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Secondary metabolites from *Morinda elliptica* leaves and root extracts and their antioxidant activities

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ABSTRACT

The isolation and purification of dichloromethane fraction of the root bark extract and leaves extract of *Morinda elliptica* using different chromatographic methods have led to a total of five metabolites. Their structures were elucidated using various spectral data and were confirmed as benzaldehyde (**1**), 2-hydroxy-1-methoxyanthracene-9,10-quinone (**2**), dehydroabietic acid (**3**), and 3-(4-ethoxy-3-methoxyphenyl)-1-phenylprop-2-en-1-one (**4**), which were extracted from the root, while methyl cinnamate (**5**) was successfully isolated from the leaves extract. The 1,1-diphenyl-2-picryl-hydrazil assay revealed that dichloromethane and methanol fractions of the leaves and root extracts demonstrated strong antioxidant activities, which were comparable with ascorbic acid. Hexane and ethyl acetate fractions showed weak antioxidant activities. This study showed that *M. elliptica* has a beneficial effect, considering the virtue of its antioxidant properties. These isolated compounds (**1**-**4**) were reported for the first time in the root bark extract of *M. elliptica* and contributed to the chemotaxonomic importance of *M. elliptica*. Dehydroabietic acid (**3**) which is claimed to be a key to active compounds in treating acute stroke, epilepsy, asthma, hypertension, gastric hypermotility, and psychoses, has further added to the medicinal value of *M. elliptica.*

Keywords: *Morinda elliptica*; secondary metabolites; root bark extract; leaves extract; antioxidant activity

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1. INTRODUCTION

The advent of drug-resistant microorganism coupled with uncontrolled side effects of modern medicine has made traditional medicine to be accepted as an alternative form of the health care system (Dahiru et al., 2010). Various plants with medicinal values that are used in different traditional medicine systems were explored for their immense potentials. The potentials of these medicinal plants in pharmacology have been revealed from the results of these studies (Wakawa and Hauwa, 2013; Palipoch, 2013). Different parts of these medicinal plants are employed in various ways in treating many health problems and ailments; leaves are taken for headache,

cholera, diarrhea, and particularly in a fever, and for loss of appetite. The pounded leaves have healed the spleen and wounds. A lotion of leaves is used for hemorrhoids and rubbed on the body after childbirth (Burkill, 1996).

Antioxidant activities of compounds are reported to play an active role in protecting the body from free radical-induced oxidative stress (Rupan et al., 2012). Oxidative stress has been known as a major causative factor in the induction of many chronic degenerative diseases (Young and Woodside, 2001). These include diabetes mellitus, atherosclerosis, and inflammatory conditions (Rosenfeld, 1998). Plants contain natural antioxidants, which scavenge for harmful free radicals from the human body. Many reports on the antioxidant activity of medicinal plants (Wang et al., 1996) are shown that the plants contain many antioxidants like polyphenol. These antioxidants are important in quenching singlets and triplet oxygen, decomposing peroxides as well as absorbing and neutralizing free radicals (Djeridane et al., 2006).

The carcinogenicity of synthetic antioxidants is being restricted for use in food or medicinal materials. As such, interest has increased considerably in finding naturally occurring antioxidants (Velioglu et al., 1988). Plants with ethnopharmacological data that indicated their ethnobotany uses for the treatment of abdominal diseases as well as antibacterial activities have been proven to contain antioxidant activities. This inspired the choice to assess the antioxidant activities of the leaves and root extracts of *Morinda elliptica*.

M. elliptica is a shrub, a genus of the family Rubiaceae, and growing wild in bushes or newly developed areas throughout the Malay peninsula (Wakawa et al., 2017). In Malay peninsular, *Morinda* comprises of nine species, which include three species of trees and shrub (*M. citrifolia*, *M. elliptica*, and *M. corneri*) and six species of climbers (*M. lacunosa*, *M. rigida*, *M. calciphila*, *M. scortechinii*, *M. umbellate*, and *M. ridleyi*) (Wong, 1984). *M. citrifolia* has been extensively studied and vast active phytochemicals are reported for many medicinal purposes (Rath et al., 1995; Wakawa et al., 2017). *M. elliptica* has also been referred to as the noni fruit (Chan-Blanco et al., 2006). However few researchers have explored the potential of this species of *Morinda* (*M. elliptica*).

