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Metabolites profiling by LC-ESI-MS/MS technique and *in-vitro* antioxidant activity of *Bauhinia madagascariensis* Desv. and *Bauhinia purpurea* L. aerial parts cultivated in Egypt: a comparative study

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Abstract: *Bauhinia madagascariensis* Desv. is a deciduous tree that is recently acclimatized in Egypt. Herein we report metabolites profile and assess antioxidant activity of *Bauhinia madagascariensis* Desv as compared to *Bauhinia purpurea* L. Secondary metabolites of *B. madagascariensis* and *B. purpurea* aerial parts methanol extracts were investigated using UPLC-ESI-QTOF-MS/MS. A total of 59 metabolites in *B. madagascariensis* and 66 metabolites in *B. purpurea* methanol extracts were identified. The identified classes in both extracts were flavonoids, phenolic, carboxylic and fatty acids, coumarins, stilbenes and acyclic diterpenoids. The most abundant class in both extracts was carboxylic acids. The total phenolic and flavonoid contents in *B. madagascariensis* and *B. purpurea* were 429.4 ± 4.17 mg (GAE)/g, 129 ± 1.83 mg (QE)/g and 353 ± 3.81 mg (GAE)/g, 72 ± 3.2 mg (QE)/g, respectively, which reflects the richness of *B. madagascariensis* in total phenolic and flavonoid contents as compared to *B. purpurea*. The antioxidant activity of the tested extracts has been evaluated using DPPH, H_2O_2 and FRAP antioxidant methods. *B. madagascariensis* extract showed stronger antioxidant capacity as compared to *B. purpurea* extract which could be assigned to the higher concentration of phenolic and flavonoid contents. The findings highlighted that *B. madagascariensis* is a good candidate for more studies against diseases originate from oxidative stress.

Keywords: *Bauhinia madagascariensis*; *Bauhinia purpurea*; UPLC-ESI-QTOF-MS/MS; Antioxidant; DPPH& FRAP.

1. INTRODUCTION

Bauhinia is a genus of the family Fabaceae, which is one of the principal families of flowering plants that are globally distributed. This genus included 300 species of trees or shrubs widely spread in hot and intermediate climatic areas of the world [1, 2]. Many of its species possess various pharmacological and therapeutic actions such as anti-inflammatory, anti-diabetic, antitumor, antimicrobial, hepatoprotective, and anti-ulcer activities [3, 4].

Bauhinia purpurea L. (*B. purpurea*) is a small tropical evergreen tree or erect shrub grows up to 17 m tall. It is native to China and India and is indigenous to Southern Asia and widely distributed throughout the world [5] Phytochemical studies on this species reported the existence of several secondary metabolites as saponins, flavonoids, phenolic compounds, triterpenoids, fatty acids, phytosterols, and alkaloids [3].

The whole plant possesses many pharmacological potentials as antidiarrheal, anti-dysentery, anti-inflammatory, antinociceptive, anti-ulcer, anti-hyperlipidemic, anti-cancer, nephroprotective, antioxidant and antiepileptic [3-6].

Bauhinia madagascariensis Desv. (*B. madagascariensis*) is endemic to Madagascar, occurring from the southern and western zones to the north, and recently is acclimatized in Egypt. It is small shrubs, dry deciduous tree with unusual, green

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and bright red flowers, its flowering stage is at late spring and early summer. The bark is used as rope and the branches are used as posts and in house construction [7]. In spite of the medical importance of Bauhinia genus, the secondary metabolites and the pharmacological potentials of *B. madagascariensis* plant are not very well known [8].

In the field of plant metabolites profiling, liquid chromatography - high resolution mass spectrometry (LC-HRMS) provides a sensitive and accurate tool for structural identification of compounds due to the resultant fragment ions by different collision energies and accurate mass measurements for corresponding elemental composition [9] When these accurate data are combined with reference compounds databases such as ReSpect (negative, 1573 records) it is possible to identify several compounds in one experiment with high accuracy [9]. Phenolics possess an antioxidant property through the deactivation of majority of free radicals, accordingly they play an important role in managing many disorders such as cardiovascular, diabetes, and cancer by management of oxidative stress [10].

This study is directed to identify the metabolites profile of *B. madagascariensis* aerial parts as compared to *B. purpurea* using UPLC-ESI-MS/MS and to compare their antioxidant potentials.

2. METHODS

2.1. Plant material

Bauhinia madagascariensis Desv. and *Bauhinia purpurea* L. aerial parts were collected in November (2019) from Mazhar botanic garden in Cairo, Egypt. They were identified and authenticated by Dr. Treasa Labib, Consultant botanic garden at El-Orman Botanical garden, Giza, Egypt. Voucher specimens were kept in department of Pharmacognosy and medicinal plants, faculty of Pharmacy –girls- Al Azhar University, Cairo, Egypt under the number Bp-2020 and Bm-2020 respectively. The plant materials were dried under ambient temperature, grounded and kept in a sealed container till the beginning of the work.

2.2. Extract preparation

The dried powdered aerial parts of *B. madagascariensis* (600 g) and *B. purpurea* (550g) were soaked and extracted exhaustively using methanol (100%) (1.5 L X 3 times). The combined extracts were filtered, evaporated to dryness in a vacuum at 45 °C and weighed to give a methanol extract of both plants (55 g and 42 g) respectively.

2.3. Phytochemical screening of *B. madagascariensis* and *B. purpurea* methanol extracts

B. madagascariensis and *B. purpurea* methanol extracts were screened to identify the presence of several phytoconstituents as phenolics, saponins, steroids, triterpenoids, tannins, flavonoids, alkaloids and anthraquinone glycosides according to the methods described in reference [11].

2.4. Total phenolic and flavonoid contents estimation

The total phenolic content (TPC) was assessed by utilizing Folin Ciocalteu assay using the method described in reference [12]. The results were calculated from a calibration curve of gallic acid standard and expressed as mg gallic acid equivalent (GAE)/g plant extract. While total flavonoid content (TFC) was determined by the pharmacopeial method described in reference [13] using quercetin as reference compound and the data were calculated from a calibration curve of quercetin standard and expressed as mg quercetin equivalent (QE)/g plant extract.

2.5. UPLC-ESI-QTOF-MS/MS

UPLC-ESI-QTOF-MS/MS was used for profiling secondary metabolites of *B. madagascariensis* and *B. purpurea* aerial parts methanol extracts according to the method adopted in reference [14] (Supplementary file).

2.6. Assessment of *in-vitro* antioxidant capacity

Various *in vitro* antioxidant methods were accustomed to assess the antioxidant capacity of *B. madagascariensis* and *B. purpurea* methanol extracts.

2.6.1. DPPH free radical scavenging assay

Free radical scavenging activity of *B. madagascariensis* and *B. purpurea* extracts against DPPH$^{\bullet}$ radicle, was evaluated spectrophotometrically by applying the method previously established [15] (Supplementary file).

2.6.2. Hydrogen Peroxide Radical (H_2O_2) Scavenging Activity

The ability of *B. madagascariensis* and *B. purpurea* extracts to deactivate hydrogen peroxide radical was assayed by applying the method adopted in reference [16] (Supplementary file).

2.6.3. Ferric reducing antioxidant power (FRAP)

The reducing power of the extracts was evaluated using the method mentioned in references [17, 18]. In this assay the ability of the extracts to reduce ferric ion to ferrous is an indicator for their possible antioxidant property (Supplementary file**).**

2.7. Statistical analysis

Linear regression analysis is the approach that was applied for estimation of TPC, TFC, FRAP and IC_{50}. All samples in all experiments were analyzed in triplicate, and the results are expressed as mean ± standard deviation.

3. RESULTS

3.1 Phytochemical screening

Qualitative phytochemical analysis of *B. madagascariensis* and *B. purpurea* methanol extracts allowed the identification of the presence of many classes of constituents as steroids, terpenoids, saponins, alkaloids, carbohydrates / glycosides, tannins, and phenolics which is in complete accordance with the reported phytochemical constituents of genus *Bauhinia* [3,19].

