Chemical composition and antibacterial activity of extracts and compounds isolated from *Citrullus colocynthis* (L.) Schrad

Rachid Chawech, Dhekra Mhalla, Mohamed Trigui, Mohamed Mihoubi, Nicolas Fabre, Raoudha Jarraya

Abstract

The aim of this study was the phytochemical screening of different extracts of leaves and stems prepared with three increasing polarity solvents (n-hexane, ethyl acetate and methanol) of *Citrullus colocynthis*. Our main goal was to investigate the antibacterial activity of different extracts and compounds isolated from ethyl acetate extract of leaves using the agar disc well-diffusion method. Founded results showed that ethyl acetate extracts of leaves and stems possess potential antibacterial activity compared to other extracts against tested Gram-positive and negative bacterial strains. A bioguided isolation of the ethyl acetate extract obtained from the leaves led to the identification of Cucurbitacin E and the Gluco-Cucurbitacin E. Moreover, a LC-MS analysis of ethyl acetate leaf extract of *C. colocynthis* permitted the identification of eight other cucurbitacins. Antibacterial activities of the isolated compounds emphasize the importance of sugar moiety. The most important minimum inhibitory concentrations (MIC) values were obtained for the ethyl acetate extract 0.625 mg/mL against *Bacillus cereus* and Glucocucurbitacin E 1.25 mg/mL against both *Bacillus cereus* and *Enterococcus faecalis*.

**Keywords:** *Citrullus colocynthis*, Antibacterial activity, LC-ESI-MS, cucurbitacin E; 2-O-β-D-glucocucurbitacin E.

1. Introduction

Food-borne illnesses associated with Gram-positive and Gram-negative bacteria including *Staphylococcus aureus*, *Escherichia coli* and *Salmonella enteritidis* present a major worldwide public health concern [1]. Furthermore, the clinical effectiveness of many existing antibiotics is threatened by the emergence of multi-drug-resistant pathogens [2] and by consequence, infectious diseases have become the leading cause of death worldwide. Bacteria are able to adapt rapidly to the presence of antimicrobial molecules and thereby inducing an increasing resistance with the antimicrobial misuse [3]. Moreover, there has been a growing interest in the development of more effective compounds from natural sources with lower toxicity against eukaryotic systems. *Citrullus colocynthis* belongs to the family of *Cucurbitaceae* which is widespread in the south of Tunisia [4]. This plant is widely used in folk medicine for the treatment of many diseases such as rheumatism [5, 6], hypertension [6] and various contagious diseases, including dermatological problems and gynecological, urinary and pulmonary infections [5-7]. Several metabolites have been isolated from the fruits of *C. colocynthis* [8-13] and it has been reported that these fruits have very interesting antibacterial and antifungal activities [7, 14-22]. In this work we report the antibacterial activities of leaves and stems extracts together with isolated products of *C. colocynthis* against six strains of bacteria, as well as the investigation of the phytochemical screening of extracts. Further analysis of the active leaves EtOAc extract of the plant by Liquid Chromatography coupled to Mass Spectrometry is also presented.

2. Materials and methods

2.1. Collection of plant material

The leaves and stems of *C. colocynthis* were collected near Ben-Guerdene, in the South-East of Tunisia, in May 2012 and authenticated by Pr. Mohamed Chaieb, Department of Botany of the University of Sfax. A voucher specimen (LCSN115) was deposited in the Herbarium of the Laboratoire de Chimie des Substances Naturelles (LCSN), Department of Chemistry, Faculty of Sciences, University of Sfax, Tunisia.
2.2. Extract preparation, separation and isolation of secondary metabolites
The air-dried leaves (300 g) and stems (240 g) were successively macerated during 24 h thrice with 2 L of n-hexane, ethyl acetate and methanol at room temperature. All the obtained extracts were filtered through a filter paper then concentrated under reduced pressure on a Büchi Rotavapor® at 40 °C, and afforded 2.5, 11.0 and 15.0 g of dry extracts for the leaves respectively, and 0.15, 3.4 and 6.7 g of dry stems extracts.

The ethyl acetate extract of leaves (2.0 g) was subjected to column chromatography on silica gel (60 Å, 40–63 µm) to afford a second compound (20 mg). Fraction 5 (80 mg, hexane: EtOAc, 100:0 to 0:100) was purified by column chromatography on silica gel (60 Å, 70–200 µm), using binary solvent systems of increasing polarity (from n-hexane: EtOAc, 100:0 to 0:100 then from EtOAc:MeOH, 7:3) to afford a first compound (50 mg). Fraction 10 (80 mg, EtOAc:MeOH, 7:3) was purified by column chromatography on silica gel (60 Å, 40–63 µm) to afford a second compound (20 mg).

