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Original Article

PHYTOCHEMICAL INVESTIGATION AND FREE RADICAL SCAVENGING ACTIVITIES OF ESSENTIAL OIL, METHANOL EXTRACT AND METHANOL FRACTIONS OF *NEPHROLEPIS BISERRATA*

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ABSTRACT

Objective: In the current study, the essential oil, methanol extract and methanol fractions (n-hexane, chloroform, ethyl acetate and n-butanol) of *Nephrolepis biserrata* L. were evaluated.

Methods: Preliminary phytochemical screening was done. The antioxidative effect was determined using 2,2diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity. Total phenol andflavonoid contents were calculated using Folin -Ciocalteau and aluminium chloride reagents. The phytochemical analysis of the essential oil, methanol extract and methanol fractions were performed by gas chromatography - mass spectrometry (GCMS).

Results: The preliminary screening confirmed the presence of active chemical constituents such as anthraquinones, alkaloids, tannins, steroids, phytosterol, saponin, triterpinoids and flavonoids. Our results also indicated that essential oil, methanol extract and methanol fractions are rich in phenolic and flavonoid contents. The GCMS analysis of our samples showed the presence of various biological important compounds. The dominant compounds are benzeneacetaldehyde, alpha.-cubebene, butyrolactone, phenol, benzyl alcohol, phenol, 2-methoxy-, 4h-pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl, 2h-pyran-2-one, 4,6-dimethyl-, catechol, benzofuran, 2,3-dihydro-, phenol, 2,4-bis(1,1-dimethylethyl), hexadecanoic acid, methyl ester, n-hexadecanoic acid, 9,12-octadecadienoic acid, methyl ester, phytol, gamolenic acid and octadecanoic acid.

Conclusion: Our results demonstrate that the essential oil, methanol extract and methanol fractions of the *N. biserrata* represent a good source of potential bioactive compounds that could be used in pharmaceutical industry.

Keywords: *Nephrolepis biserrata,* Methanol extract, Antioxidant activity, Total phenolic, Flavonoids, Phytochemicals, GCMS analysis.

INTRODUCTION

Reactive oxygen species (ROS) are oxygen-centred free radicals such as hydrogen peroxide, superoxide, hydroxyl HO^- and peroxyl ROO- [1]. Over production of ROS result in a condition known as oxidative stress, results in the disruption of cell membrane, protein denaturation, lipid peroxidation and oxidative DNA damage [2,3]. Which have generally been considered to be linked with many chronic diseases in human beings, including diabetes, cancer, cardiovascular diseases, alzheimer's disease, neurodegenerative disorders, atherosclerosis and inflammation [4-7].

Phytochemical compounds with antioxidant properties have the ability to minimize or inhibit the damages caused by ROS. The antioxidant properties of different natural product depend on the phenolic content, vitamins C and E, carotenoids, flavonoids and other phytocomponents [8]. Because of this positive role, a lot of experimental study is focused on exploiting the potential of phytochemical constituents from various plants and establishing their association with health benefits. Previous published data have established that various bioactive compounds occur naturally in plants [9,10].

Nephrolepis biserrata (Nephrolepidaceae) is a perennial fern [11]. The plant is distributed in various Southeast Asian countries including Malaysia [12,13]. Locally the plant is known as 'Paku larat', or 'Paku pedang' (Malay) [14]. *N. biserrata* occurs on the river banks, mountain slopes and tree trunks. It is used to treat jaundice by local people in Brunei [15]. *N. biserrata* is also used for the treatment of bacterial and a fungal infection such as boils, abscess, sore and blisters [16].

In Sarawak Malaysia, the plant is used for the treatment of skin disorders [12]. The paste of the leaves is also applied on the cuts and wounds [16]. However, despite the frequent utilization of *N. biserrata*, phytochemical information is not reported yet. The aim of the current study is to estimate the antioxidant activities and phytochemical analysis of essential oil, methanol extract and methanol fractions (n-hexane, ethyl acetate, chloroform and butanol) of *N. biserrata.*

MATERIALS AND METHODS

Chemicals

α,α-diphenyl-picrylhadrazyl (DPPH), and gallic acid, were purchased from Sigma. Extraction solvents, methanol, n-hexane, butanol, chloroform and ethyl acetate (HPLC grade) were purchased from Merck (Darmstadt, Germany). Lead acetate, Na_2CO_3, aluminium chloride, potassium acetate, potassium sulphate, sodium hydroxide, sodium nitroprusside, hydrogen peroxide, sulfanilic acid, glacial acetic acid, were all obtained from Merck, USA

Sample collection

N. biserrata was collected from the lower land of Papar, Sabah. Malaysia. The plant was identified and voucher of the specimen was deposited at Institute of Tropical Biology and Conservation (ITBC) Herbarium, University Malaysia Sabah. The voucher number is MDS-001.

Extraction and fractionation

Plant dry powder (60 g) was extracted with pure methanol (300 ml) using soxhlet method (50-60°C and 72h). The methanol residues were removed from the extract using a vacuum rotary evaporator. The methanol crude extract was further fractionated with n-hexane, ethyl acetate, chloroform and butanol in a separatory funnel with increasing order of polarity. The solvents were removed from the extracts under vacuum. The samples were kept at -80°C for 24 h and then lipolized using a freeze drier. The freeze dried samples were then stored in the freezer for further studies [17].