3.2. quantification of total phenolics and flavonoid contents

Phenolics and flavonoids total contents in *B. madagascariensis* and *B. purpurea* were 429.4 ± 4.17 mg (GAE)/g, 129 ± 1.83 mg (QE)/g and 353 ± 3.81 mg (GAE)/g, 72 ± 3.2 mg (QE)/g respectively. These results reflect the abundance of phenolics and flavonoids in *B. madagascariensis* significantly as compared to *B. purpurea.*

3.3. LC-ESI-MS/MS analysis of bioactive compounds in *B. madagascariensis* and *B. purpurea* extracts

Liquid chromatography–mass spectrometry (LC–MS) is a blended analytical method that gather both liquid chromatography efficient separation and mass analysis accuracy of the mass spectrometry (MS) [14].

Liquid chromatography-mass spectrometry (LC-ESI-MS/MS) technique in its negative ionization mode was used to identify the major secondary metabolites of *B. madagascariensis* and *B. purpurea* methanol extracts. It permitted the tentative identification of 59 metabolites in *B. madagascariensis* and 66 metabolites in *B. purpurea* methanol extracts. The identification relied on the comparison of the collected MS data with those of library databases and available published data. The identified compounds from both extracts (total ion chromatogram (TIC), Supplementary file) are listed in Table (1) and (2).

The identified metabolites presented in tables 1 & 2 are arranged in the order of their relative abundance in the analysed extracts among the identified compounds. The relative abundance was estimated from the sum of the peak areas of the identified metabolites. Mass spectra of the most abundant compounds in *B. madagascariensis* and *B. purpurea* extracts are illustrated in **Fig. 1, 2**.

3.4. *In-vitro* antioxidant activity

In the present work, both tested extracts showed substantial antioxidant activity for DPPH radicles in a concentration-dependent manner, *B. madagascariensis* showed IC_{50} 18.24 ± 0.82 µg/ml while *B. purpurea* extract was 50.54 ± 2.18 µg/ml which reflected the stronger antioxidant power of *B. madagascariensis* extract as compared to *B. purpurea* extract **(fig. 3).**

The antioxidant capacity of both plant extracts was further assessed by measuring their capacity to reduce H_2O_2 radicle where *B. madagascariensis* extract showed superior activity with IC_{50} 44.54 ± 2.94 µg/ml over *B. purpurea* extract which showed IC_{50} 84.94±3.46 µg/ml **(fig. 4).**

The ability of both extracts to reduce the ferric tripyridyltriazine complex to the colored ferrous tripyridyltriazine by donor electrons in the sample was also estimated. Data from this test displayed a substantial reducing activity of both tested extracts using FRAP assay expressed as mMol Fe^{+2}/g sample, and again *B. madagascariensis* extract showed stronger activity than *B. purpurea* extract as compared to ascorbic acid reference standard **(fig. 5).**

4. DISCUSSION

Phytochemical investigation of *B. madagascariensis* and *B. purpurea* methanol extracts allowed the identification of the presence of many classes of constituents including phenolics and flavonoids, moreover, quantitative estimation of total phenolic and flavonoid contents in both plant extracts reflected the richness of *B. madagascariensis* in both classes as compared to *B. purpurea* which led us to further investigate and compare the abundance of secondary metabolites in

both plants using liquid chromatography-mass spectrometry (LC-ESI-MS/MS) technique (Total ion chromatogram (TIC) for both extract are illustrated in **fig. 6, 7,8& 9** in Supplementary file)..

The identified classes in *B. madagascariensis* and *B. purpurea* methanol extracts by using LC-ESI-MS/MS in its negative ionization mode were flavonoids, phenolic, carboxylic and fatty acids, coumarins, stilbenes, and acyclic diterpenoids. The most abundant class in both extracts was carboxylic acids. The identification of the most abundant compounds was carried out as follow:

4.1. Flavonoids

The flavonoid structure often shows the existence of many chemical groups such as hydroxyl (OH), methyl (CH_3) and methoxy (OCH_3) groups. The mass fragmentation of flavonoids usually leads to the loss of neutral fragments with values (28 Da), (18 Da) and (44 Da) corresponding to the loss of CO, H_2O and CO_2 respectively, together with fragment ions of linked sugar moieties. Furthermore, the retro-Diels–Alder (RDA) type of fragmentation is one of the common flavonoid's fragmentation patterns [81].

From the peak area, the most abundant flavonols in *B. madagascariensis* were kaempferol-3-*O*-α-L-rhamnoside (23.2%) and Kaempferol-3-*O*-α -L-arabinoside (2.1%).

Kaempferol-3-*O*-α-L-rhamnoside showed a precursor ion peak $[M - H]^-$ at *m/z* 431.1926 attributed to the molecular formula $C_{21}H_{20}O_{10}$, and it gave daughter ion fragments at 285.0385 [M – 146 – H] $^-$ due to one rhamnosyl moiety loss , 385.1860 [M – 46($CO+H_2O$)–H] $^-$ and 362.8818 [M – 3×18 (-$3H_2O$)-15(Methyl group) –H] $^-$. Kaempferol-3-*O*-α-L-arabinoside exhibited a deprotonated molecular ion peak $[M - H]^-$ at *m/z* 417.212 assigned to the molecular formula $C_{20}H_{18}O_{10}$, and it gave MS^2 fragments at 286.9384 [M – 132 (arabinose moiety)–2H]$^-$, 354.9343[M–46(CO_2+H_2O)–2H] $^-$ and 371.2058[M – 46($CO+H_2O$)–H] $^-$ [82].

In *B. purpurea*, isorhamnetin-3-*O*-rutinoside presented the highest abundant flavonol (8.6%) followed by isorhamnetin-3-*O*-glucoside (1%). Isorhamnetin-3-*O*-rutinoside gave a molecular ion peak $[M - H]^-$ at *m/z* 623.1622 for the molecular formula $C_{28}H_{32}O_{16}$ and prominent fragments at 300.0310 [M – 308 (rutinoside moiety)-15(Methyl group) –H] and 315.0499 [M – 308 (rutinoside moiety) –H). Isorhamnetin-3-*O*-glucoside exhibited $[M - H]^-$ at *m/z* 477.1062 for the molecular formula $C_{22}H_{22}O_{12}$ and prominent fragments at 299.01874 [M – 162 (glucose moiety)-15(Methyl group) –2H] and 314.0335 [M – 162 (glucose moiety) –2H] [83].

The major identified flavanones in the *B. madagascariensis* involved hesperetin-7-*O*-neohesperidoside (2.1%) and naringenin (1.07%). Hesperetin-7-*O*-neohesperidoside gave deprotonated peak $[M - H]^-$ at *m/z* 609.2533 for the molecular formula $C_{28}H_{34}O_{15}$ and prominent fragment at 300.0666 [M – 308 (rhamnopyranosyl-D-glucopyranose moiety)–2H] $^-$ corresponding to the loss of both rhamnose and glucose moieties, while the major identified flavanones in the *B. purpurea* involved naringenin with $[M - H]^-$ at *m/z* 271.0629 assigned to the formula $C_{15}H_{12}O_5$ and it showed fragments at 107.0124 [M – 120 (^{1,3} A−) – 44 (-CO_2)- H] $^-$ and 151.005 [^{1,3} A−] the last two fragments were due to retro-Diels–Alder (RDA) fragmentation pattern [84].

The major identified flavones in the *B. madagascariensis* involved Baicalein-7-*O*-glucuronide (1.64%) and acacetin (1.07%). Baicalein-7-*O*-glucuronide revealed $[M - H]^-$ at *m/z* 445.0714 for the molecular formula $C_{21}H_{18}O_{11}$ and prominent fragment at 355.1051[M – 90 ($CO+CO_2+H_2O$)- H] $^-$, 399.0443[M – 46 ($CO+H_2O$)- H] $^-$. Acacetin gave $[M - H]^-$ at *m/z* 283.10 for the molecular formula $C_{16}H_{12}O_5$ and prominent fragment at 209.06032 [M – 30($2CH_3$)-44(CO_2)-H]$^-$, 225.0552[M – 30(CH_3)- 18(H_2O)-H]$^-$, 253.0469 [M – 30($2CH_3$)- H]$^-$, 268.0733[M – 15(CH_3)- H]$^-$,while the major identified flavones in the *B. purpurea* involved apigenin (2.82%) and luteolin (1.27%). Apigenin exhibited $[M - H]^-$ at 269.0825 corresponding to the molecular formula $C_{15}H_{10}O_5$ and it showed fragments at 199.0776 [M–72(CO_2+CO)- 2H]$^-$ and 227.0716 [M – 42(CH_2CO) -H]$^-$. Luteolin revealed deprotonated peak $[M - H]^-$ at 285.0738 corresponding to the molecular formula $C_{15}H_{10}O_6$ and it showed fragments at 149.0270 [M – 120 -15(CH_3)-2 H]$^-$, 227.0357 [M – 30(OCH_3)-18(H_2O) H]$^-$, 255.0548 [M – 30(OCH_3)-H]$^-$ and 270.0548 [M – 15(CH_3)- H]$^-$ [85], mass spectra of the most abundant flavonoids in *B. madagascariensis* and *B. purpurea* are illustrated in Figs.(1&2).

