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Research Article

DRYOPTERIS COCHLEATA RHIZOME: A NUTRITIONAL SOURCE OF ESSENTIAL ELEMENTS, PHYTOCHEMICALS, ANTIOXIDANTS AND ANTIMICROBIALS

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ABSTRACT

Objetive: *Dryopteris cochleata* belongs to the family of Dryopteridaceae. The rhizomes of *D. cochleata* exhibits various therapeutic values to treat many ailments such as, epilepsy, leprosy, cuts, wounds, ulcers, swelling, etc. *D. cochleata* extracts possess antimicrobial, antioxidant property and also used to treat gonorrhea, muscular pain, rheumatic, throat problems and antidote for snake and dog bites. The present investigation deals with *in vitro* antioxidant activity, estimation of phytoconstituents, antimicrobial potential of various solvent (hexane, chloroform, ethylacetate, acetone, methanol and water) extracts of *D. cochleata* rhizome and its elemental analysis.

Methods: *In vitro* antioxidant activity was done by DPPH, superoxide, nitric oxide, hydroxide, ABTS, reducing power, ferrous ion chelating and lipid peroxidation assays. Total phenolics, flavonoids, flavonol, tannins, carbohydrate, protein and ascorbic acid content were estimated as per standard method. The elemental analysis was performed by LIBS. Agar well diffusion method was employed to determine the antimicrobial activity.

Results: Acetone extract showed significant antioxidant activity, higher amount of phytoconstituents and potential antimicrobial activity when compared with other extracts. The LIBS analysis revealed the presence of many nutrient elements (such as calcium, magnesium, oxygen, carbon, silicon, aluminium, iron, strontium, nitrogen, barium, sodium, hydrogen and potassium). The result of GC–MS profile of acetone extract showed about 61 compounds.

Conclusion: This study reveals that acetone extract of *D. cochleata* posses potential antimicrobial, antioxidant property and high nutritive value that may be used to serve society.

Keywords: *Dryopteris cochleata*, antioxidant, antimicrobial activity, LIBS analysis, GC–MS.

INTRODUCTION

Research on relationship between antioxidants and prevention of non-communicable disease such as cardiovascular disease, cancer, diabetes has been increasing in recent years. Epidemiological and *in vitro* studies strongly suggest that plant food containing phytochemicals with antioxidants have potent protective effects against these diseases. However, there is a widespread agreement that some synthetic antioxidants such as BHA, BHT, TBHQ need to be replaced with natural antioxidants owing to their hazardous health risks and toxicity [1]. Therefore, it is utmost need to find out new sources of safe and economy antioxidants of natural origin. Phenolic compounds are the natural antioxidants acting such as chelating metal ions, preventing radical formation and improving the antioxidant, the antioxidant endogenous system. Probably the most important natural phenolics are flavonoids because of their broad spectrum of chemical and biological activities, including antioxidant and free radical-scavenging properties. Being widespread across the plant kingdom, medicinal plants have gained tremendous interest as potential therapeutic agents against a wide range of biological actions such as antibacterial, antiviral, anticancer, anti-inflammatory and anti-allergic activities [2]. Higher plants have developed different adaptive mechanisms to reduce oxidative damage resulting from salt stress, through the biosynthesis of a cascade of antioxidants [3]. Bearing this in mind, the present work was designed to investigate the chemical profile of *D. cochleata* rhizomes. This plant has been considered to possess potential therapeutic value which belongs to the family Dryopteridaceae. Extracts of rhizome is used for epilepsy, leprosy, blood purification, as tonic for strength, for cuts, wounds, ulcers, swelling, pains, etc., it has antifungal property and also used as an antidote, for snake and dog bites. Leaves show antibacterial activity [4]. The whole plant extract is used as gonorrhea [5]. The juice of fronds is used to treat muscular and rheumatic pain [6]. Their root decoctions are used in cuts and throat problems. *D. cochleata* leaves possess antioxidant activity with good radical scavenging properties [7]. LIBS studies is a very effective analytical technique for qualitative and quantitative elemental analysis of any sample, whether it is solid, liquids, gases, aerosols or biological sample. For multielement analysis of samples only a single shot of laser pulse is sufficient [8]. Many elements in trace amount in human body play an essential role in metabolic process [9]. Therefore, identification of elements in medicinal plant and food products are vital. This has opened the possibility to carry out a systematic survey to design the active pharmacological activities of *D. cochleata* rhizome to sort out the therapeutic phytocompounds and non-toxic natural antioxidants to replace the synthetic ones.

MATERIALS AND METHODS

Plant Collection and Extraction

Healthy *D. cochleata* rhizomes were collected from Kolli hills, Namakkal district, Tamil Nadu State, India. The plant material was identified and confirmed by Botanical Survey of India (BSI), Coimbatore, Tamil Nadu, India. (BSI/SRC/5/23/2011-12/Tech./248). 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 dried extract was used in the following tests.

Pharmacological activity analysis

Phytochemical studies

The preliminary phytochemical screening tests [10], secondary metabolites like total phenolics, flavonoid [11], flavonol [12], tannin [13] were carried out by standard methods.

Antioxidant activities

DPPH [14], ABTS [15], hydroxide [16], ferrous ion chelating activity [17], reducing power activity [13], Nitric oxide [18], superoxide [19]

Radical scavenging activity and inhibition of lipid peroxidation using TBARS [11] were assayed. Ascorbic acid, BHA and TBHQ were used as standards.

Estimation of primary metabolites

Total carbohydrate [20], proteins [21], ascorbic acid [22] contents were estimated by standard methods.

Antimicrobial activity

Four Gram-positive bacterial strains, namely *Bacillus subtilis* [MTCC 441], *Staphylococcus aureus* [MTTC 96], *Staphylococcus epidermidis* [MTCC 435] and *Enterococcus faecalis* [Clinical collection], three Gram-negative bacterial strains, namely *Klebsiella pneumoniae* [MTCC 109], *Salmonella typhi* [MTCC 98] and *Shigella flexneri* [MTCC 1457] and three fungal strains, namely *Candida albicans*, *Aspergillus niger* and *Cryptococcus neoformans* were used in this investigation. The broth cultures of each test organism were prepared by inoculating a loop-full of culture in a 5 ml of Mueller Hinton Broth (MHB) for bacteria, and Sabouraud Dextrose Broth (SDB) for fungus then incubated 14 to 16 hours at 37° C for bacteria and 3 days at room temperature for fungus.

The antimicrobial activities of different extract of *D. cochleata* were determined by agar well diffusion method. The standardized suspension culture of test organisms (50 µl) was swabbed on the molten Mueller Hinton Agar (MHA) media, for bacteria and SDB for fungus. Made four wells (5 mm diameter) on each swabbed Petri plates using sterile cork borer then each extract 50 µl was delivered into the wells and allowed one to diffuse at room temperature for 1 h. Chloramphenicol (10 µg) and fluconazole (10 µg) were used as positive control for bacteria and fungus, respectively. The bacterial plates were incubated at 37˚C for 24 h and fungus plates were incubated at room temperature for 3 days. After incubation, the plates were measured for zone of growth inhibition (in mm).

Gc–ms analysis

The qualitative and quantitative analysis of *D. cochleata* rhizome acetone extract was carried out using a cp3800 saturn 2200 Gas Chromatography–Mass Spectometer (GC–MS) system. The temperature programs were 80°–350°C at the rate of 3°C/min. Ion source temperature was 200°C and scan range was 20–500 amu. The identification of components was based on comparison of their mass spectra with those of Wiley and NIST libraries.

LIBS Studies

Sample Preparation

The *D. cochleata* rhizome was washed with tap water, prior to distilled water and shade dried. The total dried mass was grounded into a fine uniform powder. For getting the flat surface of the sample for LIBS experiment, we have prepared the pellets of the *D. cochleata* rhizome powder using tie along-with pellet making machine (KBr). This pellet was used for further LIBS studies.

LIBS Experiment

The laser source used is Nd:YAG laser (Continuum surelite III-10, USA) operating at 532 nm wavelength and capable of delivering a maximum energy of 425 mJ over a pulse duration of 4 ns at maximum pulse repetition rate of 10 Hz. The resulting spectra were analyzed using OOILIBS software. In this process, the Pulsed laser beam having 15 mJ energy was focused on the surface of *D. cochleata* rhizome sample through a 30 cm plano-convex lens, which makes the plasma on the surface of the sample in its perpendicular direction. Then the plasma cools, and characteristic emission lines from the plasma is collected at 45^0 from incident laser though a collecting lens coupled with the fiber bundle and fed to broad band spectrometer (Ocean Optics 2000+). This spectrometer is connected with the computer, where we can save the spectra of the sample. The identification of elements present in the sample is done with the help of NIST data base (National Institute of Standards and Technology, Electronic database. (Available at: http://physics.nist.gov/physrefdata/asd /linesform.html).