Extraction of essential oil

One hundred gram (100 g) of *N. biserrata* leaves were subjected to hydro distillation for 3 h using a distillation type apparatus. The

obtained essential oil was separated, dried over anhydrous sodium sulphate, and stored in a sealed vial for further analysis.

Determination of total phenolic content

The total phenolic content was determined by Folin-Ciocalteu method [18] with minor modifications. One milligram (1mg) of stock solution of our sample was prepared. Folin-Ciocalteu reagent was prepared by 10 fold dilution (ratio 1:9). Briefly, 1.5 ml of Folin-Ciocalteu reagent was mixed with 0.2 ml of assay samples in a tube and mixed. After 5 minutes, 1.5 ml sodium carbonate (60 g/l) was added to each tube and vortexed. Finally the samples were incubated in the darkness for 90 min at room temperature. The absorbance was measured at 725 nm against the blank. Gallic acid was used as a standard for the quantification of phenolic compound. Various Concentrations (0.01, 0.02, 0.04, 0.08 and 0.1 mg/ml) of gallic acid were used to plot the slandered calibration curve. The concentration of the total phenolic content was estimated as mg of gallic acid equivalent by using an equation obtained from gallic acid calibration curve. The analyses were carried out in triplicate.

Determination of total flavonoid content

The total flavonoid content was determined by aluminum chloride method [19] with minor modifications. To 0.25 ml of our sample (1mg/ml), 1.25 ml of distilled water and 0.075 ml of sodium nitrate (5%) were added in a tube, vortexed and left in the darkness for 6 min. Further 0.15 ml aluminum chloride (10%) was added to the tube, vortexed and left for 5 min at room temperature. Finally 0.5 ml of sodium hydroxide (4%) was added to the tube, followed by the addition of distilled water to obtain a final volume of 2.5 ml. The mixture in the tube was mixed. The absorbance was measured by spectrophotometer at 510 nm against the blank. Catechin was used as a reference with various concentrations (0.02, 0.04, 0.06, 0.08, 0.10 and 0.20 mg/ml). The total flavonoid content was expressed in mg of catechin equivalents per gram of extract. The analyses were carried out in triplicate.

Radical scavenging activity using DPPH method

DPPH method [20] was used for measuring the scavenging ability of essential oil, methanol extract and methanol fractions of *N. biserrata*. The stock solution of our sample was further diluted with distilled water at various ratios (5 different dilutions were prepared for essential oil, extract and fractions) based on their free radical scavenging activities.

An aliquot of each dilution (0.3 ml) was mixed with 2.7 ml of DPPH• (6×10^5 M in absolute methanol prepared) and left in the dark for 60 min. Absolute methanol was used as blank. The absorbance was measured at 512 nm using spectrophotometer. Ascorbic acid was used as standard. The radical-scavenging activity was calculated according to the formula summarized below.

$\%\ RSA = (A_{B\ control} - A_{A\ sample} / A_{B\ control}) \times 100$ (Eq. 1)

% RSA: Percentage of radical scavenging activities.

$A_{A:}$ Absorbance values of extract sample

$A_{B:}$ Absorbance values of control sample

The % DPPH radical scavenging activity was plotted against the sample/standard concentration to obtain IC_{50} value, which represents the concentration of the extract or standard antioxidant (mg/ml) required to scavenge 50% of the DPPH radical in the reaction mixture.

Preliminary phytochemical screening

The preliminary phytochemical screening of the methanol extract and methanol fractions (I mg/ml) of *N. biserrata* was carried out to evaluate the existence or the absence of various phytochemical compounds. The plant extract and fractions were analysed for alkaloids, steroids, flavonoids, triterpenoids, saponin, tannins, anthraquinones and phytosterol [21].

Gas chromatography-mass spectrometry (GCMS) analysis

Prepared samples of *N. biserrata* essential oil, methanol extract and methanol fractions were injected into a GCMS system consisting of an Agilent 7890A gas chromatograph system coupled with an Agilent 5975C mass spectrometry detector.

A capillary column HP-5MS (30 m × 0.25 mm) of 0.25 µm film thickness of coated material was used. Injector temperature was adjusted at 250°C, the temperature settings were as follow: initiate at 40°C and hold for 3 min; from 40 to 200°C at 3°C/min and then hold for 3 min.

A post-run of 5 min at 200°C was adjusted for the next injection. Pure helium gas (99.9% pure) was used as carrier gas and maintained at 1.0 ml/min constant flow rate. Gas chromatography was done in the splitless mode. Identification of compounds was carried out by referring to National Institute of Standards and Technology (NIST) library and the compositions were computed with reference to the abundance of the compounds in chromatogram. Each analysis was done in triplicate.

Data analyses

The experimental results were expressed as mean ± SD. All the assays were performed in triplicate.

RESULTS

Percentage (%) Yield

The percentage yields of methanol extract and methanol fractions (n-hexane, ethyl acetate, chloroform and butanol) of *N. biserrata* are shown in Table. 1.

