# *Carissa macrocarpa* leaves polar fraction ameliorates doxorubicin-induced neurotoxicity in rats via downregulating the oxidative stress and inflammatory markers

**Mohamed A. A. Orabi [1], Heba M. A. Khalil[2,*], Mohamed E. Abouelela[3], Dalia Zaafar[4], Yasmine H. Ahmed[5], Reham A. Naggar[6], Hamad S. Alyami[7], El-Shaymaa Abdel-Sattar[8], Katsuyoshi Matsunami[9] and Dalia I. Hamdan[10,**]**

1 Department of Pharmacognosy, College of Pharmacy, Najran University, Najran 55461, Kingdom of Saudi Arabia.

2 Department of Veterinary Hygiene and Management, Faculty of Veterinary Medicine, Cairo University, Giza, 12211, Egypt

3 Department of Pharmacognosy, Faculty of Pharmacy, Al-Azhar University, Assiut-branch, Assiut 71524, Egypt

4 Pharmacology and Toxicology Department, Faculty of Pharmacy, Modern University for Information and Technology

5 Cytology and Histology Department, Faculty of Veterinary Medicine, Cairo University, Giza, 12211, Egypt.

6 Department of pharmacology and Toxicology, college of pharmacy and Drug Manufacturing, Misr University of Science and Technology (MUST), 6th October, Giza 12566, Egypt

7 Department of Pharmaceutics, College of Pharmacy, Najran University, Najran 55461, Kingdom of Saudi Arabia

8 Department of Medical Microbiology and Immunology, Faculty of Pharmacy, South Valley University, 83523, Qena, Egypt

9 Department of Pharmacognosy, Graduate School of Biomedical and Health Sciences, Hiroshima University, 1-2-3 Kasumi, Minami-Ku, Hiroshima 734-8553, Japan

10 Department of Pharmacognosy and Natural Products, Faculty of Pharmacy, Menoufia University, Shibin Elkom, 32511, Egypt

*Corresponding author:
**Heba M. A. Khalil**,
Department of Veterinary Hygiene and Management, Faculty of Veterinary Medicine, Cairo University, Giza, 12211, Egypt, Mobile: +201013666331. E-mail: heba.ali315@gmail.com.
**Corresponding author:
**Dalia. I. Hamdan**,
Department of Pharmacognosy and Natural Products, Faculty of Pharmacy, Menoufia University, Shibin Elkom, 32511, Egypt, Mobile: +201289699978. E-mail: masmn12345.1973@gmail.com.

