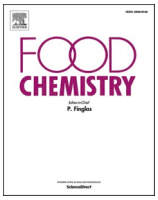
Food Chemistry 412 (2023) 135587

Contents lists available at ScienceDirect

Food Chemistry

journal homepage: www.elsevier.com/locate/foodchem



Metabolic profiling of *Ochradenus baccatus* Delile. utilizing UHPLC-HRESIMS in relation to the *in vitro* biological investigations

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ARTICLE INFO

Keywords:
Ochradenus baccatus
UHPLC/MS
Resedaceae
Desert plants
Zebra fish model
Cytotoxicity

ABSTRACT

Ochradenus baccatus Delile (Resedaceae) is a desert plant with edible fruits native to the Middle East. Few investigators have reported antibacterial, antiparasitic and anti-cancer activities of the plant. Herein we evaluated the cytotoxic activity of *O. baccatus* using four cell lines and a zebrafish embryo model. Additionally, liquid chromatography coupled with mass spectroscopy was performed to characterize the extract's main constituents. The highest cytotoxicity was observed against human cervical adenocarcinoma (HeLa), with CC_{50} of 39.1 μg/mL and a selectivity index (SI) of 7.23 ($p < 0.01$). Metabolic analysis of the extract resulted in the annotation of 57 metabolites, including fatty acids, flavonoids, glucosinolates, nitrile glycosides, in addition to organic acids. The extract showed an abundance of hydroxylated fatty acids (16 peaks). Further, 3 nitrile glycosides have been identified for the first time in *Ochradenus sp*., in addition to 2 glucosinolates. These identified phytochemicals may partially explain the cytotoxic activity of the extract. We propose *O. baccatus* as a possible safe food source for further utilization to partially contribute to the increasing food demand specially in Saharan countries.

1. Introduction

The use of plant-derived cures is a prevalent practice in many geographical regions and across different cultures, including the Middle East and Northern Africa (Amer & Mohammad, 2022). Natural ecosystems are essential resources for human existence and well-being (Martínez Pastur, Perera, Peterson, & Iverson, 2018). The therapeutic value of wild medicinal plants is attributed to their phytoconstituents and their physiological interaction with human biological functions (Okwu & Okwu, 2004). History of drug discovery provides many examples of valuable drugs discovered from traditionally used medicinal plants through rigorous *in vitro*, *in vivo* and clinical investigations.

Family Resedaceae is a small family of six genera and around 85 species distributed in temperate regions, mainly around the Mediterranean Basin. Most species grow in sunny and arid habitats, like steppes, deserts and dry slopes, and generally prefer basic soils (Martín-Bravo & Escudero, 2012). In Egypt's flora, Resedaceae is represented by 5 genera and 16 species (Boulos, 1999). Previous investigation of Resedaceae taxa has revealed wide range of biological activities, including antimicrobial, antidiabetic, anti-inflammatory, anthelminthic, and anticancer activities (Al Qurainy, Nadeem, Khan, Alansi, & Tarroum, 2013; Berrehal et al., 2010; Bhatia, Mandal, Nevo, & Bishayee, 2015; Hussein, Elkhateeb, Marzouk, Ibrahim, & Kawashty, 2013).

Ochradenus baccatus Delile is a perennial large semi-deciduous shrub

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https://doi.org/10.1016/j.foodchem.2023.135587
Received 20 August 2022; Received in revised form 21 December 2022; Accepted 25 January 2023
Available online 27 January 2023

found in sandy, stone areas in the Middle East. It is a dense shrub, approximately 0.5–2 m high, with woody bases, many fleshy green smooth branches, and greenish yellow when mature. Being tolerant to drought and salinity, it is widely distributed in desert and semidesert regions from Central-North Africa to South West Asia (Miller, 1984; Omar, 2007) (Boulos, 1999; El-Amier & Abdul-Kader, 2015). *O. baccatus* has a long history of medical usage in many countries (Alqasoumi, Soliman, Awaad, & Donia, 2012; Nawash & Ahmad Al-S, 2011). Extracts of *O. baccatus* were shown to induce a potential effect against *Plasmodium falciparum* (Al Qurainy et al., 2013). Other reported activities include antibacterial (Abdel-Sattar, Harraz, & El-Gayed, 2008; Al-Omar, Eldeeb, Mobark, & Mohammed, 2020), antioxidant (Barakat, El-Mousallamy, Souleman, & Awadalla, 1991), anti-inflammatory (Al Qurainy et al., 2013), and anticancer activities (Bhatia et al., 2015).

Ripe fruits of *O. baccatus* are consumed raw in Libya (Mahklouf, 2019) as a desert food and also as a functional food to treat stomach pain (Lotze, 2001). Moreover, many animal species in Sinai and the Arabian desert eat the fleshy fruits of *O. baccatus* and disperse the seed without biting it (Bronstein et al., 2007; Spiegel & Nathan, 2012). Preliminary phytochemical examination of the aerial parts of *O. baccatus* revealed the presence of alkaloids, flavonoids, glycosides, coumarins, saponins and steroidal compounds (Al-Omar et al., 2020; Barakat et al., 1991; Hussain & El-Oqlah, 1997). Furthermore, it was observed that the pulp of *O. baccatus* fruits is highly enriched in glucosinolates (Trabelcy et al., 2021).

This study aims to extensively characterize the metabolic fingerprint of aerial parts of *O. baccatus* extract for the first time to unravel the phytoconstituents. And importantly to validate its safety being consumed as a desert food. The toxicity profiles were evaluated both in Zebra fish embryos and cytotoxicity against different cell lines using a microculture tetrazolium (MTT) based assay.

The toxicity of the extract was tested using the zebrafish embryo model, which is widely recognized as a potential new method for chemical testing that may provide a bridge between cell and protein-based assays and mammalian testing. The model is in agreement with the principles of the 3Rs rule (reduction, refinement, replacement) in animal welfare (Ford, 2017), thereby becoming an alternative to *in vivo* rodents model for screening the toxicity and developmental toxicity (Wang, Liu, Wang, He, & Chen, 2011; Yumnamcha, Roy, Devi, & Nongthomba, 2015). The Zebrafish Embryo Toxicity (ZET) model focuses on the early stages of embryo development and is considered a more humane model compared to adult zebrafish testing (Achenbach, Leggiadro, Sperker, Woodland, & Ellis, 2020).

2. Material and methods

2.1. Plant material and extraction

The above-ground parts of *Ochradenus baccatus* Delile. were collected from Wadi El-Rashrash, a depression in the northern section of Eastern Desert (Helwan Desert), Giza Governorate, Egypt (29°27′55.10″N3 31°21′46.03″E) during May 2021. The identification of species was done according to Boulos (1999). A voucher specimen (Mans. 0181502007) was prepared and deposited in the Herbarium of Botany Department, Faculty of Science, Mansoura University, Egypt.

The plant material was dried at room temperature (30–35°C) and grinded into a powder using a blender. After that, 150 g of powdered plant material of *O. baccatus* was extracted by maceration using methanol till complete exhaustion. The filtered extract was evaporated till dryness at 50 °C using a rotary evaporator. The dried extract obtained at a yield of 7 %w/w on dry weight basis, was kept at −80 °C for further chemical and biological analyses.

2.2. LC–MS and qualitative analysis

The analysis of the *O. baccatus* extract was performed using a high-resolution LC-MS Thermo Scientific Ultimate 3000RS chromatographic system. The separation was carried out on a Waters Acquity HSS T3 column (150 × 2.1 mm i.d.; 1.8 μm, Milford, USA) at 45 °C using a linear gradient from 5 % to 70 % phase B (acetonitrile with 0.1 % formic acid) in phase A (0.1 % formic acid in Milli-Q water) for 30 min, with a flow rate of 0.4 mL/min.

The photodiode array detector recorded absorbances in 190–600 nm wavelength range with 5 nm bandwidth and 10 Hz acquisition frequency. A flow splitter was used to divert the column effluent in a proportion of 1:3 between Q-TOF MS (Bruker Impact II HD, Bruker, Billerica, MA, USA) and charged aerosol detector (CAD, Thermo Corona Veo RS) linked in parallel. The acquisition frequency for CAD was 10 Hz.

The MS analyses were operated in both positive and negative ion mode, using electrospray ionization. Linear spectra were obtained in the *m/z* 80 to *m/z* 1800 mass range, with 5 Hz acquisition frequency and the following parameters of the mass spectrometer: negative ion capillary voltage 3.0 kV; positive ion capillary voltage 4.0 kV; dry gas flow 6 L/min; dry gas temperature 200 °C; collision cell transfer time 90 μs; nebulizer pressure 0.7 bar. The obtained data were calibrated internally with sodium formate introduced into the ion source via a 20 μL loop at the start of each separation. The chromatographic data were acquired and processed using Bruker DataAnalysis 4.4 software, and metabolite structure elucidation and identification were achieved mostly using SIRIUS 4.8.2 software integrating CSI:FingerID for searching in molecular structure databases (Dührkop et al., 2019; Hoffmann, Nothias, Ludwig, Fleischauer, Gentry, Witting, Dorrestein, Dührkop, & Böcker, 2021).

2.3. Zebrafish embryo toxicity (ZET) assay

Zebrafish (*Danio rerio*) stocks of the AB strain were maintained at 28.5 °C on a 14/10 h light/dark cycle under standard aquaculture conditions, and fertilized eggs were collected via natural spawning. Embryos were reared under 14/10 h light/dark conditions in embryo medium: 1.5 mM HEPES, pH 7.1–7,3,17.4 mm NaCl, 0.21 mm KCl, 0.12 mm $MgSO_4$, and 0.18 mm $Ca(NO_3)_2$ at 28.5 °C. The 4-hpf (hours post-fertilization) embryos were placed in 48 well-plates, 5 embryos per well and then incubated in 4 different concentrations of tested extract – 10 embryos per concentration (n = 10). After 24, 48 and 72 h, embryos were checked under the microscope for any sign of cytotoxicity such as coagulation of the embryo, lack of somite formation, non-detachment of the tail and/or lack of heartbeat. Each day compound solution was changed. Two ranges of concentrations were tested, first 20–50 μg/mL and then 40–100 μg/mL.