Table 1: **The percentage yields of methanol extract and methanol fractions of *N. biserrata***

Extract/fractions	Percentage (%) yield
n-Hexane fraction	0.30 ± 0.02
Ethyl acetate fraction	1.93 ± 0.11
Chloroform fraction	0.30 ± 0.02
Butanol fraction	0.64 ± 0.10
Methanol extract	10.38 ± 0.41

Table 2: Total phenolic content of methanol extract and methanol fractions of *N. biserrata*

Extract/fractions	Total phenolic content (mg/g)
Essential oil	--
n-Hexane fraction	89.33 ± 0.72
Ethyl acetate fraction	122.33 ± 0.28
Chloroform fraction	116.83 ± 0.72
Butanol fraction	169.33 ± 4.04
Methanol Extract	127.28 ± 1.57

Total phenolic content of essential oil, methanol extract and methanol fractions

Total phenolic content was estimated according to the equation (y = 0.006x + 0.016, r2 = 0.9969) as gallic acid equivalent (mg/g extract) (Table 2). The highest phenolic content was found in butanol fraction (169.33 ± 4.04 mg/g) and methanol extract (127.28 ± 1.57 mg/g) followed by the other fractions, respectively (Table 2).

Total flavonoid content of essential oil, methanol extract and methanol fractions

Total flavonoid content was determined according to the equation (y = 0.0033x + 0.0091, r2 = 0.9989) obtained by calibration curves as catechin equivalent (mg/g extract). The highest flavonoid content was found in butanol fraction (53.19 ± 2.21 mg/g) and methanol extract (28.72± 1.82 mg/g) followed by other fractions and essential oil, respectively (Table 3).

DPPH scavenging activity of the essential oil, methanol extract and methanol fractions

The DPPH free radical scavenging activity was estimated at various concentrations. DPPH scavenging increased with the concentration of essential oil, extract and fractions of our sample. (Figure. 1)

Preliminary phytochemical screening of the methanol extract and methanol fractions

The preliminary phytochemical screening of methanol extract and methanol fractions of *N. biserrata* showed the existence of various bioactive components including anthraquinones, alkaloids, tannins, steroids, phytosterol, saponin, triterpinoids and flavonoids (Table 4).

Table 3: Total flavonoid content of essential oil, methanol extract and methanol fractions of *N. biserrata*

Extract/fractions	Total flavonoid contents (mg/g)
Essential oil	3.02 ± 0.33
n-Hexane fraction	22.53 ± 2.59
Ethyl acetate fraction	27.07 ± 0.70
Chloroform fraction	23.59 ± 2.20
Butanol fraction	53.19 ± 2.21
Methanol extracts	28.72± 1.82

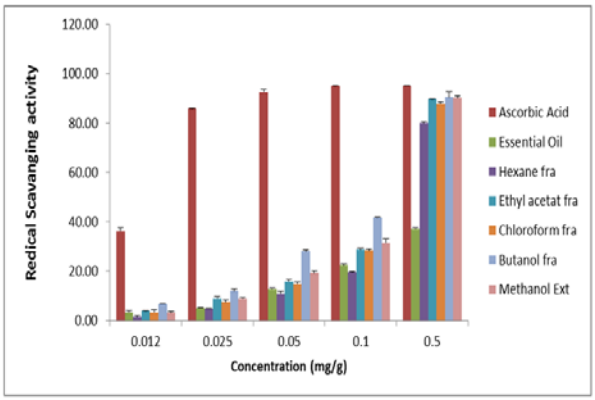

Fig. 1: Radical scavenging activity of the essential oil, methanol extract and methanol fractions of *N. biserrata*.

Table 4: The preliminary phytochemical screening of methanol extract and methanol fractions of *N. biserrata*

Biochemical	Inference				
	n-Hexane	Ethyl acetate	Chloroform	Butanol	Methanol
Alkaloids	++	+	+	+	++
Anthraquinones	-	-	-	-	+
Flavonoids	+	+	+	++	++
Phytosterol	+	+	+	++	++
Saponins	+	+	+	+	++
Tannins	+	+	+	+	+
Triterpenoids	++	++	++	++	++
Steroids	+	+	+	++	++

+ = present; ++ = Strong present, – = absent.

Phytochemical analysis by GCMS

The phytochemical compounds exist in the essential oil, methanol extract and methanol fractions of *N. biserrata* were identified by GCMS (Figure 2 to 7).

Table 5: The GCMS analysis of the essential oil, methanol extract and methanol fractions of *N. biserrata*

