



Extraction, Radical Scavenging and Antimicrobial Activity of Essential Oils of Root of *Dryopteris marginalis*

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Received: 30 May 2019;

Accepted: 7 June 2019;

Published online: 28 September 2019;

AJC-19578

The aim of the study was to characterize the chemical constituents and determine antibacterial and antioxidant activities of essential oils and three different extracts of *Dryopteris marginalis*. The root had essential oil yield of 0.36 % (w/w) in which 12 organic compounds representing 97.22% of the root oils were identified. Tyranton was found as the most abundant component with 77.571 % of the total concentration in the essential oil. The zones of inhibition of different organic extracts against the tested bacteria were found in the range of 6.5-15 mm. *Pseudomonas* was the most vulnerable with MICs of 15.62 µg/mL by both ethyl acetate and petroleum ether extract producing 3.8 mm zone of inhibition. The essential oils extracted from roots of *Dryopteris marginalis* showed maximum 85.29 % inhibition of radical scavenging at 2 mg/mL concentration. Among all root extracts, methanol extract exhibited 41.11 % inhibition at 2 mg/mL concentration.

Keywords: Essential oil, *Dryopteris marginalis* (L.), Radical scavenging, Antimicrobial activity, Alternative drug.

INTRODUCTION

In the technological revolution era, still 80 % people of the world depend on conventional sources for health remedy purposes [1]. The use of artificial agents became popular but it also brought the safety issues in front as many studies showed some respiratory tract and other health risks [2]. This is why, natural constituents are still considered trustworthy [3]. It is believed that nature may have the solution to even some incurable diseases [4]. Plants are comprised of different constituents including essential oil which can be used in different purposes. Essential oils are extracted from medicinal plants and confided to impart antimicrobial and antioxidant activities [5]. Although the presence of microbes has been discovered recently but various plants were used against them even before knowing the activity

[6,7]. Essential oils extracted from different sources have found their use in protection of food from microbial contamination, treatment in various diseases, perfumery, cosmetics, sanitary purposes, etc. [8-10]. But there are a number of resources which are still waiting to be revealed. Thus, extraction of essential oils from different natural resources has allured the attention and still has the room to discover new components with potential [11]. Among them different species of *Dryopteris* fern have shown promising results against various microbes.

There are about 250 species of *Dryopteris* fern available in different parts of Asia [12]. These ferns are favoured by the tropical weather and damp shady places, this is why it can be found in almost every corner of Bangladesh. Different investigations have showed that *Dryopteris* ferns contain variety of essential oils and show different activities against some specific

microorganisms. Previous studies have suggested that different species can be used as anthelmintic medicine [13], antipyretics [14], anticonvulsant, anti-rheumatic drugs, leprostatic agents [15], anti-tumour and immunomodulatory agents [16]. Other studies also indicated that some of the species of *Dryopteris* has already been used as anti-venom and healing snake bite wounds [17] and in the treatment of schistosomiasis [18]. A report published by Hill [19] in 1937, confirmed the presence of 'filicin' which can be used as a worm expellant.

Dryopteris marginalis is known as the marginal shield fern or marginal wood fern which is very common and available all over Bangladesh. In the present study, the chemical composition of the essential oil obtained from roots and determined anti-bacterial activity from three different extracts of roots. This study also indicates the antioxidant activities of essential oil and various organic extracts with emphasis for the possible future use of the essential oil and extracts of *Dryopteris marginalis*. To the best of our knowledge, no report has been published on the analysis of root of *Dryopteris marginalis* species yet.

EXPERIMENTAL

Plant material: Healthy, disease free plant samples were collected from Atghoria, Pabna district and Islamic University, Kushtia campus of Bangladesh in January 2015 and the species was identified by the Department of Botany, University of Dhaka, Bangladesh. The collected plants were separated from undesirable materials or plant parts and then were washed with deionized water, then they were shade-dried for four weeks. The roots were then collected and ground into coarse powder by a grinder. In an air proof container, the powder was placed and stored in low temperature and dry place till further analysis.

