as well. The present data of their contents are also provided as the basis for assessment of the preventive of role of *D. cochleata* leaves against free radical effects and will enrich the national food composition database. These observations may be used to substantiate the scientific reasoning that the free radical scavenging is indeed the mode of operation of these plants in the treatment or prevention of the onset of deadly diseases. Furthermore, studies in isolation and quantification of the compounds to elucidate their different antioxidant mechanisms and the existence of possible synergism, if any, and their effects through *in vivo* studies are needed to evaluate their natural biological function.

Acknowledgements

One of the author, Mr. A. Kathirvel gratefully acknowledges the financial assistance (University Research Fellow) rendered by the Periyar University, Salem. The authors thank Mr. D. Kumaravel, Senior Scientist, the Indian Institute of Crop Processing Technology (IICPT), Tanjore, Tamil Nadu, India, for permitting to perform GC–MS studies.

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