E. oil/extract/fractions	No	Compound name	RT	Area (%)
	1.	Benzaldehyde	11.06	0.99
E. oil	2.	Benzeneacetaldehyde	14.98	17.47
	3.	Phenylethyl Alcohol	18.35	2.19
	3.	Naphthalene	21.44	0.21
	4.	1-Cyclohexene-1-carboxaldehyde, 2,6,6-trimethyl-	23.38	0.25
	5.	1-Cyclohexene-1-acetaldehyde, 2,6,6-trimethyl-	25.05	0.23
	6.	Naphthalene, 2-methyl-	26.49	0.41
	7.	Naphthalene, 2,3-dimethyl-	31.86	0.13
	8.	3-Buten-2-one, 4-(2,6,6-trimethyl-1-cyclohexen-1-yl)-	34.86	2.52
	9.	Butylated Hydroxytoluene	35.98	46.80
	10.	3aH-Cyclopentacycloocten-3a-ol, 1,2,3,4,7,8,9,9a-octahydro-1,5,8,8-tetramethyl-, [1R-(1. alpha.,3a. beta.,9a. alpha.)]-	37.57	3.02
	11.	Naphthalene, 1,2,3,4,4a,7-hexahydro-1,6-dimethyl-4-(1-methylethyl)-	40.31	0.92
	12.	. alpha.-Cubebene	40.98	11.19
	13.	Methyl tetradecanoate	43.98	1.24
	14.	9,12,15-Octadecatrienoic acid, methyl ester, (Z,Z,Z)-	49.59	0.65
	15.	9-Hexadecenoic acid, methyl ester, (Z)-	50.02	1.51
	16.	Hexadecanoic acid, methyl ester	50.75	5.31
	17.	1,3a-Ethano-3aH-indene, 1,2,3,6,7,7a-hexahydro-2,2,4,7a-tetramethyl-, [1R-(1.	55.26	0.38

		alpha.,3a. alpha.,7a. alpha.)]-		
	18.	9,12-Octadecadienoic acid (Z,Z)-, methyl ester	55.91	0.49
	19.	cis-13-Octadecenoic acid, methyl ester	56.11	2.05
	20.	Heptadecanoic acid, 10-methyl-, methyl ester	56.96	2.05
	1.	Butanoic acid	5.68	0.81
n-Hexane	2.	Formic acid, undecyl ester	35.22	1.50
	3.	Phenol, 2,4-bis(1,1-dimethylethyl)-	36.03	2.87
	4.	Carbonochloridic acid, decyl ester	36.74	1.61
	5.	1-Heptadecene	42.48	1.13
	6.	4-Heptafluorobutyroxytridecane	42.54	2.24
	7.	Hexadecanoic acid, methyl ester	50.75	22.68
	8.	Tetracosane	55.23	31.67
	9.	9,12-Octadecadienoic acid, methyl ester	55.91	5.56
	10.	9,12,15-Octadecatrienoic acid, methyl ester, (Z,Z,Z)-	56.11	22.50
	11.	Methyl stearate	56.96	2.75
	12.	Bicyclo[5.1.0]octane, 8-methylene-	57.26	4.67
Ethyl acetate	1.	2,2-Dimethoxybutane	5.03	5.23
	2.	1,3-Benzenediol, 4-ethyl-	17.48	10.22
	3.	n-Tridecan-1-ol	30.32	3.21
	4.	Dodecanal	31.79	1.42
	5.	3-Fluoro-5-trifluoromethylbenzoic acid, 2-tridecyl ester	33.75	4.82
	6.	1,4-Benzenedicarboxylic acid, dimethyl ester	35.26	8.67
	7.	Phenol, 2,4-bis(1,1-dimethylethyl)-	36.03	15.75
	8.	Cyclopropane, nonyl-	36.75	14.14
	9.	2-Butenedioic acid (Z)-, monododecyl ester	38.45	2.96
	10.	Hexadecanoic acid, methyl ester	50.75	21.46
	11.	9,12-Octadecadienoic acid (Z,Z)-, methyl ester	55.91	2.65
	12.	9,12,15-Octadecatrienoic acid, methyl ester, (Z,Z,Z)-	56.10	7.46
	13.	Methyl stearate	56.95	2.00
Chloroform	1.	1,2-Cyclohexanedione	12.16	1.04
	2.	5-Octadecene, (E)-	35.28	2.64
	3.	Phenol, 2,4-bis(1,1-dimethylethyl)-	36.02	7.89
	4.	Pentafluoropropionic acid, undecyl ester	36.75	8.36
	5.	Tridecyl acetate	39.78	42.05
	6.	Hexadecanoic acid, methyl ester	50.75	38.02
	1.	1,4-Benzenedicarboxylic acid, dimethyl ester	35.41	2.15
Butanol	2.	Phenol, 2,4-bis(1,1-dimethylethyl)	36.03	6.14
	3.	Cyclopropane, nonyl-	36.75	1.02
	4.	Carbonic acid, tridecyl 2,2,2-trichloroethyl ester	39.15	4.07
	5.	Benzenamine, 3-(2-phenylethenyl)-,(E)	41.51	2.29
	6.	1-Octadecanesulphonyl chloride	46.19	9.59
	7.	Tetratetracontane	46.35	3.74
	8.	Hentriacontane	46.55	1.56
	9.	Hexadecanoic acid, methyl ester	50.75	40.63
	10.	9,15-Octadecadienoic acid, methyl ester, (Z,Z)-	55.91	6.49
	11.	9,12,15-Octadecatrienoic acid, methyl ester, (Z,Z,Z)-	56.11	18.68
	12.	Methyl stearate	56.96	3.64
	1.	Acetic acid	3.47	10.35
Methanol	2.	4-Cyclopentene-1,3-dione	8.33	0.68
	3.	Butyrolactone	9.29	1.84
	4.	2-Cyclopenten-1-one, 2-hydroxy-	9.78	1.94
	5.	Phenol	12.53	1.76
	6.	1,2-Cyclohexanedione	12.96	3.50
	7.	Benzyl alcohol	14.73	4.61
	8.	Benzene acetaldehyde	15.14	1.05
	9.	Phenol, 2-methoxy-	17.32	0.38
	10.	4H-Pyran-4-one, 2,3-dihydro-3,5-di hydroxy-6-methyl	19.90	9.28
	11.	2H-Pyran-2-one, 4,6-dimethyl-	22.48	0.57
	12.	Catechol	22.97	3.29
	13.	Benzofuran, 2,3-dihydro-	23.60	2.60
	14.	4-Mercaptophenol	24.04	5.78
	15.	Ethanone, 1-(2-hydroxy-5-methylphenyl)	27.67	1.30
	16.	Cyclododecane	34.13	13.84
	17.	Phenol, 2,4-bis(1,1-dimethylethyl)	36.07	0.84
	18.	2(4H)-Benzofuranone, 5,6,7,7a-tetrahydro-4,4,7a-trimethyl-, (R)	36.49	0.37
	19.	1-Undecanol, acetate	39.83	4.29
	20.	Hexadecanoic acid, methyl ester	50.80	2.07
	21.	n-Hexadecanoic acid	51.98	9.43
	22.	Heptafluorobutyric acid, n-tetradecyl ester	55.63	0.57
	23.	9,12-Octadecadienoic acid, methyl ester	55.95	9.61
	24.	Phytol	56.51	1.26
	25.	Gamolenic Acid	57.35	6.80
	26	Octadecanoic acid	58.14	0.70

