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# Triterpenes isolated from *Cleome amblyocarpa*: evaluation of antioxidant potential

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Investigation of the chemical constituents of *Cleome amblyocarpa* growing wild in Jordan resulted in the isolation and structural elucidation of two new dammarane triterpenes along with **9** other known compounds. The new compounds were identified as 17-hydroxyambylone (**1**) and 15β-acetoxy-17α-hydroxycabralealactone (**2**). The known compounds included calycopterine (**3**), 17α-hydroxycabraleahydroxylactone (**4**), cleocarpanol (**5**), ambylone (**6**), β-sitosterol (**7**), β-sitosteryl glucoside (**8**), isorhamnetin-3-*O*-rhamnoside (**9**), kaempferol-3,7-*O*-dirhamnose (**10**) and 3′-*O*-methylquercetin-3,7-di-*O*-rhamnopyranoside (**11**). Structural elucidation of all isolated compounds was based on thorough investigation of their spectroscopic data including NMR (1D and 2D), HRESIMS, IR, and UV-Vis spectroscopy. LC-MS/MS analysis *versus* a selected set of authentic samples led to the detection of 21 constituents, including **9** of the isolated compounds. Antioxidant activities of isolated pure compounds were assessed individually and compared to ascorbic acid and α-tocopherol using $DPPH^{\cdot}$ and $ABTS^{\cdot+}$ assay methods. Compounds **9–11** showed strong antioxidant activities whereas compounds **4–6** exhibited low activity. New compounds **1** and **2** demonstrated moderate $DPPH^{\cdot}$ radical scavenging power ($IC_{50}$ = 14.70 ± 2.52 and 77.90 ± 3.77 μg $mL^{-1}$, respectively) and a moderate $ABTS^{\cdot+}$ scavenging effect ($IC_{50}$ = 22.6 ± 0.04 and 78.30 ± 1.13 μg $mL^{-1}$).

## 1. Introduction

The Cleomaceae family, recently separated by Thomson and Cooke from the Capparidaceae family, comprises nine genera.[1] Among them, *Cleome* is the largest, with more than 200 species of medicinal and ecological importance. In Jordan, three species grow wild including *Cleome amblyocarpa* Barratte & Murb., *Cleome arabica* L. and *Cleome droserifolia* (Forssk.) Del.[1]

*Cleome* species are used globally in folk medicine for the treatment of many ailments like abdominal pain, rheumatism, scabies and diabetes. They are also reported to exert sudorific, irritant and sedative effects,[2,3] and several species are well recognized for their anti-inflammatory, antiseptic, antimicrobial, carminative, anthelmintic, analgesic, antioxidant, and cytotoxicity activities.[4–6] Phytochemical investigation of the selected *Cleome* species revealed the presence of diverse secondary metabolites, including terpenoids, phenols, alkaloids, flavonoids, anthraquinones, and glucosinolates.[7–13]

*Cleome amblyocarpa* Barratte & Murb. is a glandular-pubescent annual herb that grows up to 80 cm in height. It has a hairy coat that traps a layer of dust and sand grains on its erect multi-branched stem. The leaflets are elliptical, with three-foliolate lower leaves and simple upper leaves. The flowers have a light yellowish color with red-brownish tips. The fruits are flat and pendulous at maturity, with wholly tomentose seeds.[1] This species grows wild in North and Northeastern tropical Africa as well as in West and Southwest Asia. It thrives in deserts and dry shrublands and blooms once or twice a year.[14]

In traditional herbal medicine, the leaves of *C. amblyocarpa* are used for the treatment of gastric and rheumatic diseases.[15] In Tunisia, the plant is prescribed for the treatment of headaches, nausea, vomiting, and stomach pain,[16] while in Egypt, its aerial parts are valued for their antibacterial and anti-inflammatory effects.[17] Previous phytochemical studies on *C. amblyocarpa* have reported the presence of flavonoids, dammarane-type triterpenes, cembrane derivatives, stigma-4-en-3-one, lupeol, and taraxasterol.[9,18,19]

Following our thorough investigations of medicinal plants in Jordan and Mediterranean neighboring countries,[6,20–22] herein we report the isolation and characterization of two new dammarane-type triterpenes along with other nine known compounds from the aerial parts of *C. amblyocarpa* (Fig. 1).