2.4. Cell culturing, evaluation of cytotoxicity and anticancer selectivity

Cell lines were acquired from the American Type Culture Collection (ATCC): VERO (CCL-81, monkey kidney), FaDu (HTB-43, human hypopharyngeal squamous cell carcinoma), HeLa (CCL-2, human cervical adenocarcinoma), and RKO (CRL-2577, human colon cancer). The cell media (MEM and DMEM), penicillin–streptomycin solution (PS), phosphate-buffered saline (FBS) and trypsin were obtained from Corning (Tewksbury, MA, USA), while foetal bovine serum (FBS) from Capricorn Scientific (Ebsdorfergrund, Germany). Sodium dodecylsulphate (SDS) was purchased from PanReac Applichem (Darmstadt, Germany), dimethylformamide (DMF) and dimethyl sulfoxide (DMSO, p.a.) from Avantor Performance Materials (Gliwice, Poland), whereas 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) from Sigma-Aldrich (St. Louis, Missouri, United States). Cell culturing and experiments were performed using DMEM (VERO) and MEM (FaDu, HeLa, and RKO) cell media supplemented with PS (10 mL/L) and FBS (10 % for cell passaging and 2 % for experiments). Cell maintenance and testing were performed at 37 °C in the 5 % CO_2 atmosphere (CO_2 incubator, Panasonic Healthcare Co., ltd., Japan). The *O. baccatus* extract was dissolved (50 mg/mL) in DMSO (cell-culture grade, PanReac

Applichem) to obtain the stock solution for cytotoxicity evaluation.

The influence of tested extract on cell lines was assessed using MTT-based assay as previously described (Świątek et al., 2021). Briefly, serial dilution (500 – 1 μg/mL) of *O. baccatus* extract in cell media was incubated with appropriate cells seeded in 96-well plates for 72 h. Afterwards, cell media was removed, wells were washed with PBS, and MTT supplemented cell media was added, and incubation continued for 4 h. Finally, precipitated formazan crystals were dissolved using SDS/DMF/DMSO mixture, and after overnight incubation, the absorbance at 540 and 620 nm was measured with Synergy H1 Multi-Mode Microplate Reader (BioTek Instruments, Inc. Winooski, Vermont, USA). Data analysis was performed using GraphPad Prism (version 7.04), and CC_{50} values (50 % cytotoxic concentration) were calculated from dose-response curves. Furthermore, to evaluate anticancer specificity, the selectivity indexes (SI) were calculated ($SI = CC_{50}VERO/CC_{50}Cancer$, $SI > 1$ suggests anticancer selectivity).

2.5. Statistical analysis

Results of the CC_{50} values evaluation were expressed as mean ± SD ($n \geq 3$). Statistical analysis was performed using GraphPad Prism. Statistical comparison between the mean CC_{50} values obtained for cancer cell lines compared to VERO cells was performed using one-way ANOVA followed by Dunnett's post hoc test of significance wherein $p < 0.05$ and $p < 0.01$ were considered statistically significant and statistically highly significant, respectively.

3. Results and discussion:

3.1. The UHPLC-HRESIMS profiling of O. baccatus extract

The UHPLC-MS analysis of *O. baccatus* extract led to annotating a total of 57 peaks, including 18 fatty acids, 19 glycosides, 5 organic acids, 2 triterpene derivatives, 4 sugar acids, 5 nitrogenous compounds, and 2 phytohormones derivatives (Figs. 1 & 2, Table 1). Twenty peaks were identified by comparing their high resolution mass and fragmentation patterns with those reported in the relevant literature. The remaining compounds were tentatively assigned based on the high resolution mass of the precursor ions and the fragments generated via common fragmentation pathways.

3.1.1. Fatty acids

Eighteen fatty acids were annotated in the late eluting peaks (tR between 16.39 and 19.47 min). Interestingly, 16 of these peaks were annotated as hydroxy fatty acids of predominantly C_{18} chain (12 peaks) (He, Qin, Wang, & Ding, 2020). Hydroxylated fatty acids are unusual lipids that have been identified in the seed oil of some plants but were also detected in the aerial parts of different plants by LC/MS techniques

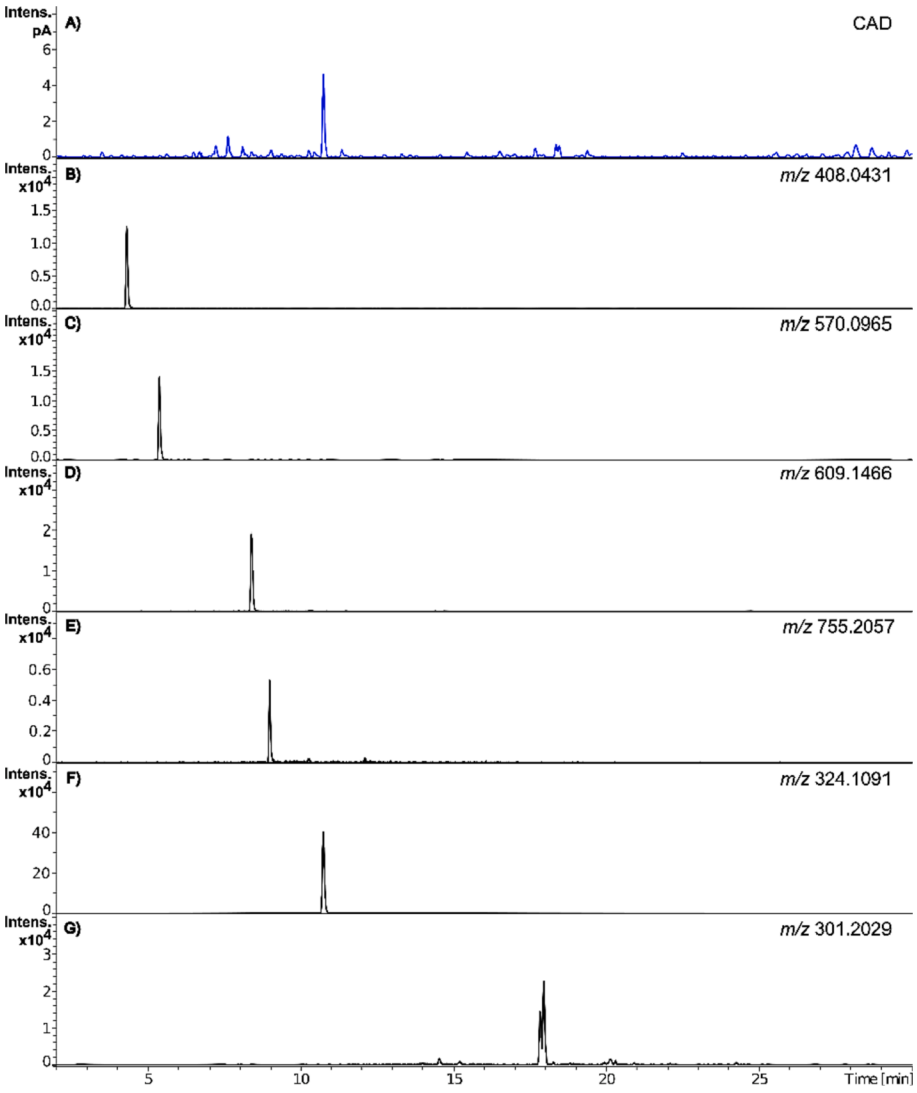

Fig. 1. UHPLC-CAD profile of *Ochradenus baccatus* methanolic extract (A). Extracted ion chromatograms (EICs) of the most representative compounds obtained from Table 1 (B-G). *m/z* 408.0431: glucotropaeolin; *m/z* 570.0965 2′′-*O*-(α-L-rhamnopyranosyloxy)benzylglucosinolate; *m/z* 609.1466 quercetin 3-*O*-hexoside-7-*O*-deoxyhexoside; *m/z* 755.2057 quercetin 3-*O*-deoxyhexosyl-hexoside-7-*O*-deoxyhexoside; *m/z* 324.1091 niazirin-type nitrile deoxyhexoside; *m/z* 301.2029 hydroxy-hexadecanedioic acid.

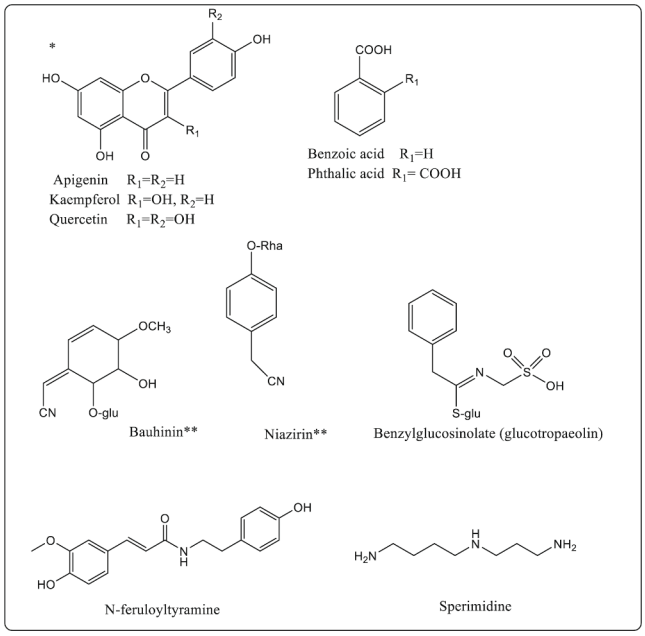

Fig. 2. Structures of phytoconstituents identified in *Ochradenus baccatus* extract by UHPLC-CAD. *Represents the aglycone part only of the flavonodial glycosides identified in Table 1. **Exact substitution pattern could not be established.

(Cahoon & Li-Beisson, 2020; Jiménez-Sánchez, Lozano-Sánchez, Rodríguez-Pérez, Segura-Carretero, & Fernández-Gutiérrez, 2016; Mahrous, Elosaily, Salama, Salama, & El-Zalabani, 2022). Tri, di and monohydroxy C_{18} fatty acids were detected with four peaks 37, 38, 39 (*m/z* 327.2181) annotated as structural isomers of trihydroxy octadecadienoic acid, which showed consecutive loss of water (−18 Da), Table 1. Similarly, peaks 41, 42 and 47 (*m/z* 329.2340) were identified as structural isomers of trihydroxy-octadecenoic acid with a similar fragmentation pattern. It is worth mentioning that differences in the fragmentation patterns between structural isomers were minimal and did not allow the determination of the hydroxylation positions. The same observation was true for C_{16} fatty acids, which were detected as mono- (peak 46), di- (peaks 44, 45) and trihydroxy (peak 43) derivatives.