Ismail and his co-researchers reported two new anthraquinones, 2-formyl-1-hydroxyanthraquinone (Ismail et al., 1997) and 2-formyl-1-hydroxy-9,10-anthraquinone (Ismail et al., 2012) along with 10 others known anthraquinones; 1-hydroxy-2-methylanthraquinone, nordamnacanthal, damnacanthal, lucidin-ω-methyl ether, rubiadin, sorandidol, morindone, rubiadin-l-methyl ether, alizarin-l-methyl ether and morindone-5-methyl ether (Ismail et al., 1997) from the root of *M. elliptica*. Apart from these, there is only a few reports on the roots of *M. elliptica*.

These anthraquinones have some applications in medicinal chemistry and have anti-cancer and antidiabetic activities (Mahapatra et al., 2015), as well as a broad spectrum of pharmacological activities, including antifungal, antibacterial, anti-inflammatory, anti-plasmodial, immunosuppressive, antioxidant, antileishmanial, analgesic and antipyretic activities (Anam et al., 2017; Matos et al., 2015). This work was designed to isolate active bioactive compounds with the view of exploring the potentials of *M. elliptica* and to add to the already existing knowledge about the medicinal efficacy of the plant.

2. MATERIALS AND METHODS

2.1 Plant materials

M. elliptica fresh root and leaves collected from uncultivated farmland in Limbang, Sarawak were used to prepare the crude extracts. They were identified and authenticated by Professor Dr. Zaini bin Assim from Faculty of Resource Science and Technology, Universiti Malaysia Sarawak. The herbarium samples were given the voucher specimen number, WH/MEL015/03, and WH/MER015/04. The studied samples were cleaned and dried at room temperature.

2.2 Extraction and isolation of secondary metabolite

The dried root bark of *M. elliptica* was grounded into a powdered form by using a laboratory grinder machine (FGR-350, Quest Scientific, New Delhi, India). The ground plant samples were macerated in four different solvents, namely hexane, dichloromethane, ethyl acetate and methanol. The sample was macerated in hexane (sample: to solvent ratio, 1:3) in an erlenmeyer flask (5L) for 5 days at room temperature. The resulting hexane solution was filtered using a Whatman filter paper (No. 4). Fresh hexane was added to the residue and allowed to stand for 72 hours and then filtered. Both the filtrates were combined, and the solvent was removed under reduced pressure below 50°C in a rotary evaporator (Heidolph Laborota 4000 efficient, Berlin, Germany) to obtain the hexane extract. The same procedures were employed to the residue by using three different polarity solvents to yield dichloromethane, ethyl acetate and methanol extracts.

The secondary metabolite was isolated and purified from the dichloromethane fractions of the leaves (5.322g) and root bark extract (5.322g) by using various chromatographic methods, which included column chromatography (silica gel 60, Merck (Germany) 70-230 mesh ASTM 0.063-0.200 mm) and radial chromatography, chromatotron (silica gel 60 PF_{254} containing gypsum, Merck (Germany), 1.07749). The elution was performed with various combinations of solvents systems, for example, hexane: dichloromethane 9:1, 7:3, or hexane: ethyl acetate 9:1, 4:1. The TLC profiles of subfractions or compounds were examined under a short-wave and long-wave UV light to identify a suitable solvents system for further isolation and purification processes. TLC profile was also used to check the purity of compounds.

2.3 Identification of secondary metabolites

The identification of secondary metabolites was employed by various spectroscopic techniques i.e., gas chromatography-mass spectrometry (GC-MS) (Shimadzu model QP 10 Plus, Tokyo, Japan), Fourier transform infrared (FTIR) spectroscopy (Nicolet iS10 SMART iTR Thermo Scientific, Waltham, USA) and nuclear magnetic resonance (NMR) (JEOL JNM- ECA 500, Tokyo, Japan). The GC-MS analysis was performed to obtain the molecular mass of the compound, which is based on mass to charge ratio (*M/z*) (Kalaiselvan et al., 2012). The FTIR analysis described by Shalini and Samparthkumar (2012) was performed. The sample was scanned in a range of 400 to 4000 cm^{-1} with a resolution of 4 cm^{-1}. The sample for NMR analysis was dissolved in acetone-

D_6 and ^1H and ^{13}C-NMR spectra were measured at 125 and 500 MHz, respectively (Efdi et al., 2010).