2.3. Physical data of isolated compounds
The purification of the ethyl acetate extract of leaves have resulted the isolation of two tetracyclic cucurbitane-type triterpenes (Fig. 1). Through the analysis of their 1H- and 13C-NMR, IR, UV and MS spectroscopic data, these two compounds were identified as cucurbitacin E [23-24] and 2-O-β-D-glucocucurbitacin E [9].

Cucurbitacin E. White amorphous powder; UV (MeOH) λ_{max}(nm): 231, 267; IR (cm⁻¹): 3360, 2979, 1630, 1681, 1718, 1664, 1391, 1231; FAB-MS: m/z 579 [M+Na]⁺ (C₃₂H₴₄O₈Na).

1H-NMR (CDCl₃, 300 MHz): δ (ppm) 6.21 (1H, d (2.1 Hz), H-1), 5.74 (1H, m, H-6), 2.06/2.37 (2H, m, H-7), 2.04 (1H, m, H-8), 3.50 (1H, brs, H-10), 2.73/3.25 (2H, d (14.1 Hz), H-12), 1.48/1.89 (2H, m, H-15), 4.36 (1H, m, H-16), 2.49 (1H, d (7.2 Hz), H-17), 0.98 (3H, s, H-18), 1.37 (3H, s, H-19), 1.42 (3H, s, H-21), 6.52 (1H, d (15.50 Hz), H-23), 7.06 (1H, d (15.5 Hz), H-24), 1.57 (3H, s, H-26), 1.54 (3H, s, H-27), 1.28 (3H, s, H-28), 1.22 (3H, s, H-29), 0.96 (3H, s, H-30), 2.01 (3H, s, HAc), 4.67 (1H, d (7.6 Hz), H-1’), 3.48 (1H, m, H-2’), 3.50 (1H, m, H-3’), 3.66 (1H, m, H-4’), 3.65 (1H, m, H-5’), 3.93 (1H, dd (2.5, 11.5 Hz), H-6’), 4.09 (1H, d (11.5), H-6’).

13C-NMR (CDCl₃, 75 MHz): δ (ppm) 125.16 ( CH, C-1), 145.26 (C, C-2), 198.83 (C, C-3), 48.03 (C, C-4), 135.60 (C, C-5), 121.30 (CH, C-6), 24.04 (CH₃C=C), 41.33 (CH, C-8), 49.03 (C, C-9), 35.37 (CH, C-10), 214.43 (C, C-11), 49.10 (CH₂, C-12), 49.19 (C, C-13), 50.63 (C, C-14), 45.56 (CH₂, C-15), 71.22 (CH, C-16), 58.23 (CH, C-17), 19.96 (CH₃C=C), 20.09 (CH₃, C-19), 78.22 (C, C-20), 25.91 (CH₁, C-21), 202.56 (C, C-22), 120.38 (CH, C-23), 151.95 (CH, C-24), 79.38 (C, C-25), 26.07 (CH₃C=C), 26.54 (CH₁, C-27), 20.13 (CH₃, C-28), 27.88 (CH₂C=C), 18.22 (CH₃C=C), 21.97 (CH₃, Acetyl), 170.36 (C, Acetyl), 100.61 (CH, C-1’), 76.80 (CH, C-2’), 72.28 (CH, C-3’), 75.86 (CH, C-4’), 69.37 (CH, C-5’), 61.71 (CH₂, C-6’).

2.4. Phytochemical screening
The preliminary phytochemical screening was performed according to Harborne methods [25]. The different crude extracts of leaves and stems of C. colocynthis were tested for the presence of flavonoids, terpenoids, alkaloids and saponins. The qualitative results are expressed as (+) for the presence and as (-) for the absence of a phytochemical type of compounds.

Each extract or batch containing products was dissolved in a low amount of solvent giving a solution (S). To this latter solution, it was added various reagents according to the following experimental protocols P₁, P₂, P₃ and P₄.