3.1.2. Glycosides

3.1.2.1. Flavonoid derivatives. Flavonoids have ubiquitous distribution within the plant kingdom. Different substitution patterns on the flavonoid backbone, as well as conjugation with acids and sugars, afford great complexity and diversity in their structure and biological activities (Yang, Liu, Yang, Gupta, & Jiang, 2018). Six different flavonoid glycosides have been annotated in *O. baccatus* extract, namely: peaks 23 (*m/z* 609.1466), 27 (*m/z* 755.2057), 29 (*m/z* 593.1517) and 30 (*m/z* 901.2387) which all showed a characteristic fragment of quercetin aglycone at *m/z* 301.0353 calculated for $C_{15}H_9O_7^-$. Meanwhile, peak 24 (*m/z* 563.1406) was annotated as apigenin *C*-hexoside-*C*-pentoside. Two fragments at *m/z* 443 $[M-120-H]^-$ and *m/z* 473 $[M-90-H]^-$ are indicative of *C*-glycosylation with a hexose, while fragments at *m/z* 383.0772 $[M-120-60-H]^-$ and *m/z* 353.0667 $[M-120-90-H]^-$ indicated the presence of additional *C*-glycosylation with a pentose sugar (Benayad, Gómez-Cordovés, & Es-Safi, 2014; Cao, Yin, Qin, Cheng, & Chen, 2014). Peak 28 was tentatively identified as kaempferol-*O*-hexoside deoxyhexoside after showing a characteristic loss of 162 Da for -*O*-hexoside at *m/z* 431.0967 $[M-162-H]^-$ and loss of 146 Da characteristic of -*O*-deoxyhexoside at *m/z* 447.0939 $[M-146-H]^-$, further confirmed by the fragment at 285.0407 $[M-162-146-H]^-$ indicating loss of both residues (Vukics & Guttman, 2010).

3.1.3. Hydroxybenzoic acid derivatives

A total of 4 hydroxybenzoic acid derivatives were characterised in the negative ion mode, including a hexose ester at *m/z* 299.0774 (peak 6) and 3 glycosides. Peaks 8 (*m/z* 315.0722) and 11 (*m/z* 315.0725) were annotated as structural isomers of dihydroxybenzoic acid hexoside that showed different base peaks at *m/z* 152.0109 $[M-163-H]^-$ for neutral loss of hexoside and 153.0193 $[M-162-H]^-$, respectively. Lastly, peak 10 (*m/z* 329.0880) was identified as hydroxy-methoxybenzoic acid hexoside in accordance with the molecular ion of $C_{14}H_{17}O_9^-$.

3.1.3.1. Glucosinolates. Glucosinolates are plant-derived sulphur compounds and occur as secondary metabolites of almost all plants of the order Brassicales (VanEtten & Tookey, 2018). Our data analysis identified two glucosinolates peaks: peak 12 (*m/z* 408.0431) for benzylglucosinolate (glucotropaeolin) and peak 15 (*m/z* 570.0965) for rhamnopyranosyloxy benzylglucosinolate which both have been recently identified in *O. baccatus* (Trabelcy et al., 2021). Both glucosinolates showed the characteristic fragment ions $[M-H-C_7H_7-N{=}C=S]^-$ at *m/z* 259.0124 and $[M-H-C_7H_7-N{=}C=O]^-$ at *m/z* 274.9896 which are common ions for glucosinolates (Zhou et al., 2017).

3.1.4. Other glycosides

Peaks 13 (*m/z* 359.0987) and 14 (*m/z* 371.0986) were identified by their major fragment ions compared to the library database as erigeside C (Zhou, Ma, Guo, Zhang, & Chang, 2019) and 1-*p*-coumaryl hexose (Mekky et al., 2015). Further, three nitrile-containing glycosides were observed: first bauhinin-type nitrile hexoside: peak 17 (*m/z* 342.1197), which showed the characteristic fragment corresponding to loss of methoxy group and a hexoside at *m/z* 150.0556 (Chen et al., 1985) and peak 31, a niazirin-type nitrile deoxyhexoside which showed its molecular ion peak at 324.1091$[M+HCOO^-]^-$ and major fragment at *m/z* 132.0449 for $[M-146-H]^-$ (Fantoukh et al., 2021). Likewise, peak 22 was identified as mandelonitrile-type cyanogenic hexoside, which exhibited molecular ion at *m/z* 310.0935. To the best of our knowledge, nitrile compounds have not been reported in *Ochradenus sp*. before. Organic nitriles have limited distribution in the plant kingdom and are usually confined to certain plant families, mainly in the form of hydrolysable glycosides that releases HCN gas. Besides, two more peaks were labelled as glycosides: dideoxyhexosyl hexoside; peak 9 ($[M-H]^-$ at *m/z* 309.1191), and benzyl alcohol pentosyl-hexoside; peak 18 ($[M+HCOO^-]^-$ at *m/z* 447.1511). Major fragments of the latter at *m/z* 147.0663 and 269.1033 correspond to loss of hexosyl and pentosyl residues, respectively.

3.1.5. Organic acids, triterpenes and derivatives

Both aliphatic and aromatic acids are the important bioactive compounds of edible and medicinal plants with a key role in metabolism and physiology (Adamczak, Ożarowski, & Karpiński, 2019). The investigated extract showed different dicarboxylic acids with both aliphatic and aromatic backbone. Peak 19 and peak 20 were aromatic dicarboxylic acids, namely, hydroxyphthalic acid (*m/z* 181.0146) and phthalic acid (*m/z* 165.0195), which were readily identifiable by their decarboxylation fragments $[M-H-44]^-$ at *m/z* 137.0239 and 121.0295, respectively. Aliphatic dicarboxylic acids included peak 26: octanedioic acid (*m/z* 173.0825), peak 32: azelaic acid (*m/z* 187.0980) and peak 33: Oxododecanedioic acid (*m/z* 243.1242) with C_8, C_9 and C_{12} aliphatic chain, respectively. Further, two triterpene glycosides (Peaks 48 and 51) were annotated as oleanolic acid derivatives at *m/z* 793.4400 and 631.3860.

3.1.6. Sugar acids

Plant biomass provides a convenient source of sugars. Four sugar

Table 1
Compounds identified in the methanolic extract of *Ochradenus baccatus* using UHPLC-QTOF-MS/MS.

No.	Compound Name	Class	tR (min)	Formula	MolecularWeight (Da)	Error (ppm)	Mσ*	*m/z* detected and adduct	Fragment ions *m/z* **	Reference
1	Mixture of polar constituents		0.8–1.4	–	–	–	–	–	–	–
2	*N*-Fructosyl pyroglutamate	Nitrogenous compounds	1.50	$C_{11}H_{17}NO_8$	291.26	0.0	15.3	290.0881 $[M-H]^-$	**200.0564** ($C_8H_{10}NO_5$), 128.0353 ($C_5H_6NO_3$)	https://doi.org/10.1016/0031–9422(82)80023-x
3	Unidentified	Nitrogenous compounds	1.54	$C_{14}H_{19}N_3O_9$	373.32	1.6	14.8	372.1043 $[M-H]^-$	243.0623 ($C_9H_{11}N_2O_6$), **128.0353** ($C_5H_6NO_3$)	
4	Adenosine	Nitrogenous compounds	1.62	$C_{10}H_{13}N_5O_4$	267.24	−0.4	23.1	268.1045 $[M+H]^+$	**268.1041** ($C_{10}H_{14}N_5O_4$), 136.0617 ($C_4H_{10}NO_4^+$)	
5	*N*-Fructosyl (iso)leucine	Nitrogenous compounds	1.78	$C_{12}H_{23}NO_7$	293.31	−1.6	14.0	294.1552 $[M+H]^+$	**276.1442** ($C_{12}H_{22}NO_6$), 258.1336 ($C_{12}H_{20}NO_5^+$), 248.1492 ($C_{11}H_{22}NO_5^+$), 230.1387 ($C_{11}H_{20}NO_4^+$)	–
6	Hexose 4-hydroxybenzoate	Hydroxybenzoic acid derivatives	2.90	$C_{13}H_{16}O_8$	300.26	−0.5	13.5	299.0774 $[M-H]^-$	**137.0244** ($C_7H_5O_3$)	
7	Trihydroxyphenyl-propanoic acid hexuronide	Sugar acids	3.08	$C_{15}H_{18}O_{11}$	374.30	−0.4	25.9	373.0778 $[M-H]^-$	**329.0878** ($C_{14}H_{17}O_9$), 167.0350 ($C_8H_7O_4$), 165.0557 ($C_9H_9O_3$), 123.0452 ($C_7H_7O_2$)	–
8	Dihydroxybenzoic acid hexoside isomer	Hydroxybenzoic acid derivatives	3.51	$C_{13}H_{16}O_9$	316.26	−0.3	1.6	315.0722 $[M-H]^-$	**152.0109** ($C_7H_4O_4$)	https://doi.org/10.1002/pca.2928
9	Dideoxyhexosyl hexoside	Glycosides	3.72	$C_{12}H_{22}O_9$	310.30	0.1	15.3	309.1191 $[M-H]^-$	**147.0663** ($C_6H_{11}O_4$)	–
10	Hydroxy-methoxybenzoic acid hexoside (vanillic acid glucoside)	Hydroxybenzoic acid derivatives	3.81	$C_{14}H_{18}O_9$	330.29	−0.7	20.7	329.0880 $[M-H]^-$	**167.0350** ($C_8H_7O_4$)	https://doi.org/10.1016/j.arabjc.2020.11.006
11	Dihydroxybenzoic acid hexoside isomer	Hydroxybenzoic acid derivatives	4.13	$C_{13}H_{16}O_9$	316.26	−1.0	15.2	315.0725 $[M-H]^-$	**153.0193** ($C_7H_5O_4$)	https://doi.org/10.1002/pca.2928
12	Benzylglucosinolate (glucotropaeolin)	Glucosinolates	4.30	$C_{14}H_{19}NO_9S_2$	409.43	−0.7	15.6	408.0431 $[M-H]^-$	328.0851 ($C_{14}H_{18}NO_6S$), 274.9908 ($C_6H_{11}O_8S_2$), **259.0130** ($C_{12}H_5NO_6$), 195.0330 ($C_{12}H_5NO_2$), 166.0325 (C_8H_8NOS)	https://doi.org/10.1016/j.phytochem.2021.112760
13	Erigeside C [1-*O*-(4-hydroxy-3,5-dimethoxybenzoyl-hexose]	Phenolic glycosides	4.51	$C_{15}H_{20}O_{10}$	360.31	−1.9	3.7	359.0987 $[M-H]^-$	**197.0455** ($C_9H_9O_5$), 182.0221 ($C_8H_6O_5$), 153.0557 ($C_8H_9O_3$), 138.0322 ($C_7H_6O_3$)	https://doi.org/10.1007/s10600-019–02797-2
14	1-*p*-Coumarylhexose	Phenolic glycosides	4.91	$C_{15}H_{18}O_8$	326.30	−2.3	28.7	371.0986* $[M+FA-H]^-$	**163.0401** ($C_9H_7O_3$), 119.0502 (C_8H_7O)	https://doi.org/10.1039/c4ra13155j
15	2′′-*O*-(Rhamnopyranosyloxy) benzylglucosinolate	Glucosinolates	5.39	$C_{20}H_{29}NO_{14}S_2$	571.57	−1.5	12.3	570.0965 $[M-H]^-$	424.0390 ($C_{14}H_{18}NO_{10}S_2$), 328.0851 ($C_{14}H_{18}NO_6S$), 290.9854 ($C_{12}H_5NO_6S$), 274.99009 ($C_{12}H_5NO_5S$), **259.0130** ($C_{12}H_5NO_6$)	https://doi.org/10.1016/j.phytochem.2021.112760
16	Benzoyloxy-hydroxypropyl hexuronic acid isomer	Sugar acids	6.95	$C_{16}H_{20}O_{10}$	372.32	−1.0	16.9	371.0987 $[M-H]^-$	**371.0987** ($C_{16}H_{19}O_{10}$), 353.0874 ($C_{16}H_{17}O_9$), 311.0772 ($C_{14}H_{15}O_8$), 249.0616 ($C_9H_{13}O_8$), 231.0510 ($C_9H_{11}O_7$), 175.0248 ($C_6H_7O_6$), 121.0512 ($C_7H_5O_2$)	–
17	Bauhinin-type nitrile hexoside	Nitrile-containing glycoside	7.11	$C_{15}H_{21}NO_8$	343.33	−0.7	16.1	342.1197 $[M-H]^-$	**150.0556** ($C_8H_8NO_2$)	https://doi.org/10.1021/np50042a008
18	Benzyl alcohol pentosyl-hexoside	Aromatic glycosides	7.11	$C_{18}H_{26}O_{10}$	402.39	−0.6	38.0	447.1511* $[M+FA-H]^-$	**269.1033** ($C_{13}H_{17}O_6$), 161.0454 ($C_6H_9O_5$)	–
19	Hydroxyphthalic acid	Aromatic dicarboxylic acids	7.22	$C_8H_6O_5$	182.13	−2.0	1.2	181.0146 $[M-H]^-$	**137.0239** ($C_7H_5O_3$)	–
20	Phthalic acid	Aromatic dicarboxylic acids	7.61	$C_8H_6O_4$	166.13	−0.7	2.5	165.0195 $[M-H]^-$	**121.0295** ($C_7H_5O_2$)	–
21	Benzoyloxy-hydroxypropyl hexuronic acid isomer	Sugar acids	8.10	$C_{16}H_{20}O_{10}$	372.32	−0.5	1.7	371.0985 $[M-H]^-$	371.0986 ($C_{16}H_{19}O_{10}$), 353.0885 ($C_{16}H_{17}O_9$), 311.0773 ($C_{14}H_{15}O_8$), **249.0618** ($C_9H_{13}O_8$), 231.0514 ($C_9H_{11}O_7$), 175.0253 ($C_6H_7O_6$), 121.0296 ($C_7H_5O_2$)	–
22			8.23	$C_{14}H_{17}NO_7$	311.29	−0.9	15.2		**132.0442** (C_8H_6NO)	–