Table 1. Secondary metabolites tentatively identified in *B. madagascariensis* extract using LC-ESI- MS/MS in negative mode.

No.	Tentatively identified compounds	RT. min.	Precursor. *m/z* $[M-H]^-$	*%	Molecular Formula	MS/MS fragments	Ref
Flavonoids							
1	Naringenin	1.16	271.0447	1.07	$C_{15}H_{12}O_5$	109.02976,123.03866,181.0377, 181.0514, 191.0261	69, 84
2	Isorhamnetin-3-*O*-glucoside	1.16	477.0458	0.2	$C_{22}H_{22}O_{12}$	133.0138,195.0622,289.013,315.0458 ,320.01483,357.0918,373.05429, 431.05838,476.96921, 476.9943	83
3	Baicalein-7-*O*-glucuronide	1.29	445.0714	1.64	$C_{21}H_{18}O_{11}$	102.9558,103.00453,131.0454,133.01 02,311.0979,341.0401,355.1051,399. 0443	37
4	Quercetin-3-*O*-xyloside	1.44	433.1666	0.1	$C_{20}H_{18}O_{11}$	132.0476, 197.0603, 297.1184, 301.07559, 387.15073	42
5	Isorhamnetin-3-*O*-rutinoside	3.20	623.1594	0.2	$C_{28}H_{32}O_{16}$	137.0249,323.0886,485.1337	83
6	Luteolin-3', 7-di-*O*-glucoside	4.15	609.1441	0.2	$C_{27}H_{30}O_{16}$	112.9832,285.1441,399.0551,447.144 1,489.1014	43
7	Quercitrin	4.3	447.1842	0.63	$C_{21}H_{20}O_{11}$	89.0213,161.0439,191.0348, 179.0346,179.0556,253.0584,271.048 3,310.9256,310.9487, 401.1821, 402.87936	48
8	Kaempferol-7-*O*-neohesperidoside	4.48	593.15	0.8	$C_{27}H_{30}O_{15}$	383.0839, 473.1105	57
9	Kaempferol-3-*O*-(6-*p*-coumaroyl)-glucoside	4.653	593.1221	0.7	$C_{30}H_{26}O_{13}$	353.0631,431.1221,473.10614	28
10	Kaempferol-3-*O*-*α*-L-rhamnoside	5.70	431.1926	23.2	$C_{21}H_{20}O_{10}$	152.0851,153.0900,161.0444,205.123 8,223.1346,285.0385, 295.0095, 362.8818, 385.1860	82
11	Luteolin-8-C-glucoside	5.93	447.0937	0.2	$C_{21}H_{20}O_{11}$	242.9398,285.04089,301.03125,327.0 460,327.0694,357.0792, 357.0594 402.86487	30
12	Apigenin 8-C-glucoside	6.24	431.0985	1.8	$C_{21}H_{20}O_{10}$	113.0205,205.1234,283.06772,311.05 25,311.0684,385.1831,430.8700,430. 8793	31
13	Rhoifolin	6.24	577.1571	1.08	$C_{27}H_{30}O_{14}$	487.1259, 575.3056	32
14	Hesperetin-7-*O*-neohesperidoside	6.29	609.1449	2.1	$C_{28}H_{34}O_{15}$	300.0277	33
15	Quercetin-4'-glucoside	6.58	463.0848	1.83	$C_{21}H_{20}O_{12}$	218.9431,258.93524,286.93277,286.9 471,300.02295,301.0340,354.92548,3 94.91696, 462.89609	33
16	Hesperetin	6.84	301.1643	0.1	$C_{16}H_{14}O_6$	112.9862,139.1124,183.13603,184.15 13,218.9491,255.22867, 300.9852, 300,9497	83
17	Luteolin-7-*O*-glucoside	7.27	447.0937	1.6	$C_{21}H_{20}O_{11}$	284.02393,285.04349, 300.0344	34
18	Kaempferol-3-*O*-*α*-L-arabinoside	8.06	417.212	2.1	$C_{20}H_{18}O_{10}$	280.9177,286.9384, 354.9343,371.2058	82
19	Daidzein-8-C-glucoside	9.07	415.1967	0.11	$C_{21}H_{20}O_9$	204.9072,206.9026,207.0543, 368.9526, 414.9583	35
20	3 5 7-trihydroxy-4'-methoxyflavone	11.09	299.0548	0.7	$C_{16}H_{12}O_6$	284.0319	83

Table 1. Contin.

No.	Tentatively identified compounds	RT. min.	Precursor. *m/z* $[M-H]^-$	*%	Molecular Formula	MS/MS fragments	Ref
21	3'-Methoxy-4',5,7-trihydroxyflavonol (Isorhamnetin)	11.35	315.0502	0.23	$C_{16}H_{12}O_7$	300.0249,315.05182	[36]
22	Luteolin	12.39	285.1147	0.1	$C_{15}H_{10}O_6$	135.0056,163.03726, 270.0484	[85]
23	Apigenin	15.07	269.1302	0.04	$C_{15}H_{10}O_5$	132.9850, 254.1195	[38]
24	Acacetin	16.32	283.10	1.07	$C_{16}H_{12}O_5$	209.06032,225.0552,253.04692, 268.0733	[39]
25	(+)-3 3' 4' 5 7-Pentahydroxyflavan	21.06	289.1783	0.22	$C_{15}H_{14}O_6$	112.9864,180.9732, 220.9590	[40]
26	Acacetin-7-*O*-Rutinoside	26.6	591.2227	0.1	$C_{28}H_{32}O_{14}$	180.9762,248.9616,316.9490,386.9558,454.9271,522.9356, 590.8800, 590.8889	[41]
Acids							
Carboxylic acid:							
27	Citric Acid	1.05	191.0207	18.2	$C_6H_8O_7$	57.0341,85.0293, 87.00706, 111.00874,129.01518,147.0295, 173.0093, 191.02019	[86]
28	Maleic Acid	1.1	115.004	5.54	$C_4H_4O_4$	71.01218, 114.99706	[87]
29	Succinic Acid	1.18	117.02	0.64	$C_4H_6O_4$	73.02809, 99.91895	[44]
30	Mucic Acid	1.18	209.0322	2.4	$C_6H_{10}O_8$	57.0339,71.0148,84.0299, 85.02793, 87.0079, 89.0235, 129.02156,133.0176,159.02483,191.0202	[45]
31	Gluconic Acid	1.19	195.0507	15.1	$C_6H_{12}O_7$	59.0126,75.00982,129.02153, 159.0305, 177.0395	[46]
32	D-(+)-Malic Acid	1.2	133.0507	0.31	$C_4H_6O_5$	71.0122, 87.0037, 89.02324, 115.00164, 132.5897	[47]
33	L-(+)-Tartaric acid	1.28	149.0450	1.21	$C_4H_6O_6$	72.992,73.02708,89.0223, 89.035, 131.0354	[88]
34	Glutaric Acid	1.63	131.0707	0.4	$C_5H_8O_4$	85.0623,85.0714, 113.0360	[57]
35	D-(+)-Galacturonic acid	9.15	193.052	0.8	$C_6H_{10}O_7$	133.02541,134.03557,137.0255,161.02365,178.0235	[57]
36	Citramalate	1.17	147.0317	0.52	$C_5H_8O_5$	87.0101,103.0418,129.01518,129.03123, 146.9872	[53]
37	2-Isopropylmalic acid	1.37	175.0618	2.13	$C_7H_{12}O_5$	85.06539,87.0084,113.0595,113.0730,114.6799,115.0405,130.9682,131.0624,157.05554,174.9615	[54]
Phenolic acid:							
38	Caffeic Acid	1.22	179.0536	1.1	$C_9H_8O_4$	59.0131,71.0154,75.00986, 85.0305, 134.041, 135.0448, 161.0472	[49]
39	Homogenentisic acid	1.42	167.0347	0.2	$C_8H_8O_4$	91.0279,108.0262,123.04784,152.00166	**[88]**
40	3-(4-hydroxyphenyl) prop-2-enoic acid	1.49	163.0384	0.6	$C_9H_8O_3$	93.03959,119.04836,163.0351,163.0666	[50]
41	2,5-Dihydroxy benzoic acid	1.49	153.0182	0.7	$C_7H_6O_4$	78.9583,108.01871,108.0295, 109.02794	[51]
42	P-Hydroxy benzoic Acid	3.2	137.0236	0.3	$C_7H_6O_3$	65.0401, 92.7426, 93.03278	[57]

Table 1. contin.