Statistical Analysis

All the results are expressed as mean values standard deviation (SD), n = 3. Data were analyzed using one way analysis of variance (ANOVA) followed by Tukey's multiple comparison *post hoc* test using SPSS software 16.0 versions. Values of $p < 0.05$ were considered statistically significant [23, 24].

RESULTS AND DISCUSSION

Table 1 clearly depicts the presence of numerous constituents, which are responsible for the antioxidant activity. Their presence was rated from copious, slight to moderately present for almost all the crude solvent extracts. An enormous variety of secondary metabolites such as alkaloids, terpenes, saponins, quinines and polyphenols are synthesized exclusively by plants for various purposes, i.e., as a chemical defense against herbivores, toxicity, microbial attack, etc. [25]. Thus, a considerable number of phytoconstituents has showed their presence in qualitative screening tests, as depicted in Table 1. This preliminary work has provided a platform to estimate various contents of those phytoconstituents and thereby the antioxidant assessments. Through the screening tests, it was able to detect the existence of both flavonoid and flavonol. Highest flavonoid content was recorded by acetone extract, which showed its significant potency when compared with other extracts (Table 2).

Table 1: Phytochemical screening of *D. cochleata* rhizome extracts in 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.

Table 2: Estimation of various contents of *D. cochleata* rhizome in different solvents.

Phytochemicals (µg/mg extract)*	Petroleum ether	Chloroform	Ethylacetate	Acetone	Methanol	Water
Total phenolic content	23.17 ± 0.54[a]	71.28 ± 0.81[c]	82.52 ± 1.42[d]	141.92 ± 1.96[e]	80.29 ± 0.20[d]	41.55 ± 0.93[b]
Total flavonoid content	20.59 ± 1.40[a]	55.36 ± 1.61[c]	81.81 ± 1.73[e]	127.23 ± 2.59[f]	62.85 ± 1.07[d]	33.70 ± 0.73[b]
Total flavonol content	15.36 ± 1.28[a]	61.25 ± 0.27[c]	137.13 ± 0.27[e]	143.54 ± 0.87[f]	97.29 ± 0.47[d]	41.19 ± 1.24[b]
Total Tannin content	13.57 ± 2.64[a]	18.19 ± 1.01[b]	42.13 ± 0.20[d]	125.07 ± 1.42[f]	66.27 ± 0.88[e]	26.97 ± 0.41[c]
Total ascorbic acid content	16.00 ± 1.16[a]	32.21 ± 0.80[c]	76.64 ± 1.08[e]	104.09 ± 0.81[f]	52.56 ± 1.52[d]	24.14 ± 0.80[b]
Total protein content	21.60 ± 2.11[a]	49.36 ± 0.74[c]	78.97 ± 0.45[e]	110.93 ± 0.74[f]	59.32 ± 0.90[d]	37.24 ± 0.74[b]
Total carbohydrate content	17.49 ± 0.61[a]	47.98 ± 0.33[b]	62.03 ± 1.42[d]	75.03 ± 4.66[e]	55.83 ± 0.73[c]	58.05 ± 0.35[c,d]

Data represent the mean ± S.D (n=3). Mean values of each row followed by different superscript letter (a-f) significantly differ (f > e > d > c > b > a) significantly differ when subject to Tukey's multiple comparison test ($p<0.05$).

The next highest was the ethylacetate (81.81±1.73 µg) extract, yet not upto the mark of the former extract. On the other hand, the lower contents were seen in water (33.70±0.73 µg) and petroleum ether (20.59±1.40 µg) extracts. Moreover, an excellent amount of phenol and flavonoid were recorded in which acetone and ethylacetate has significant contents. Total phenolic contents have also produced richest amounts about 141.92±1.96 µg for competing with the total flavonol content acetone extract, which was the highest content (143.54±0.87 µg) among the others. Hence, it can be guessed roughly that flavonols would be present if isolated, rather than flavonoids. Whatsoever, satisfactory contents were projected by almost all the crude extracts with regard to acetone. Expect tannin content, the increasing orders obtained were similar for total phenol, total flavonoid and total flavonol contents: acetone > ethylacetate > methanol > chloroform > water > petroleum ether as tabulated in Table 2. Acetone extract has replenished its effect in total tannin content too. As reported there have been a correlation demonstrated a link between antioxidant in plants with total phenolic and flavonoid content [26].

Carbohydrate content was found significant in acetone extract (75.03±4.66µg). Ethylacetate extract has given a good competence with a content of 62.03±1.42 µg. A least content was produced by petroleum ether (17.49±0.61 µg) extract, water extract has surprisingly good content of about 58.05±0.35 µg, the reason behind it may be the tendency of carbohydrates being water soluble components. Methanol extract showed a closer carbohydrate content of about 55.83±0.73 µg, which is to be noted. But contrastingly, in both protein and vitamin C content, methanol has exhibited higher contents when compared with the water extract. In fact, the chloroform extract proceeded ahead of water extract producing contents of 49.36±0.74 µg for protein and 32.21±0.80 µg for ascorbic acid. Obviously, for the above estimations, acetone had richer contents followed by ethylacetate extract. Thus, from the above discussion, for a deeper and clear understanding, it can be revised that the acetone extract has been significant throughout the estimations carried out and the next is ethylacetate except for the total tannin content. Other extracts took their own capacity being efficient in the order discussed earlier. *D. cochleata* rhizome has not only showed its ability to be used as pharmacological agent (phenolic, flavonoid, flavonol and tannin content), but also as a supplement (carbohydrate, protein and Vitamin-C) after further *in vivo* investigation. All these above records can be compared with our earlier reports on *D. cochleata* leaves, wherein it has enclosed a systematic profile of the above estimations; in which acetone has left its strong foot print too [7].

Antioxidants may offer resistance to oxidative stress by scavenging free radicals, inhibiting lipid peroxidation, terminating nitric and superoxides, etc., thus prevent the onset of deadly diseases. Thus, to document the underexploited species, strategies to assess the scavenging effects of *D. cochleata* rhizome assays such as DPPH radical scavenging activity, reducing power, nitric oxide scavenging activity, superoxide radical scavenging activity has been performed as shown in results. They are most commonly used, mainly because of their easy performing, high reproducibility and accuracy [27, 28].

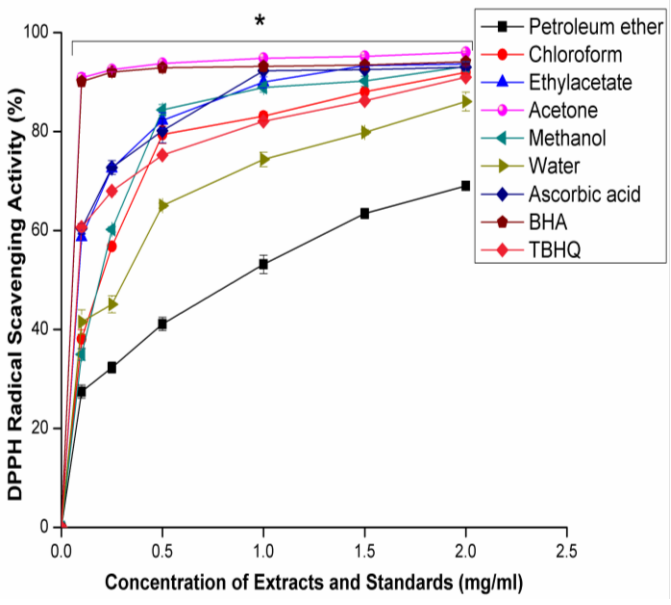

Fig. 1: DPPH radical scavenging activity. Each value represents a mean ± SD (*p < 0.05).

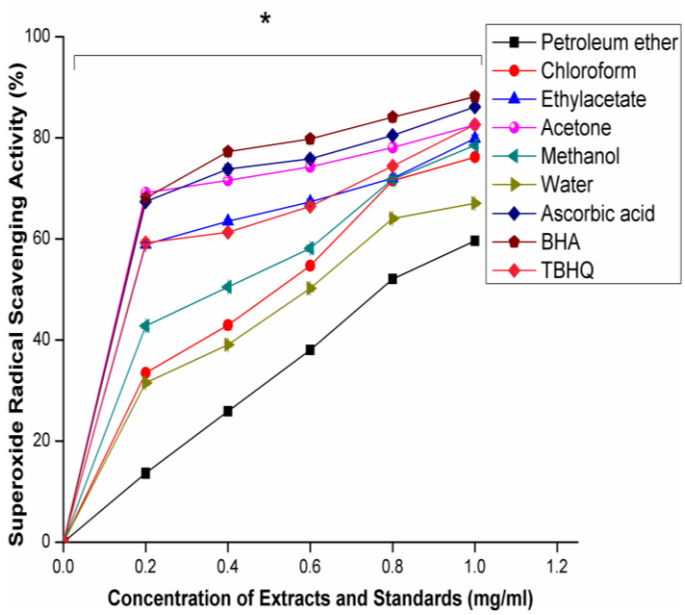

Fig. 2: Superoxide radical scavenging activity. Each value represents a mean ± SD (*p < 0.05).