(*continued on next page*)

Table 1 (*continued*)

No.	Compound Name	Class	tR (min)	Formula	MolecularWeight (Da)	Error (ppm)	Mσ*	*m/z* detected and adduct	Fragment ions *m/z* **	Reference
	Mandelonitrile-type cyanogenic hexoside	Nitrile-containing glycosides						310.0935 $[M-H]^-$		
23	Quercetin 3-*O*-hexoside-7-*O*-deoxyhexoside	Flavonoid glycosides	8.39	$C_{27}H_{30}O_{16}$	610.52	−0.8	5.1	609.1466 $[M-H]^-$	463.0886 ($C_{21}H_{19}O_{12}$), **446.0857** ($C_{21}H_{18}O_{11}$), 447.0913 ($C_{21}H_{19}O_{11}$), 301.0356 ($C_{15}H_9O_7$), 299.0201 ($C_{15}H_7O_7$)	https://doi.org/10.1002/mas.20212
24	Apigenin *C*-hexoside-*C*-pentoside	Flavonoid glycosides	8.51	$C_{26}H_{28}O_{14}$	564.49	−3.3	5.3	563.1406 $[M-H]^-$	545.1301 ($C_{26}H_{25}O_{13}$), 503.1195 ($C_{24}H_{23}O_{12}$), 473.1089 ($C_{23}H_{21}O_{11}$), 443.0984 ($C_{22}H_{19}O_{10}$), 383.0772 ($C_{20}H_{15}O_8$), **353.0667** ($C_{19}H_{13}O_7$)	https://doi.org/10.1002/jms.3803 https://doi.org/10.1002/jms.3413
25	Hydroxy(hydroxyphenyl) valeric acid hexuronide	Sugar acids	8.69	$C_{17}H_{22}O_{10}$	386.35	−0.9	19.5	385.1144 $[M-H]^-$	385.1146 ($C_{17}H_{21}O_{10}$), **267.0725** ($C_9H_{15}O_9$), 249.0618 ($C_9H_{13}O_8$), 231.0506 ($C_9H_{11}O_7$), 207.0527 ($C_7H_{11}O_7$), 113.0247 ($C_5H_5O_3$)	–
26	Octanedioic acid	Aliphatic dicarboxylic acids	8.91	$C_8H_{14}O_4$	174.19	−1.8	10.4	173.0825 $[M-H]^-$	**133.0247** ($C_8H_{13}O_4$), 129.0916 ($C_7H_{13}O_2$), 111.0813 ($C_7H_{11}O$)	–
27	Quercetin 3-*O*-deoxyhexosyl-hexoside-7-*O*-deoxyhexoside	Flavonoid glycosides	8.95	$C_{33}H_{40}O_{20}$	756.66	−2.1	36.2	755.2057 $[M-H]^-$	609.1476 ($C_{27}H_{29}O_{16}$), **446.0865** ($C_{21}H_{18}O_{11}$), 301.0353 ($C_{15}H_9O_7$), 299.0204 ($C_{15}H_7O_7$)	https://doi.org/10.1002/mas.20212https://doi.org/10.1016/S0031-9422(00)84723–8
28	Kaempferol 3-*O*-hexoside-7-*O*-deoxyhexoside	Flavonoid glycosides	9.19	$C_{27}H_{30}O_{15}$	594.52	−1.5	27.2	593.1526 $[M-H]^-$	447.0939 ($C_{21}H_{19}O_{11}$), **430.0915** ($C_{21}H_{18}O_{10}$), 431.0967 ($C_{21}H_{19}O_{10}$), 285.0407 ($C_{15}H_9O_6$), 283.0253 ($C_{15}H_7O_6$)	https://doi.org/10.1002/mas.20212
29	Quercetin 3,7-di-*O*-deoxyhexoside	Flavonoid glycosides	9.35	$C_{27}H_{30}O_{15}$	594.52	−0.9	28.2	593.1517 $[M-H]^-$	447.0924 ($C_{21}H_{19}O_{11}$), **446.0859** ($C_{21}H_{18}O_{11}$), 301.0359 ($C_{15}H_9O_7$), 299.0204 ($C_{15}H_7O_7$)	https://doi.org/10.1002/mas.20212
30	Quercetin 3-*O*-deoxyhexosyl-(*p*-coumaroyl)hexoside-7-*O*-deoxyhexoside	Flavonoid glycosides	10.28	$C_{42}H_{46}O_{22}$	902.80	−1.2	12.0	901.2387 $[M-H]^-$	755.1948 ($C_{36}H_{33}O_{18}$), 609.1465 ($C_{27}H_{29}O_{16}$), **446.0860** ($C_{21}H_{18}O_{11}$), 301.0353 ($C_{15}H_9O_7$), 300.0275 ($C_{15}H_8O_7$), 299.0204 ($C_{15}H_7O_7$)	https://doi.org/10.1002/mas.20212https://doi.org/10.1016/S0031-9422(00)84723–8
31	Niazirin-type nitrile deoxyhexoside	Nitrile-containing glycosides	10.75	$C_{14}H_{17}NO_5$	279.29	−0.6	8.0	324.1091* $[M + FA-H]^-$	**132.0449** (C_8H_6NO)	https://doi.org/10.1055/a-1240–6186
32	Nonanedioic acid (azelaic acid)	Aliphatic dicarboxylic acids	11.36	$C_9H_{16}O_4$	188.22	−2.4	2.7	187.0980 $[M-H]^-$	**187.0980** ($C_9H_{15}O_4$), 169.0875 ($C_9H_{13}O_3$), 125.0976 ($C_8H_{13}O$)	–
33	Oxododecanedioic acid	Aliphatic dicarboxylic acids	12.76	$C_{12}H_{20}O_5$	244.28	−1.6	10.8	243.1242 $[M-H]^-$	**243.1242** ($C_{12}H_{19}O_5$), 225.1136 ($C_{12}H_{17}O_4$), 207.1027 ($C_{12}H_{15}O_3$), 199.1343 ($C_{11}H_{19}O_3$), 155.1424 ($C_{10}H_{19}O$)	–
34	*N*-Feruloyltyramine	Nitrogenous compounds	13.32	$C_{18}H_{19}NO_4$	313.35	−1.0	16.2	312.1244 $[M-H]^-$	**312.1244** ($C_{18}H_{18}NO_4$), 297.1007 ($C_{17}H_{15}NO_4$)	https://doi.org/10.1016/j.phytochem.2005.07.014
35	*N*-Feruloyl-*O*-methyldopamine	Nitrogenous compounds	13.82	$C_{19}H_{21}NO_5$	343.37	−1.0	4.6	342.1351 $[M-H]^-$	**342.1351** ($C_{19}H_{20}NO_5$), 327.1114 ($C_{18}H_{17}NO_5$), 193.0742 ($C_{10}H_{11}NO_3$), 178.0510 ($C_9H_8NO_3$), 148.0530 ($C_9H_8O_2$)	https://doi.org/10.1016/j.phytochem.2005.07.014
36	Tricoumaroyl spermidine	Phytohormone derivative	15.44	$C_{34}H_{37}N_3O_6$	583.68	−2.0	9.8	584.2774 $[M + H]^+$	**438.2404** ($C_{25}H_{32}N_3O_4$), 420.2298 ($C_{25}H_{30}N_3O_3$), 292.2035 ($C_{16}H_{26}N_3O_2$), 275.1765 ($C_{16}H_{23}N_2O_2$), 204.1027 ($C_{12}H_{14}NO_2$), 147.0444 ($C_9H_7O_2$)	https://doi.org/10.1111/j.1365-313X.2008.03773.x
37	Trihydroxy-octadecadienoic acid isomer	Fatty acids	16.39	$C_{18}H_{32}O_5$	328.44	−1.2	3.3	327.2181 $[M-H]^-$	**327.2182** ($C_{18}H_{31}O_5$), 309.2076 ($C_{18}H_{29}O_4$), 291.1974 ($C_{18}H_{27}O_3$), 239.1661 ($C_{14}H_{23}O_3$), 229.1451 ($C_{12}H_{21}O_4$), 221.1191 ($C_{13}H_{17}O_3$), 211.1345 ($C_{12}H_{19}O_3$), 171.1030 ($C_9H_{15}O_3$)	https://doi.org/10.1016/j.jfca.2015.11.004 https://doi.org/10.1002/bmc.2809
38	Trihydroxy-octadecadienoic acid isomer	Fatty acids	16.75	$C_{18}H_{32}O_5$	328.44	−1.9	17.9	327.2183 $[M-H]^-$	**327.2183** ($C_{18}H_{31}O_5$), 309.2076 ($C_{18}H_{29}O_4$), 291.1974 ($C_{18}H_{27}O_3$), 239.1661 ($C_{14}H_{23}O_3$), 229.1451 ($C_{12}H_{21}O_4$), 221.1191 ($C_{13}H_{17}O_3$), 211.1345 ($C_{12}H_{19}O_3$), 171.1030 ($C_9H_{15}O_3$)	–