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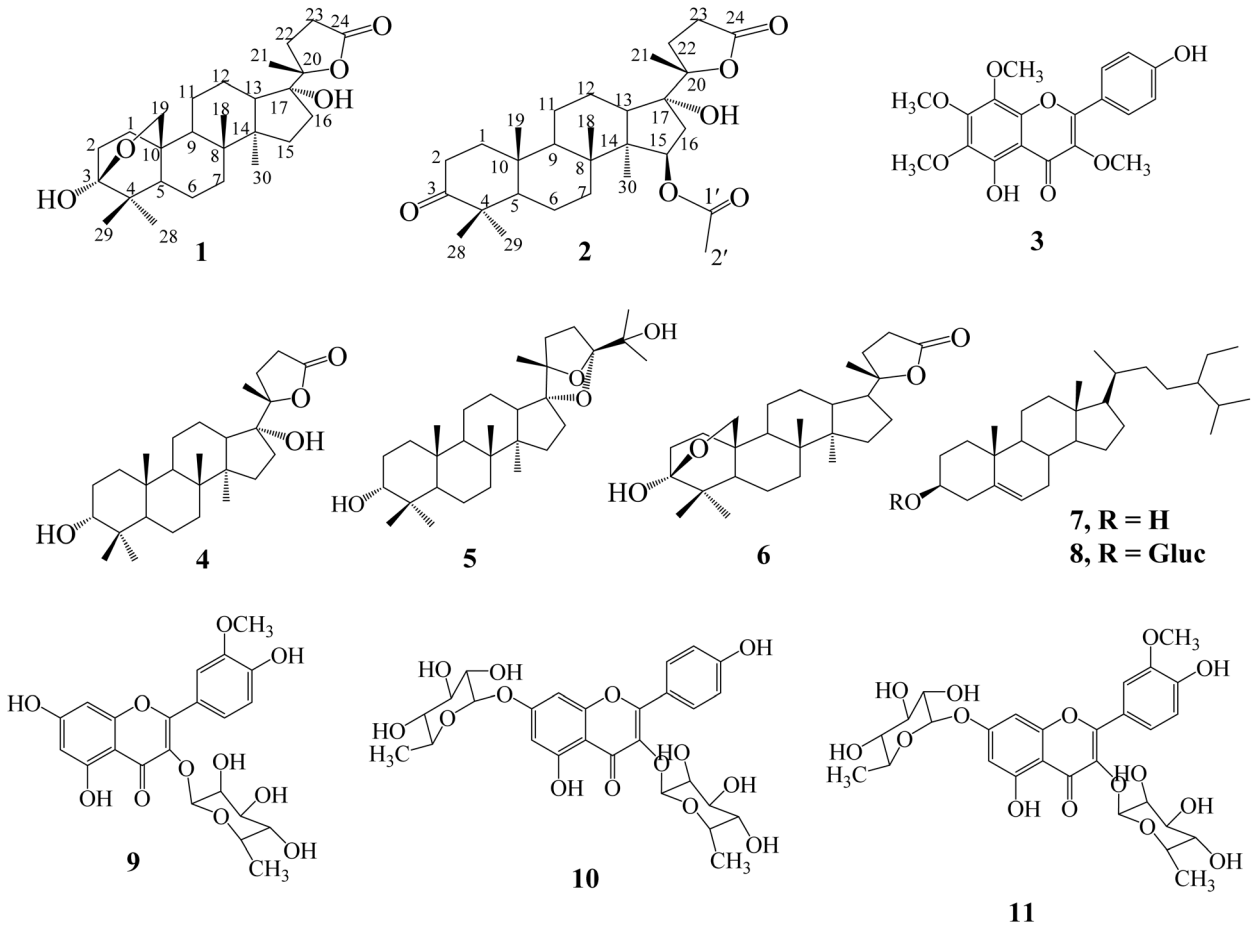


Fig. 1 Structures of compounds isolated from *C. amblyocarpa* growing wild in Jordan.

Moreover, LC-MS/MS analysis of the plant extracts *versus* selected authentic samples resulted in the detection of 21 compounds, including ten of the isolated compounds (**1–7**, **9–11**). The crude extracts (the butanol (CB) and the aqueous methanol (CM)) and the pure isolated compounds (**1–6**, **9–11**) were screened for their *in vitro* antioxidant activity using $DPPH^{\cdot}$ and $ABTS^{\cdot+}$ scavenging assay methods.

## 2. Result and discussion

The whole air-dried and defatted plant material was subjected to extraction with ethanol. After complete evaporation of the extracting solvent, the obtained dried residue was fractionated in solvents of different polarities according to the procedure described in the literature to obtain the aqueous methanol (CM) and the butanol (CB) fractions.[20,21]

### 2.1. The isolated compounds

Chromatographic separation of CM and BM fractions resulted in the isolation and structural elucidation of two new dammarane triterpenes along with nine other known compounds. The two new compounds were identified as 17-hydroxyambylone (**1**) and 15β-acetoxy-17α-hydroxycabralealactone (**2**) (Fig. 1). The other compounds were calycopterine (**3**),[23,24] 17α-hydroxycabraleahydroxylactone (**4**),[25,26] cleocarpanol (**5**),[18,25,27] ambylone (**6**),[19,28] β-sitosterol (**7**),[29] β-sitosteryl glucoside (**8**),[30] isorhamnetin-3-*O*-rhamnoside (**9**),[30] kaempferol-3,7-*O*-dirhamnose (**10**)[30] and 3,7-dirhamnose-isorhamnetin (**11**).[30] Structural elucidation of all isolated compounds was achieved based on extensive investigation of their IR, HRESIMS, UV-Vis, 1D and 2D NMR (including COSY, HMQC, and HMBC) spectral data.

Compound **1** was isolated as a white amorphous solid. Inspection of the IR spectrum of compound **1** indicated the presence of hydroxyl (3398 $cm^{-1}$), lactone (1753 $cm^{-1}$) and ether (1254 $cm^{-1}$) groups (Fig. S1). The molecular ion peak observed at *m*/*z* 445.2927 in the HREIMS of compound **1** allowed the prediction of the molecular formula $C_{27}H_{42}O_5$ ($[M-H]^-$, calcd for $[C_{27}H_{41}O_5]^-$: 445.2954). Initial analysis of the $^1H$, $^{13}C$, DEPT, HMQC, COSY and HMBC spectra of compound **1** revealed signals for 27 nonequivalent carbons including 5 methyls, 10 methylenes, one oxygenated methylene ($\delta_{C\text{-}19}$ 68.0), 3 methines, 8 quaternary carbons three of which are oxygenated ($\delta_{C\text{-}3}$ 98.2, $\delta_{C\text{-}17}$ 83.4 and $\delta_{C\text{-}20}$ 93.0), in addition to one carbonyl carbon of a lactone moiety ($\delta_{C=O}$ 176.6) (Fig. S3). The $^1H$-NMR and HMQC spectra displayed signals for 5 methyl groups resonating at $\delta_H$ 0.88 (Me-30), 0.98 (Me-18), 1.03 (Me-29), 1.13 (Me-28) and 1.43 (Me-21); double doublet signals for the oxygenated C-19 ($\delta_C$