Extraction of essential oil: The essential oil was extracted according to the method described by Viuda-Martos *et al.* [20] with some modifications. Briefly, the air dried roots (200 g) were hydrodistilled for 3, 3 and 4 h respectively using a Clevenger-type apparatus. The oily layer acquired on top of the aqueous distillate was separated and dried with anhydrous sodium sulfate. The oil acquired was stored in tightly sealed dark vials and covered with aluminum foil at 4 °C until further analysis.

Gas chromatography-mass spectrometry (GC-MS) analysis: Hewlett Packard 6890 gas chromatography was used for analysis of essential oils. In the GC-MS, HP-5 MS capillary column which is 30 m × 0.25 mm (crosslinked phenyl-methyl siloxane) in dimension and 0.25 µm of film thickness was used and was equipped with a Hewlett Packard 6890 mass selective detector. As a carrier gas helium was used in the system. Different conditions of chromatographer like ion source, ionization energy and electron current were 230 °C, 70 eV and 2 A, respectively. The temperature of column was set for 40 °C for the first 5 min and then it was increased to 280 °C at a rate of 10 °C/min. The retention indices were calculated for all volatile constituents using a homologous series of C₈-C₂₂ n-alkanes. The constituents were identified either with the comparison of the identical retention indices to literature [21] or comparing the mass spectra stored in Wiley mass spectral database (Hewlett Packard, Vienna, Austria). The relative proportion (%) was also identified for each volatile compound.

Preparation of organic extracts: Each 15 g of root powder was weighed with the electrical balance and transferred into three separate 150 mL conical flasks. Then 75 mL of methanol, ethyl acetate and petroleum ether was added in each flask respectively and placed on a shaker at room temperature for 7 days. The crude extracts were then filtered by passing the extracts through Whatman no. 1 filter paper and the solvents were evaporated by vacuum rotary evaporator at 45 °C. After that the extracts were diluted to 7.81, 15.62, 31.25, 62.5, 125, 250, 500, 1000 µg/mL. All the extracts were stored in refrigerator at 4 °C in sterile for further use [22].

Microbial strains and culture: Different microorganism strains were provided by Bangladesh Council of Scientific and Industrial Research (BCSIR), Rajshahi, Bangladesh and Department of Biotechnology and Genetic Engineering, Islamic University, Kushtia, Bangladesh. The antimicrobial activity of essential oil was tested against five different microorganisms. Among them, *Staphylococcus aureus* and *Bacillus cereus* were Gram-positive strains while *Salmonella typhi*, *Pseudomonas* and *E. coli* were Gram-negative bacteria. All the strains were stored in their appropriate agar slants at 4 °C and cultured at 37 °C on nutrient agar or nutrient broth mediums.

Antimicrobial bioassay: *in vitro* Antimicrobial activities of the test samples were carried out by disc diffusion method [23-25]. Filter paper discs (5 mm diameter) of various extract (methanol, ethyl acetate, petroleum ether) were prepared by impregnating blank sterile paper discs to the respective extracts with different concentrations (7.81, 15.62, 31.25, 62.5, 125, 250, 500 and 1000 µg/mL) and residual solvents were completely evaporated. Discs containing the test materials were placed on nutrient agar medium uniformly seeded with the test bacteria. Standard discs of kanamycin (10 µg/discs) and blank discs (impregnated with solvents followed by evaporation) were used as positive and negative control respectively. These plates were then kept at low temperature (4 °C) for 24 h to allow maximum diffusion of test samples and then incubated at 37 °C for 24 h to allow maximum growth of the bacteria. The antibacterial activity of the test agents was determined by measuring the diameter of zone of inhibition in millimeter. Each assay in this experiment was replicated three times. Minimum inhibitory concentration (MIC) values of the extracts showing significant results (methanol and ethyl acetate extract) were determined in the present study following the serial dilution technique [26].

