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Preliminary Phytochemical and GC-MS Analyses of Methanolic Extract of *Blechnum orientale* L. Collected From Kothiyar, Kanyakumari District, Tamil Nadu, India

[1] Fredrick Raja E. and [2] John Peter Paul J*

[1] Research Scholar (Reg. No. 19211282261029), Department of Botany, St. Xavier's College (Autonomous), Palayamkottai - 627 002, Tamil Nadu. Affiliated to Manonmaniam Sundaranar University, Abishekapatti, Tirunelveli- 627 012, Tamil Nadu, India.

[2]*Assistant Professor of Botany & Director, Centre for Advanced Research in Plant Sciences (CARPS), St. Xavier's College (Autonomous), Palayamkottai – 627 002, Tamil Nadu, India.

ABSTRACT

The aim of the study was to screen the phytochemicals of methanolic extract of *Blechnum orientale* L. collected from Kothiyar, located in Kanyakumari district, Tamil Nadu, India. The preliminary phytochemical analysis was carried out by Harborne method, followed by GC-MS analysis. In the preliminary phytochemical analysis of *Blechnum orientale* L., the presence of twelve different types of secondary metabolites such as alkaloids, anthocyanin, anthraquinones, cardiac glycosides, coumarin, diterpenes, emodins, flavonoids, saponins, steroids, tannins and triterpenoids were reported in methanolic extract. GC-MS spectrum of methanolic extract of *Blechnum orientale* L. showed 20 different major peaks which indicated the presence of 20 compounds. The prevailing compounds in methanol extract were toluene (1.927min), thiazole, 4,5-dihydro-2-methyl- (2.249min), ethylbenzene (2.466min), benzene, 1,3-dimethyl- (2.523min), phosphine, acetyldimethyl- (2.684min), guanethidine (4.584min), 2-propanamine, n-methyl-n-nitroso- (5.303min), 4h-pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl- (5.435min), diethylene glycol hexyl ether (6.333min), 2,2-dimethylpropanoic acid, nonyl ester (6.655min), 8-thiabicyclo[3.2.1]oct-2-ene (7.156min), 4h-pyrazole, 3-tert-butylsulfanyl-4,4-bistrifluoromethyl- (9.217min), 1,3-benzenediol, 4-propyl- (11.250min), cyclohexanone, 2-methyl-5-(1-methylethenyl)- (13.775min), 2,4-hexadienal, (E,E)- (14.210min), n-hexadecanoic acid (14.966min), phytol (16.451min), 2(1h)-naphthalenone, octahydro-4a-methyl-7-(1-methylethyl)-, (4a.alpha.,7.beta.,8a.beta.)- (16.602min), 7-pentadecyne (16.659min) and 1,4-bis (trimethylsilyl) benzene (20.186min) respectively.

Keywords: Phytochemicals, Pteridophytes, GC-MS, *Blechnum orientale,* NIST

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***Address for Correspondence:**

John Peter Paul J Assistant Professor of Botany & Director, Centre for Advanced Research in Plant Sciences (CARPS), St. Xavier's College (Autonomous), Palayamkottai – 627 002, Tamil Nadu, India.

INTRODUCTION

Pteridophytes existed from Paleozoic era and they have faced many stochastic disturbances that led them to adapt to many serious changes of environment[1]. Hence, ferns are expected to comprise numerous effective secondary metabolites than other plants. Many useful phytochemicals or secondary metabolites for instance, alkaloids, flavonoids, phenols, steroids, triterpenoids, varied amino acids and fatty acids have been reported in the ferns[2]. Besides, they also contain unique phytochemicals, yet not found in higher plants[3].