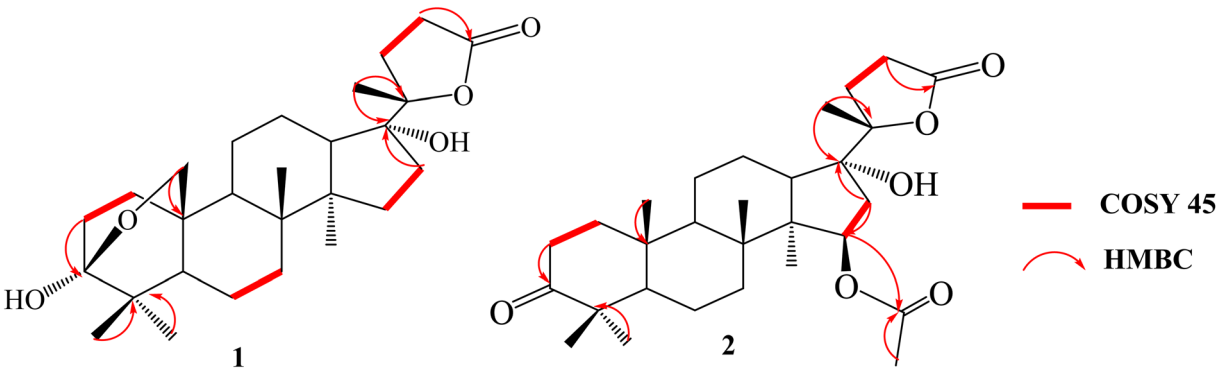


Fig. 2 HMBC and $^{1}H-^{1}H$ COSY correlation of compounds **1** and **2**.

68.0) methylene protons at $\delta_H$ 4.25 (1H, dd, $J$ = 8.8, 2.7 Hz, H-19a) and 3.75 (1H, dd, $J$ = 8.8, 1.6 Hz, H-19b) in addition to overlapping peaks in the range $\delta_H$ 1.42–2.22 assigned to the aliphatic protons of compound **1** (Fig. S2).

The combination of all these spectral data suggested that compound **1** is a damarane triterpene that is very similar to the structure of the damarane triterpenoid ambylone (compound **6**), differing only in the detection of an extra hydroxyl group in compound **1**.[19,28] The location of this hydroxyl group was suggested to C-17 of the skeleton based on the disappearance of the methine signal $\delta_{H\text{-}17}$ 1.24 observed in compound **6**. This assignment was further supported by the down field shift observed for C-17 in compound **1** ($\delta_C$ 83.4) when compared to C-17 in compound **6** ($\delta_C$ 49.1). Careful inspection of the different 2D NMR spectra including COSY, HMQC and HMBC (Fig. 2 and S4–S6) allowed the correct assignment of all oxygenated carbons in compound **1** ($\delta_{C\text{-}3}$ 98.2, $\delta_{C\text{-}17}$ 83.4 and $\delta_{C\text{-}20}$ 93.0). The HMBC spectrum of compound **1** revealed long range correlations between H-19 protons ($\delta_H$ 3.75 & 4.25) and each of C-10 ($\delta_C$ 32.6), C-5 ($\delta_C$ 49.5) and C-1 ($\delta_C$ 35.5), thus confirming oxygenation of C-19 and the substitution pattern of ring A. The location of the extra hydroxyl group at C-17 was evidenced from the long-range correlations observed between H-21 protons ($\delta_H$ 1.43) and each of C-20 ($\delta_C$ 93.0) and C-17 ($\delta_C$ 83.4) in addition to the correlation between H-16 ($\delta_H$ 1.52) and C-17. These correlations allowed identification of compound **1** as 17-hydroxyambylone.

Compound **2** was obtained from subFr. CMIII-3-sub-3 as pure white amorphous solid. The molecular formula $C_{29}H_{44}O_6$ of **2** was characterized based on its HRESIMS, which revealed a molecular ion peak at $m/z$ 487.3042 $[M-H]^-$ (calcd for $[C_{29}H_{43}O_6]^-$: 487.3060). The IR absorption bands at 3494, 1767, 1740, 1648 and 1295 $cm^{-1}$ indicated the presence of hydroxyl, lactone, ketone, ester, and ether groups, respectively (Fig. S7).

The 1D spectra (Fig. S8–S11) and 2D (Fig. 2 and S12–S14) spectra ($CDCl_3$) of compound **2** closely resembled those of the known dammarane triterpene 17α-hydroxycabralealactone (Takhi *et al.*, 2011), except for the presence of an extra acetyl group. The $^{1}H$-NMR spectrum (Fig. 2 and S8) revealed 6 methyl singlets assigned to Me-21 ($\delta_H$ 1.47), Me-28 ($\delta_H$ 1.17), Me-19 ($\delta_H$ 1.09), Me-29 ($\delta_H$ 1.04), Me-18 ($\delta_H$ 1.01) and Me-30 (0.97). An additional methyl singlet signal resonating at 2.14 was attributed to the $CH_3CO$ protons. The overlapping peaks detected upfield the spectrum in the range $\delta_H$ 1.29–2.69 were typical of the different aliphatic protons of the suggested skeleton.[4] However, the proton spectrum revealed a multiplet signal integrating for one proton and resonating at $\delta_H$ 3.81 (1H, m) that was assigned to H-15, where the acetyl group location was suggested.