P₁: Protocol of Liebermann: to 1 mL of (S) is added 0.2 mL of EtOH-H₂O (1-1) mixture in the presence of some drops of HCl with Mg chips. When the coloration of solution varies from Orange to red-violet, it is considered the presence of flavonoids, terpenoids, alkaloids and saponins.

P₂: Protocol of Liebermann: to 1 mL of (S) is added 0.2 mL of acetic anhydride and 4 drops of concentrated H₂SO₄. The
change of coloration from violet to blue-green indicates the presence of sterols and (or) triterpenoids.

Ps: Protocol of Mayer reaction: to 1 mL of (S) is added 0.5 mL of HCl (0.1 N) in addition to 5 drops of Mayer reagent. The appearance of a white precipitate in the concentrated liquid is significant of the presence of alkaloids.

Pn: Protocol of presence of saponins: approximately 1 mL of solvent containing extract is mixed with little amount of distilled water, then, vigorously shaken for 2 min: the Formation of froth indicates the presence of saponins.

2.5. Total polyphenol content
The total phenolic content of C. colocynthis extracts was determined using the Folin–Ciocalteu method described by Heimler with some modifications [28]. To 125 µL of the diluted sample extract, 0.5 mL of deionized water and 125 µL of the Folin–Ciocalteu reagent were added. The mixture was kept for 6 min and then 1.25 mL of a 7% aqueous Na2CO3 solution was added. The final volume was adjusted to 3 mL with water. After 90 min, the absorbance was measured at 760 nm against water as a blank. The amount of total phenolics was expressed as gallic acid equivalents (GAE, mg of gallic acid/g of extract) through the calibration curve of gallic acid.

2.6. Total flavonoid content
The total flavonoid content of extracts was determined according to the method described by Akrout et al, which relies on the formation of a flavonoid–aluminum complex, possessing a maximum absorbance at 430 nm [27]. The calibration curve was made using quercetin. Approximately 1 mL of diluted sample was mixed with 1 mL of 2% aluminum trichloride (AlCl3) methanolic solution. After incubation at room temperature for 15 min, the absorbance of the reaction mixture was measured at 430 nm with a Shimadzu Jenway 6320D spectrophotometer and the total flavonoid content was determined using the Folin–Ciocalteu method described by Tagg and McGiven [29] and broth microdilution protocol of Mayer reaction: to 1 mL of (S) is added 0.5 mL of HCl (0.1 N) in addition to 5 drops of Mayer reagent. The appearance of a white precipitate in the concentrated liquid is significant of the presence of alkaloids.

2.8. Determination of antibacterial activity
Antibacterial activity of C. colocynthis extracts and isolated products was performed by agar disc well-diffusion method as described by Tagg and McGiven [28] and broth microdilution assay. For the agar well-diffusion assay, fresh bacteria suspension (100 µL) was adjusted to 10⁶ CFU/mL and was inoculated on a surface of agar plates. Moreover, we have punched in the inoculated agar medium with sterile Pasteur pipettes wells having a 6 mm diameter. Then, the extracts were added to each well. The negative controls consisted of 20% DMSO and 50% ethanol which were used to dissolve the plant extracts and isolated cucurbitacins. Gentamicin (15µg/well) was also used as positive control in order to determine the sensitivity of each bacterial strain. The plate was allowed to stand for 2 h at 4 °C to permit the diffusion of the extracts followed by incubation at 37 °C for 24 h. The antibacterial activity is measured by determining the zones of inhibition (clear zone around the well) against the tested microorganisms. All tests were repeated three times.

Minimum inhibitory concentrations (MICs) were determined according to Eloff [29] in sterile 96-well microplates with a final volume in each microplate well of 200 µL. A twofold serial dilution of the extract was prepared in the microplate wells over the range 0.078-10 mg/mL. To each test well was added 10 µL cell suspension to final inoculum concentrations of 10⁶ CFU/mL. The plates were then covered with the sterile plate covers and incubated at 37 °C for 24 h. Gentamicin was used as a standard antibiotic at a concentration of 15 µg/well. The MIC was defined as the lowest concentration of the extract at which the microorganism does not demonstrate visible growth after incubation. As an indicator of microorganism growth, 25 µL of 0.5 mg/mL p-iodonitrotetrazolium chloride (INT), dissolved in sterile water, were added to the wells and incubated at 37 °C for 30 min. The lowest concentration of extract or compound showing no growth was taken as its minimal inhibitory concentration MIC. For the determination of the Minimum bactericidal concentrations (MBC), 5 µL from each well that showed no change in colour was plated on nutrient agar and incubated at 37 °C for 24 h. The lowest concentration that yielded no growth after this sub-culturing was taken as the MBC, indicating that > 99.9% of the original inoculum was killed.