Ferns co-existed with human beings for years and influenced millions of human lives as traditional medicinal cures or treatments for as carid disease, bleeding, trauma, burning, diarrhea, cold and many more in some countries[4]. In the recent years, many medicinal ferns were analyzed and reported to have various bioactivities such as anti-oxidant[5], anti-tumor[6], anti-HIV[7], anti-microbial[8], anti-inflammatory[9] and anti-viral[10]. Nowadays there is global regeneration of medicinal plants research and great emphasis is being laid on exploring bioactive compounds and biological activities of plants owing to the natural origin, cost effectiveness and lesser side effects[11]. This traditional claim provoked many researchers to investigate various phytochemicals on its pharmacological values that include bioactivities[5]. Recently, GC-MS studies have been increasingly applied for the analysis of medicinal plants as this technique has proved to

be a reliable method for the analysis of various phytochemicals. Hence, the aim of the present study was to analyse the preliminary phytochemical and GC-MS analyses in the methanolic extract of *Blechnum orientale* L.

MATERIALS AND METHODS

Collection of Plant sample

The plant material selected for the present study was *Blechnum orientale* L. which belongs to the family Blechanaceae was collected from Kothiyar, located in Kanyakumari district, Tamil Nadu, India, during the month of December, 2016 and identified and confirmed by Pteridophyte flora of the Western Ghats – South India[12]. The collected materials were washed thoroughly with tap water to remove the sediment particles. Then the samples were brought in polythene bag to the laboratory, followed by washed using distilled water. They were stored in refrigerator for further use[13].

Preparation of methanol extract

For the preparation of methanolic extract, the plant specimens were washed thoroughly and placed on blotting paper and spread out at room temperature in the shade condition for drying. The shade dried samples were grounded to fine powder using a tissue blender. The powdered samples were then stored in the refrigerator for further use. 30g powdered samples were packed in Soxhlet apparatus and extracted with methanol for 8h separately [14].

Preliminary phytochemical analysis

The methanolic extract was tested for alkaloids, anthocyanin, anthraquinones, cardiac glycosides, coumarin, diterpenes, emodins, flavonoids, saponins, steroids, tannins and triterpenoids. Phytochemical screening of the methanolic extract was carried out according to the standard methods [15].

Test for alkaloids

1ml of 1% HCl was added to the 2ml of extract in a test tube and was treated with few drops of Mayer's reagent. A creamy white precipitate indicates the presence of alkaloids.

Test for anthocyanin

2ml of extract was added with 1ml of 2N NaOH and heated for 5min. the formation of bluish green colour indicated the presence of anthocyanin.

Test for anthraquinones

2ml extract was mixed with benzene and 1ml 10% ammonia solution was added. The presence of a pink, red or violet color indicates the anthraquinones.

Test for cardiac glycosides

Take 2ml extract, 2ml of glacial acetic acid, 1ml of Conc. sulphuric acid and few drops of 5% ferric chloride. The formation of brown ring indicates the presence of cardiac glycosides.

Test for coumarins

1ml of extract was added with 1ml of 1N NaOH. The test tubes were kept in boiling water bath for few minutes and shaken well. The appearance of yellow colour indicates the presence of coumarins.

Test for diterpenes

1ml of extract was added to 1ml of distilled water and 10 drops of copper acetate solution. A emerald green color indicates the presence of diterpenes.

Test for emodins

1ml of plant extract was added to 2ml of NH_4OH and 3ml of benzene. A red color indicates the presence of emodins.

Test for flavonoids

A few drops of 1% NH_3 solution was added to 2 ml of extract in a test tube. A yellow Coloration indicates the presence of flavonoids.

Test for saponins

2ml of extract was shaken vigorously with 5ml distilled water to obtain stable persistent foam. The formation of emulsion indicates the presence of saponins.

Test for steroids

1ml extract was added with 2ml of chloroform and 1ml of sulphuric acid. The formation of reddish brown ring indicates the presence of steroids.

Test for tannins

To 2ml extract, 1ml of distilled water and 1-2 drops of ferric chloride solution was added and observed for brownish green or a blue black coloration indicates the presence of tannins.