No.	Tentatively identified compounds	RT. min.	Precursor. *m/z* $[M-H]^-$	*%	Molecular Formula	MS/MS fragments	Ref
43	3,4-dihydroxy benzoic acid	4.86	153.0199	0.21	$C_7H_6O_4$	84.9934,112.9864	[34]
44	Rosmarinic acid	7.75	359.1536	0.04	$C_{18}H_{16}O_8$	359.1492, 359.15231	[52]
Fatty Acids:							
45	Suberic Acid	1.38	173.0816	0.33	$C_8H_{14}O_4$	115.0310	[55]
Coumarins:							
46	6,7-Dihydroxycoumarin	1.49	177.0389	0.4	$C_9H_6O_4$	75.0072,89.0259,129.01485	[83]
47	Scopoletin	7.14	191.0343	0.35	$C_{10}H_8O_4$	104.0271,148.00838, 176.00929	[56]
48	Daphnetin	10.7	177.0542	1.02	$C_9H_6O_4$	117.0324,118.03984,121.0285, 145.03011, 162.0327	[89]
49	Esculin	16.92	339.2003	2.18	$C_{15}H_{16}O_9$	119.0493,176.0024,219.0667	[58]
50	7-Hydroxy-4-methylcoumarin (Hymecromone)	27.05	174.9577	0.2	$C_{10}H_8O_3$	86.99153,130.96202,130.96364,146.95874	[89]
Stilbenes:							
51	E-3,4,5'-Trihydroxy-3'-glucopyranosyl Stilbene (Astringin)	8.57	405.1178	0.12	$C_{20}H_{22}O_9$	151.00056,153.01508,195.0668, 242.05734,243.05809,243.06911,297.06024,297.0712,357.13141, 359.21344	[59]
Acyclic diterpenoids:							
52	Geranylgeranyl pyrophosphate ammonium salt	1.42	449.1982	1.02	$C_{20}H_{36}O_7P_2$	207.1397,209.1201,225.1399,269.13919,313.0972,381.0805	[60]
Other:							
53	Hinokitiol	1.23	163.0622	0.1	$C_{10}H_{12}O_2$	73.02922,101.0183,101.0280,119.0423,145.0122	[61]
54	Galactitol	1.41	181.0679	1.37	$C_6H_{14}O_6$	59.0104,89.0254,101.0231,138.0190, 138.0530,163.0677	[62]
55	3,4-Dihydroxymandelate	4.23	183.1376	0.12	$C_8H_8O_5$	183.0994,183.13379	[57]
56	D-(+)-Raffinose	5.98	503.1184	0.13	$C_{18}H_{32}O_{16}$	339.0851,457.2068	[63]
57	Gamma-Terpinene	6.82	135.0444	0.14	$C_{10}H_{16}$	92.02656,93.0294,93.0381,108.02199	[64]
58	Sinapyl aldehyde	11.13	207.0644	0.1	$C_{11}H_{12}O_4$	133.0273,164.044,188.9409,192.04233	[65]
59	D-(+)-Trehalose	13.85	341.1762	0.1	$C_{12}H_{22}O_{11}$	136.0543,228.92551,296.92877,326.0492	[66]

*percentage of abundance was calculated from the peak areas (peak area of the compound / total peaks area of the identified secondary metabolites X 100).

4.2. Carboxylic, phenolic and fatty acids

Acids upon mass fragmentation suffers from the loss of neutral fragments with values (28 Da), (18 Da), (44 Da), (42 Da) corresponding to CO, H_2O, CO_2, and CH_2CO respectively, together with fragments of alkyl groups. The most abundant carboxylic acids in *B. madagascariensis* was citric acid (18.2%), it revealed molecular ion peak $[M-H]^-$ at *m/z* 191.0207 for the molecular formula $C_6H_8O_7$ and major fragments at 85.0295$[M-88(2CO_2)+18(H_2O)-2H]^-$, 111.00874$[M-44(CO_2)+36(2H_2O)-H]^-$, 129.01518$[M-44(CO_2)\&18(H_2O)-H]^-$ and 173.00928$[M-18(H_2O)\ H]^-$ [86]. Gluconic acid was the second abundant one (15.1%) with precursor ion $[M-H]^-$ at *m/z* 195.0507 assigned for $C_6H_{12}O_7$ and daughter ions at 129.0215 $[M-(42(CH_2CO)+18(H_2O)-H]^-$, 159.0305$[M-36(2H_2O)-H]^-$ and 177.0395$[M-18(H_2O)-H]^-$, while maleic acid represented 5.54%

which was identified based on published [86] and library database.