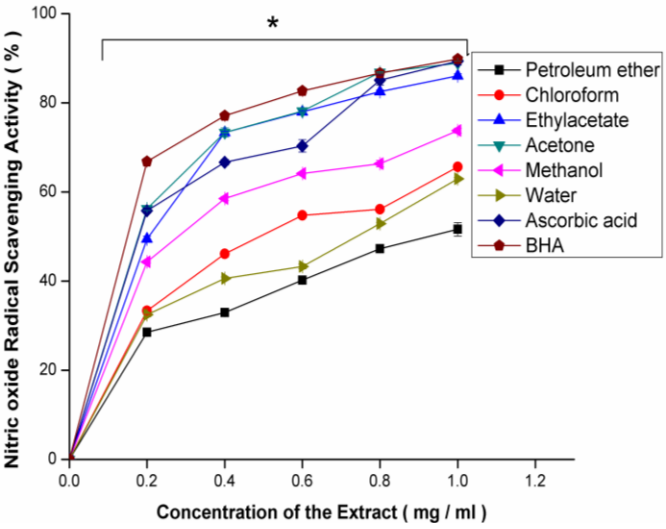

Fig. 3: Nitric oxide radical scavenging activity. Each value represents a mean ± SD (*p < 0.05).

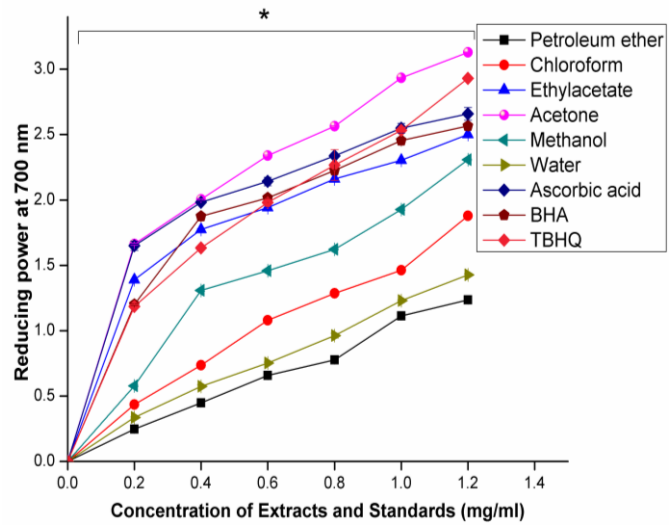

Fig. 4: Reducing power activity. Each value represents a mean ± SD (*p < 0.05)

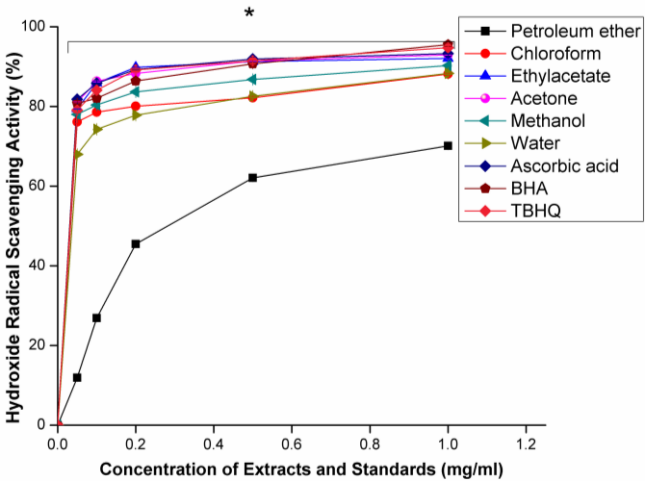

Fig. 5: Hydroxide radical scavenging activity. Each value represents a mean ± SD (*p < 0.05).

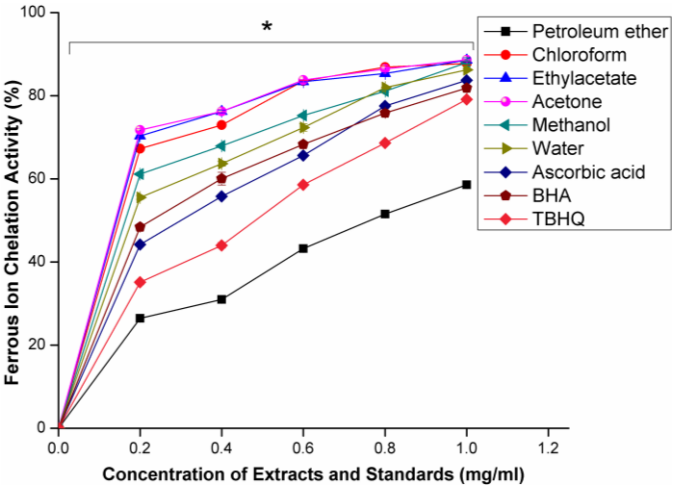

Fig. 6: Ferrous ion chelating activity. Each value represents a mean ± SD (*p < 0.05).

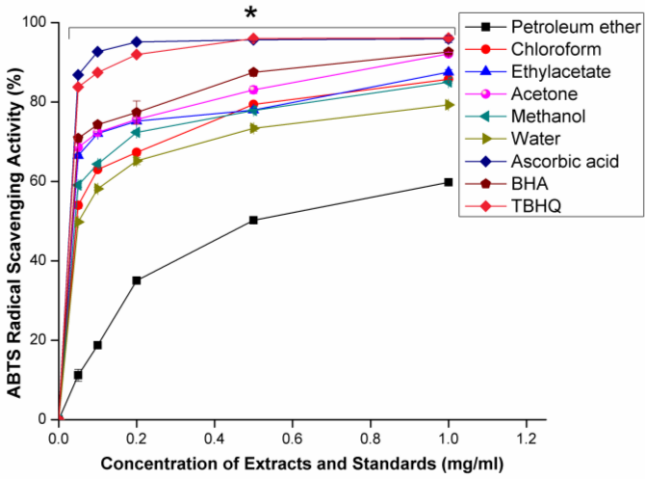

Fig. 7: ABTS radical scavenging activity. Each value represents a mean ± SD (*p < 0.05)

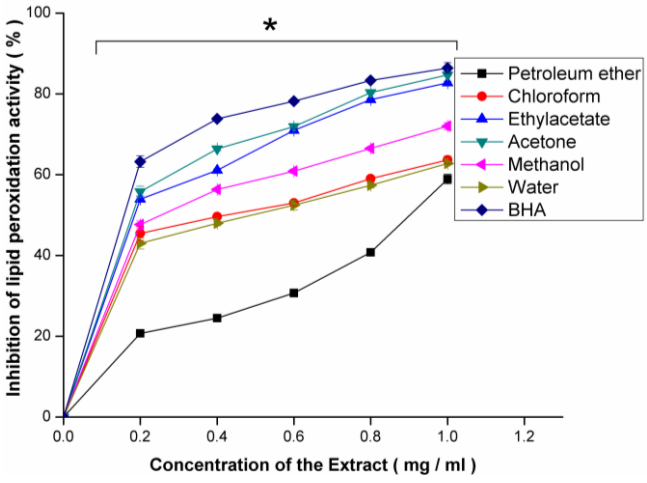

Fig. 8: Inhibition of lipid peroxidation. Each value represents a mean ± SD (*p < 0.05).

Overall, the increasing order of activity for all the antioxidant assays were exactly portraying the efficiencies of various extracts with respect to their own polarity of constituents got dissolved in them. Our results of various free radical-scavenging assays have established the antioxidant potential of the various *in vitro* systems, compared with suitable standards such as BHA, ascorbic acid and TBHQ. As given in the results, to a great surprise, acetone extract was found to be more significant than the standard proving its potency, thereby being highly effective than the synthetic standard (Fig. 1). The DPPH scavenging values for investigated extracts/fractions were expressed as IC_{50}, the IC_{50} values for different extracts were 191.1, 81.31, 54.1, 183.7, 203.6, 862 µg for chloroform, ethylacetate, acetone, methanol, water and petroleum ether among which the potent scavenger was acetone fraction. Striking evidence is that the potency of BHA was ~0.04 µg lesser than the acetone fraction in its inhibition concentration. The next standard ascorbic acid (84.45 µg) also exhibited lesser activity than ethylacetate (81.31) and thereby acetone fraction. There was observed a gradual decrease in absorption with increase in concentration as the intensity of DPPH decreased owing to scavenging of the electrons by the extracts. Likewise, superoxide radical scavenging activity has produced good results with significant IC_{50} values, among which there has been a close competition between ascorbic acid (standard), acetone and next the BHA (Fig. 2). An antioxidant being such potent than the standards should be appreciable, since it scavenges the superoxide radicals that induce tissue damage by reacting with the biological membranes. The inhibition effects of the extracts by scavenging the super oxides evidenced by the color intensity of PMS decreasing. This nitric oxide assay shows the effective electron donating property of the extracts portraying their antioxidant capacity. The absorption values were found to decrease as the extract scavenges the nitric oxide, with increase in concentration (Fig. 3). IC_{50} value of acetone extract (176 µg) was found to be potent against with BHA (149.49 µg) and ascorbic acid (176.14 µg). For a note the IC_{50} values of chloroform, ethylacetate, methanol and water extracts are tabulated in Table 3. Nitric oxide radical ($NO^{\bullet}$) is a highly reactive molecule that participates in signal transduction in the cardiovascular and immune systems. It is often characterized by contrasting actions as it can exhibit antioxidant and pro-oxidant functions.