The spectral data observed in the $^{13}C$-NMR and DEPT ($CDCl_3$) experiments of **2** (Fig. S9–S11) were in total agreement with the suggested 17α-hydroxycabralealactone skeleton, with the extra acetyl groupe positioned at C-15. These spectra revealed signals for 29 carbon including 7 Me's, 9 $CH_2$'s, 3 CH's, one CH–O moiety ($\delta_C$ 68.3, assigned for C-15), 3 quaternary carbons in addition to other 3(C=O) groups comprising one ketone ($\delta_C$ 218.2), one lactone ($\delta_C$ 178.4) and one acetyl ($\delta_C$ 176.7) carbons. However, the location of the acetyl group at C-15 was confirmed by detailed analysis of the 2D-NMR spectra (Fig. 2 and S12–S14). The strong $^{1}H-^{1}H$ correlation observed in the COSY spectrum between H-15 ($\delta_H$ 3.81) and H-16 ($\delta_H$ 2.34) in addition to the long-range correlations observed in the HMBC spectrum between H-15 ($\delta_H$ 3.81) and each of C-16 ($\delta_C$ 32.5), C-14 ($\delta_C$ 49.9) and C-1′ ($\delta_C$ 176.7) confirmed the attachment of the acetyl group to C-15. Other important correlations observed between H-16 ($\delta_H$ 2.34) and C-17 ($\delta_C$ 83.9), H-21 ($\delta_H$ 1.47) with each of C-20 ($\delta_C$ 92.9) and C-17 ($\delta_C$ 83.9) in addition to the correlation observed between H-2′ ($\delta_H$ 2.14) and C-1′ ($\delta_C$ 176.7) helped in confirming the oxygenation pattern of rings D and E of the proposed structure. Based on these data, compound **2** was identified as 15β-acetoxy-17α-hydroxareabralealactone. Table 1 lists the $^{1}H$ and $^{13}C$-NMR ($CDCl_3$) chemical shifts for compounds **1**, **2** and **6** (known compound ambylone) while Fig. 2 shows the important COSY and HMBC correlations in both new compounds **1** and **2**.

The ECD spectra of compounds **1** and **2** were recorded with a Jasco J-720 spectropolarimeter in chloroform (Fig. S15). The ECD values for **1** and **2** showed positive (300 nm) cotton effects.

Further qualitative analysis of the CB and **CM** fractions on LC-MS/MS revealed the presence of 21 compounds including **10** of the isolated compounds. Identification of these constituents was based on comparing their mass spectra with our built-in library that includes 59 authentic secondary metabolites isolated and identified in our laboratories. Table 2 lists the compounds identified in both fractions.

Table 1 $^{1}$H NMR (400 MHz) and $^{13}$C (100 MHz) spectroscopic data ($CDCl_3$, $\delta$ in ppm, $J$ in Hz) for compounds **1**, **2** and the known compound **6** (ambylone)

| | **1: 17-hydroxyambylone** | | **2: 15β-acetoxy-17α-hydroxareabralealactone** | | **6**: ambylone | |
|---|---|---|---|---|---|---|
| # | $\delta_H$ ($J$ in Hz) | $\delta_C$ | $\delta_H$ ($J$ in Hz) | $\delta_C$ | $\delta_H$ ($J$ in Hz) | $\delta_C$ |
| **1** | 1.25 (1H, m)<br>1.51 (1H, m) | 35.5 | 1.18 (1H, m)<br>1.51 (1H, m) | 39.9 | 1.19 (1H, m)<br>1.50 (1H, m) | 33.3 |
| **2** | 1.48 (1H, m)<br>2.67 (1H, m) | 29.5 | 1.40 (1H, m)<br>2.33 (1H, m) | 34.0 | 1.47 (1H, m)<br>2.68 (1H, m) | 29.2 |
| **3** | — | 98.2 | — | 218.2 | — | 98.0 |
| **4** | — | 40.5 | — | 47.4 | — | 40.5 |
| **5** | 1.90 (1H, m) | 49.5 | 1.95 (1H, m) | 47.4 | 1.85 (1H, m) | 49.7 |
| **6** | 1.37 (1H, m)<br>2.46 (1H, m) | 19.8 | 1.36 (1H, m)<br>2.05 (1H, m) | 19.6 | 1.36 (1H, m)<br>2.56 (1H, m) | 19.7 |
| **7** | 1.15 (1H, m)<br>1.29 (1H, m) | 29.7 | 1.15 (1H, m)<br>1.33 (1H, m) | 33.9 | 1.16 (1H, m)<br>1.24 (1H, m) | 29.5 |
| **8** | — | 39.8 | — | 40.8 | — | 39.2 |
| **9** | 1.51 (1H, m) | 46.2 | 1.42 (1H, m) | 50.0 | 1.48 (1H, m) | 45.1 |
| **10** | — | 32.6 | — | 36.8 | — | 35.6 |
| **11** | 1.21 (1H, m)<br>1.95 (1H, m) | 22.6 | 1.21 (1H, m)<br>1.60 (1H, m) | 21.9 | 1.21 (1H, m)<br>1.60 (1H, m) | 22.6 |
| **12** | 1.50 (1H, m)<br>1.95 (1H, m) | 29.3 | 1.50 (1H, m)<br>1.92 (1H, m) | 22.8 | 1.50 (1H, m)<br>1.92 (1H, m) | 27.0 |
| **13** | 1.58 (1H, m) | 45.1 | 1.55 (1H, m) | 46.1 | 1.55 (1H, m) | 43.3 |
| **14** | — | 49.9 | — | 49.9 | — | 50.0 |
| **15** | 1.90 (1H, m)<br>1.14 (1H, m) | 32.5 | 3.81 (1H, m) | 68.3 | 1.95 (1H, m)<br>1.18 (1H, m) | 31.2 |
| **16** | 1.54 (1H, m)<br>1.80 (1H, m) | 23.0 | 1.78 (1H, m)<br>1.97 (1H, m) | 32.5 | 1.78 (1H, m)<br>1.97 (1H, m) | 25.3 |
| **17** | — | 83.4 | — | 83.9 | 1.24 (1H, m) | 49.1 |
| **18** | 0.98 (3H, s) | 15.3 | 1.01 (3H, s) | 16.1 | 0.91 (3H, s) | 15.2 |
| **19** | 4.25 ($1H_a$, dd, 8.8, 2.7);<br>3.75 ($1H_b$, dd, 8.8, 1.6) | 68.0 | 1.09 (3H, s) | 15.4 | 4.27 ($1H_a$, dd, 8.8, 2.7);<br>3.76 ($1H_b$, dd, 8.8, 1.6) | 68.0 |
| **20** | — | 93.0 | | 92.9 | | 90.0 |
| **21** | 1.43 (3H, s) | 22.9 | 1.47 (3H, s) | 22.9 | 1.38 (3H, s) | 22.5 |
| **22** | 1.48 (1H, m)<br>2.53 (1H, m) | 35.5 | 1.48 (1H, m)<br>2.00 (1H, m) | 29.2 | 1.48 (1H, m)<br>2.57 (1H, m) | 35.6 |
| **23** | 2.56 (1H, m)<br>2.65 (1H, m) | 26.8 | 2.53 (1H, m)<br>2.62 (1H, m) | 29.6 | 2.52 (1H, m)<br>2.63(1H, m) | 25.4 |
| **24** | — | 176.7 | — | 178.4 | — | 176.4 |
| **25** | — | — | — | — | — | — |
| **26** | — | — | — | — | — | — |
| **27** | — | — | — | — | — | — |
| **28** | 1.13 (3H, s) | 29.1 | 1.17 (3H, s) | 26.7 | 1.05 (3H, s) | 26.8 |
| **29** | 1.03 (3H, s) | 18.5 | 1.04 (3H, s) | 21.0 | 1.00 (3H, s) | 18.4 |
| **30** | 0.88 (3H, s) | 16.5 | 0.97 (3H, s) | 16.9 | 0.88 (3H, s) | 16.9 |
| **1′** | — | — | — | 176.7 | — | — |
| **2′** | — | — | 2.14 (3H, s) | 23.4 | — | — |