## Supplementary Material

### List of contents

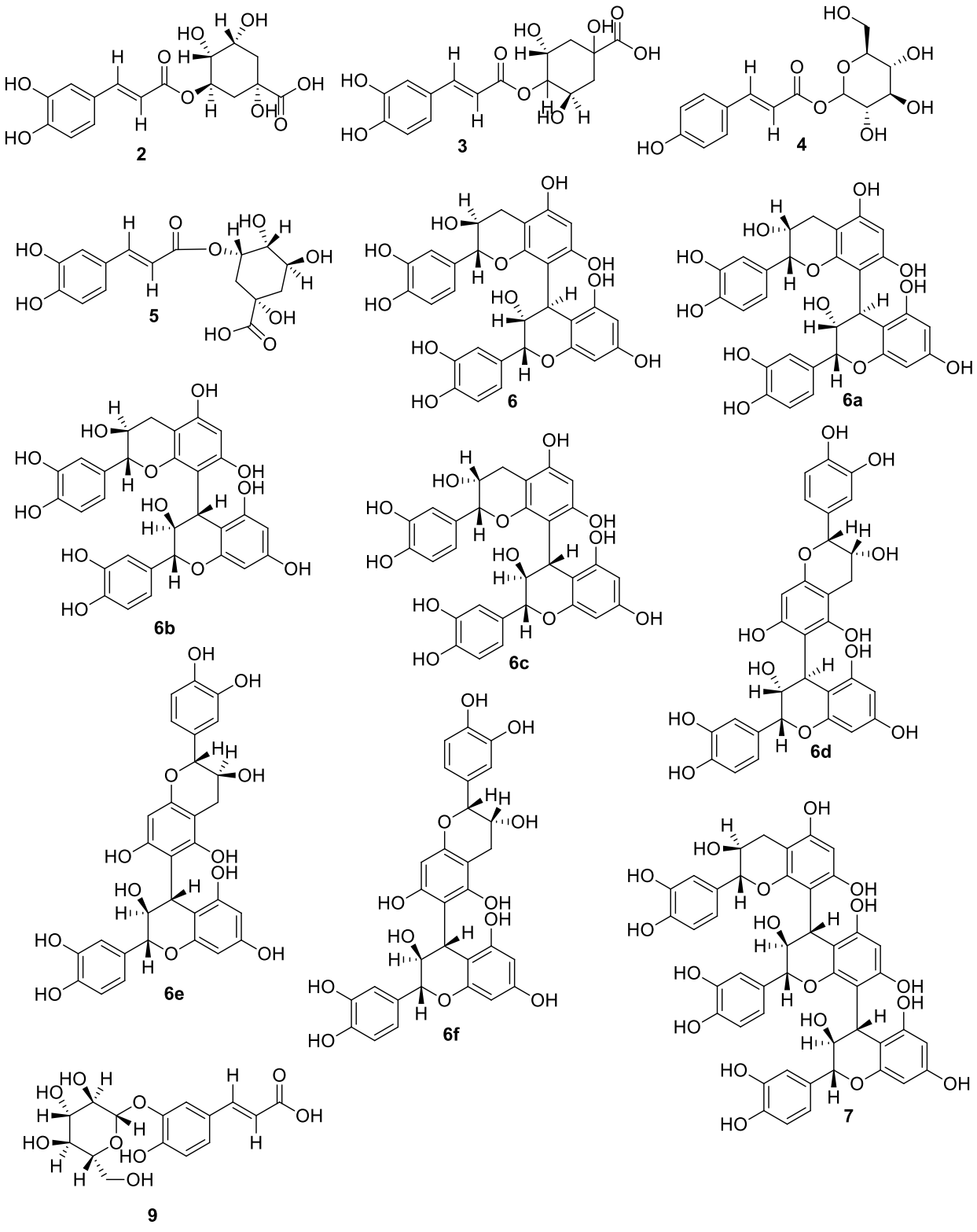


**Figure S1**. Structures of compounds evaluated by molecular docking simulation.

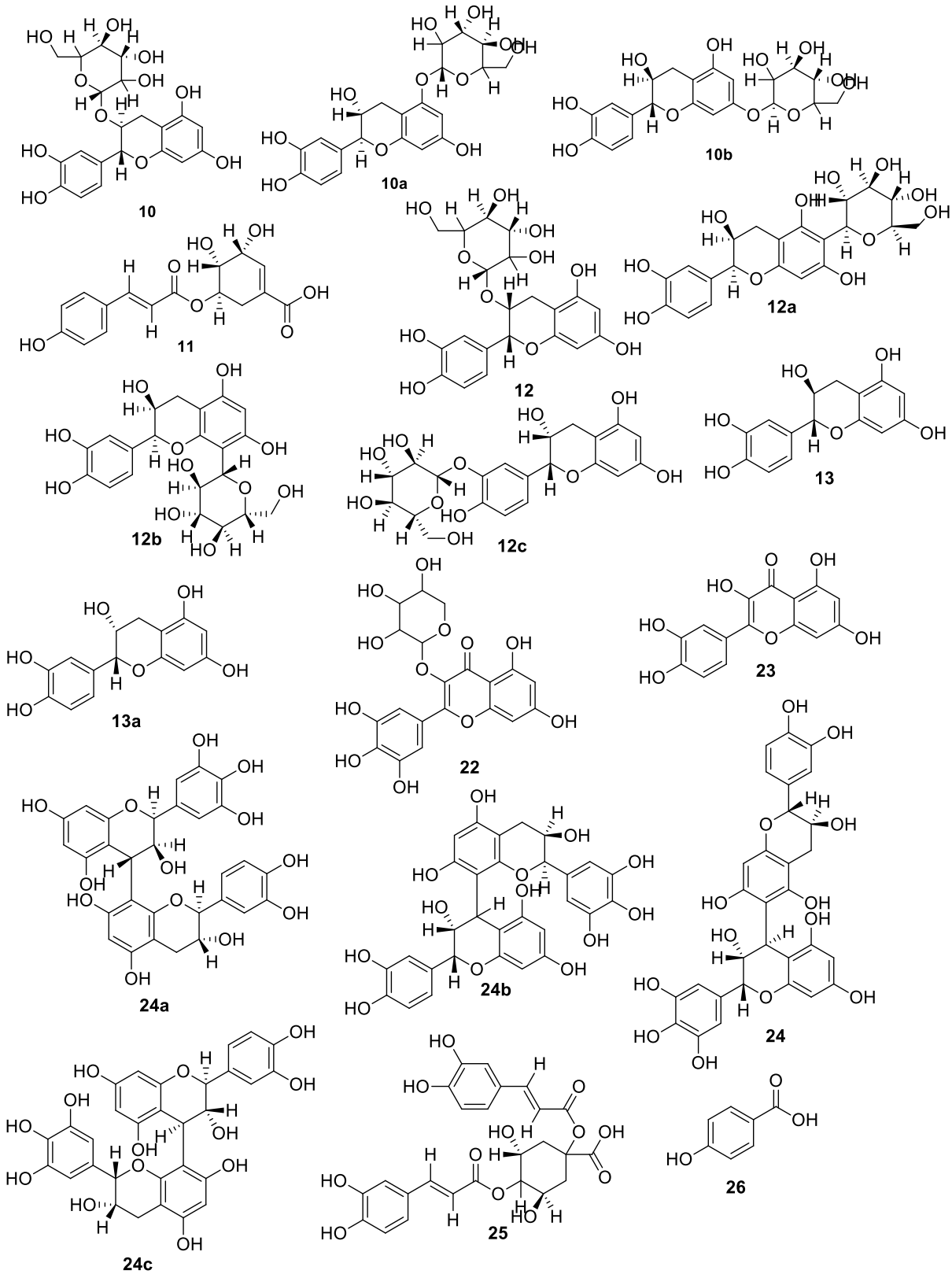


**Figure S2**. Structures of compounds evaluated by molecular docking simulation: Continued

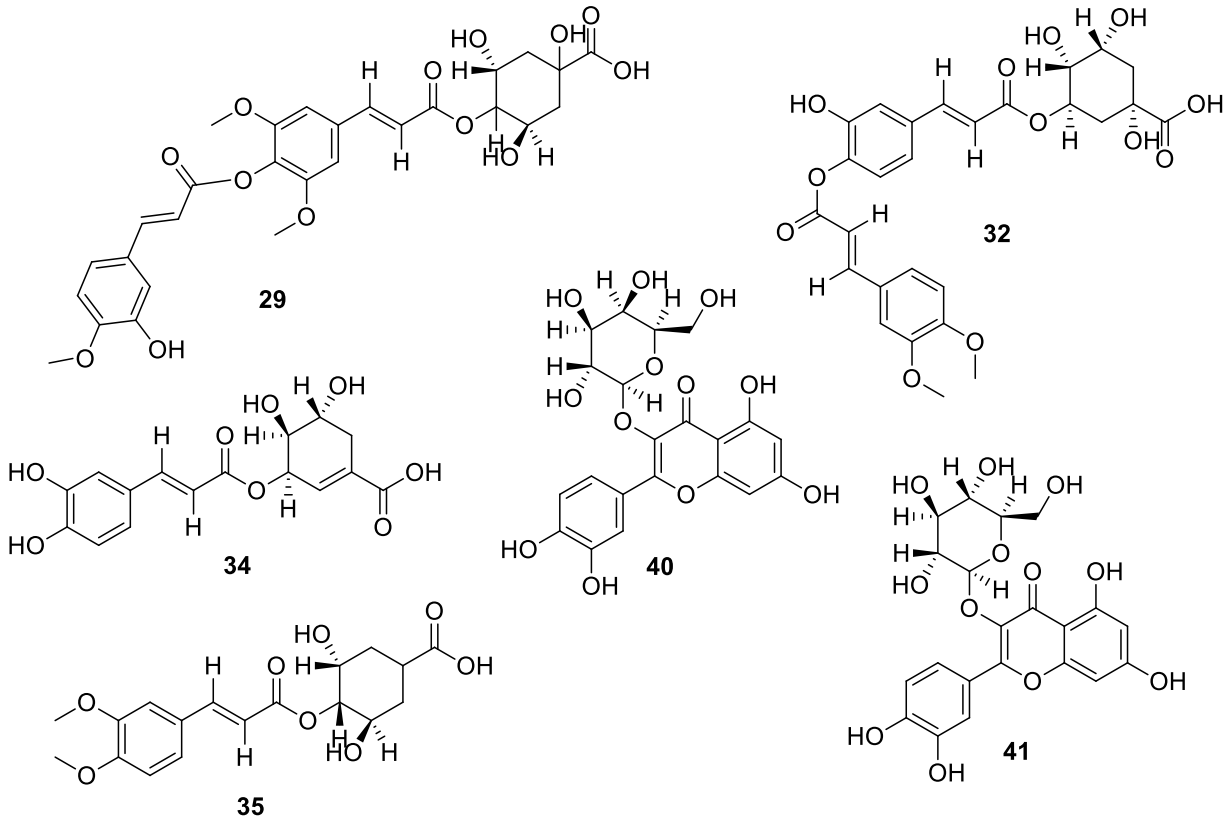


**Figure S3**. Structures of compounds evaluated by molecular docking simulation: Continued

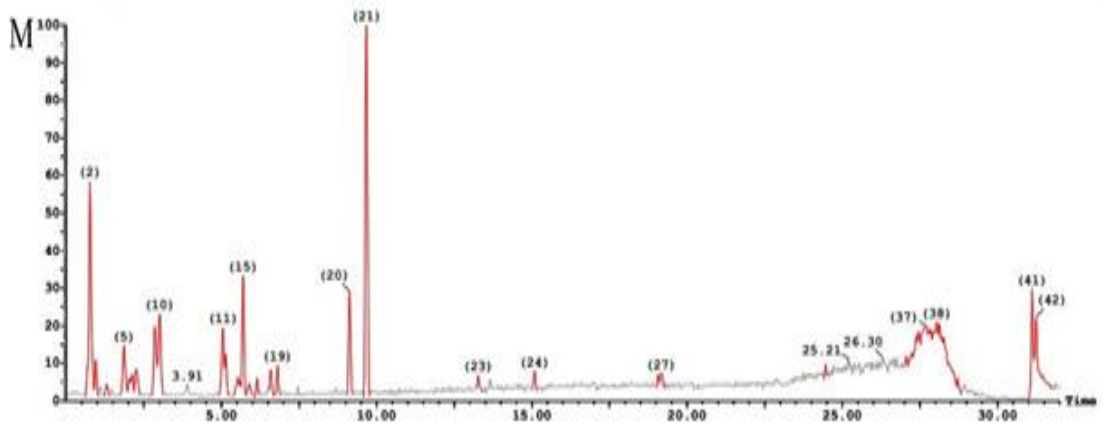


**Figure S4.** TIC of *C. macrocarpa* polar fraction of the leaves using UPLC-ESI-MS/MS in negative ionization mode.

**Table S1.** Docking score of detected polyphenolics and their isomers against TACE enzyme

| No. | Compound Name | TNF-activating converting enzyme (TACE) | |
|---|---|---|---|
| | | Score (kcal/mol) | RMSD refine (Å) |
| **2** | Chlorogenic acid | -12.6813 | 0.91 |