All the extracts exhibited NO scavenging effects in which the maximum inhibition was produced by the acetone extract, obviously. Scavengers of nitric oxide compete with oxygen leading to reduced production of nitric oxide [29]. Thus, the important effects of the various extracts of scavenging the free radicals has been made possible and attempts can be made on the pharmacological effects of *D. cochleata* rhizome.

Table 3: Scavenging activities and reducing power activity IC_{50} values compared with standards like ascorbic acid, BHA and TBHQ.

Various Solvent Extracts and Standards	Scavenging Activities and Reducing power IC_{50} (µg)							
	DPPH	Nitric oxide	Hydroxide	Superoxide	Ferrous ion chelation	ABTS	Lipid peroxidation (TBARS)	Reducing power
Petroleum ether	862.00	716.70	281.16	767.88	753.49	429.27	895.82	446.70
Chloroform	191.10	309.95	32.21	513.06	146.82	47.13	416.03	241.22
Ethylacetate	81.31	203.30	30.08	170.81	139.89	37.54	182.54	69.73
Acetone	54.10	176.00	29.01	141.49	137.76	36.47	175.08	54.50
Methanol	183.70	272.30	32.74	377.12	161.75	40.74	247.05	171.57
Water	203.60	527.99	34.87	582.36	178.28	50.87	480.54	334.78
Ascorbic acid	84.45	176.14	25.81	144.69	294.49	27.94	-	56.86
BHA	54.14	149.49	29.54	146.29	218.79	34.87	155.89	65.05
TBHQ	74.30	135.63	31.14	163.35	476.81	27.95	194.27	79.66

For measurement of the reductive ability, we investigated the Fe^{3+} to Fe^{2+} transformation for all the extracts using the method of Oyaziu (1986) [13], witnessing the electron donating capacity. Moreover, the reducing capacity of any phytocompounds may serve as a significant indicator of its potential antioxidant activity. The results indicated the antioxidant properties of almost all the extracts, in which the acetone extract has provided its efficiency with IC_{50} value is 54.5 µg. There has been observed a gradual increase in the absorption values with increasing concentration, for all the extracts (Fig. 4). In this activity, ethylacetate extract showed (69.73 µg) lesser activity than the BHA (65.05 µg). In the reducing power assay, the presence of antioxidants in the extracts results in the reduction of the Fe^{3+}/ferricyanide complex to its ferrous form. There can be made positive correlation between the total phenol and reducing power activity. Acetone extract had a significant total phenolic content (141.92±1.96 µg), which reasoned the excellent reducing power activity. Also it can be correlated with total flavonoid, total tannin and flavonol content.

The hydroxide radical scavenging activity of *D. cochleata* rhizome in various solvent extracts in different concentration (0.05, 0.1, 0.2, 0.5 and 1 mg/ml) is shown in Fig. 5. If the potent hydroxide radical scavenging activity in acetone extract (92.94 ± 0.03%) at maximum followed by ethylacetate extract (92.08 ± 0.08%) compared with ascorbic acid (93.30 ± 0.08%), BHA (95.50 ± 0.40%) and TBHQ (94.73 ± 0.07%). The methanol (90.25 ± 0.12%) and chloroform (88.14 ± 0.29%) has moderate percent of scavenging activity (Fig. 5). The least activity was shown in water (88.30 ± 0.22%) and petroleum ether extracts (70.09 ± 0.19%) at 1 mg/ml concentration. Hydroxyl and hydroperoxide radicals were initiating the free radical chain reaction [16]. The presence of transition metal ions in biological system, it will produce hydroxide radicals in Fenton type reaction. *D. cochleata* rhizome acetone extract has higher ferrous ion chelating capacity, the 200 µg of acetone extract chelation around 71.75±0.35 % of ferrous ion (Fig. 6). The metal ion chelating potential of the extract/compound is related to spatial conformation, position and number of electron donating groups [30, 31]. ABTS radical scavenging activity measured on the green color $ABTS^{\bullet-}$ radical scavenge the bioactive compounds present in extracts at 734 nm (Fig. 7). The acetone extract has higher ABTS radical scavenging activity, e.g., 50 µg of acetone extract (68.60±0.17%) scavenge radical when compared with other extracts such as ethylacetate (66.52±0.37%), methanol (59.07±0.52%), chloroform (53.99±0.81%), water (49.78±0.29%) and petroleum ether (11.18±1.53%) extracts. Petroleum ether has least ABTS radical scavenging activity owing to very low amount of phenolic and flavonoid content. Polyphenolic compounds and ABTS radical scavenging activities shows good correlation between the rate law and stoichiometric factor [32].

The measure of thiobarbituric acid reactive substance (TBARS) has been widely used in studies of anti-lipid peroxidation activity of natural phytochemicals. It is noteworthy that almost at all concentration, all the extracts, displayed the best inhibitory effect against lipid peroxidation and thus being capable of inhibiting TBARS formation (Fig. 8). Acetone showed the potent anti-lipid peroxidation activity among the others with respect to its standard (ascorbic acid and BHA), whose IC_{50} values were 175.08 and 155.88 µg, respectively. This is the only assay which showed lesser activity in contrast to the standard.

The results of antimicrobial activity of various solvent extracts of rhizome of *D. cochleata* were done by agar well diffusion (Table 4). All extracts portrayed good to moderate antimicrobial

activity against most of the tested human pathogens. Acetone extract, contributed significantly high activity against all tested pathogens and maximum activity was observed in *E. faecalis* (24 mm) followed by *C. albicans* (20 mm). Ethylacetate extract expressed good antimicrobial activity against most of the tested microorganisms highest activity detected in *C. albicans* (17 mm) followed by *E. faecalis* (14 mm). Moderate antimicrobial activity was observed in methanol, chloroform and petroleum ether extracts. Water extract showed least activity against all tested human pathogens. Nil activity was recorded in standard drug fluconazole at the tested concentration this may be due to the resistance of the fungal strains. The findings of our results are comparable with earlier reports of Thomas [33] who screened the antibacterial activity of petroleum ether, acetone, methanol and water extracts of leaves of *D. cochleata* against some pathogenic bacterial strains.

Among all the tested extracts acetone extract exhibited significantly high antibacterial activity, which strengthen the outcome of the present study. Lee *et al.* [34] has studied that the antibacterial activity of various solvent crude extracts and isolated components from the rhizome *D. crassirhizoma* by disc method. The tested extracts and compounds were highly active against Gram-positive bacteria (such as, methicillin-resistant *Staphylococcus aureus*, *Streptococcus mutans* and *Bacillus subtilis*). The extracts and compounds were not active against fungi which supported our findings.

Table 4: Antimicrobial activity of *D. cochleata* rhizome in various solvent extracts by agar well diffusion method.