### 2.2. Determination of total phenol (TPC) and total flavonoid (TFC) contents

Both, the (CM) and (**CB**) fractions were assayed for their TPC and TFC according to the procedure listed in the literature.[22] The TPC was determined based on the regression equation $Y = 7.9501x + 0.0389$, $R^2 = 0.9993$, established with gallic acid, where $Y$ is the absorbance at 765 nm and $x$ refers to the TPC of the tested fraction. Similarly, the TFC was determined using $AlCl_3$ method described in the literature,[6,20] and quantitative estimation was based on the linear regression equation $Y = 0.6416x + 0.0007$, $R^2 = 0.999$, established with quercetin, where $Y$ is the absorbance at 510 nm and $x$ is the TFC in the tested fraction. As revealed in the results shown in Table 3, **CB** fraction is richer in both phenolic compounds (103.53 ± 0.50 GAE) and flavonoids (448.82 ± 6.30 QE) when compared to the **CM** fraction. These findings supported the antioxidant activity results, in which **CB** fraction showed higher $DPPH^{\cdot}$ and $ABTS^{\cdot+}$ scavenging potentials when compared to the **CM** fraction.

### 2.3 Antioxidant activity

The two crude fractions (CB and CM) and their isolated pure compounds (**1–6**, **9–11**) were assayed for their antioxidant

 

Table 2 The LC- MS/MS analysis data of detected metabolites from the CB and CM extracts of *C. amblyocarpa* from Jordan[a]