2.4 Antioxidant activity

Assessment of the antioxidant activities of the plant extract on the free radical scavenging effect of DPPH was employed using the procedures described by Khong et al. (2006). Ascorbic acid was used as standard and a working solution was prepared in methanol. The stock solution of the extracts (5 mg/mL) for antioxidant activity was prepared by dissolving a known amount of the dry extract in methanol (98% w/v). A suitable dilution was used to prepare the working solution of each extract with various concentrations (1, 10, 50, 100, and 500 mg/mL). Exactly 0.0024 g of DPPH was dissolved in 100 mL methanol and 1 mL of the solution was added with 2 mL of the sample solution and the standard solution in separate test tubes. The solution mixtures were kept in dark for 30 minutes. UV spectrophotometer (Jasco V-630, Tokyo, Japan) was used to measure the absorbance of the sample at 517 nm. A mixture of 1 mL methanol and 0.002% DPPH was used as blank and the absorbance was recorded. The samples were performed in triplicates.

2.5 Statistical analysis

From the data, the IC_{50} of the plant was calculated by using statistical software (GraphPad prism 3.02, California, USA) and the mean was expressed at a 95 % level of confidence.

3. RESULTS AND DISCUSSION

3.1 Isolation and identification of secondary metabolite from root bark extract

Four compounds were isolated from the root bark extract (5.322g) of *M. elliptica* by several chromatographic techniques. The structures of these compounds were identified as benzaldehyde (**1**) (10.4 mg), 2-hydroxy-1-methoxyanthracene-9,10-quinone (**2**) (9.4 mg), dehydroabietic acid (**3**) (6.4 mg), and 3-(4-ethoxy-3-methoxyphenyl)-1-phenylprop-2-en-1-one (**4**) (6.8 mg). The structures were elucidated based on the IR, ^1H-NMR, ^{13}C-NMR, and MS spectral data and also by comparison of their spectroscopic data with the reported data. These compounds **1**, **2**, **3,** and **4** were isolated and identified for the first time in the root bark extract of *M. elliptica.*

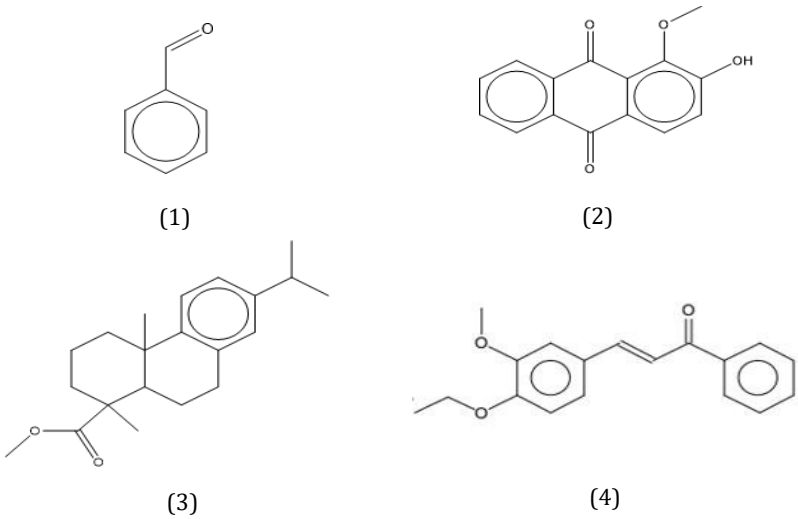

Figure 1. Structure of benzaldehyde (**1**), 2-hydroxy-1-methoxyanthracene-9,10-quinone (**2**), dehydroabietic acid (**3**) and 3-(4-ethoxy-3-methoxyphenyl)-1-phenylprop-2-en-1-one (**4**)

Compound **1** was obtained as a white yellowish compound. It was fractionated by column chromatography and eluted with hexane: $CHCl_3$ (9:1). TLC analysis showed that the R_f value of compound **1** was 0.23. It had a melting point of 50-51°C. GC-MS [C_7H_6O] (S.I. 82.9%) 106 (M^+); IR V_{max} cm^{-1}: 3011, 1699, 1592, 1015; MS *m/z* (% rel. int): 108 (10), 107 (90), 106 (97), 103 (6), 79 (5), 78 (18), 77 (100), 74 (12), 63 (7), 52 (14), 51 (40), 50 (25), 49 (7), 39 (8); ^1H-NMR (500 MHz, $CDCl_3$) δ (ppm): 9.85 (1H, s, CHO), 7.44 (2H, s, H3/H5), 7.73 (2H, s, H2/H6), 7.46 (1H, s, H4); ^{13}C-NMR (500 MHz, $CDCl_3$) δ (ppm): 191.70 (C-7), 136.98 (C-1), 134.35 (C-4), 129.98 (C-2/C-6), 129.12 (C-3/C-5).