| **3** | Cryptochlorogenic acid | -13.3260 | 0.93 |
| **4** | coumaroyl-5-*β*-glucose | -11.9755 | 1.07 |
| **5** | 5-*O*-Caffeolyquinic acid | -13.6537 | 0.96 |
| **6** | Procyanidin Bl | -16.2139 | 2.66 |
| **6a** | Procyanidin B2 | -17.5405 | 1.82 |
| **6b** | Procyanidin B3 | -13.9250 | 1.60 |
| **6c** | Procyanidin B4 | -17.5085 | 0.84 |
| **6d** | Procyanidin B5 | -19.9566 | 1.77 |
| **6e** | Procyanidin B6 | -21.9152 | 1.25 |
| **6f** | Procyanidin B8 | -17.0187 | 1.92 |
| **7** | Procyanidin C2 | -13.2742 | 1.80 |
| **9** | Caffeic acid 3-glucoside | -12.8895 | 1.44 |
| **10** | Catechin 3-*O*- *β* -D-glucopyranoside | -18.5367 | 1.69 |
| **10a** | Catechin 5-*O*- *β* -D-glucopyranoside | -17.1693 | 1.31 |
| **10b** | Catechin 7-*O*- *β* -D-glucopyranoside | -15.3866 | 1.57 |
| **11** | 4-*O*-*p*-Coumaroylshikimic acid | -11.6970 | 1.24 |
| **12** | Epicatechin 3-*O*- *β* -D-glucopyranoside | -20.6389 | 1.34 |