| No. | RT [min] | *m/z* meas* | Mwt calcd | Name | Class# | Molecular formula[a] | CB* | CM* |
|---|---|---|---|---|---|---|---|---|
| **1** | 2.25 | 153.0228 | 154.0301 | 2,5-Dihydroxybenzoic acid | PA | $C_7H_6O_4$ | + | — |
| **2** | 2.63 | 137.0247 | 138.0317 | 4-Hydroxybenzoic acid | PA | $C_7H_6O_3$ | + | + |
| **3** | 4.94 | 609.1436 | 610.1509 | Luteolin-7,3′-di-*O*-glucoside | Fl | $C_{27}H_{30}O_{16}$ | + | — |
| **4** | 5.28 | 609.1462 | 610.1535 | 3-Glu-7-RhaQuercetin | Fl | $C_{27}H_{30}O_{16}$ | + | — |
| **5** | 5.55 | 593.1487 | 594.1585 | 3-Rha-7-RhaQuercetin | Fl | $C_{27}H_{30}O_{15}$ | + | — |
| **6** | 5.78 | 563.1416 | 564.1488 | Apiin | Fl | $C_{26}H_{28}O_{14}$ | + | — |
| **7** | 5.81 | 463.0884 | 464.0957 | Hyperoside | Fl | $C_{21}H_{20}O_{12}$ | + | + |
| **8** | 5.90 | 413.1452 | 414.1525 | β-Sitosterol (**7**) | St | $C_{29}H_{50}O$ | + | + |
| **9** | 6.14 | 593.1513 | 594.1585 | 3-*O*-Neohesperidoside kaempferol | Fl | $C_{27}H_{30}O_{15}$ | + | + |
| **10** | 6.33 | 577.154 | 578.1612 | Kaempferol-3,7-*O*-dirhamnose (**10**) | Fl | $C_{27}H_{30}O_{14}$ | + | + |
| **11** | 6.51 | 607.1644 | 608.1731 | 3′-*O*-methylquercetin-3,7-di-*O*-rhamnopyranoside (**11**) | Fl | $C_{28}H_{32}O_{15}$ | + | + |
| **12** | 7.53 | 285.0401 | 286.0474 | 3,6,3′,4′-Tetrahydroxyflavone | Fl | $C_{15}H_{10}O_6$ | + | — |
| **13** | 7.74 | 461.1064 | 462.1137 | Isorhamnetin-3-*O*-rhamnoside (**9**) | Fl | $C_{22}H_{22}O_{11}$ | + | + |
| **14** | 9.97 | 299.0539 | 300.0612 | Hispidulin | Fl | $C_{16}H_{12}O_6$ | + | + |
| **15** | 10.80 | 299.0539 | 300.0612 | Ladanetin | Fl | $C_{16}H_{12}O_6$ | + | + |
| **16** | 14.02 | 373.0903 | 374.0976 | Calycopterine (**3**) | DT | $C_{19}H_{18}O_8$ | + | + |
| **17** | 16.02 | 445.2897 | 446.2969 | 17-Hydroxyambylone (**1**) | DT | $C_{27}H_{42}O_5$ | — | + |
| **18** | 18.43 | 473.2840 | 474.2914 | Cleocarpanol (**5**) | DT | $C_{30}H_{50}O_4$ | — | + |
| **19** | 20.26 | 487.2993 | 488.3066 | 15β-acetoxy-17α-hydroxycabralealactone (**2**) | DT | $C_{29}H_{44}O_6$ | — | + |
| **20** | 22.01 | 429.2944 | 430.3017 | Ambylone (**6**) | DT | $C_{27}H_{42}O_4$ | — | + |
| **21** | 23.28 | 431.3171 | 432.3173 | 17α-hydroxycabraleahydroxylactone (**4**) | DT | $C_{27}H_{44}O_4$ | — | + |

[a] All are reported as $[M–H]^-$; *CB: butanol fraction, CM: aqueous methanol fraction, #Class of secondary metabolite detected: PA: phenolic acid; Fl: flavonoid; DT: dammarane triterpene, St: sterol.

Table 3 Results for TPC (mg $g^{-1}$ GAE) and TFC (mg $g^{-1}$ QE) of the CB and CM fractions. Data are expressed as the mean ± SD of three independent experiments

| Fraction/compound | TPC | TFC |
|---|---|---|
| CB | 103.53 ± 0.50 | 448.82 ± 6.30 |
| CM | 64.00 ± 1.47 | 120.53 ± 0.00 |

Table 4 Results for the $DPPH^{\cdot}$ and $ABTS^{\cdot+}$ antioxidant $IC_{50}$ (μg $mL^{-1}$) values for extracts CB, CM, pure isolated constituents (**1–6**, **9–11**) and reference compounds. Data are expressed as the mean ± SD of three independent experiments

| | $IC_{50}$ (μg $mL^{-1}$) | |
|---|---|---|
| Fraction/Compound | $DPPH^{\cdot}$ | $ABTS^{\cdot+}$ |
| CB | 16.00 ± 0.50 | 7.50 ± 2.50 |
| CM | 18.00 ± 0.05 | 11.00 ± 0.50 |
| **1** | 14.70 ± 2.52 | 22.6 ± 0.04 |
| **2** | 77.90 ± 3.77 | 78.30 ± 1.13 |
| **3** | 7.10 ± 0.37 | 7.09 ± 0.70 |
| **4** | 168.00 ± 1.97 | n.d |
| **5** | 271.00 ± 6.14 | 193.00 ± 1.80 |
| **6** | n.d | n.d |
| **9** | 10.50 ± 1.24 | 7.97 ± 0.58 |
| **10** | 8.32 ± 0.85 | 2.38 ± 0.11 |
| **11** | 2.55 ± 0.07 | 1.25 ± 0.04 |
| Ascorbic acid | 1.58 ± 0.035 | 1.78 ± 0.06 |
| α-tocopherol | 1.79 ± 0.01 | 2.33 ± 0.01 |

activities, using the $DPPH^{\cdot}$ and $ABTS^{\cdot+}$ methods, results are shown in Table 4 (Fig. S59 and S60). Compounds **9**, **10**, and **11** exhibited strong antioxidant activities, with compound **11** being significantly more potent than compounds **9** and **10**. This enhanced activity may be attributed to the greater number of hydroxyl groups in compound **11** compared to the other two compounds (**9** and **10**). The new compounds **1** and **2** showed moderate $DPPH^{\cdot}$ radical scavenging power ($IC_{50}$: 14.70 ± 2.52 and 77.90 ± 3.77 μg $mL^{-1}$, respectively), with their activities being markedly lower than those observed for the positive controls; ascorbic acid and α-tocopherol ($IC_{50}$: 1.58 ± 0.035 and 1.79 ± 0.01 μg $mL^{-1}$, respectively). Compounds **1** and **2** also exhibited moderate $ABTS^{\cdot+}$ scavenging effect ($IC_{50}$: 22.6 ± 0.04 and 78.30 ± 1.13 μg $mL^{-1}$, respectively), which was also lower than those observed for the tested reference compounds, ascorbic acid and α-tocopherol ($IC_{50}$: 1.78 ± 0.06 and 2.33 ± 0.01 μg $mL^{-1}$, respectively).