Organism tested	Various solvent extracts (mm)*						
	Petroleum ether	Chloroform	Ethyl acetate	Acetone	Methanol	Water	Control
B. subtilis	3.67 ± 0.58^{a}	10.33 ± 0.58^{b}	11.67 ± 0.58^{b}	15.33 ± 0.58^{c}	11.33 ± 1.15^{b}	4.00 ± 1.00^{a}	$19.5 \pm 0.55^{d\ \#}$
E. faecalis	6.33 ± 0.58^{a}	13.67 ± 0.58^{b}	14.00 ± 1.00^{b}	24.33 ± 1.53^{c}	$10.00 \pm 0^{a,b}$	7.00 ± 2.00^{a}	$22.50 \pm 2.26^{c\ \#}$
K. pneumoniae	4.67 ± 1.53^{a}	11.33 ± 0.58^{b}	11.67 ± 1.15^{b}	17.33 ± 1.53^{c}	10.00 ± 1.00^{b}	5.00 ± 1.00^{a}	$20.0 \pm 0.89^{c\ \#}$
S. aureus	07.67 ± 1.53^{b}	$11.00 \pm 1.00^{c,d}$	11.33 ± 1.15^{d}	15.67 ± 0.58^{e}	$8.67 \pm 1.15^{b,c}$	0.33 ± 0.58^{a}	$25.50 \pm 0.55^{f\ \#}$
S. epidermis	$4.00 \pm 1.00^{a,b}$	1.33 ± 0.58^{a}	9.33 ± 0.58^{b}	16.33 ± 1.53^{d}	6.00 ± 0^{b}	0.33 ± 0.58^{a}	$27.17 \pm 2.64^{e\ \#}$
S. flexneri	05.67 ± 0.58^{a}	9.00 ± 1.00^{b}	$10.33 \pm 0.58^{b,c}$	11.67 ± 0.58^{c}	9.67 ± 0.58^{b}	6.67 ± 0.58^{a}	$25.17 \pm 0.75^{d\ \#}$
P. vulgaris	5.00 ± 1.00^{a}	11.67 ± 0.58^{b}	10.67 ± 2.08^{b}	15.67 ± 0.58^{c}	10.0 ± 1.00^{b}	9.00 ± 1.00^{b}	$40.50 \pm 0.84^{d\ \#}$
C. albicans	14.33 ± 0.58^{b}	$16.67 \pm 1.15^{c,d}$	17.00 ± 1.00^{d}	20.67 ± 0.58^{e}	$14.67 \pm 0.58^{b,c}$	0^{a}	-†
A. niger	8.67 ± 1.15^{b}	8.33 ± 1.53^{b}	10.00 ± 0^{b}	10.33 ± 0.58^{b}	8.67 ± 0.58^{b}	0^{a}	-†
C. neoformans	7.00 ± 1.73^{b}	9.67 ± 1.15^{c}	14.67 ± 0.58^{d}	20.00 ± 0^{e}	14.67 ± 0.58^{d}	0^{a}	-†

= Streptomycin (10 µg/ml); † = Fluconazole (10 µg/ml)

*Data represent the mean ± S.D (n=3). Mean values of each row followed by different superscript letter significantly differ (e > d > c > b > a) when subject to Tukey's multiple comparison test ($p<0.05$)

The genera of Dryopteris possess good antimicrobial property. Many species in these genera were screened for their antimicrobial activity (*D. filix* [35, 36], *D. crassirhizoma* [33, 37, 38], *D. cochleata* [39], *D. chrysocoma* [40], *D. cochleata* [33] and *D. syrmatica* [41]), which supported results of the present investigations. In contrary, the study of Voravuthikunchai et al. [42] results revealed that *D. syrmatica* extracts showed no antimicrobial activity against all tested pathogens at the tested concentration, which was dissimilar with the present findings. All the tested rhizome extracts of *D. cochleata* possessed good antimicrobial property that may be used as a traditional medicine to treat the infectious diseases.

From the GC–MS results, it can be noted that enormous numbers of compounds have been identified tentatively (Fig. 9 and Table 5). For instance, many aliphatic ester and ketones, heterocyclic, alkaloids, alkanes, terpene, phenolics, flavonoids, silane compounds have been identified. The retention time at 9.75, 10.06, 14.12, 16.13, 20.95, 21.54 and 26.05 were considered important which were responsible for the phenolics, flavonoids with molecular weight 110, 170, 194, 302, 182, 224, 328, respectively. Higher antioxidant of acetone extract may be due to (i) higher quantity of secondary metabolites (ii) compound solubility higher in acetone than other solvents [23].

Minerals are involved in structural components of human tissues, resources of acid-base balance and maintain the body fluids, transport of gases and muscle contractions [22]. The LIBS spectrum of *D. cochleata* rhizome sample in wavelength range 200-500 and 550-900 nm in air atmosphere are shown in Fig. 10 and 11, respectively. LIBS spectra results clearly designate the presence of calcium (315.9, 317.9, 370.6, 393.2, 396.7, 422.6, 430.2, 442.5, 443.4, 445.4, 487.7, 558.9, 606.0, 612.2, 616.2, 643.8, 646.1, 714.6 and 854.1 nm), magnesium (279.4, 285.1 and 383.7 nm), oxygen (407.7, 777.3 and 844.6 nm), carbon (2290.6 and 247.8 nm), silicon (251.1 and 585.8 nm), aluminium (308.2 and 309.33 nm), iron (239.5, 240.4, 358.1, 371.9, 373.7 and 438.3 nm) strontium (421.1 and 460.7 nm), nitrogen (567.4, 746.7 and 867.9 nm), barium (455.3, 493.3, 553.9, 599.3, 649.6 and 719.9 nm), sodium (589.4 and 821.7 nm), hydrogen (656.2 nm) and potassium (404.3, 766.4, 769.9 and 794.8 nm) were identified using OOILIBS software.

Calcium is an important element for bones, nail, hair, teeth development and nerve, muscle and heart functions. It is activates prothrombin to thrombin, blood coagulation, enzyme activation, membrane permeability, muscle contraction, normal transmission of nerve impulses and muscles by regulating endo-exoenzymes and blood pressure[43, 44]. Calcium deficiency causes rickets, back pain, osteoporosis, indigestion, irritability, premenstrual tension and cramping of the uterus [45]. Magnesium is essential element for activation of many enzymes (cofactors) such as phosphate-transferring enzyme myokinase, diphophopyridinenucleotide kinase and creatine kinase, act as a cofactor for thymine pyrophosphate [43]. Magnesium prevents heart diseases, requirement in plasma, extra cellular fluid, maintaining osmotic equilibrium, nucleotide participation, etc. [46].Iron is essential for formation of hemoglobin, myoglobin and cytochrome [47]. It is an essential compound of many enzymes (cytochrome c, c_1, a_1, etc.,), respiration [48]. Its main role was myelination of spinal

cord, cerebellar folds in brain (white part) and neurotransmitter synthesis act as cofactor [49].

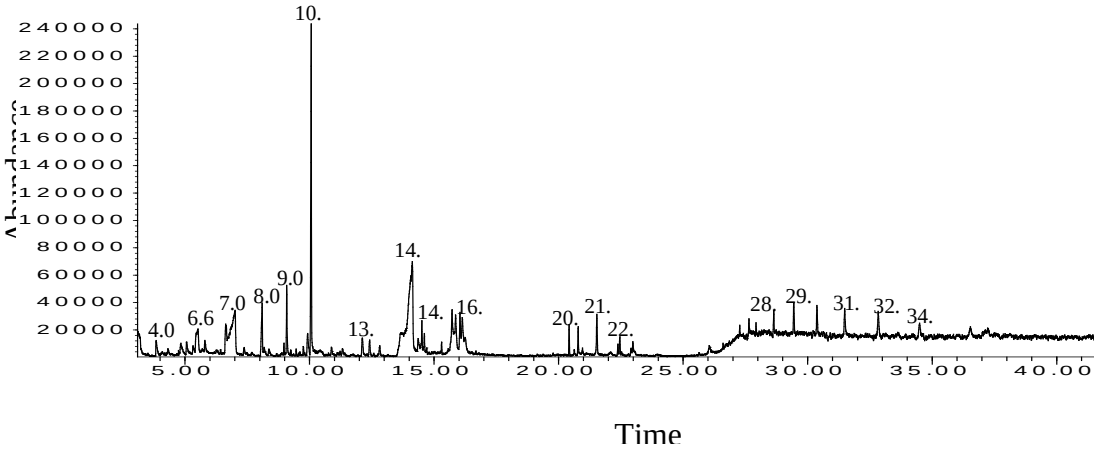

Fig. 9: Chromatogram obtained from the GC-MS in the extract of *D. cochleata* rhizome