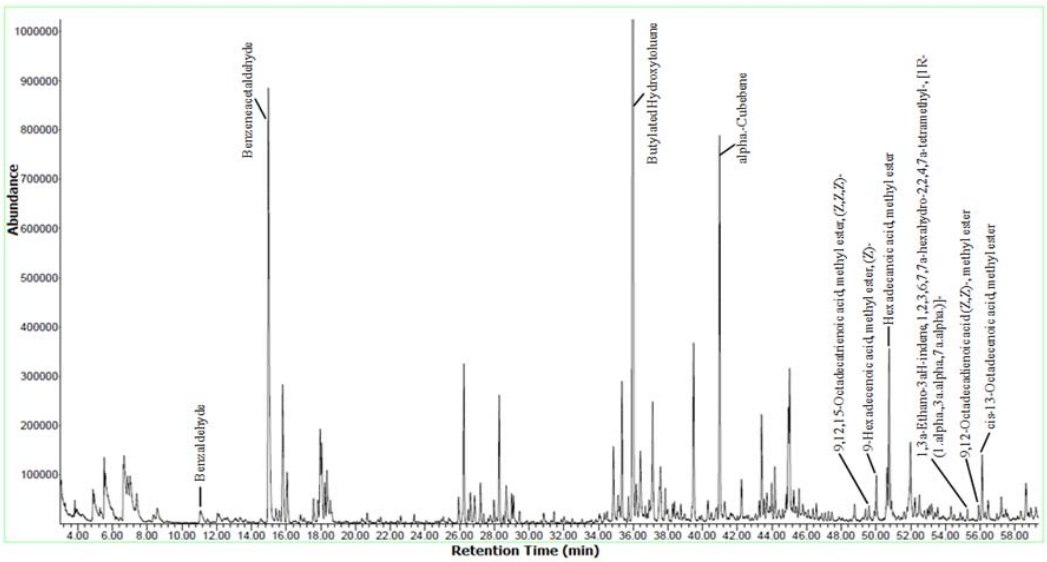

Fig. 2: A typical gas chromatogram of the chemical constituents of essential oil of *N. biserrata*.

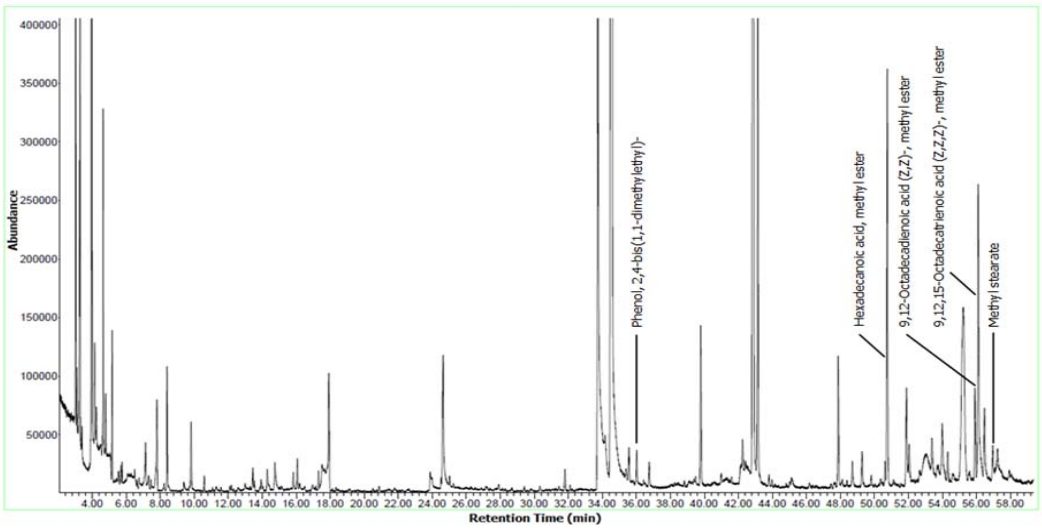

Fig. 3: A typical gas chromatogram of the chemical constituents of n-hexane fraction of *N. biserrata* methanol extract

