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ORIGINAL ARTICLE

Liquid Chromatography High-Resolution Mass Spectrometry Analysis, Phytochemical and Biological Study of Two Aizoaceae Plants: A New Kaempferol Derivative from *Trianthema portulacastrum* L.

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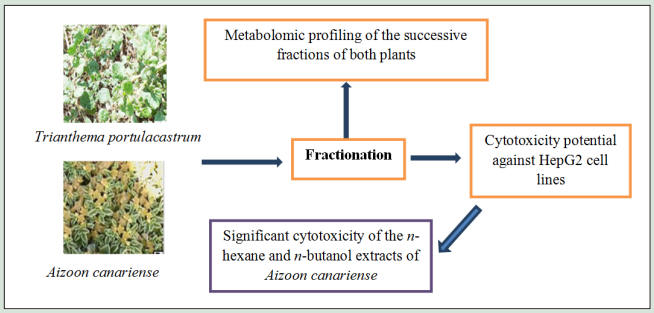
ABSTRACT

Background: Natural remedies used for the treatment of liver diseases have a major concernworldwide. **Objectives:** Evaluation of the cytotoxic potential of successive fractions of two Aizoaceae plants; *Trianthema portulacastrum* L. and *Aizoon canariense* L. against human hepatocellular carcinoma (HepG2) cell lines. Moreover, metabolomic profiling of the successive fractions of both plants was carried out. **Materials and Methods:** Cytotoxic activity of successive fractions of the two plants against hepatocellular carcinoma (HCC) HepG2 cell lines were evaluated using viability assay, whereas metabolomic profiling was carried out using Liquid Chromatography High-Resolution ElectroSpray Ionization Mass Spectrometry (LC-HR-ESI-MS). **Results:** Significant cytotoxic activity of the *n*-hexane and *n*-butanol extracts of *A. canariense* (24.7 ± 3.5 and 55.3 ± 4.9 µg/mL, respectively) is recorded. On the other hand, metabolomic profiling of both plants resulted in dereplication of 27 metabolites belonging to different chemical classes; for example, sterols, flavonoids, diterpenes, triterpenes, tetraterpenes, alkaloids, lignans, hydrocarbons, and nucleosides. Phytochemical study of the biologically active fractions resulted in the isolation of one new compound; kaempferol-3-O-(2″-O-β-D-glucopyranosyl)-6″-O-E-feruloyl-β-D-glucopyranosid (T1). Biological testing of the isolated compounds indicated significant activity of T1 against HCC (IC_{50} = 7.19 ± 0.27 µg/mL). **Conclusion:** Phytochemicals isolated from *T. portulacastrum* L and *A. canariense* L. may be responsible for their cytotoxic activity against HCC HepG2 cell lines.

Key words: Aizoon, cytotoxic activity, human hepatocellular carcinoma cell lines, liquid chromatography high-resolution ElectroSpray ionization mass spectrometry, *Trianthema*

SUMMARY

- Metabolomic profiling and evaluation of the cytotoxic potential of successive fractions of *Trianthema portulacastrum* L. and *Aizoon canariense* L. are performed. The cytotoxic activity is evaluated against human hepatocellular carcinoma (HepG2 cell lines). Kaempferol-3-*O*-(2″-*O*-β-D-glucopyranosyl)-6″-*O*-E-feruloyl-β-D-glucopyranosid is isolated as a new compound from *Trianthema portulacastrum* L. herb.



Abbreviations Used: DNP: Dictionary of natural products; HCC: Hepatocellular carcinoma; LC-HR-ESI-MS: Liquid chromatography high-resolution ElectroSpray ionization mass spectrometry; TOF: Time-of-flight; UPLC: Ultra performance liquid chromatography.

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INTRODUCTION

After cardiovascular disease, cancer is the second leading cause of death amongst the non-communicable diseases.[1] Hepatocellular carcinoma (HCC) is one of the most fatal malignancies in Egypt, and this may be due to the spread of hepatitis B and C infections, the overuse of pesticides or aflatoxin contamination, especially in rural areas.[2] Chemotherapy, hormonal therapy, radiotherapy, or surgery used for the treatment of cancer, have many side effects.[3] Natural remedies have a long history for the treatment of liver diseases, and medicinal plants and natural products are continuously used all over the world.[4] The most prominent chemotherapeutics of plant origin, which were obtained either directly through isolation or derived from lead structures, are the indole alkaloids vincristine and vinblastine, podophyllotoxin derivatives etoposide and teniposide.[5] Desert plants have effective defense systems that allow them to withstand the stressful conditions for survival; bioactive secondary metabolites are one of these mechanisms.[6]

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Family Aizoaceae which are commonly known as ice plant, carpet weeds, or stone plants includes about 130 genera and 1200 species.[7] Many of Aizoaceae plants are reported for their antifungal, antibacterial,[8,9] acaricidal activity against *Rhipicephalus annulatus*[10] and antioxidant activity.[11]