Table 5: Tentative identification of active compounds by GC-MS analysis

S. No.	*RT*	*Peak area*	*Name*	*Molecular Formula*	*Compound Nature*
1.	3.8417	0.8937	2-Propenoic acid, methyl ester	$C_4H_6O_2$	Aliphatic ester
2.	4.8362	1.0214	Furfural	$C_5H_4O_2$	Heterocyclic compounds
3.	5.0648	0.7022	2-Furanmethanol	$C_5H_6O_2$	Heterocyclic compounds
4.	5.322	0.4175	Urea, trimethyl-	$C_4H_{10}N_2O$	Aliphatic amide
5.	5.5163	2.4766	Phosphine, triethyl-	$C_5H_{15}P$	Phosphine
6.	5.8021	0.5312	2-Hydroxy-2-cyclopenten-1-one	$C_5H_6O_2$	Cyclic ketone
7.	6.6365	1.8343	4-Octanone, 2-methyl-	$C_9H_{18}O$	Aliphatic ketone
8.	6.728	0.8012	Methanamine, N-methoxy-	C_2H_7NO	N-substituted amine
9.	7.008	7.5544	Propane, 2-fluoro-2-methyl-	C_4H_9F	Aliphatic halide
10.	7.3795	0.3112	Piperazine, 1,4-dimethyl-	$C_6H_{14}N_2$	Heterocyclic compounds
11.	8.0939	2.2486	2,4(1H,3H)-Pyrimidinedione, 5-methyl-	$C_5H_6N_2O_2$	Heterocyclic compounds
12.	8.1797	0.5468	3-Furancarboxylic acid, methyl ester	$C_6H_6O_3$	Heterocyclic compounds
13.	8.3683	0.485	6,6-dideutrononen-1-ol-3	$C_9H_{16}D_2O$	Unsaturated alcohol
14.	8.9741	0.3146	2-Propanamine, N-methyl-N-nitroso-	$C_4H_{10}N_2O$	Nitroso compound
15.	9.0884	2.049	4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl-	$C_6H_8O_4$	Heterocyclic compounds
16.	9.7514	0.2556	1,2-Benzenediol	$C_6H_6O_2$	Phenolic compound
17.	9.9228	1.2287	2-Undecyl-tetrahydropyran	$C_{16}H_{32}O$	Heterocyclic compounds
18.	10.0657	9.5759	3,4,5-trihydroxy benzoic acid (Gallic acid)	$C_7H_6O_5$	Phenolic compound
19.	10.883	0.3536	1-(Methylthio)-3-pentanone	$C_6H_{12}OS$	Aliphatic ketone
20.	11.3231	0.2929	cis-4-Nonene	C_9H_{18}	Alkene
21.	12.1232	1.0293	1-Ethyl-3-methylcyclohexane (c,t)	C_9H_{18}	Cyclic hydrocarbon
22.	12.4147	0.7123	Methyl 4-pentynoate	$C_6H_8O_2$	Aliphatic ester
23.	12.8205	0.5412	2,3-Diazabicyclo[2.2.1]hept-2-ene, 1-phenyl-	$C_{11}H_{12}N_2$	Azo compound
24.	13.6606	2.7749	2,2-Di(hydroxymethyl)butyl allyl ether	$C_9H_{18}O_3$	Hydroxy compound
25.	13.7635	1.7197	(tetrahydroxycyclopentadienone)tricarbonyliron (0)	$C_8H_4FeO_8$	Hydroxy compound
26.	14.1293	16.5205	Ferulic acid	$C_{10}H_{10}O_4$	Phenolic compound
27.	14.3636	0.886	β-D-Glucopyranose	$C_6H_{12}O_6$	Carbohydrate
28.	14.5122	1.1248	5,5,9 – trimethyl-3-methylene-2,3,5,6,7,8,9,9a-octahydro-1H-benzo[7]annulene	$C_{15}H_{24}$	Cyclic hydrocarbon
29.	14.6094	0.5651	Bicyclo[7.2.0]undec-4-ene, 4,11,11-trimethyl-8-methylene-, [1R-(1R*,4E,9S*)]-	$C_{15}H_{24}$	Bicyclic compound
30.	15.3066	0.2724	1,6,10-Dodecatrien-3-ol, 3,7,11-trimethyl-, [S-(Z)]-	$C_{15}H_{26}O$	Alkene
31.	15.7239	3.426	3-(o-Azidophenyl)propanol	$C_9H_{11}N_3O$	Azide compound

32.	15.8668	2.7368	(S)-(+)-5-sec-Butyl-2-pyrimidinol	$C_8H_{12}N_2O$	Hetrocyclic compound
33.	16.0439	1.7595	D-(-)-Quinic acid	$C_7H_{12}O_6$	cyclic polyol
34.	16.1354	2.5896	Quercetin	$C_{15}H_{10}O_7$	Flavonoid
35.	16.2554	0.9874	6-Desoxy-l-altritol	$C_6H_{14}O_5$	Carbohydrate
36.	20.4218	0.6542	Hexadecanoic acid, methyl ester	$C_{17}H_{34}O_2$	Aliphatic saturated ester
37.	20.7876	0.8885	n-Hexadecanoic acid	$C_{16}H_{32}O_2$	Aliphatic saturated carboxylic acid
38.	20.9591	0.2976	Ethanone, 1-(2,6-dihydroxy-4-methoxyphenyl)-	$C_9H_{10}O_4$	Phenolic compound
39.	21.5421	1.4945	Aspidinol (or) 1-(2,6-dihydroxy-4-methoxy-3-methoxyphenyl)-1-butanone	$C_{12}H_{16}O_5$	Phenolic compound
40.	22.3879	0.315	9-Eicosyne	$C_{20}H_{38}$	Alkyne
41.	22.4622	0.5883	10-Octadecenoic acid, methyl ester	$C_{19}H_{36}O_2$	Aliphatic unsaturated ester
42.	22.9137	0.2948	8-Heptadecyne, 1-bromo-	$C_{17}H_{31}Br$	Alkyne
43.	22.9823	0.7361	9-Octadecenoic acid (Z)-	$C_{18}H_{34}O_2$	Aliphatic unsaturated acid
44.	26.0572	0.4608	Cyclopenta[c]furo[3',2':4,5]furo[2,3-h][1]benzopyran-1,11-dione, 2,3,6a,9a-tetrahydro-3-hydroxy-4-methoxy-, [3S-(3.α.,6a.α,9a.α.)]-	$C_{17}H_{12}O_7$	Phenolic compound
45.	26.6058	0.3791	Octadecane, 3-ethyl-5-(2-ethylbutyl)-	$C_{26}H_{54}$	Alkane
46.	26.7144	1.7648	Gibberellin A3	$C_{19}H_{22}O_6$	Tetracyclic diterpene acid
47.	26.863	0.5888	Bis(trimethylsiloxy)methylsilane	$C_7H_{22}O_2Si_3$	Silane
48.	27.0002	0.984	Silicic acid, diethyl bis(trimethylsilyl) ester	$C_{10}H_{28}O_3Si_3$	Silane ether
49.	27.2002	1.5827	1,1,1,3,5,5,5-Heptamethyltrisiloxane	$C_7H_{22}O_2Si_3$	Siloxane
50.	27.2745	1.6854	Sulfurous acid, pentadecyl 2-propyl ester	$C_{18}H_{38}O_3S$	Alkyl sulphonic ester
51.	27.366	1.3072	Silane, 1,4-phenylenebis[trimethyl]-	$C_{12}H_{22}Si_2$	Silane
52.	27.5546	0.9322	Cyclotrisiloxane, hexamethyl-	$C_8H_{18}O_3Si_3$	Cyclic siloxane
53.	27.646	1.6554	Di-(2-ethylhexyl)phthalate	$C_{24}H_{36}O^{2-}_4$	Aromatic carboxylic acid
54.	27.9318	1.2509	N-ethyl-1,3-dithioisoindoline	$C_{10}H_9NS_2$	Alkaloid
55.	28.6405	1.2276	Indole-2-one	C_8H_5NO	Alkaloid
56.	29.4463	1.6901	Eicosane	$C_{20}H_{42}$	Alkane
57.	30.9095	0.4249	2-Ethylacridine	$C_{15}H_{13}N$	Alkaloid
58.	31.4867	1.3105	n-Octadecane	$C_{18}H_{38}$	Alkane
59.	36.539	0.2182	Silicic acid, diethyl bis(trimethylsilyl) ester	$C_{10}H_{28}O_4Si_3$	Siloxane
60.	37.1392	0.2973	1,1,3,3,5,5-hexamethyl-cyclohexasiloxane	$C_6H_{18}O_3Si_3$	Cyclic siloxane
61.	37.2306	0.0714	2-Ethylacridine	$C_{15}H_{13}N$	Alkaloid