Compound 1 was identified as benzaldehyde, which was based on the available spectral data. This structure elucidation of the compound was also confirmed by comparison with literature data (Tan et al., 2014; Verma et al., 2017). The IR spectrum of compound 1 indicated similarity with the IR spectrum of benzaldehyde reported by Tan et al. (2014). The NMR (1H-, 13C-) data was also supported by Verma et al. (2017) and Tan et al. (2014). Benzaldehyde, which occurs naturally, is widely used in the preparation of various aniline dyes, perfumes, flavoring, and pharmaceuticals. It has been used directly as a flavoring agent in foods (Molina et al., 2014). Verma et al. (2017) reported that a high amount of benzaldehyde is extracted from leaf of *Prunus persica* (commonly known as peach).

Compound **2** was isolated as a yellow substance. It was fractionated using chromatotron and eluted with hexane: ethyl acetate (9.8:0.2). The TLC analysis showed R_f value of 0.65. It has a melting point of 179-180°C; GC-MS [$C_{15}H_{10}O_4$] (S.I. 85.4%) 254 (M^+); IR V_{max} cm^{-1}: 3402, 3013, 1669, 1577, 1276, 982, 839; MS *m/z* (% rel. int): 355 (1), 341 (2), 281 (4), 255 (9), 253 (54), 235 (38), 209 (14), 207 (100), 206 (13), 180 (9), 152 (14), 138 (20), 126 (18), 105 (8), 77 (10), 63 (7), 49 (6); ^1H-NMR (500 MHz, acetone D_6) δ (ppm): 8.19-8.20 (2H, d, H-5/H-8), 7.87 (1H, d, H-3), 7.65 (2H, d, H-6/H-7), 7.30

(1H, d, H-4), 3.92 (3H, s, OMe); ^{13}C-NMR (500 MHz, $CDCl_3$) δ (ppm): 181.92 (C-9), 181.60 (C-10), 161.67 (C-2), 148.79 (C-1), 136.83 (C-13), 133.09 (C-6), 133.09 (C-7), 129.95 (C-14), 126.84 (C-3), 126.10 (C-11), 126. 31 (C-12), 125.43 (C-5), 125.03 (C-8), 121 34 (C-4), 60.42 (1-OCH_3).

Both NMR (^{1}H-, ^{13}C-) data were similar to the reported 2-hydroxy-1- methoxyanthracene-9,10-quinone by Wu et al. (2009). The data was further supported by the similar stretching and bending characteristics in the IR spectrum of 2-hydroxy-1-methoxyanthracene-9,10-quinone. Thus, compound **2** was confirmed as 2-hydroxy-1-methoxyanthracene-9,10-quinone. The chemical diversity and biological activities of anthraquinones and their derivatives recently attracted the attention of industries, particularly pharmaceuticals, food colorants, and clothes dyeing (Fouillaud et al., 2016).