(*continued on next page*)

Table 1 (*continued*)

No.	Compound Name	Class	tR (min)	Formula	MolecularWeight (Da)	Error (ppm)	Mσ*	*m/z* detected and adduct	Fragment ions *m/z* **	Reference
39	Trihydroxy-octadecadienoic acid isomer	Fatty acids	16.92	$C_{18}H_{32}O_5$	328.44	−2.1	17.3	327.2184 $[M-H]^-$	**327.2184** ($C_{18}H_{31}O_5$), 309.2080 ($C_{18}H_{29}O_4$), 291.1965 ($C_{18}H_{27}O_3$), 269.1773 ($C_{15}H_{25}O_4$), 251.1652 ($C_{15}H_{23}O_3$), 211.1338 ($C_{12}H_{19}O_3$), 183.1400 ($C_{11}H_{19}O_2$)	–
40	Trihydroxy-octadecenoic acid isomer	Fatty acids	17.69	$C_{18}H_{34}O_5$	330.46	−1.4	6.5	329.2338 $[M-H]^-$	**329.2338** ($C_{18}H_{33}O_5$), 311.2230 ($C_{18}H_{31}O_4$), 293.2126 ($C_{18}H_{29}O_3$), 229.1449 ($C_{12}H_{21}O_4$), 211.1344 ($C_{12}H_{19}O_3$), 171.1031 ($C_9H_{15}O_3$)	–
41	Trihydroxy-octadecenoic acid isomer	Fatty acids	17.83	$C_{18}H_{34}O_5$	330.46	−1.9	22.0	329.2340 $[M-H]^-$	**329.2340** ($C_{18}H_{33}O_5$), 311.2251 ($C_{18}H_{31}O_4$), 293.2135 ($C_{18}H_{29}O_3$), 229.1447 ($C_{12}H_{21}O_4$), 211.1345 ($C_{12}H_{19}O_3$), 171.1034 ($C_9H_{15}O_3$)	–
42	Hydroxy-hexadecanedioic acid isomer	Fatty acids	17.83	$C_{16}H_{30}O_5$	302.41	−2.7	15.0	301.2029 $[M-H]^-$	**301.2029** ($C_{16}H_{29}O_5$), 283.1923 ($C_{16}H_{27}O_4$), 265.1815 ($C_{16}H_{25}O_3$), 257.1736 ($C_{14}H_{25}O_4$), 239.2023 ($C_{15}H_{28}O_2$), 221.1919 ($C_{15}H_{25}O$)	–
43	Hydroxy-hexadecanedioic acid isomer	Fatty acids	17.96	$C_{16}H_{30}O_5$	302.41	−2.9	15.5	301.2029 $[M-H]^-$	**301.2029** ($C_{16}H_{29}O_5$), 283.1924 ($C_{16}H_{27}O_4$), 265.1817 ($C_{16}H_{25}O_3$), 257.1736 ($C_{14}H_{25}O_4$), 239.2023 ($C_{15}H_{28}O_2$), 221.1919 ($C_{15}H_{25}O$)	–
44	Dihydroxy-palmitic acid isomer	Fatty acids	18.38	$C_{16}H_{32}O_4$	288.42	−0.3	4.7	289.2374 $[M+H]^+$	289.2375 ($C_{16}H_{33}O_4$), 271.2270 ($C_{16}H_{31}O_3$), **253.2163** ($C_{16}H_{29}O_2$), 235.2056 ($C_{16}H_{27}O$), 217.1953 ($C_{16}H_{25}$)	–
45	Dihydroxy-palmitic acid isomer	Fatty acids	18.48	$C_{16}H_{32}O_4$	288.42	−1.0	5.1	289.2376 $[M+H]^+$	289.2375 ($C_{16}H_{33}O_4$), **271.2269** ($C_{16}H_{31}O_3$), 253.2161 ($C_{16}H_{29}O_2$), 235.2057 ($C_{16}H_{27}O$), 217.1946 ($C_{16}H_{25}$)	–
46	Hydroxy-oxohexadecanoic acid	Fatty acids	19.22	$C_{16}H_{30}O_4$	286.41	−0.6	4.2	287.2218 $[M+H]^+$	269.2111 ($C_{16}H_{29}O_3$), **251.2004** ($C_{16}H_{27}O_2$), 233.1901 ($C_{16}H_{25}O$), 215.1793 ($C_{16}H_{23}$)	–
47	Trihydroxy-octadecenoic acid isomer	Fatty acids	19.22	$C_{18}H_{34}O_5$	330.46	−2.3	17.0	329.2342 $[M-H]^-$	**329.2344** ($C_{18}H_{33}O_5$), 311.2242 ($C_{18}H_{31}O_4$), 293.2125 ($C_{18}H_{29}O_3$), 229.1447 ($C_{12}H_{21}O_4$), 211.1344 ($C_{12}H_{19}O_3$), 201.1142 ($C_{10}H_{17}O_4$), 199.1353 ($C_{11}H_{19}O_3$)	–
48	3-*O*-hexuronyl-28-*O*-hexosyl-oleanolic acid	Triterpene glycoside	19.41	$C_{42}H_{66}O_{14}$	794.97	−2.6	20.1	793.4400 $[M-H]^-$	**793.4401** ($C_{42}H_{65}O_{14}$), 631.3868 ($C_{36}H_{55}O_9$), 613.3759 ($C_{36}H_{53}O_8$), 587.3982 ($C_{35}H_{55}O_7$), 569.3863 ($C_{35}H_{53}O_6$), 455.3548 ($C_{30}H_{47}O_3$)	–
49	Dihydroxy-octadecadienoic acid	Fatty acids	22.50	$C_{18}H_{32}O_4$	312.44	−1.9	18.2	313.2379 $[M+H]^+$	313.2379 ($C_{18}H_{33}O_4$), 295.2269 ($C_{18}H_{31}O_3$), **277.2163** ($C_{18}H_{29}O_2$), 259.2058 ($C_{18}H_{27}O$), 241.1949 ($C_{18}H_{25}$)	
50	Triacetyl-androstenetriol	Phytohormone derivatives	24.58	$C_{25}H_{38}O_6$	434.57	−1.5	25.2	433.2602 $[M-H]^-$	**433.2602** ($C_{25}H_{37}O_6$), 415.2511 ($C_{25}H_{35}O_5$), 287.2230 ($C_{16}H_{31}O_4$), 269.2122 ($C_{16}H_{29}O_3$), 163.0409 ($C_9H_7O_3$), 145.0293 ($C_9H_5O_2$), 117.0353 (C_8H_5O)	
51	3-*O*-hexuronyl-oleanolic acid	Triterpene glycoside	25.59	$C_{36}H_{56}O_9$	632.83	−1.4	11.5	631.3860 $[M-H]^-$	**631.3860** ($C_{36}H_{55}O_9$), 613.3764 ($C_{36}H_{53}O_8$), 571.3646 ($C_{34}H_{51}O_7$), 555.3696 ($C_{34}H_{51}O_6$), 509.3649 ($C_{33}H_{49}O_4$), 455.3538 ($C_{30}H_{47}O_3$)	
52	Hexadecanedioic acid (thapsic acid)	Fatty acids	25.98	$C_{16}H_{30}O_4$	286.41	−1.1	2.3	285.2075 $[M-H]^-$	**285.2075** ($C_{16}H_{29}O_4$), 267.1969 ($C_{16}H_{27}O_3$), 241.2180 ($C_{15}H_{29}O_2$), 223.2070 ($C_{15}H_{27}O$)	
53		Fatty acids	26.26	$C_{18}H_{30}O_3$	294.43	−1.6	76.5			

(*continued on next page*)

Table 1 (*continued*)