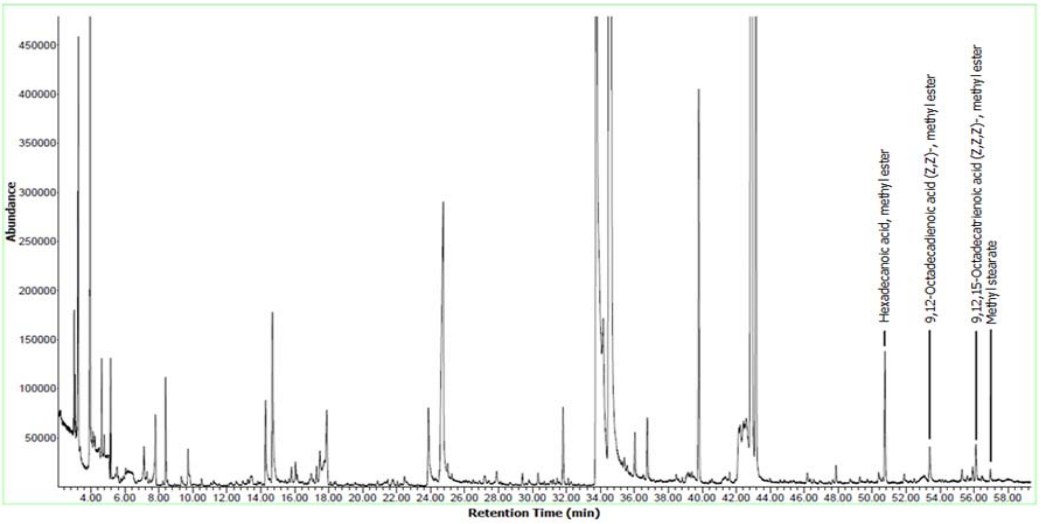

Fig. 4: A typical gas chromatogram of the chemical constituents of ethyl acetate fraction of *N. biserrata* methanol extract

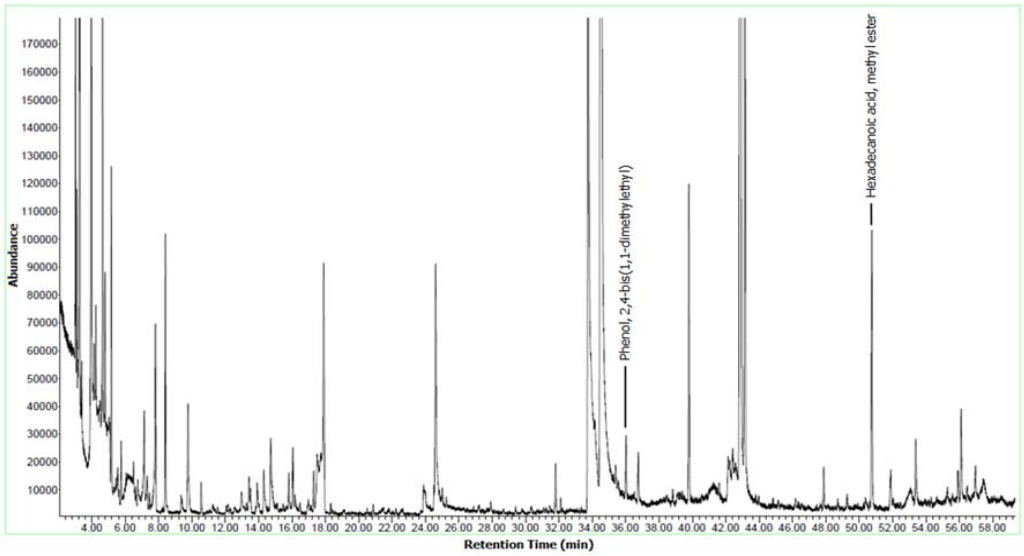

Fig. 5: A typical gas chromatogram of the chemical constituents of chloroform fraction of *N. biserrata* methanol extract