2.9. LC-MS profiles
LC-MS profile of EtOAc crude extract of C. colocynthis leaves was acquired using an ULTIMATE 3000 UHPLC system (Thermo Finnigan) coupled to an ion trap MS (LCQ DECA XP Max, Thermo Finnigan) equipped with an ESI source functioning in the positive ion mode. Plant extract was separated using an UPLC BEH C18 Phenomenex column (150 × 4.6 mm i.d., 5 µm, Waters) with a gradient composed of 80% of water (+0.1% formic acid) to 100% of CH3CN over 70 min at a flow rate of 0.8 mL/min. The data were acquired over a m/z range of 100-2000 in full scan mode. The capillary and cone voltages were set to 2.5 kV and 40 V, respectively. The source temperature was maintained at 120 °C, and the desolvation and cone gas flows were set to 900 L/hr at 350 °C and 20 L/hr, respectively.

3. Results and discussion
3.1. Phytochemical screening
Table 1 summarizes all the phytochemical screening of extracts. The obtained results revealed the presence of a large amount of terpenoids in the ethyl acetate extracts. In parallel, methanolic extracts showed a medium concentration of terpenoids and conversely, the n-hexane extracts showed
disability of these compounds. Flavonoids were found in both methanolic and ethyl acetate extracts but alkaloids are only found in the methanolic extracts. The saponins were not detected in any extracts. According to Uma et al [17], a phytochemical screening of leaf extracts show the presence of alkaloids, flavonoids and terpenoids.

<table>
<thead>
<tr>
<th>Table 1: Phytochemical screening of C. colocynthis (L.) leaves and stems extracts.</th>
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<tr>
<td></td>
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<td></td>
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<tr>
<td>Flavonoids</td>
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<tr>
<td>Terpenoids/ steroids</td>
</tr>
<tr>
<td>Alkaloids</td>
</tr>
<tr>
<td>Saponins</td>
</tr>
</tbody>
</table>

+++: Strong positive test; ++: Low positive test; +: Weak positive test; –: Negative test.

3.2. Total polyphenol and flavonoid content

Table 2 reports the polyphenols and flavonoids contents in the six extracts considered in this study. The results were expressed either as mg of gallic acid (polyphenols) and quercetin (flavonoids) equivalent per g of extract. The highest values were found in the ethyl acetate and methanol extracts of leaves and stems, amounted to 323, 314, 260 and 240 mg in MS, ML, ES and EL respectively. flavonoids amounted to 270, 250, 150 and 147 mg in ML, MS, ES and EL respectively. The n-hexane extracts do not contain polyphenols or flavonoids.

<table>
<thead>
<tr>
<th>Table 2: Total polyphenols and flavonoids content in C. colocynthis (L.) leaves and stems extracts.</th>
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<tr>
<td></td>
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<tr>
<td></td>
</tr>
<tr>
<td>Polyphenols *</td>
</tr>
<tr>
<td>Flavonoids *</td>
</tr>
</tbody>
</table>

a: (mg GAE/g): mg of gallic acid equivalent per g of dry plant extract.

4. Antibacterial Activity

C. colocynthis extracts were screened for their antibacterial activity against a panel of bacterial strains (three Gram-positive and three negative bacteria). Antibacterial potential of leaves and stems extracts was assessed in terms of their inhibition zone of bacterial growth using well diffusion method. The results of the antibacterial activity showed various degrees of inhibition against the 6 tested bacteria as presented in table 3. We have observed that the C. colocynthis (L.) part and the type of solvent can have a great impact on the antibacterial activity. Among the tested extracts, the ethyl acetate ones showed strong inhibition compared to other extracts on all tested bacterial strains. The stem hexane and methanol extracts showed a broad-spectrum for antibacterial activity compared to the leaves extracts. Our results revealed that Gram positive bacteria such as B. cereus, S. aureus and E. faecalis were more sensitive as compared with E. coli, S. enteritidis and P. aeruginosa. The growth inhibition zone measured ranged from 12.1 to 29.5 mm for all the sensitive bacteria, and ranged from 7 to 12.6 mm for Gram-negative strains. The observed differences in the inhibition zones could be probably due to cell membrane permeability or other genetic factors. The outer membrane of the Gram-negative bacteria acts as a barrier to many environmental substances including antibiotics [30].