## 3. Conclusion

Phytochemical investigation of the butanol (CB) and aqueous methanol (CM) fractions obtained from the crude extract of *C. amblyocarpa* from Jordan resulted in the isolation and identification of two new dammarane triterpenes, namely 17-hydroxyambylone (**1**) and 15-acetoxy-17-hydroxycabralealactone (**2**) in addition to other **9** known compounds. LC-MS/MS analysis of the crude extracts *versus* a selected set of authentic samples led to the detection of 22 constituents. The crude fractions, new compounds **1** and **2**, and previously untested known compounds **3–6**, **9–11** were all assayed for their $DPPH^{\cdot}$ and $ABTS^{\cdot+}$ scavenging power. Among the tested compounds, isorhamnetin-3-*O*-rhamnoside (**9**), kaempferol-3,7-*O*-dirhamnose (**10**) and 3′-*O*-methylquercetin-3,7-di-*O*-rhamnopyranoside (**11**) exhibited exceptionally strong antioxidant potentials in both assay methods that were close to the activity

observed for the reference controls. 3′-*O*-methylquercetin-3,7-di-*O*-rhamnopyranoside (**11**) was significantly more potent due to the substitution pattern of this flavonol glycoside. Moreover, the new compounds, **1** and **2**, exhibited moderate antioxidant potentials in both assay methods, with compound **1** being more active than compound **2**. This could mainly be attributed to the presence of the free 3-OH and 17-OH hydroxyl groups, as these groups, although aliphatic, provide accessible hydrogen donor sites. For compound **2**, the replacement of the 3-OH group with a carbonyl group in addition to the acetylation at C-15, together eliminate the potential hydrogen donation capacity, increase steric hindrance and hydrophobicity. These structural features reduce radical accessibility, therefore explaining the lower $DPPH^{\bullet}$ and $ABTS^{\bullet+}$ radical scavenging power of compound **2** compared to **1**.

Despite that the current results revealed moderate activity of the new compounds, further pharmacological screening of the plant material and the pure constituents may help in revealing their undiscovered therapeutic potentials.

# 4. Experimental

## 4.1. General

$^1$H-NMR spectra were recorded on a Bruker Avance III 400 MHz spectrometer with TMS as an internal standard. $^{13}$C-NMR spectra were recorded at 100 MHz. High resolution mass spectra (HRESIMS) were acquired by electrospray ionization in the positive mode technique using a Bruker APEX-4 Mass spectrometer. TLC was performed on silica gel 60 GF254 pre-coated glass plates (0.25 or 0.50 mm in thickness, Macherey-Nagel). Compounds were visualized under UV light or spraying with sulfuric acid-anisaldehyde spraying reagent followed by heating. Circular dichroism (CD) spectra were recorded with a Jasco J-720 spectropolarimeter (0.1 nm resolution, 10 accumulations, 1 cm cell) in chloroform. Blank measurement of chloroform provided no ICD effects in the region examined.

LC-MS/MS was conducted on a Bruker Daltonik Impact II ESI-Q-TOF System equipped with a Bruker Daltonik Elute UPLC system (Bremen, Germany), both in the positive $[M + H]^+$ and negative $[M–H]^-$ electrospray ionization modes. Apollo II ion Funnel electrospray source was used to power this instrument. The capillary capacity was 2500 V, the nebulizer gas pressure was 2.0 bar, the dry gas (nitrogen) flow rate was 8 L min$^{-1}$, and the dry temperature was 200 °C. The mass resolution was 50 000 FSR (Full Sensitivity Resolution), while the TOF repetition rate was up to 20 kHz. The mass accuracy was <1 ppm.

## 4.2. Plant material

*C. amblyocarpa* Barratte & Murb. was collected from Wadi Rum region during the full flowering stage (April-2020). The taxonomic identity of the plant was certified by Prof. Dr Jamil N. Lahham (Department of Biology, Faculty of Science, Yarmouk University, Irbid, Jordan). A reference specimen (CA/C/2020) was stored in Prof. Mahmoud A. Al-Qudah Laboratory, Yarmouk University, Irbid, Jordan.

## 4.3. Extraction and fractionation

Air dried and powdered plant material (10 kg) was subjected to extraction with petroleum ether to remove fatty acids and waxes (20 L, 10 days, RT). Then, secondary metabolites were extracted from the defatted dried plant reside by maceration in ethanol at room temperature (5 times, 7 days each). Then, the combined and dried ethanolic extract (approximately 500 g) was partitioned as described in the literature[6,20–22] to afford the aqueous methanol (CM; 129.1 g) and butanol (CB; 115.96 g) fractions.

## 4.4. Determination of TPC, TFC and antioxidant activity

The TPC and TFC of CB and CM fractions were evaluated according to the Folin–Ciocalteu technique and aluminum chloride assay method, respectively, according to the procedure described in the literature.[22] Data are expressed as the mean ± SD of three independent experiments.

The antioxidant activity of both fractions and isolated compounds (**1–6**, **9–11**) was assessed using the $DPPH^{\bullet}$ and $ABTS^{\bullet+}$ assay methods, as outlined in the literature.[6,22] Data are expressed as the mean ± SD of three independent experiments.