Compound **3** was isolated as orange substance with a melting point of 246-248°C. It was fractionated by using chromatotron and was eluted with hexane: $CHCl_3$ (9:1) as the mobile phase solvent. The TLC showed a single spot with R_f value of 0.82; GC-MS [$C_{21}H_{30}O_2$] (S.I. 87.2%) 314 (M^+); IR Vmax cm^{-1}: 2918, 1711, 1431, 1221, 1091; MS *m/z* (% rel. int): 429 (1), 400 (1), 355 (3), 315 (5), 314 (11), 29 (12), 281 (6), 240 (16), 239 (100), 209 (6), 197 (8), 141 (7), 128 (8), 90 (6), 72 (8), 43 (10); ^{1}H-NMR (500 MHz, $CDCl_3$) δ (ppm): 3.65 (3H, s, OMe), 1.86 (2H, s, H-7), 1.67 (7H, m, H-14), 1.55 (4H, d, H-2), 1.42 (2H, d, H-1), 1.33 (9H, d, H-16), 1.12 (3H, d, H-20); ^{13}C-NMR (500 MHz, $CDCl_3$) δ (ppm): 178.19 (C-18), 146.62 (C-13), 129.31 (C-14), 87.61 (C-15), 84.24 (C-8), 74.56 (C-12), 52.64 (C-21), 49.40 (C-9), 47.91 (C-5), 46.33 (C-4), 37.50 (C-1), 37.19 (C-3), 35.80 (C-10), 31.94 (C-7), 29.78 (C-16), 23.99 (C-11), 22.40 (C-17), 21.75 (C-6), 16.22 (C-2), 14.14 (C-20).

Based on the spectral data, compound **3** was elucidated as dehydroabietic acid. The ^{1}H-NMR and ^{13}C-NMR data of compound **3** were also supported by the reported NMR data for dehydroabietic acid by Koutsaviti et al. (2017). As a result, compound **3** was confirmed as dehydroabietic acid (**3**).

Compound **3** is a typical compound of a class that is common to the genus *Pinus* and was reported for the first time as a natural product of plant origin to the genus *Pinus* (Pinaceae) (Koutsaviti et al., 2017). This work thus made it the second to be isolated as a natural product of plant origin. The isolated compound from root bark of *M. elliptica* may improve medicinal efficacy of *M. elliptica*. Dehydroabietic acid (**3**) has demonstrated the enhanced anticancer activity in cervical carcinoma cells, hepatocellular carcinoma cells or breast cells (Gonzalez et al., 2010). Previous studies have demonstrated that dehydroabietic acid (**3**) and some derivatives are chemical modulators, which make compound **3** as a new scaffold in treating acute stroke, epilepsy, asthma, hypertension, gastric hypermotility and psychoses (Gonzalez et al., 2010).

Compound **4** was isolated as pale-yellow substance with a melting point of 106-107°C. It was fractionated with chromatotron using hexane: ethyl acetate (4:1) as eluting solvent. The TLC analysis showed a single spot with R_f value of 0.26; GS-MS [$C_{18}H_{18}O_3$] (S.I. 86.3%) 282 (M^+); IR V_{max} cm^{-1}: 2943, 2844, 1708, 1585, 1422, 1266, 1210, 1113, 1038, 949, 818, 654; MS *m/z* (% rel. int): 415 (1), 400 (1), 355 (3), 341 (2), 285 (2), 284 (6), 283 (18), 282 (100), 281 (70), 254 (6), 251 (48), 236 (15), 220 (10), 205 (16), 178 (8), 165 (10), 151 (12), 118 (10), 91 (18), 63 (14), 44 (11); ^{1}H-NMR (500 MHz, acetone D_6) δ (ppm): 8.03 (2H, m, H-10), 8.01 (2H, m, H2'/H-6'), 7.85 (2H, m, H-3'/H-5'), 7.36 (1H, m, H-2), 7.36 (2H, s, H-11), 6.62 (1H, d, H-4'), 4.30 (3H, s, H-7), 3.96 (3H, s, OMe), 1.22-1.27 (2H, d, H-8); ^{13}C-NMR (500 MHz, acetone D_6) δ (ppm): 183.31 (C-12), 149.17 (C-3), 149.17 (C-4), 145.18 (C-10), 136.96 (C-1'), 134.36 (C-4'), 129.98 (C-3'/C-5'), 126.95 (C-2'/C-6'), 126.07 (C-1), 121.74 (C-6), 121.05 (C-11), 118.90 (C-5), 112.81 (C-2), 64.82 (C-7), 60.65 (C-9), 15.32 (C-8).