<table>
<thead>
<tr>
<th>Table 3: Antibacterial activity of C. colocynthis leaves and stems extracts</th>
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<tr>
<td></td>
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<tr>
<td></td>
</tr>
<tr>
<td>Leaves</td>
</tr>
<tr>
<td>Hexane</td>
</tr>
<tr>
<td>EtOAc</td>
</tr>
<tr>
<td>MeOH</td>
</tr>
<tr>
<td>Stems</td>
</tr>
<tr>
<td>Hexane</td>
</tr>
<tr>
<td>EtOAc</td>
</tr>
<tr>
<td>MeOH</td>
</tr>
<tr>
<td>Gentamicin*</td>
</tr>
<tr>
<td>DMSO 20%</td>
</tr>
</tbody>
</table>

ZI*: Diameter of inhibition zone including.

Gentamicin*: standard antibiotic at a concentration of 15 µg/well

5. Antibacterial activity of isolated terpenoids

Based on its inhibitory activity, the EtOAc extract prepared from the leaves was subjected to biofractionation and purification using column chromatography. Bioassay-guided isolation of EtOAc extract led to the isolation of two cucurbitacins: the Cucurbitacin E and the Gluco-Cucurbitacin
E. The resulting compounds were further tested on the selected bacterial strains (Table 4). Cucurbitacin E did not show any inhibitory activity. The Gluco-cucurbitacin E showed potent antibacterial activity against Gram-positive bacteria with MIC values ranging from 1.25 to 2.5 mg/mL and moderate antibacterial activity against Gram-negative ones with MIC values of 5 mg/mL. Enterobacteriaceae, such as P. aeruginosa, have been classified as antimicrobial-resistant organisms of concern in healthcare facilities. In our study, fractions are more effective than the isolated constituents probably due to some additive effects of number of constituents present in the plant extract. Since a bioactive individual can change its property in the presence of other bio-components, present in the fraction resulting in increased activity i.e. the synergistic effect [31, 32]. The antimicrobial activity varied significantly across the geographic location, organs, maturation and climate. It was reported previously that fruits of Tunisian C. colocythis extracts were active against both Gram-positive and Gram-negative bacterial strains but extracts prepared from Pakistan plants displayed inhibitory effect only against Bacillus subtilis [7, 17]. In our study, the cucurbitacin E was inactive at the tested concentration of 10 mg/mL against the tested bacteria however it was reported by Ali et al [33] a highest inhibition effect by cucurbitacin E at 500 mg/mL, while cucurbitacin B and I showed moderate effect against Staphylococcus aureus and Klebsiella pneumonia. It is clear that this activity was to be in dose dependent manner. The strong antibacterial activity of the glycosylated cucurbitacin E compared to cucurbitacin E is due to the sugar moiety, which may increases the water solubility of these compounds.

### Table 4: Determination of MIC and MBC values of C. colocythis ethyl acetate leaf extract and isolated terpenoids Cucurbitacin E and Gluco-Cucurbitacin E

<table>
<thead>
<tr>
<th>Extract /Compound</th>
<th>Minimum Inhibitory Concentrations MIC (mg/mL)</th>
<th>Minimum Bactericidal Concentration MBC in (mg/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>B. cereus</td>
<td>S. aureus</td>
</tr>
<tr>
<td>EtOAc</td>
<td>0.625(a)</td>
<td>5</td>
</tr>
<tr>
<td></td>
<td>1.25(b)</td>
<td>10</td>
</tr>
<tr>
<td>Cucurbitacin E</td>
<td>&lt;5</td>
<td></td>
</tr>
<tr>
<td>Gluco-Cucurbiticine E</td>
<td>1.25</td>
<td>2.5</td>
</tr>
<tr>
<td>Gentamicin(c)</td>
<td>0.001</td>
<td>0.002</td>
</tr>
<tr>
<td></td>
<td>0.002</td>
<td>0.004</td>
</tr>
</tbody>
</table>

**MIC**: Minimum inhibitory concentrations (mg/mL).  
**MBC**: The Minimum bactericidal concentration (as mg/mL)  
**Genta**: Gentamicin was used as a standard antibiotic at a concentration of 15 µg/mL.  
-d: activity not detected