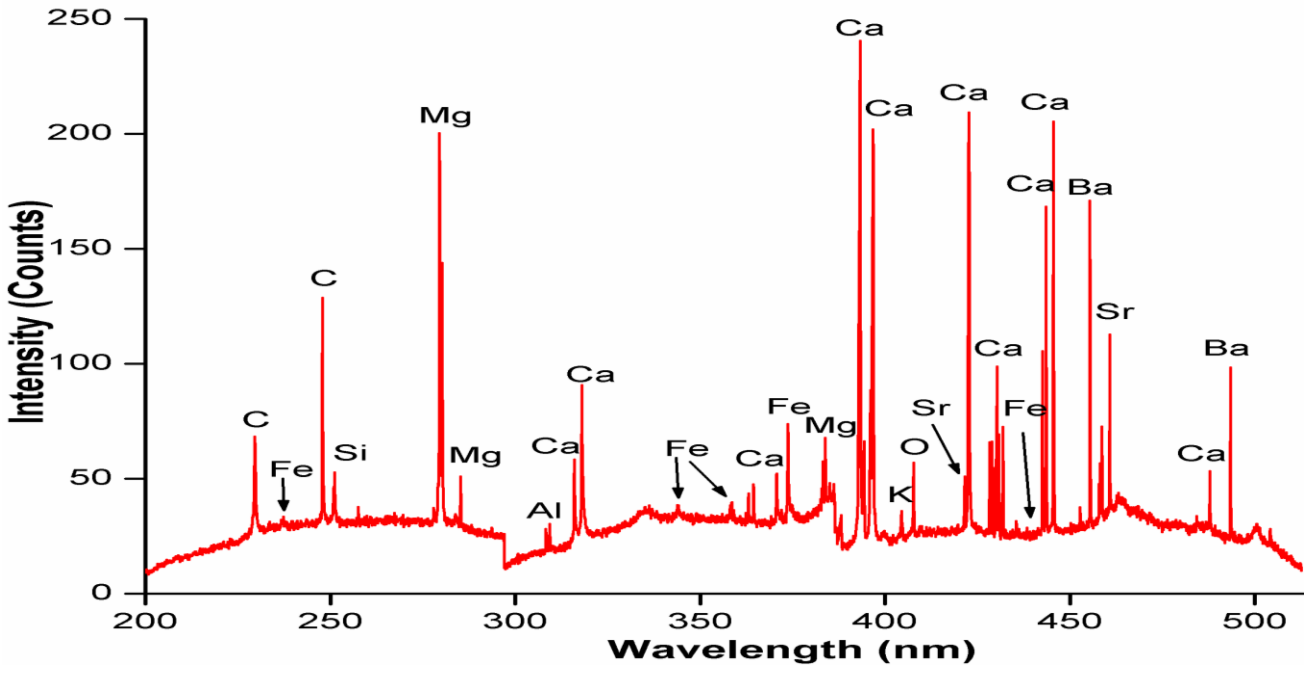

Fig. 10: LIBS spectra in air atmosphere in the spectral range 200–500 nm (laser energy 15 mJ, gate delay 1.5 µs)

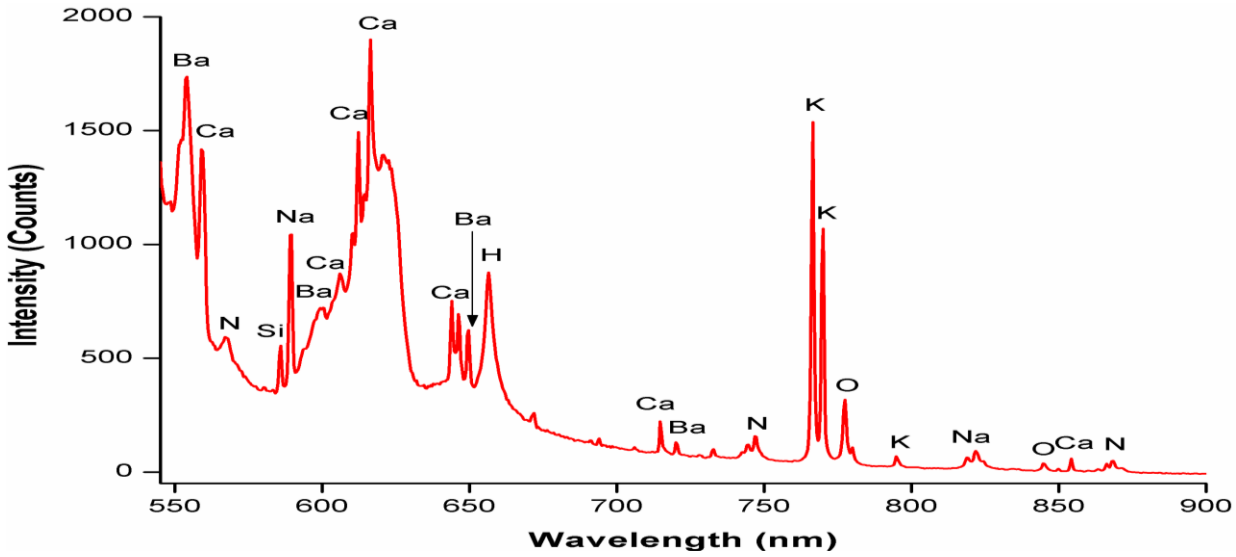

Fig. 11: LIBS spectra in air atmosphere in the spectral range 550–900 nm.

Iron deficiency causes anemia, weakness, depression, poor resistance to infection [50, 51]. Potassium is essential for intracellular fluid, acid-base balance, osmotic pressure regulation, conduction of nerve impulse, transfer of phosphate from ATP to pyruvic acid, cellular enzyme reactions, prevent hyperkalemia and Addison's diseases [43]. Sodium is primary source for extracellular fluids, maintain acid-base balance, osmotic pressure and plasma volume. It prevents irritability of muscles, cell permeability, nerves activation, muscle function, membrane potential maintenance, nerve pulse transmission, absorption process for monosaccharides, amino acids, pyrimidines and bile salts [43, 52]. Its diuretic nature and Na plays an important role in the transport of metabolites. The Na/K ratio for food is an important factor in prevention of hypertension arteriosclerosis, were Na enhances and K depresses blood pressure [53].

Silicon is an important element for structure of collagenous connective tissues and bone formation [54]. It is a necessary constituent of some mucopolysaccharides, hyaluronic acid and chondroitin-4-sulfate. This compound is important for tissue connectivity [55]. It plays an essential role in bone calcification and glycosaminoglycan metabolism in cartilage and connective tissue [43]. Strontium is essential for bone, teeth calcification and higher incidence for carious teeth [52]. Sodium, potassium, magnesium, calcium, manganese, copper, zinc and iodine daily intake reduces individual risk factors and prevent cardiovascular disease for both human beings and animals [56].

From the above evidences, it can be elucidated that *D. cochleata* rhizome consists enormous variety of pharmacological constituents, therapeutic phytocompounds responsible for its potent antioxidant, antibacterial, antifungal activity and nutrient elements.

CONCLUSION

The antioxidant capacities, protein, carbohydrate, vitamin contents etc of *D. cochleata* rhizome accomplished with several *in vitro* biochemical assays, antibacterial, antifungal activities have been clearly portrayed. Among the crude extracts evaluated, acetone showed the highest antioxidant capacity, which may be a valuable source of natural antioxidants. A strong correlation studies, supporting *in vitro* assays, various contents has implied that antioxidants in these plants were capable of scavenging free radicals and reducing oxidants. Moreover, GC–MS analysis showed the existence of various antioxidant compounds with variable chemical structure. For identification of plant nutrition, we have recorded the LIBS spectrum which shows presence of nutrient element calcium, magnesium, silicon, iron, barium, aluminium, sodium and potassium. Hence, higher antioxidant potential and nutrient elements of *D. cochleata* rhizome indicates that the plant may be used as a replacement source of synthetic antioxidants and nutrient stuff. All the fruitful results encourages, *in vivo* studies, further isolation and purification of bioactive components to explore their therapeutical potential too.

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REFERENCES

1. Safer AM, Nughamish AJ. Hepatotoxicity induced by the antioxidant food additive BHT in rat: An electron microscopical study. Histol and Histopathol 1999;4:391-406.
2. Carla DG, Mascolo N, Izzo AA, Capasso F. Flavonoids: old and new aspects of a class of natural therapeutic drugs. Life Sci 1999;65:337-353.
3. Falleh H, Jalleli I, Ksouri R, Ksouri R, Boulaaba M, Guyot S, Magne C. Abdelly C. Effect of a salt treatment on phenolic compounds and antioxidant activity of two mesembry anthemum edule provenances. Plant Physiol and Biochem 2012;52:1-8.
4. Heena P, Achaleshwar B. *In vitro* antimicrobial activity of fronds (leaves) of some important pteridophytes. J Microbiol Antimicrobial 2010;2(2):19-22.
5. Singh S, Dixit RD, Sahu TR. Some medicinally important Pteridophytes of Central India, Int J forest Usufructs Manag 2003;4(2):41-51.
6. Kunjani J, Ananda RJ. Ethnobotanical studies on some lower plants of the central development region. Nepal Ethanobot Leaf 2008;2(1):832-840.
7. Kathirvel A, Sujatha V. Phytochemical studies, antioxidant activities and identification of active compounds using GC-MS of *Dryopteris cochleata* leaves. Arab J Chem 2012;(http://dx.doi.org/10.1016/j. arabjc.2012.03.018).
8. Cremers DA, Chinni RC. Laser-induced breakdown spectroscopy - capabilities and limitations. Appl Spect Rev 2009;44(6):457-506.
9. Dell BLO, Sunde RA. Handbook of nutritionally essential mineral element, Academic Press. 1997;15-31.
10. Onwukeame DM, Ikuegbvweha TB, Asonye CC. Evaluation of phytochemical Constituents, antibacterial activities and effects of exudates of *Pycanthus angolensis* weld warb