No.	Compound Name	Class	tR (min)	Formula	MolecularWeight (Da)	Error (ppm)	Mσ*	*m/z* detected and adduct	Fragment ions *m/z* **	Reference
	Hydroxy-octadecatrienoic acid isomer							293.2127 [M−H]⁻	**293.2124** ($C_{18}H_{29}O_3$), 275.2018 ($C_{18}H_{27}O_2$), 265.2163 ($C_{17}H_{29}O_2$), 235.1709 ($C_{15}H_{23}O_2$), 211.1337 ($C_{12}H_{19}O_3$), 183.1393 ($C_{11}H_{19}O_2$), 171.1025 ($C_9H_{15}O_3$)	
54	Hydroxy-octadecatrienoic acid isomer	Fatty acids	26.55	$C_{18}H_{30}O_3$	294.43	−1.1	20.1	293.2125 [M−H]⁻	**293.2125** ($C_{18}H_{29}O_3$), 275.2018 ($C_{18}H_{27}O_2$), 223.1342 ($C_{13}H_{19}O_3$), 195.1392 ($C_{12}H_{19}O_2$)	
55	Hydroxy-octadecadienoic acid isomer	Fatty acids	27.90	$C_{18}H_{32}O_3$	296.45	2.0	67.8	295.2273 [M−H]⁻	**295.2274** ($C_{18}H_{31}O_3$), 277.2166 ($C_{18}H_{29}O_2$), 183.1392 ($C_{11}H_{19}O_2$), 171.1026 ($C_9H_{15}O_3$)	
56	Hydroxy-octadecadienoic acid isomer	Fatty acids	28.19	$C_{18}H_{32}O_3$	296.45	2.6	3.7	295.2271 [M−H]⁻	**295.2271** ($C_{18}H_{31}O_3$), 277.2165 ($C_{18}H_{29}O_2$), 195.1385 ($C_{12}H_{19}O_2$), 183.1388 ($C_{11}H_{19}O_2$)	
57	Palmitoyl-(sulfodeoxyhexosyl)-glycerol	Glycosyl-monoacylglycerols	28.74	$C_{25}H_{48}O_{11}S$	556.71	2.3	10.4	555.2832 [M−H]⁻	**555.2832** ($C_{25}H_{47}O_{11}S$), 317.0534 ($C_9H_{17}O_{10}S$), 299.0439 ($C_9H_{15}O_9S$), 225.0068 ($C_6H_9O_7S$)	
58	Oxo-octadecadienoic acid	Fatty acids	29.47	$C_{18}H_{30}O_3$	294.43	4.3	5.7	293.2110 [M−H]⁻	**293.2111** ($C_{18}H_{29}O_3$), 275.2001 ($C_{18}H_{27}O_2$), 249.2209 ($C_{17}H_{29}O$), 195.1736 ($C_{13}H_{23}O$), 179.1077 ($C_{11}H_{15}O_2$), 167.1082 ($C_{10}H_{15}O_2$)	

* Isotopic pattern fit factor (mσ).
** Numbers in bold indicate the base peak; in round brackets the corresponding formulas.
FA: Formic acid.

acids derivatives were assigned in *O. baccatus* extract as hexauronic acid derivatives, namely peaks 7, 16, 21 and 25 at *m/z* 373.0778, 371.0987, 371.0985, 371.0986 and 385.1144, respectively.

3.1.7. Nitrogenous compounds

Five peaks were annotated as nitrogenous compounds. In detail, peak 2 ($[M-H]^-$ at *m/z* 290.0881) and peak 34 ($[M-H]^-$ at *m/z* 312.1244) were identified as *N*-fructosyl pyroglutamate and *N*-feruloyltyramine, respectively, (Nahrstedt, Walther, & Wray, 1982; Zamble, Hennebelle, Sahpaz, & Bailleul, 2007). Likewise, *N*-Feruloyl-*O*-methyldopamine, peak 35 was characterized by the molecular ion peak $[M-H]^-$ at *m/z* 342.1351(King & Calhoun, 2005). Further, peak 4 was assigned as adenosine ($[M+H]^+$ at *m/z* 268.1045) and had a daughter ion at *m/z* 136.0617 ($C_4H_{10}NO_4^+$). On the other hand, peak 5 was assigned as an isoleucine derivative, with *m/z* 294.1552 and major fragment of *m/z* 276.1442 by the loss of H_2O molecule (Johnstone & Rose, 1985).

3.1.8. Phytohormone derivatives

Brassinosteroids are endogenous phytohormones that promote plant growth and normal development (Kumari & Hemantaranjan, 2018). As polyhydroxylated sterols, these compounds can exist in a free form, esterified with organic acids, sulphate or in the form of glucosides (Hussain et al., 2020). In our analysis, peak 50 was described as a brassinosteroid derivative at *m/z* 433.2602 with androstenetriol backbone esterified with 3 molecules of acetic acid.

A polyamine derivative, Peak 36 (*m/z* 584.2774) was identified as tricoumaroyl spermidine based on comparison with literature database (Grienenberger et al., 2009). Notably, spermidine is a plant hormone that regulates plant growth and was found conjugated with phenolic acids in several plant families (Aylanc, Tomás, Russo-Almeida, Falcão, & Vilas-Boas, 2021; Chen, Shao, Yin, Younis, & Zheng, 2019).

3.2. Zebrafish embryo toxicity assay

Perturbed development can manifest as morphological malformations, behavioural abnormalities or death of the embryos. Zebrafish embryos develop externally and are optically transparent. Using simple microscopic techniques, numerous effects can be assessed non-invasively over the course of development (Pecio, Otify, Saber, El-Amier, Shalaby, Kozachok, Elmotayam, Świątek, Skiba, & Skalicka-Woźniak, 2022; Truong, Harper, & Tanguay, 2011).

It was shown that *O. baccatus* extract showed no toxic effects at low concentrations tested. The first sign of toxicity was noticed after 48 h incubation at the concentration of 75 μg/mL when 2 out of 10 larvae exhibited shortened tails as well as heart malformation. At the higher concentration (100 μg/mL), much more body malformations were noticed on the second day.

3.3. Cytotoxic activity of O. baccatus extract

The results of cytotoxicity studies are presented in Table 2. The anticancer selectivity was assessed with reference to normal (non-cancerous) VERO cells. The *O. baccatus* extract showed a selective antineoplastic effect on all tested cancer cell lines with the highest activity (CC_{50} 39.1 μg/mL) and selectivity (SI 7.23, $p < 0.01$) observed

Table 2
The cytotoxicity of *Ochradenus baccatus* extract on different cell lines.

	VERO	FaDu		HeLa		RKO	
	CC_{50}	CC_{50}	SI	CC_{50}	SI	CC_{50}	SI
***O. baccatus* extract**	282.77 ± 12,19	98.99 ± 15.59	2.86	39.1 ± 1,67	7.23	199.38 ± 44.95	1.42

CC_{50} – 50 % Cytotoxic concentration (μg/mL), Mean ± SD; concentration decreasing cellular viability by 50 %; SI – Selectivity Index (SI = CC_{50}VERO/CC_{50}Cancer)

against human cervical adenocarcinoma-derived cells (HeLa). The human hypopharyngeal squamous cell carcinoma cells (FaDu) were less sensitive to tested extracts, however, still significant selectivity was observed (SI 2.86, $p < 0.01$). Noteworthy, as shown in Fig. 3, at 125 μg/mL, *O. baccatus* extract induced almost 100 % cytotoxic effect of HeLa, whereas, in the case of FaDu cells, the median cellular viability was 33 %.

Thoppil, Harlev, Mandal, Nevo, and Bishayee (2013) studied the antitumor activities of extracts from selected desert plants against human hepatocellular carcinoma cells (HepG2). They reported that *O. baccatus* aqueous extracts showed CC_{50} values of 1.51 and 0.83 mg/mL after 24 and 48 h incubation, respectively (Thoppil et al., 2013). In our research, the methanolic extract showed significantly higher toxicity toward all tested cell lines. Unfortunately, Thoppil et al. (2013) didn't report the chemical analysis of the tested extract, and thus, it is impossible to elucidate if the different composition was responsible for lower *in vitro* cytotoxicity. Interestingly, another study performed by Khan, Khan, Adil, and Alkhathlan (2022) reported that *O. baccatus* methanolic extract showed higher cytotoxicity towards HepG2, decreasing the cellular viability by 28.4 % at 50 μg/mL concentration after 24 h of exposure. Little has been reported concerning the toxicity of other *Ochradenus* species. Ali et al. (2016) reported that *Ochradenus arabicus* crude ethanolic extract shows a cytotoxic effect on adenocarcinoma breast cancer cells (MCF-7) with a CC_{50} of 562 μg/mL. The limitations of both aforementioned studies focusing on the toxicity of *O. baccatus* extracts were the use of only one cancer cell type and the lack of any information concerning the activity towards normal cells, making it impossible to assess the anticancer selectivity. That is why our study design not only took into consideration a more comprehensive panel of cancer types but also the use of normal cells for comparison and evaluation of selectivity.

Studies on *in vivo* toxicity of *O. baccatus* ethanol extracts (1000 – 4000 mg/kg) in male Wistar rats did not show any symptoms of acute toxicity, no diarrhoea, haematuria, restlessness, uncoordinated muscle movements, or respiratory distress were observed, and no mortality was reported during 24 h of observation ($LD_{50} > 4000$ mg/kg). The *O. baccatus* ethanol extract was also administered (100, 200 or 400 mg/kg) during a 65-day treatment period in rats without showing any effect on the body weights, and biochemical tests performed afterwards showed no indication of organ-related toxicity, with normal serum transaminases (ALT and AST) level indicating the lack of hepatotoxicity, as well as normal urea and creatinine confirming the absence of nephrotoxicity. During the studies on the influence of *O. baccatus* treatment on reproductive organs of male rats, no significant changes in the relative weight of the testes, seminal vesicles and ventral prostate were observed, as well as serum testosterone, lutrophin (LH), prolactin (PRL), and follicle-stimulating hormone (FSH) levels remained unchanged compared to control (Soliman, Donia, Awaad, Alqasoumi, & Yusufoglu, 2012). Al-Omar et al. (2020) also studied the acute toxicity of *O. baccatus* ethanol extract (1000 – 4000 mg/kg) in male albino rats and reported no significant toxicity after 24 h. Additionally, oral treatment with 50 mg/kg or 100 mg/kg of ethanol extract using an orogastric cannula for consecutive 30 days resulted in no significant differences in the serum levels of urea, creatinine, glucose, ALT, AST, total protein and cholesterol, however, the serum levels of triglycerides were significantly decreased in animals that received 100 mg/kg of the extract. Moreover, the histopathological evaluation of rats' livers and kidneys also indicated no significant changes compared to the control group (Al-Omar et al., 2020).