Trianthema portulacastrum L. is an Indian medicinal plant that exhibits anti-hepatotoxic activity,[12] anti-hepatocarcinogenic potential[13] and chemopreventive activity against breast cancer.[14] Ecdysone is a major constituent of *T. portulacastrum* L. in addition to leptorumol, trianthenol, 5,2'-dihydroxy-7-methoxy-6,8-dimethylflavone, 3-acetylaleuritolic acid, 3,4-dimethoxy cinnamic acid, 5-hydroxy-2-methoxybenzaldehyde, betacyanin, *and p*-methoxybenzoic acid.[15]

Aizoon canariense L.(Aizoaceae) is a perennial plant distributed in North Africa, Canary Islands, Mediterranean, Arabian Peninsula, south Iran, Pakistan, and Afghanistan.[16] Few studies concerning the secondary metabolite content and the biological potential of this plant have been previously reported.

This paper was designed to evaluate the cytotoxic potential of different fractions of two Aizoaceae plants. In addition, the study of the metabolic pattern of both plants using LCHRMS was conducted. Furthermore, isolation and identification of the major components of the active fractions using different chromatographic and spectroscopic techniques and evaluation of their cytotoxic potential against HepG2 cell lines were carried out.

MATERIALS AND METHODS

General experimental

Bruker Avance III 400 MHz with AEON Nitrogen-Free Magnet and BBFO Smart Probe (Bruker AG, Switzerlaznd) was used to record ^{1}H and ^{13}C NMR spectra 400 MHz and 100 MHz, respectively. Data acquisition and processing were performed using Topspin 3.1 Software. Liquid Chromatography High-Resolution Electrospray Ionization Mass Spectrometry (LC-HR-ESI-MS) metabolomic analyses was performed using an Acquity Ultra Performance LC system connected to a Synapt G2 HDMS quadrupole time-of-flight hybrid mass spectrometer (Waters, Milford, USA).

Plant materials

Entire herbs of *T. portulacastrum* L. and *A. canariense* L. were collected in the flowering stage in spring and summer 2015 and 2017, from Cairo-Suez Canal road, East desert, Egypt and identified by Prof. Dr. Abdel Haleem Abdel Motagaly, Agriculture Museum, Giza, Egypt. The voucher specimen's numbers (R-Trian-10) and (R-Aizo) were given to *T. portulacastrum* L. and *A. canariense* L., respectively, and deposited at the Botanical Herbarium, Agriculture Museum, Dokki, Egypt.

Extraction

Air-dried powdered plant material of *T. portulacastrum and A. canariense* (1 kg each) was separately exhaustively extracted with 70% ethanol (3 L × 5 times). The extracts were then concentrated under reduced pressure at 45°C. The alcoholic extract residues (116 and 120 g) of *T. portulacastrum* and *A. canariense,* respectively, were subjected to sequential solvent partitioning using *n*-hexane (3 × 500 ml), ethyl acetate (4 × 300 ml) and *n*-butanol (3 × 200 ml). Each solvent fraction was dried under vacuum till dryness.

Cytotoxic activity testing

The human HCC (HepG2) were obtained from the Tissue Culture Unit at VACSERA. Trypan blue dye, dimethyl sulfoxide, and crystal violet were purchased from Sigma (St. Louis, Mo., USA). Fetal Bovine serum, HEPES buffer solution, RPMI-1640, DMEM, gentamycin, 0.25% Trypsin-EDTA, and L-glutamine were purchased from Lonza. Cell line propagation and cytotoxicity evaluation were performed as previously described.[17,18]

Metabolomic analysis of different extracts of *Trianthema portulacastrum* and *Aizoon canariense*

Metabolomic analysis of the different fractions (*n*-hexane, ethyl acetate, and *n*-butanol) of *T. portulacastrum* and *A. canariense* was performed according to Abdelmohsen *et al*.[19] using analytical techniques of LC-HR-ESI-MS. Detection of the metabolites was performed using ESI MS in both the positive and negative modes. Data were processed and extracted using MZmine 2.20.[20] Chromatogram builder and deconvolution followed the detection of mass ion peaks. The local minimum search algorithm was processed, and isotopes were recognized by the isotopic peaks grouper. An adduct and complex search were done. Then, the processed data set was submitted to peak identification and molecular formula prediction. Both +ve and −ve ionization mode data sets were dereplicated against METLIN and Dictionary of natural products (DNP) databases. Hits from the database were retrieved using Chem-Bio Finder version 13.