Minimum inhibitory concentration (MIC): Minimum inhibitory concentrations (MICs) of essential oils from different extracts of ethyl acetate, petroleum ether and methanol were tested by the methods described by Gur *et al.* [27] with slight modification. The cultured bacterium was taken into the screw cap tubes. Deionized water (10 mL) was added to the screw cap tube and whirled the mixture for homogenous mixing. This suspension was used as inoculums. The optical density (OD) was measured with the colorimeter and an optical density of 10⁶ to 10⁸ CFU/mL for the test organisms was confirmed. For the determination of MIC, nutrient broth medium was prepared by using peptide digest of animal tissue 5 g/L, sodium chloride 1.5 g/L, beef extract 1.5 g/L, yeast extract 1.5 g/L and distilled water 1000 mL. For the test, 13 g/1000 mL of nutrient broth media was taken in a conical flask. The media was properly

TABLE-1
CHEMICAL COMPOSITION OF THE ESSENTIAL OIL ISOLATED FROM THE ROOT OF *D. marginalis*

No.	Compound name	Molecular weight (mw)	Retention index (RI)	Peak area (%)
1	3-Ethyl-3-buten-2-one	98	721	0.924
2	Methacide	92	794	5.273
3	4-Hydroxy-2-pentanone	102	817	2.177
4	Tyranton	116	845	77.571
5	Iso-octyl alcohol	130	995	0.399
6	Tridecene	182	1304	0.795
7	Pyrimidine-2,4(1 <i>H</i> ,3 <i>H</i>)-dione-5-amino-6-nitroso	156	1365	0.597
8	9-Eicosene	280	2017	1.093
9	Pentanoic acid	306	2255	2.938
10	Muscalure	332	2315	1.270
11	Di chloro acetic acid	366	2423	0.923
12	2,6-Bis(<i>t</i> -butyl)-4-(di-methyl benzyl) phenol	324	2455	3.260
Total				97.22 %

dissolved with the distilled water and then sterilized in an autoclave for 15 min with 121 °C and 15 lbs/inch² pressure. After autoclaving the media was cooled for some time and poured into the autoclaved screw cap tubes in the laminar air flow cabinet. It was then ready for inoculation of microorganism with different concentration of extracts.

DPPH assay: On the basis of the scavenging activities of stable 2,2-diphenyl-1-picrylhydrazyl (DPPH, Sigma-Aldrich, St. Louis, USA) free radical, the DPPH radical scavenging activity of the essential oil and extracts was measured [28]. Various concentrations of test extracts (0.1 mL) were added to 2.9 mL of 0.004 % (w/v) methanol solution of DPPH. The incubation period was 30 min at room temperature and at 517 nm, the absorbance was monitored against a blank. Inhibition percent of free radical of DPPH (I %) was calculated as follows:

$$I (\%) = \frac{A_{\text{blank}} - A_{\text{sample}}}{A_{\text{blank}}} \times 100$$

where A_{blank} is the absorbance of the control reaction (containing all reagents except the test compound) and A_{sample} is the absorbance of test compound.

Statistical analysis: Each experiment was run in triplicate and main values were calculated with SD (standard deviation). One-way analysis of variance (ANOVA) and Duncan's multiple range tests were carried out to determine significant differences ($p < 0.05$) between the means by SPSS version 11.0 and EXCEL program.

RESULTS AND DISCUSSION

Chemical composition of the essential oils: The hydro-distillation of roots of *D. marginalis* gave the golden yellow oil with yield of 0.36 % (w/w). Composition identification was confirmed by comparing the data obtained from the samples used in this study with the result reported by Adams [21]. GC-MS analysis of the essential oil led to the identification of 12 compounds, representing 97.22 % of the total oil. The detected major compounds were tyranton (77.571%), methacide (5.273%), 2,6-bis(*t*-butyl)-4-(dimethyl benzyl) phenol (3.260 %), pentanoic acid (2.938 %), 4-hydroxy-2-pentanone (2.177 %) and muscalure (1.270 %). The identified compounds are listed in Table-1 according to their elution order.