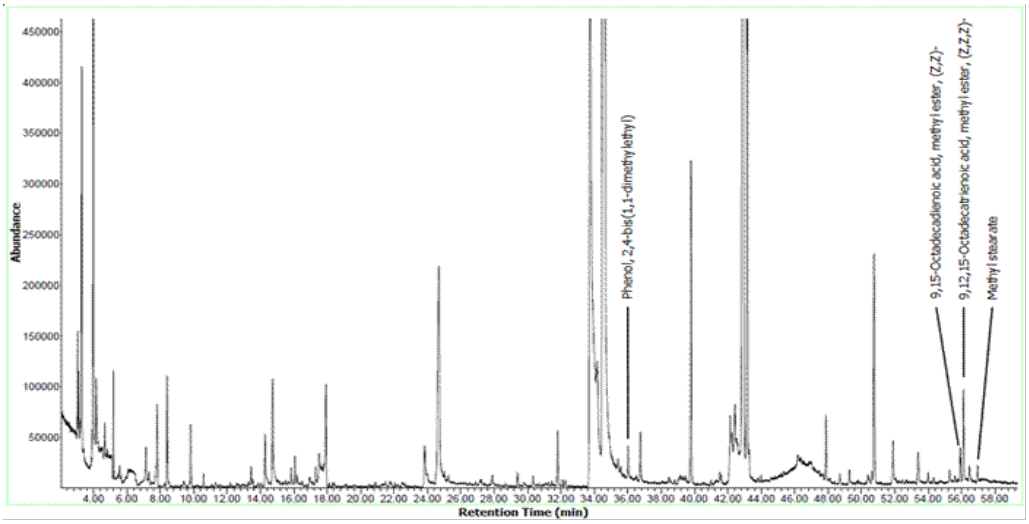

Fig. 6: A typical gas chromatogram of the chemical constituents of butanol fraction of *N. biserrata* methanol extract

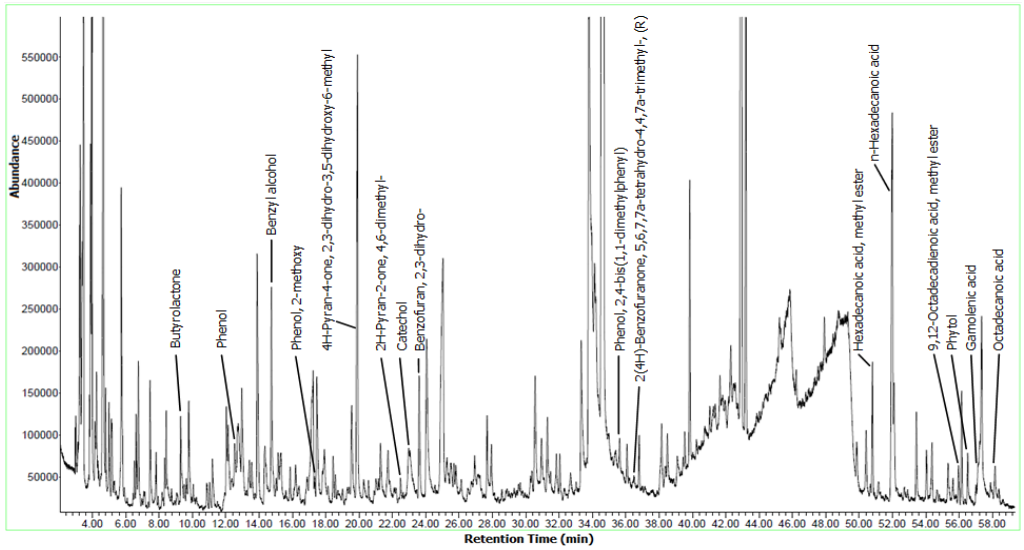

Fig. 7: A typical gas chromatogram of the chemical constituents of methanol extract of *N. biserrata*

The retention time (RT), percentage composition (%) and compounds name of the essential oil, methanol extract and methanol fractions of *N. biserrata* are indicated in Table 5. The identified bioactive compounds including, benzeneacetaldehyde, butylated hydroxytoluene, alpha.-cubebene, butyrolactone, phenol, benzyl alcohol, phenol, 2-methoxy-, 4h-pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl, 2h-pyran-2-one, 4,6-dimethyl-, catechol, benzofuran, 2,3-dihydro-, phenol, 2,4-bis(1,1-dimethylethyl), hexadecanoic acid, methyl ester, n-hexadecanoic acid, 9,12-octadecadienoic acid, methyl ester, phytol, gamolenic acid and octadecanoic acid.

DISCUSSION

Phenolic compounds present in various plants have attained considerable attentions due to their potential antioxidant property. Phenolics undergo a complex redox reaction with the phosphotungstic and phospho-molybdic acids found in the Folin-Ciocalteu reagent [22]. The data obtained in the present study indicated that the phenolic compound level is high in the butanol fraction and methanol extract followed by ethyl acetate, chloroform and n-hexane fractions. Antioxidant activity of the essential oil, methanol extract and methanol fractions of *N. biserrata* was also compared with total phenolic content and it was further found that radical scavenging effects were directly proportional to the phenolic content occur in our sample. Flavonoids are low molecular mass polyphenolics compounds, synthesized by plants as defense mechanism against infection [23]. The compounds show several biological activities including antioxidant and anti-inflammatory actions [24,25]. The n-hexane fraction and essential oil showed lower flavonoid values as compared to methanol extract and ethyl acetate, chloroform and butanol fractions (Table 3).

DPPH scavenging assay has been used for investigating antioxidant activities of food and medicinal plants due to its stability [26-28]. This assay determines the scavenging of stable radical species DPPH by antioxidant compounds occur in the essential oil, methanol extract and its fraction. Methanol extract of *N. biserrata* showed promising antioxidant activity with IC_{50} value of 0.25 mg/g while butanol fraction has maximum antioxidant activity with IC_{50} value of 0.22 mg/g followed by ethyl acetate, chloroform, n-hexane fraction and essential oil with IC_{50} values of 0.26, 0.27, 0.30 and 0.70 mg/g. The positive control, ascorbic acid indicated maximum scavenging effect at very low concentration with IC_{50} value of 0.060 mg/g. (Figure 1).