| **12a** | Epicatechin 6-C-glucoside | -19.2057 | 1.30 |
| **12b** | Epicatechin 8-C-glucoside | -19.1597 | 0.93 |
| **12c** | Epicatechin-3'-*O*-glucoside | -14.7443 | 1.74 |
| **13** | Catechin | -14.2871 | 1.02 |
| **13b** | Epicatechin | -14.1969 | 0.84 |
| **22** | Myricetin-3-*O*-xyloside | -18.2396 | 1.15 |
| **23** | Quercetin | -13.1148 | 1.19 |
| **24a** | Epigallocatechin-(4 *β* -8)-catechin | -18.0768 | 1.27 |
| **24b** | Epicatechin (4 *β*.8) epigallocatechin | -18.3569 | 1.97 |
| **24c** | Catechin-(4*α*-8)-(-)-epigallocatechin | -18.0083 | 1.44 |
| **24** | Epigallocatechin-(4 *β* -6)-(+)-catechin | -14.8778 | 1.82 |
| **25** | 1,4-Dicaffeoylquinic acid | -15.0462 | 1.10 |
| **29** | Feruloyl-*O*-sinapoylquinic acid | -15.8150 | 1.08 |
| **32** | Dimethoxycinnamoyl-*O*-caffeoylquinic acid | -13.6193 | 1.00 |
| **34** | 3-Caffeoylshikimic acid | -15.6133 | 0.89 |
| **35** | Dimethoxycinnamoylquinic acid | -13.1423 | 1.48 |
| **40** | Hyperoside | -19.9102 | 1.47 |
| **41** | Isoquercetin | -16.0728 | 1.10 |

**UPLC-ESI-MS-MS metabolites charctrization**

*Organic phenolic acids and their derivatives*

Hydroxybenzoic acid (**26**) and syringic acid (**28**) were detected at different retention times as reported in Table 1. Compounds **2**, **3**, **5**, and **34** that showed a molecular ion at *m/z* 353 were detected at retention times 1.87, 2.03, 2.16, 15.06, and 16.52, respectively. Compounds **2** and **5** produced $MS^2$ fragments at *m/z* 191 ([quinic acid−H]), 179 ([Caffeic acid−H]), 161 ([caffeic acid−$H_2O$−H]), hence, assigned as 3-O-caffeolyquinic acid and 5-O-caffeolyquinic acid, respectively. Where 4-O-caffeolyquinic acid was designated for compound **3** with characteristic $MS^2$ fragments at *m/z* 191, 173 [191−$H_2O$], and 161 [1].