## 4.5. Isolation and purification of secondary metabolites

Fraction CM (129.1 g) was absorbed on silica gel (150 g, 230–400 mesh) and then subjected to column chromatography in a column filled with the same adsorbent (600 g, 230–400 mesh) and eluted with a gradient combination of *n*-hexane/EtOAc of increasing polarity. A total of 211 eluent fractions (500 mL each) were collected and then consolidated into five main groups (CMI – CMV) based on their TLC behavior. These groups were then subjected to a combination of CC, TLC or crystallization to separate their constituents. Fr. CMI (2.5 g) was subjected to CC on a column packed with silica gel (50 g, 230–400 mesh) and eluted with a gradient of *n*-hexane/EtOAc affording four subfractions (SubFr. CMI-1 to SubFr. CMI-4). SubFr. CMI-2 (500 mg) was purified by crystallization in MeOH to yield **3** (70 mg). Subjecting Fr. CMII (10.80 g) to CC (250 g silica gel, 230–400 mesh) eluted with a gradient mixture of *n*-hexane/EtOAc afforded five subfractions (SubFr. CMII-1 to SubFr. CMII-5). SubFr. CMII-3 (610 mg) was purified by crystallization in MeOH to yield **4** (55 mg). SubFr. CMII-4 (700 mg) was purified on CC (packed with 5 g silica gel, 230–400 mesh) eluted with *n*-hexane/EtOAc to yield **5** (122.0 mg). Fraction CMIII (41.7 g) was also subjected to CC (200 g silica gel, 70–230 mesh) eluted with *n*-hexane/EtOAc mixture of increasing polarity (5% stepwise) to produce six subfractions (SubFr. CMIII-1 to SubFr. CMIII-6). SubFr. CMIII-3 (6.5 g) was chromatographed on a silica gel column (150 g, 230–400 mesh) eluted with *n*-hexane/$CH_2Cl_2$ gradient mixture and four subfractions were obtained (SubFr. CMIII-3-sub-1 to SubFr. CMIII-3-sub-4). Consequently, subFr. CMIII-3-sub-1 (1.5 g) was purified on CC (75 g silica gel, 230–400 mesh, eluent: gradient of Bz/EtOAc mixture) to obtain pure **6** (25 mg). SubFr. CMIII-3-sub-2 (1.0 g) was purified by CC (50 g silica gel, 230–400 mesh eluent: gradient of Bz/EtOAc mixture) to obtain **1** (20 mg). Similarly, **2** (40 mg) purification was achieved upon subjecting subFr. CMIII-3-sub-3 (1.75 g) to CC (75 g silica gel, 230–400 mesh)

using Bz/EtOAc as an eluent. Compound **7** (1.40 g) was recovered from Fr. CMIV (8.0 g) upon crystallization in MeOH. Fraction CMV (34 g) was subjected to CC (250 g silica gel, 70–230 mesh, eluent system: *n*-hexane/EtOAc) to generate seven subfractions (subFr. CMV-1 to subFr. CMV-7). SubFr. CMV-4 (2.0 g) was separated on CC (150 g silica gel, 230–400 mesh) eluted with *n*-hexane/EtOAc mixture of increasing polarity, to yield **8** (711 mg).

The butanol fraction was treated in a similar way. Briefly, CB (115.96 g) fraction was absorbed on silica gel (150 g, 70–230 mesh) and then chromatographed on a column packed with the same adsorbent (550 g) and eluted with a gradient mixture of $CHCl_3$/MeOH of increasing polarity. The elution process afforded 100 fractions (500 mL each) that were consolidated into three major groups according to their TLC behavior (CBI – CBIII). Each group was then subjected to a combination of CC, TLC and recrystallization to separate its pure constituents. Fr. CBI (30.5 g) was separated on a silica gel column (250 g, 70–230 mesh) eluted with a gradient mixture of $CHCl_3$/MeOH afforded 4 subfractions (subFr. CBI-1 to subFr. CBI-4). Further chromatographic separation of subFr. CBI-2 (6.5 g) on CC (150 g silica gel, 230–400 mesh, eluent system: gradient of Bz/EtOAc) yielded other four subfractions (subFr. CBI-sub-2-1 to subFr. CBI-2-sub-4). Purification of subFr. CBI-2-sub-2 (1.5 g) on CC (75 g silica gel, 230–400 mesh; eluent: EtOAc/MeOH of increasing polarity) afforded **9** (25 mg). SubFr. CBI-2-sub-3 (0.5 g) was purified on a sephadex LH-20 column to obtain **10** (50 mg). Fr. CBIII (3.0 g) was separated on a silica gel column (170 g, 70–230 mesh; eluent system: isocratic elution with EtOAc) affording **11** (28.5 mg).

**4.5.1. 17α-hydroxyambylone (1).** Compound **1** was obtained from SubFr. CMIII-3-sub-2 as pure white amorphous solid. IR (KBr) $\nu$ ($cm^{-1}$): 3398, 2966, 1753, 1254. $R_f = 0.48$ (5% MeOH/$CHCl_3$) and 0.77 (60% EtOAc/Bz). $^1$H-NMR and $^{13}$C-NMR ($CDCl_3$) $\delta$ ppm, see Table 1. HRESIMS *m*/*z* 445.2927 $[M–H]^-$ (calcd for $[C_{27}H_{41}O_5]^-$: 445.2954).

**4.5.2. 15β-acetoxy-17α-hydroxycabralealactone (2).** Compound **2** was obtained from subFr. CMIII-3-sub-3 as pure white amorphous solid. IR (KBr) $\nu$ ($cm^{-1}$): 3494, 2947, 1767, 1740, 1648, 1455, 1295, $R_F = 0.56$ (5%MeOH/CF) and 0.45 (30%EtOAc/Bz). $^1$H-NMR and $^{13}$C-NMR ($CDCl_3$) $\delta$ ppm, see Table 1. HRESIMS *m*/*z* 487.3042 $[M–H]^-$ (calcd for $[C_{29}H_{43}O_6]^-$: 487.3060).

## Conflicts of interest

The authors declare no conflicts of interest.

## Data availability

Data will be made available on request.

Supplementary information is available. See DOI: https://doi.org/10.1039/d5ra04113a.

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