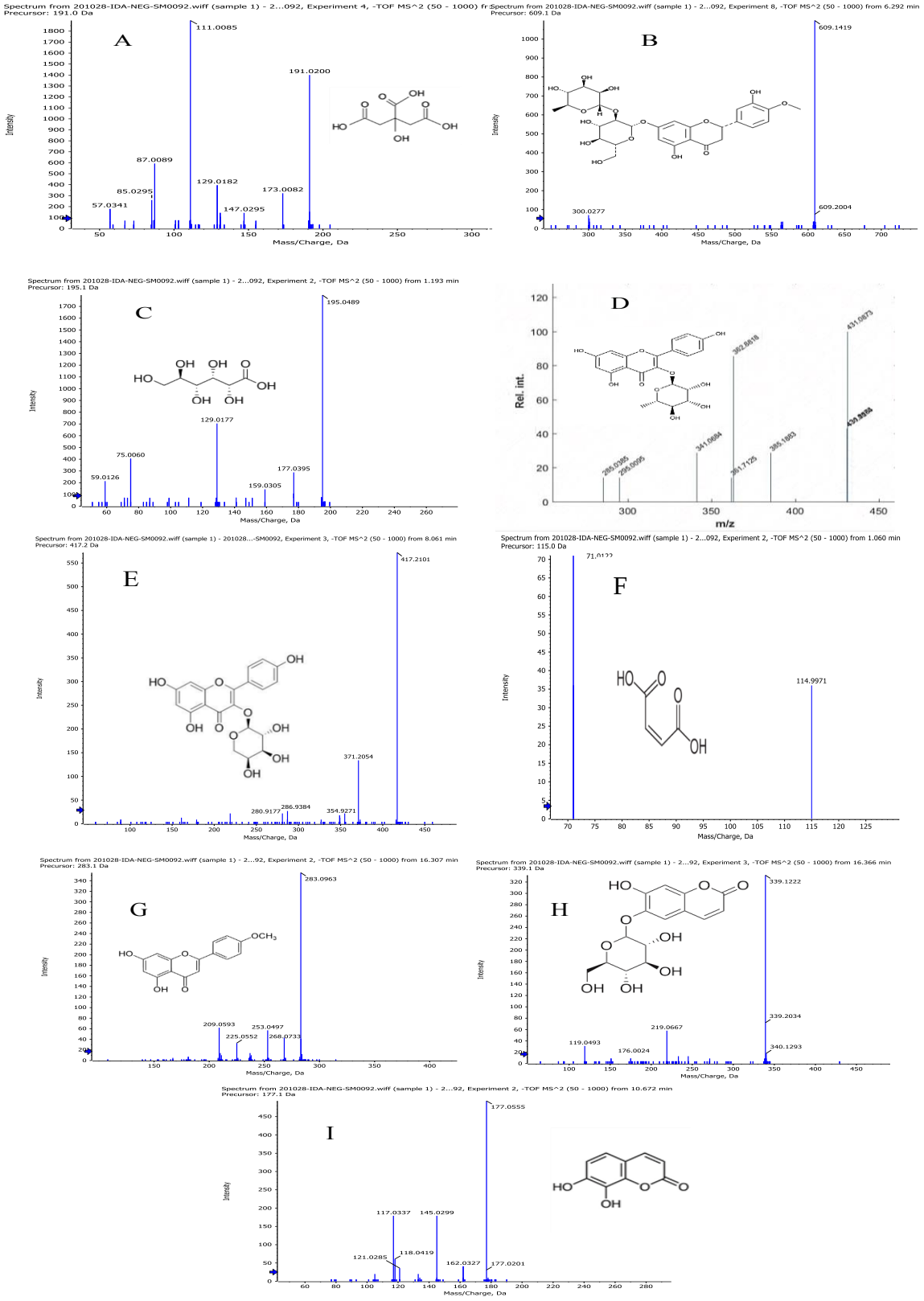

Figure 1. Mass spectra of the major identified compounds in *B. madagascariensis* using LC-ESI-MS/MS. (A: citric acid, B: Hesperetin-7-*O*-neohesperidoside, C: Gluconic acid, D: Kaempferol-3-*O*-α-L-rhamnoside, E: Kaempferol-3-*O*-α-L-arabinoside, F: Maleic Acid, G: Acacetin, H: Esculin and I: Daphnetin).

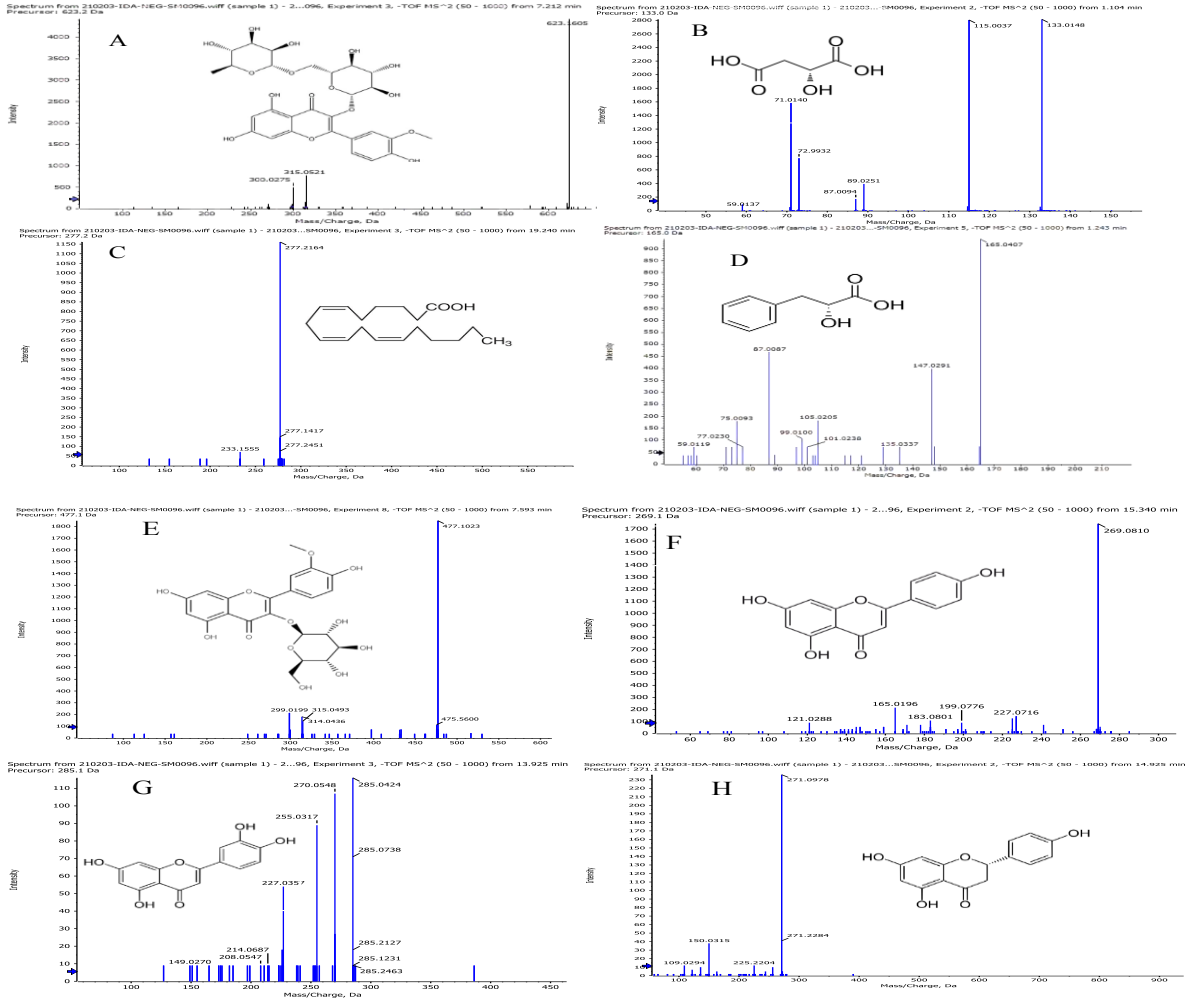

Figure 2. Mass spectra of major identified compounds in B. purpurea using LC-ESI-MS/MS. (A: Isorhamnetin-3-*O*-rutinoside, B: D-(+)-Malic acid, C: γ-Linolenic acid , D: D-3-Phenyllactic acid ,E: Isorhamnetin-3-*O*-glucoside , F: Apigenin , G: Luteolin, H: Naringenin).

D-(+)-Malic acid (Butanedioic acid) was the most abundant carboxylic acid in *B. purpurea* (27.67%) with precursor ion $[M - H]^-$ at *m/z* 133.0131 assigned for $C_4H_6O_5$ formula and showed fragments at 71.0122$[M –44(CO_2) +18(H_2O) - H]^-$, 89.0232 $[M – 44(CO_2) - H]^-$ and 115.0016 $[M – 18(H_2O) - H]^-$. Gluconic acid was the second abundant one representing 8%. D-(+)-Galacturonic acid was the third abundant one (4.04%). It exhibited $[M – H]^-$ at *m/z* 193.0499 corresponding to the molecular formula $C_6H_{10}O_7$ and showed fragments at 132.0225 $[M –(42(CH_2CO)+ 18(H_2O) -2H]^-$, 133.0254 $[M –(42(CH_2CO)+ 18(H_2O) -H]^-$,137.0255[M -54 $(3H_2O)$ $-2H]^-$, 161.0236 $[M – 28(CO)-4H]^-$, 178.0235$[M –15(CH_3)- H]^-$ and 192.4388$[M – 2H]^-$. Maleic acid (4%) revealed deprotonated ion $[M – H]^-$ at *m/z* 115.0034 assigned for $C_4H_4O_4$ formula. It also displayed MS^2 fragments at 71.01375$[M –44(CO_2) - H]^-$ and 114.99706 $[M –2 H]^-$ [87].

The major detected phenolic acid in *B. madagascariensis* was caffeic acid (1.1%) with deprotonated peak $[M – H]^-$ at *m/z* 179.0536 assigned for $C_9H_8O_4$ and MS^2 fragments at 71.0154 $[M –44(CO_2)-36(H_2O)-28(CO) -H]^-$, 134.0415$[M – 44(CO_2) -2H]^-$, 135.0448$[M –44(CO_2) -H]^-$ and 161.0472 $[M - 18(H_2O) -H]^-$. Phenolic acid with the highest concentration in *B. purpurea* was phenyllactic acid (4.8%) with $[M – H]^-$ *m/z* at 165.0405 assigned for $C_9H_{10}O_3$ and daughter fragment ions at 75.0653 $[M – 44(CO_2)-28(CO)-18(H_2O) -H]^-$, 77.0230$[M–88(2CO_2) -H]^-$,101.02113$[M–28(CO)-36(2H_2O) -H]^-$, 105.0220 $[M–(42(CH_2CO)+18(H_2O)-H]^-$ and 147.03706$[M - 18(H_2O) -H]^-$ [88].