Additionally, 3-O-caffeoylshikimic acids has been proposed for **34**, depending upon $MS^2$ fragments (at *m/z* 179, 161) and published data [2]. Another caffeolyquinic acid derivative, diacetoxy-5-methoxyphenyl) acroyl-O-*p*-coumaroyl-O-caffeoylquinic acid (**30**) was identified by the aid of MS, $MS^2$ data (Table 1), and reported literature [3].

Other hydroxycinnamate derivatives such as 5-O-*p*-coumaroylshikimic and 4-O-*p*-coumaroylshikimic acids have been suggested for peaks **8** and **11**. $MS^2$ fragmentation exhibited a base peak at *m/z* 163 due to [*p*-coumaric acid−$H_2O$], as well as other characteristic fragments [3]. Moreover, peak **25**, which was detected at retention time 6.58 showed deprotonated molecular ion at *m/z* 515 and distinctive fragments at *m/z* 353 [M−162] and 179 that are matched with the reported literature of dicaffeoylquinic acid (**25**). Feruloyl-O-sinapoylquinic acid (**29**) was designated for peak detected at *m/z* 573 $[M-H]^-$, based on $MS^2$ fragments (397 [M−ferulic acid−H], 223 [sinapic acid−H], 173 [quinic acid−$H_2O$−H]) as compared with the literature [2]. Besides, compounds, dimethoxycinnamoyl-O-caffeoylquinic acid (**32**) and its isomer (**33**) were observed at different retention times 13.30 and 15.66 but exhibited similar deprotonated molecular ions at *m/z* 543 and characteristic fragment ions at *m/z* 353 [M−dimethoxycinnamoyl−$H_2O$] and 173 that attributed to dimethoxycinnamoyl moiety. In addition, dimethoxycinnamoylquinic acid was suggested for peak **35** that was detected in positive ionization mode at *m/z* 383 ($R_t$ 16.59) [2]. Moreover, hydroxycinnamate glycosides were detected in the current study at retention times 2,13 and 2.87, that are corresponding to peaks **4** and **9**, respectively. Where peaks **4** was suggested to be coumaric acid hexoside, due to molecular ions at *m/z* 325 $[M-H]^-$ which produced fragment ions at *m/z* 187, 163, and 145 in accordance with the literature [4]. Also, caffeic acid hexoside was assigned for peak **9** depending upon its molecular ion at *m/z* 343 $[M+H]^+$ with distinctive fragments at 326, 311, and 285, which in agreement with the reported data [5].

*Flavonoids aglycone and glycosides*

Flavonoids are considered one of the major polyphenolics detected in the polar fractions of the species under investigation. Peak **14** and **15** provided the same deprotonated molecular ion at *m/z* 755 $[M-H]^-$ with different $MS^2$ fragments, which were 593 [M−162, sugar unit] and 285 [M−308, rutinoside unit] for peak **14** that assigned as kaempferol-O-hexoside-O-rutinoside. However, quercetin-O-deoxyhexoside-O-deoxyhexosyl-hexoside was proposed for peak **15** that produced fragment ions at m/z 609 [M−146, deoxyhexosyl unit] and 301 $[M-146-308]^-$, deoxyhexosyl and rutinoside units]. Other kaempferol derivatives were kaempferol-O-

deoxyhexoside-O-deoxyhexosyl-hexoside isomers, which were designated for peaks **17** and **18**, respectively. Both compounds provided the same molecular ions (*m/z* 739 $[M-H]^-$) and $MS^2$ fragmentation patterns (*m/z* 593 and 285). Moreover, peaks **19** and **20** were tentatively identified as quercetin-O-deoxyhexosyl-hexoside isomers based on their molecular ion at *m/z* 609 $[M-H]^-$ and $MS^2$ fragment at *m/z* 301 due to loss of rutinoside unit (−308 amu). Also, the other isomer exhibited fragment ion at *m/z* 465 in positive ionization mode due to loss of deoxyhexosyl unit (−146 amu). All the previous results were confirmed upon comparison with the literature [1].