Antimicrobial activity: The antimicrobial activity of different extracts of roots of *Dryopteris marginalis* is shown

in Table-2 in a comparative way with standard antibiotic disc-kanamycin (10 µg/discs). The observation of this study stated that, the extract from methanol showed the highest zone of inhibition of 12 mm against *S. aureus* followed by 15 mm and 10 mm against *E. coli* and *B. cereus*, respectively. The extract from ethyl acetate produced satisfactory sensitivity of 9.6, 9.7, 6.5 and 7 mm against *E. coli*, *Pseudomonas*, *B. cereus* and *S. aureus*, respectively whereas petroleum ether extracts possessed relatively poor antibacterial activity. All the extracts were somehow effective against *E. coli*, *B. cereus* and *S. aureus* whereas *S. typhi* was completely resistant against all extracts but *Pseudomonas* was partially prohibitive against the methanol extract. Positive control produced significant zones of inhibition contrary to all the tested bacteria while no zone was formed by negative control. In comparison to antibiotic, though the activity of different extracts of roots of *Dryopteris marginalis* was very close to kanamycin, but the availability, ease in extraction and comparative low cost refer it as a potential antibiotic candidate.

TABLE-2
ANTIBACTERIAL ACTIVITIES OF THREE
DIFFERENT ROOT EXTRACTS OF *D. marginalis*

Tested bacteria	Diameter of zone of inhibition (mm)			
	Kanamycin (10 µg)	Ethyl acetate	Petroleum ether	Methanol
<i>E. coli</i>	19	9.6	8.8	15
<i>Pseudomonas</i>	21	9.7	9.3	–
<i>S. typhi</i>	19	–	–	–
<i>S. aureus</i>	19	6.5	7	12
<i>B. cereus</i>	20	7	7	10

Minimum inhibitory concentration: MIC values of various extracts were found between 7.81-1000 µg/mL. The best MIC value was observed 15.62 µg/mL against *Pseudomonas* by both ethyl acetate and petroleum ether extract producing 3.8 mm zone of inhibition while *S. typhi* showed complete resistance within the range against all extracts (Table-3). In case of *E. coli*, methanol extract showed comparatively good result (MIC value 62.5 µg/mL) than the other two extract (125 and 250 µg/mL, respectively in petroleum ether and ethyl acetate extracts). *S. aureus* and *B. cereus* exhibited similar type of result in both ethyl acetate and petroleum ether extract. In both extracts, the MIC value was 125 µg/mL. But the previous one showed MIC value of 31.25 µg/mL whereas *B. cereus* showed that of 62.5 µg/mL in methanol extract.

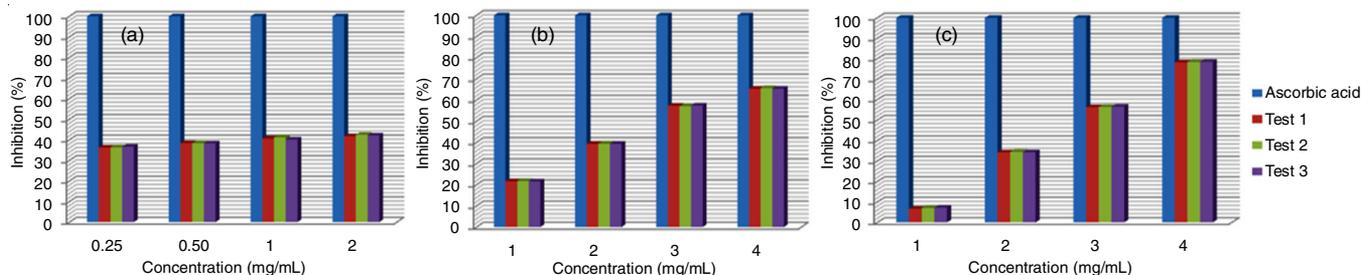


Fig. 1. DPPH radical scavenging activities of (a) methanol extract, (b) ethyl acetate root extracts, (c) petroleum ether root extracts of *D. marginalis*