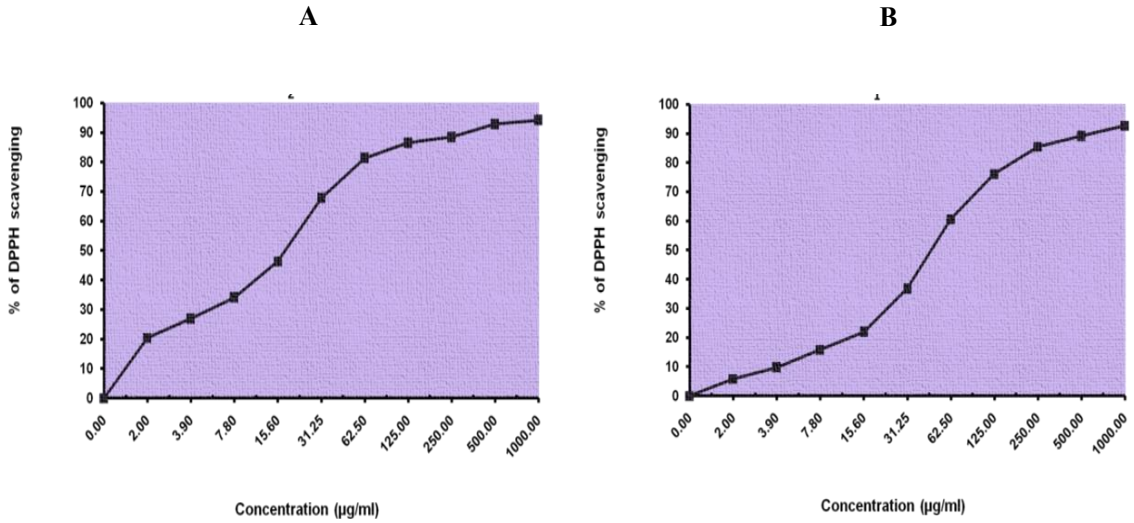

Figure 3. DPPH• scavenging assay of aerial parts methanol extract (A) *B. madagascariensis* (B) *B. purpurea*.

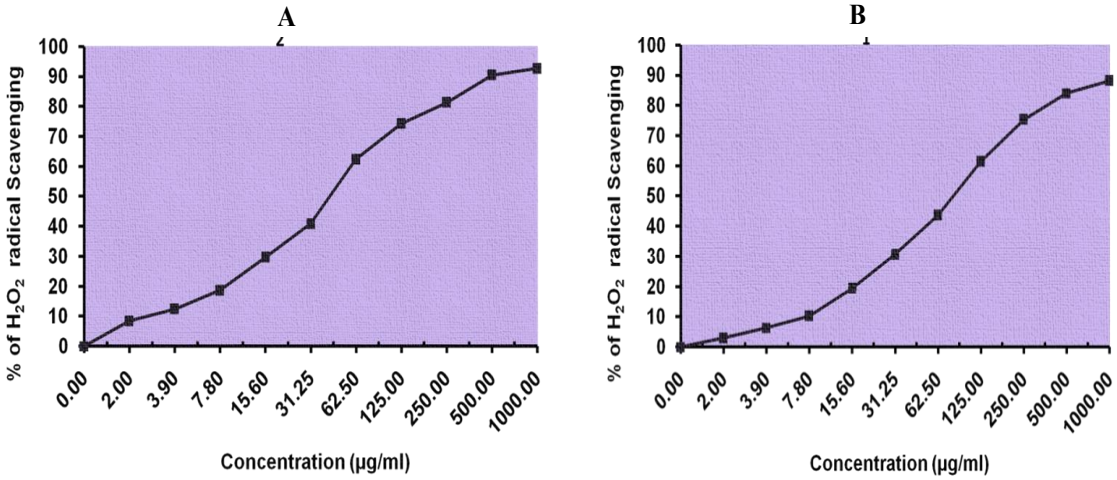

Figure 4. H_2O_2 radical scavenging assay of aerial parts methanol extract **(**A) *B. madagascariensis (*B) *B. purpurea*.

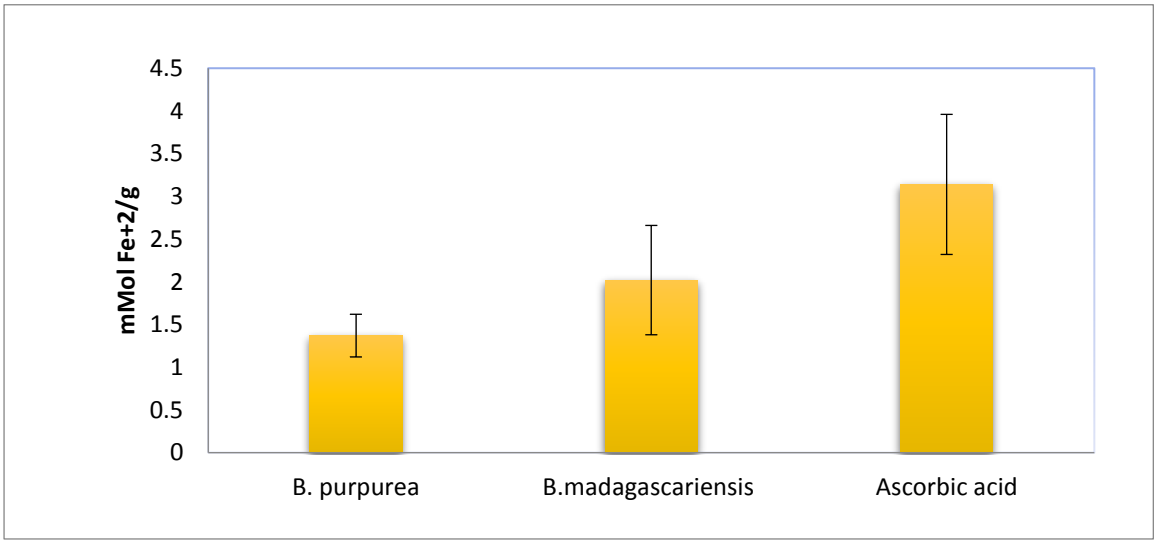

Figure 5. FRAP assay of *B. madagascariensis* and *B. purpurea* aerial parts methanol extract.

The major fatty acid in *B. purpurea* was γ-linolenic acid (19.46%), which is poly unsaturated fatty acid, it revealed $[M - H]^-$ at *m/z* 277.2178 assigned to $C_{18}H_{30}O_2$ and daughter ion at 233.1555$[M -44(CO_2) -H]^-$.

4.3. Coumarins

The major detected coumarin in *B. madagascariensis* was esculin (2.18%). It revealed precursor ion $[M - H]^-$ at *m/z* 339.2003 assigned to $C_{15}H_{16}O_9$ formula and fragments at 119.0493 $[M -$ 162(glucose moiety)-56(2CO)-2H$]^-$,176.0024[M– 162(glucose moiety)-2H$]^-$, 219.0667 $[M -44(CO_2)$ -56(2CO)-18(H_2O)-2H$]^-$,The second abundant one was daphnetin (1.02%) with $[M - H]^-$ appeared at *m/z* 177.0542 assigned to $C_9H_6O_4$ and fragments at 117.0324 [M–42(CH_2CO)-18(H_2O)-H$]^-$, 121.0285[M -56(2CO)- H$]^-$ and 145.03011[M -28 (CO)- 4H$]^-$ [89], while the major detected coumarin in *B. purpurea* was daphnetin (1%) which showed similar fragments as in *B. madagascariensis*.