Compound **4** was identified as 3-(4-ethoxy-3-methoxyphenyl)-1-phenylprop-2-en-1-one based on spectral data and comparison with the published data (Anam et al., 2017). Flavonoids and isoflavonoids are vital in defending the plant systems against microorganisms, animals and insects (Nasir et al., 2012; Sahu et al., 2012). 3-(4-Ethoxy-3-methoxyphenyl)-1-phenylprop-2-en-1-one (**4**) has been identified as a chalcone (benzylideneacetophones), which belongs to the largest class of metabolites (Anam et al., 2017). It has been reported to exhibit anti-cancer activity, antidiabetic activity (Mahapatra et al., 2015) and various pharmacological activities, including antifungal, antibacterial, anti-inflammatory, anti-plasmodial, immunosuppressive, antioxidant, antileishmanial, analgesic and anti pyretic activities (Anam et al., 2017; Matos et al., 2015). It has nonlinear optical and luminescence properties (Sun et al., 2013; Komarova et al., 2015; Yang et al., 2015).

3.2 Isolation and identification of secondary metabolite from leave extract

A pure compound was isolated from the dichloromethane fraction of the leaves extract. It was fractionated using column chromatography with hexane: $CHCl_3$ (3:2) chromatotron and eluted with hexane: $CHCl_3$ (4:1). TLC analysis of the compound gave a R_f value of 0.43. It was obtained as a white crystal substance with a melting point of 36-38°C; GC-MS [$C_{10}H_{10}O_2$] (SI 96%) 162 (M^+); IR V_{max} cm^{-1}: 2971, 1688,1631,1304, 1220, 1044, 876; MS *m/z* (% rel. int): 162 (58), 147 (2), 134 (4), 131 (100), 115 (3), 103 (68), 98 (1), 91 (5), 76 (6), 77(40), 63 (6), 51 (24), 39 (3), 27(1); ^{1}H-NMR (500 MHz, $CDCl_3$) δ (ppm): 7.69 (1H, d, H-2), 7.64 (4H, d, H-4/H-5), 7.43 (1H, s, H-6), 6.50 (1H, d, H-3), 3.79 (3H, s, H-1); ^{13}C-NMR (500 MHz, $CDCl_3$) δ (ppm): 167.13 (C-8), 144.88 (C-2), 134.66 (C-7), 129.13 (C-5/C-5'), 128.88 (C-4/C-4'), 128.21 (C-6), 117.68 (H-3), 51.68 (H-1). Based on this spectral data, it was identified as methyl cinnamate (2-propenoic acid, 3-phenyl-, methyl ester) (**5**). The IR and the NMR spectral data of methyl cinnamate were also supported by Shaza and Juamaa (2016).

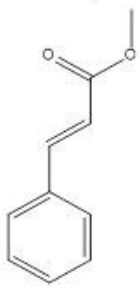

Figure 2. Structure of methyl cinnamate

Methyl cinnamate (Figure 2) was reported to possess antitumor properties, anti-inflammatory, antimicrobial activities (Sawicka et al., 2012), and anticancer activity (Kadhim et al., 2016). It was reported as a compound of low toxicity, which is used as flavoring and inhibit mitochondrial pyruvate transport (Hoskins, 1984). Methyl cinnamate is the main constituent of cinnamon oil (Xing et al., 2012). The

cinnamon oil demonstrates *in vitro* and *in vivo* antifungal activities that inactivate some postharvest pathogens, and are the causal agents of jujube (orange fruit), including *Rhizopus nigricans*, *Aspergillus flavas* and *Penicillium expansum*. The cinnamon oil exhibited stronger antifungal activity against *A. flavus* and *P. expansum* as compared to that of *R. nigricans*.

3.3 Antioxidant activity

In this study, free radical scavenging activities of leaves and roots extracts of *M. elliptica* from different solvent systems of varying polarity were evaluated. The IC_{50} values of the leaves extract of *M. elliptica* in different solvents resulted in different antioxidant activity (Table 1). This study revealed that dichloromethane and methanol fractions of the leaves extract exhibited strong antioxidant activities with IC_{50} values of 86.60 µg/mL and 35.00 µg/mL, respectively, which were comparable with that of the positive control, ascorbic acid (IC_{50} value of 64.93 µg/mL). Both hexane and ethyl acetate leaves extracts exhibited weak antioxidant activities (IC_{50} >100 µg/mL).

A similar result was observed in the root extracts. Table 2 shows the IC_{50} values of the root extract of *M. elliptica* in different solvents with different measures of antioxidant activity. The dichloromethane and methanol fractions showed stronger antioxidant activities with IC_{50} values of 42.08 µg/mL and 52.70 µg/mL, respectively, compared with ascorbic acid (IC_{50} = 66.47 µg/mL) (Mariya and Reena, 2017). However, both hexane and ethyl acetate fractions of root extracts demonstrated weak antioxidant activities with IC_{50} values >100 µg/mL. The result of this study indicated that the antioxidant activity of the methanol and dichloromethane fractions of the plant extracts of *M. elliptica* was comparable to that of the positive control.