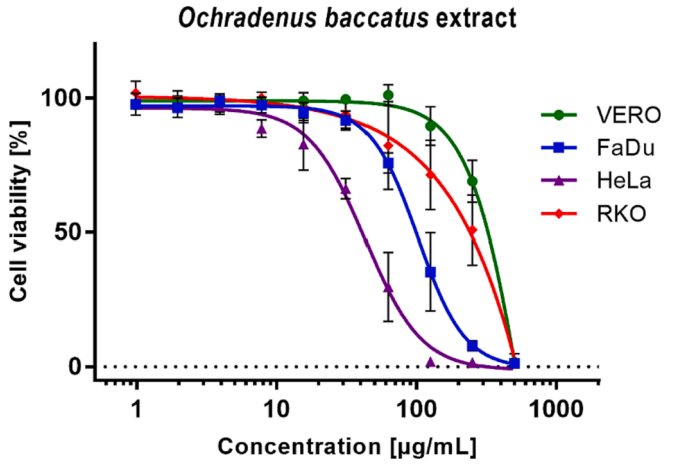

Fig. 3. Cytotoxicity profile of *Ochradenus baccatus* extract on different cell lines.

Considering the data on *in vivo* toxicity, it can be concluded that the administration of *O. baccatus* extracts is safe and has no toxic effects in animal models (Al-Omar et al., 2020; Soliman et al., 2012). Our results indicate that *O. baccatus* methanolic extract shows a significant and selective anticancer activity against cervical adenocarcinoma (HeLa) cells and, to a lower extent, towards hypopharyngeal squamous cell carcinoma (FaDu) cells. Among compounds identified in *O. baccatus* extract used in this study, glucosinolates have been long known for their antiproliferative and chemopreventive activity against cancer cell lines (Esfandiari et al., 2017; Liu et al., 2018). Apart from glucosinolates, the extract contained three nitriles (peaks 17, 22 and 31) which have not been thoroughly investigated except for few studies (Kupke et al., 2016; Unsal Tan & Zengin, 2022) but are considered as plant products with great potential due to the chemical activity of the conjugated nitrile group. Therefore, we believe this extract should undergo more extensive studies to evaluate compounds or mechanisms responsible for the observed antineoplastic effects.

4. Conclusion

Arising from the concept of sustainable development, utilization and searching for new food sources deemed necessary to cope with the increasing food demands. Herein, we investigated the underutilised desert plant *O. baccatus* as a possible sufficiently safe nutraceutical ingredient. Our results suggest that the methanolic extract of the aerial parts of *O. baccatus* possessed an acceptable antiproliferative activities toward cervical adenocarcinoma (HeLa) and hypopharyngeal squamous carcinoma (FaDu) cells with significant selectivity towards the cancer cells. This effect can be attributed to its high content of flavonoids, nitrile glycosides, fatty acids and glucosinolates, known for their cytotoxic activities, which are represented in the metabolomics profile of the extract by 57 metabolites. Therefore, further investigation of the rich metabolome of *O. baccatus* is highly recommended to identify novel anticancer compounds from this orally safe desert food.

CRediT authorship contribution statement

Łukasz Pecio: Writing – review & editing, Methodology, Investigation, Writing – original draft. **Solomiia Kozachok:** Investigation. **Fatema R. Saber:** Conceptualization, Investigation, Methodology, Writing – original draft, Writing – review & editing. **Maria Garcia-Marti:** Investigation, Methodology, Writing – review & editing. **Yasser El-Amier:** Conceptualization, Investigation, Methodology, Resources, Writing – original draft. **Engy A. Mahrous:** Investigation, Visualization, Writing – review & editing. **Łukasz Świątek:** Methodology, Investigation, Writing – original draft, Writing – review & editing. **Anastazja Boguszewska:** Methodology. **Adrianna Skiba:** Methodology, Investigation, Writing – review & editing. **Ahmed H. Elosaily:** Investigation, Methodology, Writing – review & editing. **Krystyna Skalicka-Woźniak:** Conceptualization, Resources, Writing – review & editing. **Jesus Simal-Gandara:** Conceptualization, Investigation, Resources, Writing – review & editing.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

Acknowledgements

The work was supported by Medical University of Lublin DS28. Funding for open access charge: Universidade de Vigo/CISUG. The authors would like to acknowledge Dr Mariusz Kowalczyk and Dr Dariusz Jędrejek for developing LC–MS analysis methods and carrying out LC-MS data acquisition.

References

Abdel-Sattar, E., Harraz, F. M., & El-Gayed, S. H. (2008). Antimicrobial activity of extracts of some plants collected from the Kingdom of Saudi Arabia. *Medical Science Monitor, 15*(1).

Achenbach, J. C., Leggiadro, C., Sperker, S. A., Woodland, C., & Ellis, L. D. (2020). Comparison of the Zebrafish Embryo Toxicity Assay and the General and Behavioral Embryo Toxicity Assay as New Approach Methods for Chemical Screening. *Toxics, 8*(4), 126.

Adamczak, A., Ożarowski, M., & Karpiński, T. M. (2019). Antibacterial activity of some flavonoids and organic acids widely distributed in plants. *Journal of Clinical Medicine, 9*(1), 109.

Al Qurainy, F., Nadeem, M., Khan, S., Alansi, S., & Tarroum, M. (2013). Efficient regeneration of a potential medicinal plant *Ochradenus baccatus* Delile from cotyledon and shoot axis. *Pakistan Journal of Botany, 45*(2), 501–505.

Ali, M. A., Farah, M. A., Al-Hemaid, F. M., Abou-Tarboush, F. M., Al-Anazi, K. M., Wabaidur, S., … Lee, J. (2016). Assessment of biological activity and UPLC–MS based chromatographic profiling of ethanolic extract of *Ochradenus arabicus*. *Saudi Journal of Biological Sciences, 23*(2), 229–236.

Al-Omar, M. S., Eldeeb, H. M., Mobark, M. A., & Mohammed, H. A. (2020). Antimicrobial activity and histopathological safety evidence of *Ochradenus baccatus* Delile: A medicinally important plant growing in Saudi Arabia. *Pharmacognosy Research, 12*(2), 131–136.

Alqasoumi, S. I., Soliman, G., Awaad, A. S., & Donia, A. (2012). Anti-inflammatory activity, safety and protective effects of *Leptadenia pyrotechnica*, *Haloxylon salicornicum* and *Ochradenus baccatus* in ulcerative colitis. *Phytopharmacology, 2*(1), 58–71.

Amer, H. M., & Mohammad, A. A. (2022). Medicinal plants and their validation challenges in traditional Egyptian medicine. *Journal of Applied Pharmaceutical Science, 12*(3), 23–33.

Aylanc, V., Tomás, A., Russo-Almeida, P., Falcão, S. I., & Vilas-Boas, M. (2021). Assessment of bioactive compounds under simulated gastrointestinal digestion of bee pollen and bee bread: Bioaccessibility and antioxidant activity. *Antioxidants, 10*(5), 651.

Barakat, H., El-Mousallamy, A., Souleman, A., & Awadalla, S. (1991). Flavonoids of *Ochradenus baccatus*. *Phytochemistry, 30*(11), 3777–3779.

Benayad, Z., Gómez-Cordovés, C., & Es-Safi, N. E. (2014). Characterization of flavonoid glycosides from fenugreek (*Trigonella foenum-graecum*) crude seeds by HPLC–DAD–ESI/MS analysis. *International Journal of Molecular Sciences, 15*(11), 20668–20685.

Berrehal, D., Khalfallah, A., Kabouche, A., Kabouche, Z., Karioti, A., & Bilia, A. (2010). Flavonoid glycosides from *Randonia africana* Coss. (Resedaceae). *Biochemical Systematics and Ecology, 38*(5), 1007–1009.

Bhatia, D., Mandal, A., Nevo, E., & Bishayee, A. (2015). Apoptosis-inducing effects of extracts from desert plants in HepG2 human hepatocarcinoma cells. *Asian Pacific Journal of Tropical Biomedicine, 5*(2), 87–92.

Boulos, L. (1999). Flora of Egypt. *Egypt: Al Hadara Publishing, 1*, 419.

Bronstein, J. L., Izhaki, I., Nathan, R., Tewksbury, J. J., Spiegel, O., Lotan, A., … Green, R. (2007). Fleshy-fruited plants and frugivores in desert ecosystems. *Seed Dispersal: Theory and its Application in a Changing World*, 148–177.

Cahoon, E. B., & Li-Beisson, Y. (2020). Plant unusual fatty acids: Learning from the less common. *Current Opinion in Plant Biology, 55*, 66–73.

Cao, J., Yin, C., Qin, Y., Cheng, Z., & Chen, D. (2014). Approach to the study of flavone di-*C*-glycosides by high performance liquid chromatography-tandem ion trap mass spectrometry and its application to characterization of flavonoid composition in Viola yedoensis. *Journal of Mass Spectrometry, 49*(10), 1010–1024.

Chen, C.-C., Chen, Y.-P., Hsu, H.-Y., Lee, K.-H., Tani, S., & McPhail, A. T. (1985). Bauhinin, a new nitrile glucoside from *Bauhinia championii*. *Journal of Natural Products, 48*(6), 933–937.

Chen, D., Shao, Q., Yin, L., Younis, A., & Zheng, B. (2019). Polyamine function in plants: Metabolism, regulation on development, and roles in abiotic stress responses. *Frontiers in Plant Science, 9*, 1945.

Dührkop, K., Fleischauer, M., Ludwig, M., Aksenov, A. A., Melnik, A. V., Meusel, M., … Böcker, S. (2019). SIRIUS 4: A rapid tool for turning tandem mass spectra into metabolite structure information. *Nature Methods, 16*(4), 299–302.

El-Amier, Y., & Abdul-Kader, O. (2015). Vegetation and species diversity in the northern sector of Eastern Desert, Egypt. *West African Journal of Applied Ecology, 23*(1), 75–95.

Esfandiari, A., Saei, A., McKenzie, M. J., Matich, A. J., Babalar, M., & Hunter, D. A. (2017). Preferentially enhancing anti-cancer isothiocyanates over glucosinolates in broccoli sprouts: How NaCl and salicylic acid affect their formation. *Plant Physiology and Biochemistry, 115*, 343–353.

Fantoukh, O. I., Wang, Y.-H., Parveen, A., Hawwal, M. F., Al-Hamoud, G. A., Ali, Z., … Khan, I. A. (2021). Profiling and Quantification of the Key Phytochemicals from the Drumstick Tree (*Moringa oleifera*) and Dietary Supplements by UHPLC-PDA-MS. *Planta Medica, 87*(05), 417–427.