(mysristicaeceae) on corneal ulcers in rabbit. Trop J Pharm Res 2007;6(2):725-730.
11. Barreira JCM, Ferreira ICFR, Oliveira MBPP, Pereira JA. Antioxidant activities of the extracts from chestnut flower, leaf, skins and fruit. Food chem 2008;107:1106-1113.
12. Grubesic RJ, Vukovic J, Kremer D, Vladimir-knezevic S. Spectroscopic method for polyphenols analysis. Prevalidation and application on plantogo L. species. J Pharm Biomed anal 2005;39:837-842.
13. Oyaizu M. Studies on product of browning reaction prepared from glucose amine. Jap J Nat 1986;44:307-315.
14. Barros L, Baptista P, Ferreira ICFR. Effect of *Lactarius piperatus* fruiting body maturity stage on antioxidant activity measured by several biochemical assays. Food and Chem Toxicol 2007;4:1731-1737.
15. Re R, Pellegrini N, Proteggenate A, Pannala A, Yang M, Rice - Evan C. Free radical Biol Med 1999;26:1231-1237.
16. Halliwell B, Gutteridge JM, Cross CE. Free radicals, antioxidants and human disease: where are now? J Lab Clinic Med 1992;119:598 - 620.
17. Glucin I. The antioxidant and radical scavenging activities of black pepper (*Piper nigrum*) seeds. Int J Food Sci Nutr 2005;56:491-499.
18. Sfahlan AJ, Mahmoodzadeh A, Hasanzadeh A, Heidari R, Jamei R. Antioxidant and antiradicals in almond hull and shell (*Amygdalus communis* L.) as a function of genotype. Food Chem 2009;115:529-533.
19. Liu F, Ooi V, Chang ST. Free radical scavenging activities of mushroom polysaccharide extracts. Life Sci 1997;60:763-771.
20. Hedge JE, Hofreiter BT, Whistler RL, Miller JNB. In: Carbohydrate chemistry, 17th edition, Academic Press, New york, 1962;11-12.
21. Lowry OH, Rosebrough NJ, Farr AL, Randall RJ. Protein measurement with the Folin Phenol Reagent. J Biol Chem 1951;193:265.
22. Omaye KA, Reddy CE. Cross Enhanced lung. Dystrophy in Vitamin-E deficient rabbits. J Biol Chem 1962;237:916-921.
23. Kathirvel A, Sujatha V. *In vitro* assessment of antioxidant and antibacterial properties of *Terminalia chebula* Retz. Leaves, Asian Pacif J Trop biomed 2012;S788-S795.
24. Kathirvel A, Sujatha V. Phytochemical analysis and antioxidant activity of *Barringtonia acutangula* (L.) Gaertn. leaves. Int J Pharm Pharm Sci 2012;4(2):277-281.
25. Wink M. Plant breeding: Importance of plant secondary metabolites for protection against pathogens and herbivores. Theor Appl Genet 1988;75:225-233.
26. Razab R, Abdul Aziz A. Antioxidants from tropical herbs. Nat Prod Comm 2010;5(3):441–445.
27. Ma X, Wu H, Liu L, Yao Q, Wang S, Zhan R, Xing S, Zhou Y. Polyphenolic compounds and antioxidant properties in mango fruits. Sci Horticul 2011;129:102-107.
28. Vijaya Kumar Reddy C. Sreeramulu D, Raghunath M. Antioxidant activity of fresh and dry fruits commonly consumed in India. Food Res Int 2010;43:285-288.
29. Marcocci L, Packer L, Droy-Lefai MT, Sekaki A, Gardes-Albert M. Antioxidant action of *Ginkgo biloba* extracts EGb 761. Meth Enzymol 1994;234:462-475.
30. Khokhar S, Owusu apenten RK. Iron binding characteristics of phenolic compounds: some tentative structure-activity relations. Food Chem 2003;81(1):133-140.
31. Dhankhar S, Sharma M, Ruhil S, Balhara M, Kumar M., chhillar AK. Evaluation of antimicrobial and antioxidant activities of bougainvillea spectabilis. Int J Pharm Pharm Sci 2013;5(3):178-182
32. Henriquez C, Aliaga C, Lissi E, Kinetics profiles in the reaction of ABTS derived radicals with simple phenols and polyphenols, J Chilean Chem Society 2004;49:65-67.
33. Thomas T. Preliminary antibacterial and phytochemical evaluation of *Dryopteris cochleata* (D.Don) C.Chr. J Glob Pharm Technol 2012;3(6):4-8.
34. Lee HB, Kim JC, Lee SM. Antibacterial activity of two phloroglucinols, flavaspidic acids AB and PB, from *Dryopteris crassirhizoma.* Arch Pharmacal Res 2009;32(5):655-659.
35. Mandal A, Mondal AK. Studies on antimicrobial activities of some selected ferns and lycophytes in Eastern India with special emphasis on ethno-medicinal uses. Afr J Plant Sci 2011;5(7):412-420.
36. Na Phatthalung P, Chusri S, Voravuthikunchai SP. Thai ethnomedicinal plants as resistant modifying agents for combating Acinetobacter baumannii infections. BMC Complem Alter Med 2012;12(56):2-8.
37. Shin SL. Functional components and biological activities of Pteridophytes as healthy biomaterials. Chungbuk National University, Cheongju, Korea, PhD Diss. 2010;75-83.
38. Ban SH, Kim JE, Pandit S, Jeon JG. Influences of *Dryopteris crassirhizoma* Extract on the Viability, Growth and Virulence Properties of *Streptococcus mutans.* Molecules 2012;17:9231-9244.
39. Parihar P, Parihar L, Bohra A. *In vitro* antibacterial activity of fronds (leaves) of some important pteridophytes. J Microbiol Antimicrobial 2010;2(2):19-22.
40. Jahan N, Ahmad M, Mehjabeen Zia-ul-Haq M, Mehboob Alam S, Qureshi M. Antimicrobial screening of some medicinal plants of Pakistan. Pak J Bot 2010;42(6):4281- 4284
41. Asolkar LV, Kakkar KK, Chakre OJ. Second Supplement to Glossary of Indian Medicinal Plants with Active Principles Part I (A-K). Publications and Informations Division, CSIR, New Delhi, 1992;414.
42. Voravuthikunchai S, Lortheeranuwat A, Jeeju W, Sririrak T, Phongpaichit S, Supawita T. Effective medicinal plants against enterohaemorrhagic *Escherichia coli* O157:H7. J Ethnopharmacol 2004;94:49-54.
43. Murray RK, Granner DK, Mayes PA, Rodwell VW. Harper's Biochemistry, 25th Edition, McGraw-Hill, Health Profession Division, USA, 2000.
44. Soetan KO, Olaiya CO, Oyewole OE. The importance of mineral elements for humans, domestic animals and plants: A review. Afr J Food Sci 2010;4(5):200-222.
45. Hasling C, Sondergard K, Charles P, Moselkiloe L. Calcium metabolism in postmenopausal osteoporotic woman is determined by dietary calcium and coffee intake. J Nut 1991;23:1119 -1126.
46. Scelig M. Cardiovascular consequences of Mg deficiency and loss; pathogenesis, prevalence and manifestations. Am j cardiol 1989;63(14):1101 -1102.
47. Chandra RK. Micro-nutrients and immune functions: An overview. Annals of the New York Academy science, 1990;587:9-16.
48. Malhotra VK. Biochemistry for Students. Jaypee Brothers Medical Publishers, 20th Edition, New Delhi, India, 1998.
49. Larkin EC, Rao CA. Importance of fetal and neonatal iron; adequacy for normal development of central nervous system, In: Brain behavior and iron in the infant diet (Dobbing J ed), London, UK. 1990;43-63.
50. Gaeta A, Robert Hider C. The crucial role of metal ions in neuro degeneration; the basis for promising therapeutic strategy. Brit J Pharmacol 2005;146:1041-1059.
51. Weight LM, Jalobes P, Noakes TD. Dietary Iron deficiency and sports anemia. Brit J Nut 1992;68:253-260.
52. Hays VW, Swenson MJ. Minerals and Bones. In: Dukes Physiology of Domestic Animals, 20th Edition, 1985;449-466.
53. Saupi N, Zakira MH, Bujang JS. Analytic chemical composition and mineral content of yellow velvet leaf (*Limnocharis flava* L. Buchenau). J Appl Sci 2009;9(16):2969-2974.
54. Birchall JD, Bellia JP, Roberts NB. On the mechanisms underlying the essentiality of silicon-interactions with aluminium and copper. Coord Chem Rev 1996;149:231-240.
55. Carlisle EM. Essentiality and function of silicon. In Hoekstra *et al.,* Eds. Trace Elements Metabolism in Animals, 2nd Edtiton, University Park Press, Baltimore, 1974.
56. Anke M, Groppel B, Kronemann H, Bratter P, Schramel P. Trace element analysis chemistry and medicinal Biology, Berlin, 1984;3: 421.