Table 1. IC_{50} values of *M. elliptica* leaves extracts

Crude extract	Calibration equation		IC_{50} (µg/mL) (mean+ SD)
Control	y = 3.023X + 12.93	R^2 = 0.9945	64.93 ± 2.40
Hexane	y = 0.505X – 7.197	R^2 = 0.9824	>100*
Dichloromethane	y = 1.299X + 17.85	R^2 = 0.9831	86.60 ± 1.90**
Ethyl acetate	y = 0.386X – 9.610	R^2 = 0.9981	>100*
Methanol	y = 0.946X + 11.32	R^2 = 0.9853	35.00 ± 2.10**

Note: experiments performed in triplicate (Mariya and Reena, 2017)
*IC_{50} > 100 µg/mL = weak activity; ** IC_{50} < 100 µg/mL = strong activity

Table 2. IC_{50} values of *M. elliptica* root extracts

Crude extract	Calibration equation		IC_{50} (µg/mL) (mean+ SD)
Control Hexane	y = 2.858X + 10.72 y = 1.128X – 5.074	R^2 = 0.9979 R^2 = 0.9959	66.47 ± 1.80 >100*
Dichloromethane	y = 0.714X + 1.522	R^2 = 0.9982	42.08 ± 1.50**
Ethyl acetate	y = 0.386X – 9.610	R^2 = 0.9981	>100*
Methanol	y = 1.225X + 8.831	R^2 = 0.9978	52.70 ± 1.50**

Note: experiments performed in triplicate (Mariya and Reena, 2017)
*IC_{50} > 100 µg/mL = weak activity; ** IC_{50} < 100 µg/mL = strong activity

This result is congruent to the work of Subramaniam et al. (2003) in which they reported that Mengkudu (*M. elliptica*) extract showed high superoxide scavenging assay (80%) as compared to superoxide dismutase (SOD) standard. The strong antioxidant activity exhibited by the methanol extract agrees with the work of Mariya and Reena (2017) in which they reported that methanolic extract was most effective in all the methods used for the antioxidant study of plant extract and the weak antioxidant exhibited in the ethyl acetate fraction in both the extracts. These results are incongruent with the findings of Padalia et al. (2015). The difference observed in the activity of the different fractions of the extract was not well understood. However, it could be attributed to the active compounds extracted in the different solvents due to their polarity. Due to the scavenging activities of antioxidants, they may be useful in the management of such chronic degenerative disorders. Ascorbic acid deficiency results in a striking pathological change. The reduction in the number of intracellular substances causes the endothelial wall of the capillaries to weaken (Prakash et al., 2009). Due to the depletion of the immune system, the naturally occurring antioxidants in plants as free scavenging radicals may be necessary to complement the deficiency of ascorbic acid for their availability and affordability. These natural antioxidants are responsible for preventing or inhibiting the deleterious consequences of oxidative stress. Free radicals like ascorbic acid play a vital role in the forming of intracellular substances throughout the body by impairing the formation of free radicals (Aqil et al., 2006).

4. CONCLUSION

In conclusion, the root bark extract and leaves extract of *M. elliptica* have successfully isolated five metabolites. From the root bark extract and leaves extract. Compounds (**1**-**4**) were reported for the first time in the root bark extract of *M. elliptica* and contributed to the chemotaxonomic importance

of this plant. Dehydroabietic acid is claimed to be a key to active compounds in treating hypertension, acute stroke, asthma, epilepsy, gastric hypermotility as well as psychoses (Gonzalez et al., 2010); and has further added to the medicinal value of *M. elliptica.*

Besides, the dichloromethane and methanol fractions in both leaves and root extracts showed strong antioxidant activities comparable to ascorbic acid. This showed that *M. elliptica* has beneficial effects, according to its strong antioxidant properties. Although *M. elliptica* is a medicinal plant that is used by people, it is still unexplored to a larger extent and has not much scientific validation of its efficacy in traditional medicine. Research should be encouraged to explore the vast potential of this plant.

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