### 6. Identification of terpenoids compounds from leaf ethyl acetate extract of C. colocythis

The ethyl acetate extract of C. colocythis leaves exhibited a high content of terpenoids compounds (cucurbitacins), and a significant antibacterial activity against the pathogenic strains. These compounds possess a broad range of potent biological activities, driving largely for their cytotoxic and anti-tumor properties [34-42]. Therefore, the aim of this part was to tentatively identify cucurbitacins by LC-ESI-MS analysis. The Fig. 2 shows the LC-PAD-MS chromatogram of the leaves ethyl acetate extract. A close examination of the UV spectra of individual peaks suggested the presence of compounds belonging to the family of cucurbitacins (Table 5, Fig. 2) owing to the presence of two absorption bands ranging between 220-255 nm and 270-320 nm. By comparing of the MS and MS² spectra obtained with those of literature data, ten cucurbitacins were tentatively identified. The main peaks at tR 18.35 and 32.98 min exhibited the [M+Na]⁺ ions of 2-O-β-D-glucopyranosyl-cucurbitacin L (m/z 701), 2-O-β-D-glucopyranose-cucurbitacin B (arvenin I) (m/z 743), 6'-acetyl-2-O-β-D-glucopyranose-cucurbitacin E (m/z 783), dihydroisocucurbitacin B-25-acetyl (m/z 583), 2-O-β-D-glucopyranosyl-cucurbitacin E-16-acetyl (m/z 783) and 25-p-coumaroyl-3'-acetyl-2-O-β-D-glucopyranosyl-cucurbitacin I (m/z 887) [42].

**Fig 2**: LC-PDA-(ESI+) MS chromatogram of the ethyl acetate extract of the leaves of C. colocythis
The phytochemical screening of different extracts of the stems and leaves of *C. colocynthis* revealed the presence in large amount of terpenoids in the ethyl acetate extracts. Furthermore the results obtained in this study clearly demonstrate that *C. colocynthis* leaf ethyl acetate extract exhibited potential antibacterial activity against Gram-positive bacteria such as *B. cereus*, *S. aureus*, *E. faecalis*. Gluco-cucurbitacin E showed also potent antibacterial activity against Gram-positive bacteria and moderate antibacterial activity against Gram-negative ones. The antibacterial activity of the EtOAc extract of the leaves of *C. colocynthis* could be attributed to the synergy of action of the ten cucurbitacins identified by LC-PDA-MS analysis.

### 7. Conclusion

### 8. Acknowledgments

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### 9. References


### Table 5: LC–PDA-ESI-MS/MS data selected compounds of the ethyl acetate extract of *C. colocynthis* leaves.

<table>
<thead>
<tr>
<th>Tr (min)</th>
<th>UV λ. max (nm)</th>
<th>m/z [M+Na]+</th>
<th>MS/MS</th>
<th>Molecular Formula</th>
<th>(MW) g/mol</th>
<th>Compounds</th>
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<tr>
<td>1</td>
<td>8.06</td>
<td>255, 302</td>
<td>585</td>
<td>463, 422, 348</td>
<td>C_{36}H_{44}O_{8} 556</td>
<td>2-O-β-D-glucopyranose-(22-27)-hexanocucurbitacin I</td>
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<td>2</td>
<td>10.68</td>
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<td>699</td>
<td>577,536, 348</td>
<td>C_{34}H_{32}O_{12} 590</td>
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<td>3</td>
<td>10.85</td>
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<td>701</td>
<td>682, 578, 539, 348</td>
<td>C_{36}H_{42}O_{12} 578</td>
<td>2-O-β-D-glucopyranosyl-cucurbitacin L</td>
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<tr>
<td>4</td>
<td>18.35</td>
<td>242, 310</td>
<td>741</td>
<td>680, 579, 559, 348</td>
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<td>2-O-β-D-glucopyranosyl-cucurbitacin E</td>
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<td>5</td>
<td>19.11</td>
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<td>7</td>
<td>25.27</td>
<td>220, 241</td>
<td>583</td>
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<td>Dihydroisocucurbitacin B-25-acetyl</td>
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<td>887</td>
<td>741, 683, 348</td>
<td>C_{30}H_{40}O_{12} 556</td>
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<tr>
<td>10</td>
<td>32.98</td>
<td>240, 270</td>
<td>579</td>
<td>519, 422, 348</td>
<td>C_{32}H_{44}O_{8} 556</td>
<td>Cucurbitacin E</td>
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