4.4. Other Compounds

There were some other identified compounds in *B. madagascariensis* as galactitol (1.37%) with molecular ion peak $[M - H]^-$ located at *m/z* 181.0679 assigned to $C_6H_{14}O_6$ and showed MS^2 at 59.0104 [M-18(H_2O)-86 ($C_4H_6O_2$)-H$]^-$, 89.0254[M-18(H_2O)-74($C_3H_6O_2$)-H$]^-$, 101.0231[M-18(H_2O)-62($C_2H_6O_2$)-H$]^-$ and 163.0677 $[M -18(H_2O)- H]^-$. While in *B. purpurea* sucrose (2.06%) was detected. It revealed precursor ion $[M - H]^-$ at *m/z* 341.1103 assigned to $C_{12}H_{22}O_{11}$ and revealed MS^2 fragments at 89.02563[M-162(glucose moiety)-90($C_3H_6O_3$)-H$]^-$, 135.0482 [M–162(glucose moiety)-44(CO_2)-H$]^-$,143.0320 [M–162(glucose moiety)-36($2H_2O$)-H$]^-$, 179.0535[M–162(glucose moiety)-H$]^-$, 281.0917[M– 42(CH_2CO)-18(H_2O)-H$]^-$ and 340.0068[M– 2H$]^-$ [90].

Reactive oxygen species (ROS) are highly reactive molecules that results from various metabolic activity and immune response under normal physiological condition. Alteration or increase in ROS and free radicals generation leads to oxidative stress, which can cause massive damage to human cells and tissues resulting in many health problems such as cardiovascular disease, aging, neurodegenerative diseases, mutations and cancer.[91]

In-vitro antioxidant methods differ in their action mechanisms thus provide more understanding about the extract's antioxidant capacities. The tested extracts in the current work have been estimated by DPPH, H_2O_2 and FRAP antioxidant methods.

DPPH theory depends on the estimation of the capability of the extracts to neutralize the DPPH• radicals which result in a change of the solution colour from deep violet to light yellow associated with decrease in the value of the absorbance at 517 nm [92]. Data are calculated as IC_{50}, which corresponding to the extract concentration that causes 50% decrease of DPPH activity. Low values of IC_{50} corresponding to potent antioxidant capacity. Herein the results showed that *B. madagascariensis* and *B. purpurea* extracts exhibited antioxidant activity as reflected by the calculated IC_{50}, but *B. madagascariensis* revealed stronger antioxidant capacity as compared to *B. purpurea*. Similarly *B. madagascariensis* extract showed superior activity against H_2O_2 radicle and the strongest activity in FRAP assay.

The antioxidant significant activity of the studied extracts could be attributed to their phenolic and flavonoid contents. The higher concentration of phenolics in *B. madagascariensis* extract as compared to *B. purpurea* extract could be the reason for its superiority in antioxidant capacity.

These data are supported by pervious reports that discussed the relationship between antioxidant power and phenolic constituents in the plant [93, 94].

Akter M. et al. [95] reported that kaempferol-3-*O*-α-L-rhamnoside isolated from *Pithecellobium dulce* leaves exhibited strong antioxidant activity in DPPH radical scavenging. Moreover, it efficiently suppressed 2, 2'-azobis (2-amidinopropane) dihydrochloride (AAPH) induced oxidation in DNA and human erythrocyte model. It is worth to mention that citric acid which is the most abundant compound in *B. madagascariensis* acts as an oxygen scavenger and retards unsaturated lipid/free fatty acid oxidation by inhibition of essential agents like metals-based oxygen scavengers. Additionally, it has anti-inflammatory activity, and it improves the immune system and protects both the liver and the brain [96].

Table 2. Secondary metabolites tentatively identified in *B. purpurea* extract using LC-ESI-MS/MS in negative mode.

No.	Tentatively identified compounds	RT. min.	Precursor. *m/z* [M-H]$^-$	*%	Molecular Formula	MS/MS fragments	Ref
Flavonoids							
1	Okanin-4'-*O*-glucoside	1.06	448.9874	0.16	$C_{21}H_{22}O_{11}$	240.9699,286.9874,312.9674, 315.00464, 344.9747, 386.9093	67
2	Quercetin-3-*O*-xyloside	1.4	433.13	0.04	$C_{20}H_{18}O_{11}$	132.0476, 197.0603, 297.1184, 301.07559, 387.15073,433.0501	42
3	Myricetin	1.41	317.0526	0.26	$C_{15}H_{10}O_8$	133.01244, 191.02719, 213.00081, 272.9291	68
4	Myricitrin	3.36	463.148	0.69	$C_{21}H_{20}O_{12}$	125.0206, 149.0474, 169.0489, 286.9305, 293.07965,354.9296, 394.9175, 462.8996	20
5	eriodictyol-7-*O*-glucoside	4.23	449.1068	0.07	$C_{21}H_{22}O_{11}$	179.0051, 259.05585, 269.05103, 287.0712, 381.13773	70
6	Eriodictyol-7-*O*-neohesperidoside (Neoeriocitrin)	4.26	595.1666	0.12	$C_{27}H_{32}O_{15}$	407.1342, 549.155	71
7	Quercitrin	4.7	447.186	0.26	$C_{21}H_{20}O_{11}$	89.0213, 161.0439, 191.0348, 179.0346, 179.0556, 253.0584, 271.0483, 310.9256, 310.9487, 401.1821, 402.87936	48
8	Kaempferol-3-Glucuronide	4.73	461.1684	0.7	$C_{21}H_{18}O_{12}$	161.0622, 179.06674, 324.9170, 392.9028, 415.15512	72
9	Kaempferol-3-*O*-*α*-L-Rhamnoside	5.7	431.1511	0.04	$C_{21}H_{20}O_{10}$	152.0851, 153.0900, 161.0444,205.1238, 223.1346, 385.1850	21
10	Quercetin-3,4'-*O*-di-β-glucopyranoside	5.74	625.1402	0.89	$C_{27}H_{30}O_{17}$	300.0248, 300.0250	26
11	Kaempferol-7-neohesperidoside	5.9	593.1733	0.04	$C_{27}H_{30}O_{15}$	457.20038, 547.18311, 547.2310	57
12	Naringenin	5.92	271.1069	0.05	$C_{15}H_{12}O_5$	109.0294, 150.0315, 225.2204	69
13	Luteolin-3', 7-di-*O*-glucoside	6.197	609.1474	0.27	$C_{27}H_{30}O_{16}$	284.02737,285.0419, 609.10168	43
14	Rhoifolin	6.2	577.17	0.17	$C_{27}H_{30}O_{14}$	487.1259, 575.3056,	32
15	Hesperetin	7.06	301.2028	0.04	$C_{16}H_{14}O_6$	183.1063, 201.1166, 255.2250	57
16	Isorhamnetin-3-*O*-rutinoside	7.21	623.1605	8.6	$C_{28}H_{32}O_{16}$	300.03107, 315.0499	22
17	Isorhamnetin-3-*O*-glucoside	7.59	477.1062	1	$C_{22}H_{22}O_{12}$	285.1069, 299.01874, 314.0335, 314.0465	22
18	Daidzein-8-C-glucoside	7.8	415.2	0.07	$C_{21}H_{20}O_9$	278.91101, 306.9137	35
19	Phlorizin	9.95	435.1667	0.2	$C_{21}H_{24}O_{10}$	258.09091, 273.11346,	73
20	Kaempferol-3-*O*-*α*-L-arabinoside	10.02	417.1513	0.08	$C_{20}H_{18}O_{10}$	166.0243, 218.9556, 286.9411, 354.9191, 354.9345, 402.12735	21
21	Acacetin-7-*O*-rutinoside (Linarin)	10.77	591.2112	0.16	$C_{28}H_{32}O_{14}$	316.9430, 407.1567, 439.16885, 546.8373, 528.8772	41
22	3 5 7-trihydroxy-4'-methoxyflavone (Kaempferide)	11.09	299.1	0.11	$C_{16}H_{12}O_6$	284.0347, 299.06036	22
23	3'-Methoxy-4',5,7-Trihydroxyflavonol	11.3	315.15	0.02	$C_{16}H_{12}O_7$	300.03082	36
24	Formononetin	11.4	267.0655	0.03	$C_{16}H_{12}O_4$	114.9880, 252.02963, 253.0449	74
25	Luteolin	13.93	285.07	1.27	$C_{15}H_{10}O_6$	149.0270,208.547,214.0687,227.0357,255.0548,270.0548	23
26	Apigenin	15.34	269.0825	2.82	$C_{15}H_{10}O_5$	121.0288,165.0196,183.0801,199.0776,227.0716	38