Phytochemical components found in the plants are known to be biologically active compounds and they are responsible for various activities including antioxidant, anti-inflammatory, antimicrobial, antifungal, and anticancer [29,30]. The preliminary phytochemical screening of methanol extract and its fractions showed the presence of active chemical constituents such as anthraquinones, alkaloids, tannins, steroids, phytosterols, saponins, triterpinoids and flavonoids. (Table 4). Anthraquinones have been reported with antioxidant activities. The compounds are also used as laxatives and for the treatment of fungal skin disease [31]. Our results indicate that anthraquinones are detected only in the methanol extract of *N. biserrata*. This might be the reason that the plant is used for the skin treatment by the local people in Sarawak, Malaysia [12].

Alkaloids and tannins have been reported with several pharmacological properties including anti-cholinergic, vasodilating, anti-hypertensive, anti-bacterial and anti-viral [32,33]. The data in the present studies indicate the presence of alkaloids and tannins in methanol extracts and methanol fractions. This might be the reason that *N. biserrata* is used for the treatment of infectious diseases [16]. Steroids are isolated from various plants including *Phoenix dactylifera,* and *Prunus armeniucaa* [34]. The compounds have been reported with anti-inflammatory properties [35]. Saponins exhibit coagulating and cholesterol binding activities [36]. Phytosterols are crucial in lowering blood cholesterol [37]. Triterpenoids have been reported with anti-inflammatory, analgesic and anti-pyretic properties [38]. According to the present data steroids, saponins, phytosterol and triterpenoids are present in methanol extract and methanol fractions of *N. biserrata*. This might be the reason that the plant is used for wound cleaning, and jaundice [15,16].

The GCMS analysis of essential oil, methanol extract and methanol fractions indicated the presence of various bioactive compounds. Benzeldehye is an aromatic aldehyde and has been detected in the essential oil from flowering *Marrubium vulgare* L [39]. The compound has been reported with antitumor properties [40]. Benzeneacetaldehyde is an aromatic aldehyde with antioxidant and antiinflammatory activities [41]. Butyrolactone is a ketone. The derivatives of the compound has been also isolated from the tubers of *Pleione bulbocodioides* [42] and reported with antioxidant and analgesic activities [43, 44]. Phenol is a simplest phenolic compound. It has been reported in the ethanol bark extract of *Ficus religiosa linn* with antioxidant, antiseptic and antibacterial properties [45]. Benzyl alcohol is an aromatic alcohol and has been reported with antioxidant and antimicrobial activities in the solvent extract of *Alpinia galangal* and *Ocimum basilicum L* leaves extract [46-49]. Phenol, 2-methoxy- is a phenolic compound. The compound has been also found in ethanol bark extract of *Ficus religiosa linn*. The antimicrobial activity of the compound has been reported [45]. 4H-pyran-4-one, 2,3-dihydro-3,5-di hydroxy-6-methyl is a flavonoid. The compound has been also isolated from the aqueous methanol extract of *Vitex negundo* and ethanol extract of *Cyperus rotundus* leaves. The antimicrobial, anti-inflammatory and antiproliferative properties of the compound have been reported [50,51]. 2H-pyran-2-one, 4,6-dimethyl- is pyrone and the derivatives of the compound has been also detected in *Helichrysum italicum* ssp, microphyllum and reported with antiinflammatory and antiviral properties [52]. Catechol is alcoholic compound and has been reported with anti-inflammatory properties [53]. Benzofuran 2,3-dihydro is a benzofuran and has been also found in the ethanol leaf extract of *Tabebuia rosea* and reported with antihelminthic, anti-inflammatory and antidiarrheal activities [54]. Phenol, 2,4-bis(1,1-dimethylethyl) is alkylated phenol and has been reported with antioxidant activity in *Plumbago zeylanica* [55]. Hexadecanoic acid, methyl ester and n-hexadecanoic acid are commonly known as palmitic acid ester and palmitic acid. These fatty acids have been also detected in the extract of *Vitex negundo* and reported with antioxidant, and hypocholesterolemic activities [50]. Phytol is diterpen alcohol and has been reported with anticancer, anti-inflammatory and hepatoprotective properties in *Hybanthus enneaspermus* [50,56]. Gamolenic Acid (gamma-linolenic acid) is omega 6 fatty acid and has been reported with anti-inflammatory properties [57]. Octadecanoic acid (stearic acid) is a fatty acid and has been reported with antioxidant and antimicrobial activity in *ficus religiosa linn extract* [45].

CONCLUSION

Current work described the evaluation of antioxidant activities as well as phytochemical composition of essential oil, methanol extract and methanol fractions of *N. biserrata*. To the best of our knowledge it was the first time that the analysis of phytochemical constituents and antioxidant activates of the *N. biserrata* are determined. It is demonstrated that the essential oil, methanol extract and methanol fraction (n-hexane, ethyl acetate, chloroform and butanol) of the plant contained different phytochemical compounds and possessed diverse antioxidant properties. Hence, the essential oil, extract and fractions *of N. biserrata* represent a source of potential antioxidants that could be used in pharmaceutical industry.

CONFLICT OF INTERESTS

Declared None

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