Additionally, compounds **40** and **41** belong to quercetin derivatives and have been identified as quercetin-3-O-galactoside, and quercetin-3-O-glucoside, respectively, as depicted from their MS, $MS^2$ data and comparison with literature [6]. Where, compounds **40** and **41** provided the same molecular ion at *m/z* 465 $[M+H]^+$ and $MS^2$ fragments at *m/z* 301, 300, 257, 255, 229, 179, and 151.

Furthermore, compound **22** is belonging to flavonoid glycoside [3] that was tentatively identified as myricetin pentoside [7] based on its molecular ions at *m/z* 449 $[M-H]^-$. Myricetin pentoside showed characteristic $MS^2$ fragment at 317 [M−132]. Finally, flavonoid aglycone such as quercetin has been proposed for peaks **23**.

*Flavan-3-ol derivatives*

*C. macrocarpa* is considered a main source of falvan-3-ol bioactive metabolites. From these interesting molecules, compound **13** has been identified as (epi) catechin based on a molecular ion at *m/z* 289 $[M-H]^-$, $MS^2$ fragmentation (*m/z* 245, 205, 203, 187, 179, and 161) and comparison with literature [8]. Furthermore, compounds **6**, **7**, **21,** and **31** belong to (epi)catechin derivatives of B-type; thus, (epi)catechin dimer was assigned for peaks **6**, **21**, and **31** based on deprotonated molecular ion at *m/z* 577 $[M-H]^-$, $MS^2$ fragmentation (*m/z* 425, 289) and comparison with the literature [1]. However, peak **7** that showed pseudo-molecular ion at *m/z* 865 $[M-H]^-$, and fragmentation patterns at *m/z* 451, 425, 407, and 289, were tentatively identified as (epi)catechin trimer [1].

Moreover, compounds **10**, **12**, and **16** provided the same deprotonated molecular ion at *m/z* 451 $[M-H]^-$ and different $MS^2$ fragments at *m/z* 408, 393, 351, 337, 301, 273, and 245 for compound **10** that suggested to be catechin 3-O-glucoside. However, epicatechin 3-O-glucoside and its isomer were designated for compounds **12** and **16** with $MS^2$ fragments at *m/z* 391, 343, 301, 287, 273, and 247 [9].

Compounds **24** and **27** were detected at the same molecular ion at *m/z* 593 $[M-H]^-$ and showed $MS^2$ fragments at *m/z* 557, 467, 441, 425, 407, and 289, hence proposed to be (epi)gallocatechin-(epi)catechin [7].

*Sterols and triterpenes*

Stigmasterol (**39**) was detected methanol soluble fraction [10,11]. Furthermore, the current study detected several triterpenoid aglycones at different retention times, such as compounds **36**, **37**, and **38**. Where compounds **36**, **37**, and **38** provided the same molecular ion at *m/z* 455 $[M-H]^-$,

$MS^2$ fragment ions (*m/z* 439, 419, 411, 410, 407, and 397) and designated as ursolic acid, carissic acid, and oleanolic acid upon comparison with literatures [12–15].

*Miscellaneous*

Lignans such as carinol (*m/z* 377 $[M-H]^-$ and $MS^2$ at 333, 271, 257, 163, and 119) was assigned for compound (**1**) depending on a comparison of its data with that previously reported [13]. Compound **42** with *m/z* 621 $[M-H]^-$ and distinctive fragment ion at 501 was assigned as 2(R)-26-([(2E)-3-(4-hydroxy-3-methoxyphenyl)-1-oxo-2-propen-1-yl]oxy)-2,3-dihydroxypropylester [16]. Finally, compound **43** that detected at retention time at 31.25 possessed molecular ion at *m/z* 429 $[M-H]^-$ and 431 $[M+H]^+$ with characteristic $MS^2$ fragments at m/z 205, 191, 177, 149, 121, hence assigned as α-tocopherol [17].

## References


1. Souilem F, Dias MI, Barros L, Calhelha RC, Alves MJ, Harzallah-Skhiri F, et al. Amantagula fruit (Carissa macrocarpa (Eckl.) A. DC.): nutritional and phytochemical characterization. Plant Foods Hum Nutr. 2019;74(1):76–82.