TABLE-3
LIST OF MINIMUM INHIBITORY CONCENTRATION
FOR DIFFERENT TESTED BACTERIA

Tested bacteria	Ethyl acetate extract (µg/mL)	Petroleum ether extract (µg/mL)	Methanol extract (µg/mL)
<i>E. coli</i>	250	125	62.5
<i>Pseudomonas</i>	15.62	15.62	> 1000
<i>S. thyphi</i>	> 1000	> 1000	> 1000
<i>S. aureus</i>	125	125	31.25
<i>B. cereus</i>	125	125	62.5

DPPH radical scavenging activities: Free radical scavenging properties of different extracts from roots of *D. marginalis* and essential oils are presented in Fig. 1. Higher percentage of inhibition value indicates higher antioxidant activity [29]. Essential oils extracted from roots showed higher scavenging ability of DPPH radicals of 85.29 % inhibition at 2 mg/mL concentration (Fig. 2) when compared to those of various extracts of *D. marginalis* showing maximum inhibition around 40 % at the same concentration.

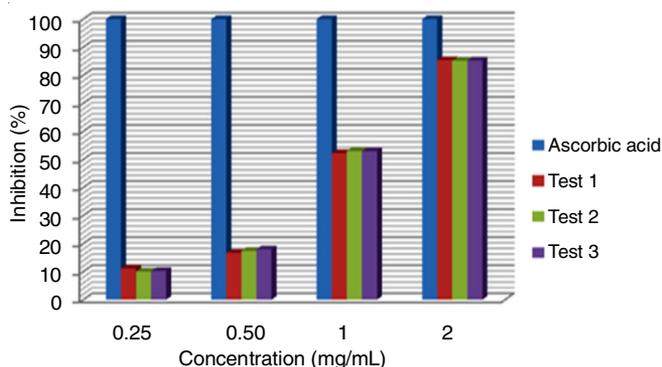


Fig. 2. DPPH radical scavenging activities of root essential oils of *D. marginalis*

Likewise, among all root extracts, methanolic extract showed significant antioxidant property of 41.11 % inhibition at the concentration of 2 mg/mL (Fig. 1a). Ethyl acetate root extract exerted comparatively low radical scavenging activity around 39.05 % inhibition at concentration of 2 mg/mL (Fig. 1b), whereas petroleum ether root extract showed the least radical scavenging activity of 33.8 % inhibition at same concentration (Fig. 1c).

The antioxidative properties in natural sources have been reported to be mostly due to phenolic compounds [30-33] suggesting that root extracts exhibiting a promising antioxidant activity may be because of the presence of phenolic compounds.

The antioxidant property of extract of *Dryopteris filixmas* has been previously reported by Ali *et al.* [34]. According to them, the antioxidative activity of the extract of *D. filixmas* obtained by polar organic solvent (methanol) was greater than those of the extracts obtained by non-polar organic solvents (petroleum ether, chloroform). The present study indicated the similar antioxidative property of *D. marginalis*. Other studies showed that few species of fern such as *D. affinis* and rhizome of *D. cochleata* provided potent antioxidant activities [35,36].

Conclusion

The outcome of present study suggests that the chemical composition of hydrodistilled oil obtained from root of *Dryopteris marginalis* representing 12 compounds containing 97.22 % of total components, having oxygenated monoterpenes and sesquiterpene hydrocarbons and their oxygenated derivatives. These compounds have enormous potential to strongly inhibit microbial pathogens. The root extracts of *Dryopteris marginalis* were found to be effective against test bacteria, can be used as antibacterial agents in designing and developing new drugs. Further analysis of purification of active compounds of *D. marginalis* will provide a better understanding of the antibacterial mechanism.

ACKNOWLEDGEMENTS

The authors are grateful to Prof. Dr. M. Oliur Rahman, Department of Botany, University of Dhaka, Bangladesh for his expert opinion to detect *D. marginalis* species.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this article.

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