Test for triterpenoids

2ml extract was mixed with 2ml of $CHCl_3$ in a test tube. 3ml Conc. H_2SO_4 was carefully added along the wall of the test tube to form a layer. An interface with a reddish brown coloration confirms the presence of triterpenoids

Gas Chromatography-Mass spectrometry (GC-MS)

The GC-MS analysis was carried out using GC model Clarus 680, Mass Spectrometer Clarus 600 (EI) Perkin Elmer, Gas Chromatography was equipped and coupled to a mass detector TurboMass 5.4.2 spectrometer with an Elite-5MS, (100% Dimethyl ply siloxane), 30.0m × 250µm df capillary column. The instrument was set to an initial temperature of 60°C and maintained at this temperature for 2min. At the end of this period, the oven temperature was raised upto 300°C, at the rate of an increase of 10°C/min and maintained for 6min. Injection port temperature was ensured as 250°C and Helium flow rate as 1ml/min. The ionization voltage was 70eV. The samples were injected in split mode as 10:1. Mass Spectral condition solvent delay 2min, transfer temperature 240°C, source temperature 240°C and scanning range was set at 50-600Da. The chemical constituents were identified by GC-MS[12].

Interpretation of mass spectrum of 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. The retention time, compound name, molecular formula and molecular weight and area percentage of the test materials were ascertained.

RESULTS AND DISCUSSION

In the present study, preliminary phytochemical analysis of *Blechnum orientale* L. showed twelve different types of secondary metabolites (alkaloids, anthocyanin, anthraquinones, cardiac glycosides, coumarin, diterpenes, emodins, flavonoids, saponins, steroids, tannins and triterpenoids) in methanolic extract. GC-MS spectrum of methanol extract of *Blechnum orientale* L. was identified and the active compounds with their retention time (RT), molecular formula, molecular weight and structure were

presented. GC-MS spectrum of methanol extract of *Blechnum orientale* L. showed 20 different major peaks which indicated the presence of 20 compounds. The prevailing compounds in methanol extract were toluene (1.927min), thiazole, 4,5-dihydro-2-methyl- (2.249min), ethylbenzene (2.466min), benzene, 1,3-dimethyl- (2.523min), phosphine, acetyldimethyl- (2.684min), guanethidine (4.584min), 2-propanamine, n-methyl-n-nitroso- (5.303min), 4h-pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl- (5.435min), diethylene glycol hexyl ether (6.333min), 2,2-dimethylpropanoic acid, nonyl ester (6.655min), 8-thiabicyclo[3.2.1]oct-2-ene (7.156min), 4h-pyrazole, 3-tert-butylsulfanyl-4,4-bistrifluoromethyl- (9.217min), 1,3-benzenediol, 4-propyl- (11.250min), cyclohexanone, 2-methyl-5-(1-methylethenyl)- (13.775min), 2,4-hexadienal, (E,E)- (14.210min), n-hexadecanoic acid (14.966min), phytol (16.451min), 2(1h)-naphthalenone, octahydro-4a-methyl-7-(1-methylethyl)-, (4a.alpha.,7.beta.,8a.beta.)- (16.602min), 7-pentadecyne (16.659min) and 1,4-bis (trimethylsilyl) benzene (20.186min) respectively (Figure-1 and Table-1).

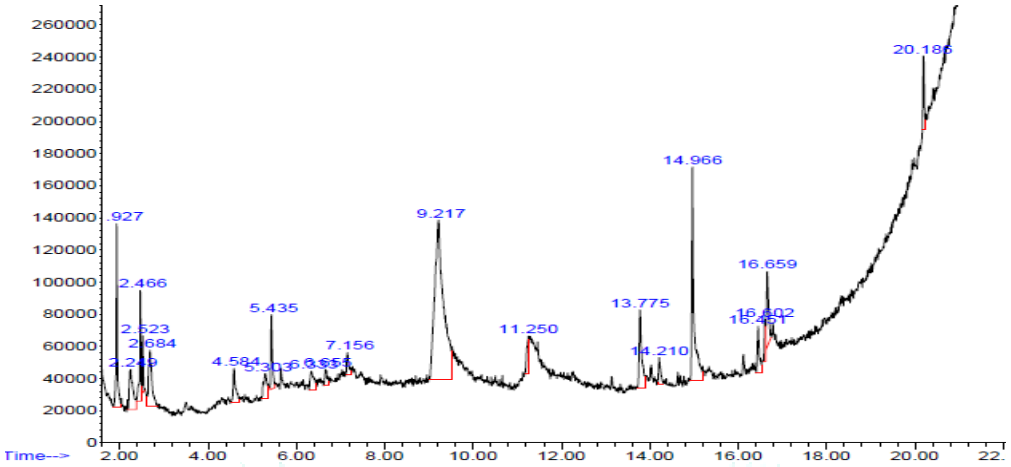

Figure-1: GC-MS spectrum analysis of methanolic extract of *Blechnum orientale* L.