Ford, K. A. (2017). Refinement, Reduction, and Replacement of Animal Toxicity Tests by Computational Methods. *ILAR Journal, 57*(2), 226–233.

Grienenberger, E., Besseau, S., Geoffroy, P., Debayle, D., Heintz, D., Lapierre, C., … Legrand, M. (2009). A BAHD acyltransferase is expressed in the tapetum of Arabidopsis anthers and is involved in the synthesis of hydroxycinnamoyl spermidines. *The Plant Journal, 58*(2), 246–259.

He, M., Qin, C.-X., Wang, X., & Ding, N.-Z. (2020). Plant unsaturated fatty acids: Biosynthesis and regulation. *Frontiers in Plant Science*, 390.

Hoffmann, M. A., Nothias, L.-F., Ludwig, M., Fleischauer, M., Gentry, E. C., Witting, M., Dorrestein, P. C., Dührkop, K., & Böcker, S. (2021). Assigning confidence to structural annotations from mass spectra with COSMIC.

Hussain, H. H., & El-Oqlah, A. A. (1997). Chemical constituents of plants growing in Bahrain. *International Journal of Pharmacognosy, 35*(2), 147–149.

Hussain, M. A., Fahad, S., Sharif, R., Jan, M. F., Mujtaba, M., Ali, Q., … Ajayo, B. S. (2020). Multifunctional role of brassinosteroid and its analogues in plants. *Plant Growth Regulation, 92*(2), 141–156.

Hussein, S. R., Elkhateeb, A., Marzouk, M. M., Ibrahim, L. F., & Kawashty, S. A. (2013). Phytochemical investigation of *Oligomeris linifolia* (Vahl), Macbr. (Resedaceae). *Biochemical Systematics and Ecology, 49*(2013), 73–76.

Jiménez-Sánchez, C., Lozano-Sánchez, J., Rodríguez-Pérez, C., Segura-Carretero, A., & Fernández-Gutiérrez, A. (2016). Comprehensive, untargeted, and qualitative RP-HPLC-ESI-QTOF/MS2 metabolite profiling of green asparagus (*Asparagus officinalis*). *Journal of Food Composition and Analysis, 46*, 78–87.

Johnstone, R., & Rose, M. (1985). Mass spectrometry of amino acids and their derivatives. In *Chemistry and Biochemistry of the Amino Acids* (pp. 480–524). Springer.

Khan, M., Khan, M., Adil, S. F., & Alkhathlan, H. Z. (2022). Screening of potential cytotoxic activities of some medicinal plants of Saudi Arabia. *Saudi Journal of Biological Sciences, 29*(3), 1801–1807.

King, R. R., & Calhoun, L. A. (2005). Characterization of cross-linked hydroxycinnamic acid amides isolated from potato common scab lesions. *Phytochemistry, 66*(20), 2468–2473.

Kumari, A., & Hemantaranjan, A. (2018). Morpho-physiological attributes of Wheat (*Triticum aestivum* L.) genotypes as influenced by brassinosteroids under heat stress. *Journal of Pharmacognosy and Phytochemistry, 7*(6), 2111–2115.

Kupke, F., Herz, C., Hanschen, F. S., Platz, S., Odongo, G. A., Helmig, S., … Lamy, E. (2016). Cytotoxic and genotoxic potential of food-borne nitriles in a liver in vitro model. *Scientific reports, 6*(1), 1–11.

Liu, P., Behray, M., Wang, Q., Wang, W., Zhou, Z., Chao, Y., & Bao, Y. (2018). Anti-cancer activities of allyl isothiocyanate and its conjugated silicon quantum dots. *Scientific Reports, 8*(1), 1084.

Lotze, J. (2001). Handbook of Arabian Medicinal Plants. *Northeastern Naturalist, 8*(3), 378.

Mahklouf, M. (2019). Ethnobotanical study of edible wild plants in Libya. *European Journal of Ecology, 5*(2), 30–40.

Mahrous, E. A., Elosaily, A. H., Salama, A. A., Salama, A. M., & El-Zalabani, S. M. (2022). Oral and Topical Anti-Inflammatory Activity of *Jatropha integerrima* Leaves Extract in Relation to Its Metabolite Profile. *Plants, 11*(2), 218.

Martín-Bravo, S., & Escudero, M. (2012). Biogeography of flowering plants: A case study in mignonettes (Resedaceae) and sedges (Carex, Cyperaceae). *Global Advances in Biogeography, 13*, 257–290.

Martínez Pastur, G., Perera, A. H., Peterson, U., & Iverson, L. R. (2018). Ecosystem services from forest landscapes: An overview. *Ecosystem Services from Forest Landscapes*, 1–10.

Mekky, R. H., del Mar Contreras, M., El-Gindi, M. R., Abdel-Monem, A. R., Abdel-Sattar, E., & Segura-Carretero, A. (2015). Profiling of phenolic and other compounds from Egyptian cultivars of chickpea (*Cicer arietinum* L.) and antioxidant activity: A comparative study. *RSC Advances, 5*(23), 17751–17767.

Miller, A. (1984). *revision of Ochradenus*. Notes-Royal Botanic Garden Edinburgh.

Nahrstedt, A., Walther, A., & Wray, V. (1982). Sarmentosin epoxide, a new cyanogenic compound from Sedum cepaea. *Phytochemistry, 21*(1), 107–110.

Nawash, O. S., & Ahmad Al-S, H. (2011). The most important medicinal plants in Wadi Araba desert in South West Jordan: A review article. *Advances in Environmental Biology*, 418–426.

Okwu, D., & Okwu, M. (2004). Chemical composition of *Spondias mombin* Linn plant parts. *Journal of Sustainable Agriculture and Environment, 6*(2), 140–147.

Omar, S. (2007). Vegetation of Kuwait Kuwait Institute for Scientific Research. *Kuwait, 161*.

Pecio, Ł., Otify, A. M., Saber, F. R., El-Amier, Y. A., Shalaby, M. E., Kozachok, S., Elmotayam, A. K., Świątek, Ł., Skiba, A., & Skalicka-Woźniak, K. (2022). *Iphiona mucronata* (Forssk.) Asch. & Schweinf. A Comprehensive Phytochemical Study via UPLC-Q-TOF-MS in the Context of the Embryo-and Cytotoxicity Profiles. *Molecules, 27*(21), 7529.

Soliman, G. A., Donia, A. E. R. M., Awaad, A. S., Alqasoumi, S. I., & Yusufoglu, H. (2012). Effect of *Emex spinosa*, *Leptadenia pyrotechnica*, *Haloxylon salicornicum* and *Ochradenus baccatus* extracts on the reproductive organs of adult male rats. *Pharmaceutical Biology, 50*(1), 105–112.

Spiegel, O., & Nathan, R. (2012). Empirical evaluation of directed dispersal and density-dependent effects across successive recruitment phases. *Journal of Ecology, 100*(2), 392–404.

Świątek, Ł., Sieniawska, E., Mahomoodally, M. F., Sadeer, N. B., Wojtanowski, K. K., Rajtar, B., … Zengin, G. (2021). Phytochemical Profile and Biological Activities of the Extracts from Two *Oenanthe* Species (*O aquatica* and *O. silaifolia*). *Pharmaceuticals, 15*(1), 50.

Thoppil, R. J., Harlev, E., Mandal, A., Nevo, E., & Bishayee, A. (2013). Antitumor activities of extracts from selected desert plants against HepG2 human hepatocellular carcinoma cells. *Pharmaceutical Biology, 51*(5), 668–674.

Trabelcy, B., Chinkov, N., Samuni-Blank, M., Merav, M., Izhaki, I., Carmeli, S., & Gerchman, Y. (2021). Investigation of glucosinolates in the desert plant *Ochradenus baccatus* (Brassicales: Resedaceae). Unveiling glucoochradenin, a new arabinosylated glucosinolate. *Phytochemistry, 187*, Article 112760.

Truong, L., Harper, S. L., & Tanguay, R. L. (2011). Evaluation of embryotoxicity using the zebrafish model. In *Drug safety evaluation* (pp. 271–279). Springer.

Unsal Tan, O., & Zengin, M. (2022). Insights into the chemistry and therapeutic potential of acrylonitrile derivatives. *Archiv der Pharmazie (Weinheim), 355*(3), 2100383.

VanEtten, C. H., & Tookey, H. L. (2018). Glucosinolates. In M. Rechcigl (Ed.), *Handbook of naturally occurring food toxicants* (pp. 15–30). CRC Press.

Vukics, V., & Guttman, A. (2010). Structural characterization of flavonoid glycosides by multi-stage mass spectrometry. *Mass Spectrometry Reviews, 29*(1), 1–16.

Wang, S., Liu, K., Wang, X., He, Q., & Chen, X. (2011). Toxic effects of celastrol on embryonic development of zebrafish (Danio rerio). *Drug and Chemical Toxicology, 34*(1), 61–65.

Yang, B., Liu, H., Yang, J., Gupta, V. K., & Jiang, Y. (2018). New insights on bioactivities and biosynthesis of flavonoid glycosides. *Trends in Food Science & Technology, 79*, 116–124.

Yumnamcha, T., Roy, D., Devi, M. D., & Nongthomba, U. (2015). Evaluation of developmental toxicity and apoptotic induction of the aqueous extract of Millettia pachycarpa using zebrafish as model organism. *Toxicological & Environmental Chemistry, 97*(10), 1363–1381.

Zamble, A., Hennebelle, T., Sahpaz, S., & Bailleul, F. (2007). Two new quinoline and tris (4-hydroxycinnamoyl) spermine derivatives from Microdesmis keayana roots. *Chemical and Pharmaceutical Bulletin, 55*(4), 643–645.

Zhou, Y., Li, P., Brantner, A., Wang, H., Shu, X., Yang, J., … Bian, B. (2017). Chemical profiling analysis of Maca using UHPLC-ESI-Orbitrap MS coupled with UHPLC-ESI-QqQ MS and the neuroprotective study on its active ingredients. *Scientific Reports, 7*(1), 1–14.

Zhou, J., Ma, J.-P., Guo, T., Zhang, J.-B., & Chang, J. (2019). Phenol Glycosides from Root Bark of Phellodendron chinense. *Chemistry of Natural Compounds, 55*(4), 743–744.