Table 2. Contin

No.	Tentatively identified compounds	RT. min.	Precursor. *m/z* [M-H]$^-$	*%	Molecular Formula	MS/MS fragments	Ref
27	Acacetin	16.59	283.108	0.03	$C_{16}H_{12}O_5$	209.06032, 225.0552, 253.04692, 268.0733, 268.03247	39
Acids							
Carboxylic Acids:							
28	Maleic Acid	1.16	115.0034	4	$C_4H_4O_4$	71.01375,114.99706	25
29	Mucic Acid	1.17	209.1197	0.04	$C_6H_{10}O_8$	57.0339,71.0148,84.0299, 85.02793, 87.007, 89.0235, 129.0215, 133.0176, 159.0248, 191.0202	45
30	Succinic Acid	1.19	117.0205	0.72	$C_4H_6O_4$	73.02809, 99.91895	75
31	Citric Acid	1.2	191.0575	2	$C_6H_8O_7$	57.0341,85.0293, 87.00706, 111.00874, 129.01518, 147.0295, 173.00928	24
32	D-(+)-Malic acid	1.2	133.0131	27.67	$C_4H_6O_5$	59.0137, 71.0122, 72.9934, 87.0037, 89.02324, 115.00164, 132.5897	47
33	Gluconic Acid	1.21	195.051	8	$C_6H_{12}O_7$	59.0126,75.00982, 129.02153, 159.0305, 177.0395	46
34	Glyceric acid	1.24	105.0197	0.24	$C_3H_6O_4$	72.99574, 75.01387	76
35	Ketoisoleucine	1.77	128.9589	1.04	$C_6H_{10}O_3$	128.95996	77
36	Gibberellin A4	2.57	331.1051	0.54	$C_{19}H_{24}O_5$	153.0199, 168.0371, 169.05573	78
37	L-(+)-Tartrate	7.76	149.0243	0.08	$C_4H_6O_6$	121.0294, 149.02725	26
38	D-(+)-Galacturonic acid	8.9	193.0499	4.04	$C_6H_{10}O_7$	132.0225, 133.02541, 134.03557,137.0255, 161.02365,178.0235, 192.4388	89
39	Isocitrate	9.77	191.0362	0.06	$C_6H_8O_7$	147.0419	79
40	Gibberelin A3	10.76	345.1867	0.04	$C_{19}H_{22}O_6$	300.9856,309.2077	78
41	DL-2-Hydroxyvaleric acid	10.61	117.0343	0.03	$C_5H_{10}O_3$	99.9239, 100.93481, 116.92402, 117.0325	87
42	2-Isopropylmalic acid	1.35	175.0607	0.92	$C_7H_{12}O_5$	85.06539, 87.0084, 113.0595, 113.0730, 114.6799, 115.0405, 130.9682,131.0624, 157.05554, 174.9615	54
43	Citramalate	1.16	147.067	0.12	$C_5H_8O_5$	87.0101, 103.0418, 129.01518, 129.03123, 146.9872	53
Phenolic acid:							
44	Caffeic Acid	1.2	179.0373	0.15	$C_9H_8O_4$	59.0131,71.0154,75.00986,85.0305,134.0415, 135.044, 161.0472	49
45	D-3-Phenyllactic acid	1.24	165.0405	4.8	$C_9H_{10}O_3$	75.00653, 77.0230, 87.01008, 99.0100, 101.02113, 105.0220, 147.03706	26
46	3-(4-Hydroxyphenyl) Prop-2-Enoic Acid (4-Coumaric acid)	1.97	163.0415	0.2	$C_9H_8O_3$	119.05019, 162.5155	57
47	Homogenentisic acid	2.18	167.0331	0.22	$C_8H_8O_4$	122.0351, 123.04061	80
48	trans-Cinnamate (Cinnamic acid)	2.3	147.0466	0.32	$C_9H_8O_2$	103.05304	81
49	3-(4-Hydroxy-3-Methoxyphenyl) Prop-2-EnoicAcid	2.27	193.048	0.39	$C_{10}H_{10}O_4$	134.03595, 137.0244, 149.0652, 178.02513	57
50	2-Hydroxyphenylacetic acid (Benzeneacetic acid)	3.06	151.0381	0.13	$C_8H_8O_3$	106.0416, 107.05109	82
51	P-Hydroxy Benzoic Acid	3.34	137.0238	0.95	$C_7H_6O_3$	65.0401, 92.7426, 93.03278	43
52	3,4-DihydroxyBenzoic Acid	4.96	153.0176	0.09	$C_7H_6O_4$	67.0186, 109.0231, 109.0334, 135.00671	34
53	Rosmarinic acid	6.13	359.0975	0.17	$C_{18}H_{16}O_8$	359.09549	52

Table 2. Contin

No.	Tentatively identified compounds	RT. min.	Precursor. *m/z* [M-H]$^-$	*%	Molecular Formula	MS/MS fragments	Ref
54	Sodium Deoxycholate	6.75	391.1625	0.08	$C_{24}H_{40}O_4$	183.0991, 322.9135	83
55	5-Methoxysalicylic acid	7.19	167.0345	0.51	$C_8H_8O_4$	108.0191, 124.0153, 152.00912	84
Fatty Acid:							
56	γ-Linolenic acid	19.24	277.2178	19.46	$C_{18}H_{30}O_2$	227.1417,233.1555	85
55	3-Hydroxy-3-Methylglutaric acid	1.29	161.0814	0.09	$C_6H_{10}O_5$	57.0360, 99.04777, 101.0240, 143.0301	86
Coumarin:							
58	Daphnetin	10.64	177.0543	1	$C_9H_6O_4$	117.03384,118.0414, 121.0285, 145.02997, 162.032	27
59	Esculin	16.82	339.1249	0.05	$C_{15}H_{16}O_9$	119.0493,176.0024,219.0667	58
Xanthine:							
60	Xanthine	8.9	151.0399	0.26	$C_5H_4N_4O_2$	91.0183, 92.02638, 95.0116, 108.02222, 136.01355	88
Stilbene:							
61	E-3,4,5'-Trihydroxy-3'-glucopyranosyl stilbene.	8.5	405.1774	0.41	$C_{20}H_{22}O_9$	151.00056, 153.0150, 195.0668, 242.05734, 243.05809, 243.0691, 297.06024, 297.071, 357.1314, 359.21344	59
Acyclic diterpenoids:							
62	Geranylgeranyl pyrophosphate ammonium salt	1.44	449.209	0.05	$C_{20}H_{36}O_7P_2$	207.1397, 209.1201, 225.1399, 269.13919, 313.0972, 381.0805	60
Other:							
63	Sucrose	1.34	341.1103	2.06	$C_{12}H_{22}O_{11}$	89.02563, 101.0224, 115.0067, 135.04828, 143.03204, 179.0403, 179.0535, 205.0739, 281.0917, 340.0068	28
64	Sinapyl aldehyde	11.03	207.0655	0.54	$C_{11}H_{12}O_4$	133.0273, 164.044, 188.9409, 192.04233	65
65	Maltitol	11.2	343.1559	0.14	$C_{12}H_{24}O_{11}$	121.0278, 189.09166, 237.1115, 311.13074	90
66	D-(+)-Trehalose	11.9	341.1385	0.16	$C_{12}H_{22}O_{11}$	121.0301, 205.0974, 219.10239	66

*percentage of abundance was calculated from the peaks areas (peak area of the compound / total peaks area of the identified secondary metabolites X 100).

5. CONCLUSIONS

The present study revealed that *B. madagascariensis* and *B. purpurea* aerial parts contain flavonoids, phenolic, carboxylic and fatty acids, coumarins, stilbenes and acyclic diterpenoids as secondary metabolites. The most abundant class of constituents in both extracts was carboxylic acids. *B. madagascariensis* contained higher percentage of phenolic and flavonoid contents as compared to *B. purpurea*. Both extracts exhibited antioxidant capacity against free radicles, where *B. madagascariensis* extract showed superior antioxidant activity as compared to *B. purpurea* extract which could be assigned to its higher concentrations of phenolic and flavonoid contents. However, more studies are required to isolate the active constituents responsible for their antioxidant potentials and also further studies are required to identify their pharmacological activities against diseases originate from oxidative stress and molecular mechanism behind these activities, which may be valuable for the discovery of new phytochemicals with higher antioxidant potential and health benefits.

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