Table-1: GC-MS spectrum analysis of methanolic extract of *Blechnum orientale* L.

RT	COMPOUND NAME	MF	MW
1.927	Toluene	C_6H_5-CH_3	92.14
2.249	Thiazole, 4,5-dihydro-2-methyl-	C_3H_3NS	85.12
2.466	Ethylbenzene	C_8H_{10}	106.16
2.523	Benzene, 1,3-dimethyl-	C_6H_6	78.02
2.684	Phosphine, acetyldimethyl-	CH_5P	48.02
4.584	Guanethidine	$C_{10}H_{22}N_4$	198.31
5.303	2-Propanamine, N-methyl-N-nitroso-	$C_4H_{10}N_2O$	102.13
5.435	4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl-	$C_6H_8O_4$	144.12
6.333	Diethylene glycol hexyl ether	$C_{10}H_{22}O_3$	190.28
6.655	2,2-Dimethylpropanoic acid, nonyl ester	$C_{15}H_{30}O_2$	242.40
7.156	8-Thiabicyclo[3.2.1]oct-2-ene	$C_7H_{10}S$	126.21
9.217	4H-Pyrazole, 3-tert-butylsulfanyl-4,4-bistrifluoromethyl-	$C_7H_{12}H_2$	124.187
11.250	1,3-Benzenediol, 4-propyl-	$C_9H_{12}O_2$	152.19
13.775	Cyclohexanone, 2-methyl-5-(1-methylethenyl)-	$C_{10}H_{16}O$	152.23
14.210	2,4-Hexadienal, (E,E)-	C_6H_8O	96.12
14.966	n-Hexadecanoic acid	$C_{16}H_{32}O_2$	256.42
16.451	Phytol	$C_{20}H_{40}O$	296.53
16.602	2(1H)-Naphthalenone, octahydro-4a-methyl-7-(1-methylethyl)-, (4a.alpha.,7.beta.,8a.beta.)-	$C_{14}H_{24}O$	208.38
16.659	7-Pentadecyne	$C_{15}H_{28}$	208.38
20.186	1,4-Bis(trimethylsilyl)benzene	$C_{12}H_{22}Si_2$	222.47

RT: Retention Time; MF: Molecular Formula; MW: Molecular Weight;

CONCLUSION

In the present study, phytochemicals of methanolic extract of *Blechnum orientale* L. were extracted. The preliminary phytochemical analysis of *Blechnum orientale* L. showed the presence of twelve different types of secondary metabolites such as alkaloids, anthocyanin, anthraquinones, cardiac glycosides, coumarin, diterpenes, emodins, flavonoids, saponins, steroids, tannins and triterpenoids. GC-MS spectrum of methanolic extract of *Blechnum orientale* L. showed toluene, thiazole, 4,5-dihydro-2-methyl-, ethylbenzene, benzene, 1,3-dimethyl-, phosphine, acetyldimethyl-, guanethidine, 2-propanamine, n-methyl-n-nitroso-, 4h-pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl-, diethylene glycol hexyl ether, 2,2-dimethyl propanoic acid, nonyl ester, 8-thiabicyclo[3.2.1]oct-2-ene, 4h-pyrazole, 3-tert-butylsulfanyl-4,4-bistrifluoro methyl-, 1,3-benzenediol, 4-propyl-, cyclohexanone, 2-methyl-5-(1-methylethenyl)-, 2,4-hexadienal, (E,E)-, n-hexadecanoic acid, phytol, 2(1h)-naphthalenone, octahydro-4a-methyl-7-(1-

methylethyl)-, (4a.alpha.,7.beta.,8a.beta.)-, 7-pentadecyne and 1,4-bis (trimethylsilyl) benzene.

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CONFLICT OF INTEREST

The author declares that she has no conflict of interest.

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