2. Jaiswal R, Matei MF, Subedi P, Kuhnert N. Does roasted coffee contain chlorogenic acid lactones or/and cinnamoylshikimate esters? Food Res Int. 2014;61:214–27.

3. Ben Said R, Hamed AI, Mahalel UA, Al-Ayed AS, Kowalczyk M, Moldoch J, et al. Tentative characterization of polyphenolic compounds in the male flowers of Phoenix dactylifera by liquid chromatography coupled with mass spectrometry and DFT. Int J Mol Sci. 2017;18(3):512.

4. Alakolanga A, Siriwardane A, Kumar NS, Jayasinghe L, Jaiswal R, Kuhnert N. LC-MSn identification and characterization of the phenolic compounds from the fruits of Flacourtia indica (Burm. F.) Merr. and Flacourtia inermis Roxb. Food Res Int. 2014;62:388–96.

5. Pereira E, Pimenta AI, Calhelha RC, Antonio AL, Verde SC, Barros L, et al. Effects of gamma irradiation on cytotoxicity and phenolic compounds of Thymus vulgaris L. and Mentha x piperita L. LWT-Food Sci Technol. 2016;71:370–7.

6. Lin L-Z, Chen P, Harnly JM. New phenolic components and chromatographic profiles of green and fermented teas. J Agric Food Chem. 2008;56(17):8130–40.

7. Jaiswal R, Jayasinghe L, Kuhnert N. Identification and characterization of proanthocyanidins of 16 members of the Rhododendron genus (Ericaceae) by tandem LC–MS. J Mass Spectrom. 2012;47(4):502–15.

8. Kang J, Price WE, Ashton J, Tapsell LC, Johnson S. Identification and characterization of phenolic compounds in hydromethanolic extracts of sorghum wholegrains by LC-ESI-MSn. Food Chem. 2016;211:215–26.

9. Yuzuak S, Ballington J, Xie D-Y. HPLC-qTOF-MS/MS-based profiling of flavan-3-ols and dimeric proanthocyanidins in berries of two muscadine grape hybrids FLH 13-11 and FLH 17-66. Metabolites. 2018;8(4):57.

10. Petta V, Gkiozos I, Strimpakos A, Syrigos K. Histones and lung cancer: are the histone deacetylases a promising therapeutic target? Cancer Chemother Pharmacol. 2013;72(5):935–52.

11. Zhou Y-X, Xin H-L, Rahman K, Wang S-J, Peng C, Zhang H. Portulaca oleracea L.: a review of phytochemistry and pharmacological effects. BioMed Res Int. 2015;2015.

12. Moodley R, Chenia H, Jonnalagadda SB, Koorbanally N. Antibacterial and anti-adhesion activity of the pentacyclic triterpenoids isolated from the leaves and edible fruits of Carissa macrocarpa. J Med Plants Res. 2011;5(19):4851–8.

13. Patel S. Food, pharmaceutical and industrial potential of Carissa genus: an overview. Rev Environ Sci Biotechnol. 2013;12(3):201–8.

14. Kaunda JS, Zhang Y-J. The genus Carissa: An ethnopharmacological, phytochemical and pharmacological review. Nat Prod Bioprospecting. 2017;7(2):181–99.

15. Jiao Q-S, Xu L-L, Zhang J-Y, Wang Z-J, Jiang Y-Y, Liu B. Rapid characterization and identification of non-diterpenoid constituents in Tinospora sinensis by HPLC-LTQ-orbitrap MSn. Molecules. 2018;23(2):274.

16. Yu J-Q, Xian Y-X, Geng Y-L, Wang D-J, Zhou H-L, Wang X. Anti-liver cancer constituents from the thorns of Gleditsia sinensis. Phytochem Lett. 2015;13:343–7.

17. Izhar S, Ahmed D. Carissa opaca: A plant with great potential for future drugs for degenerative and infectious diseases. ChemistrySelect. 2016;1(12):3005–11.