Chromatographic isolation of the main components of *Trianthema portulacastrum* extracts

n-Hexane fraction (21 g) was subjected to fractionation by Vacuum LC using silica gel for column (90 g, 25 cm × 3.5 cm) eluting with petroleum ether (pet. ether) with increasing increments of ethyl acetate (EtOAc) and collecting 100 ml fractions. Fractions were monitored using TLC and *n*-hexane–EtOAc (8:2) solvent system. Similar fractions were pooled together to give three main subfractions. Subfraction 1 (300 mg) was chromatographed on a silica gel column using pet. ether-EtOAc system in 1% increments to isolate compound T2 (50 mg). Subfraction 2, (1.250 g) was similarly re-chromatographed to give compounds T3 and T4 mixture 400 mg white powder. Subfraction 3 (1.4 g) was re-chromatographed using silica gel column eluting with dichloromethane-methanol (DCM-MeOH) with 1% increments to give compound T5 (15 mg).

The ethyl acetate fraction (3 g) was chromatographed over silica gel using gradient elution technique with EtOAc-MeOH solvent system followed by chromatographic isolation over Sephadex LH-20 column using MeOH as eluent to afford compound T6 (10 mg), compound T7 (20 mg) and compound T1 (20 mg).

Chromatographic isolation of the main components of *Aizoon canariense* extracts

n-Hexane fraction (12 g) was similarly chromatographed as hexane fraction of *T. portulacastrum* to give two main fractions. Fraction A (228 mg) was re-chromatographed over the silica gel column using pet. ether-EtOAc in 1% increment till 8% to give compound A8 (25 mg). Fraction B (260 mg) was similarly rechromatographed on silica gel column to give compound A9 and A10 (100 mg) as a mixture.

EtOAc (3 g) and *n*-butanol extracts (3 g) were pooled and chromatographed on silica gel column using DCM-MeOH. Subfractions 15–20 were pooled and chromatographed using Sephadex LH-20 column to yield compounds A11 (20 mg) and A12 (10 mg).

Acid hydrolysis of compound T1

Acid hydrolysis of compound T1, silylation of the produced sugar part and GC-MS analysis and identification were performed according to Tewtrakul *et al*.[21]

RESULTS AND DISCUSSION

Cytotoxic activity testing of different extracts of *Trianthema portulacastrum* L. and *Aizoon canariense* L. against human hepatocellular carcinoma cell lines

Herein, two plants belonging to family Aizoaceae were tested for cytotoxic activity against HCC HepG2 cell lines. Results reveal the higher activity of the ethanol extract of *A. canariense* L (IC_{50} = 50.9 ± 6.3) when compared to the ethanolic extract of *T. portulacastrum* L (IC_{50} = 84.6 ± 7.9) [Table 1]. Tracing the cytotoxic activity in the successive fractions of each species indicates greater activity for the *n*-hexane, EtOAc and *n*-butanol extracts of *A. canariense* (24.7 ± 3.5, 93.8 ± 8.7 and 55.3 ± 4.9, respectively) than similar successive fractions of *T. portulacastrum* L (103 ± 8.4, 207 ± 19.8 and >500, respectively) [Table 1]. Many reports have discussed the anticancer activity of Aizoaceae plants. *T. portulacastrum* afforded considerable chemoprevention against breast cancer,[22] and *Sesuvium portulacastrum* against Ehrlich ascites carcinoma.[23]

Metabolomic analysis of different extracts of *Trianthema portulacastrum* and *Aizoon canariense*

Previous studies reported the isolation of variable compounds from *T. portulacastrum* L.[15] However, no reports were found discussing the metabolomics content of *A. canariense*. Herein, LC-HR-MS analysis for dereplication purposes was adopted for the identification of metabolites from different fractions of *T. portulacastrum* and *A. canariense*. The dereplication study of the metabolites against the DNP and METLIN databases resulted in the identification of 24 compounds from different extracts of *T. portulacastrum* and 12 compounds from successive extracts of *A. canariense* [Table 2]. Metabolites identified from different extracts represent different chemical skeletons such as; flavonoids, sterols, hydrocarbons, lignans, diterpenes, triterpenes, tetraterpenes, nucleosides and alkaloids, where flavonoids are the most predominant class in both plants. LC-HRMS profile of *T. portulacastrum* indicates the presence of ten flavonoids; (2',3,3',4'-Tetrahydroxychalcone-3'-methylether (2), sesuvioside c (6), leptorumol (7), 7,8-dimethoxyflavanone (10), 2',5,7-trihydroxyflavanone (12), 2',5-dihydroxy-7-methoxy-6,8-dimethylflavone (16), 2',4'-dihydroxychalcone 17, ferulic acid (19), astragalin (23), sesuvioside f (25), six sterols; stigmasta-5,22-diene-3,7,11-triol (1), stigmasta-5,25-diene-3,7-diol-3-ketone (3), β-sitosterol glycoside (13), 20-hydroxyecdysone (15), stigmast-7-en-3-ol (26) and β-sitosterol (27), three hydrocarbons; tridecane (20), 1-O-(6-deoxy-6-sulfoglucopyranosyl) glycerol-3-tetradecanoyl (22) and 1-eicosanol (24), one lignan; Apteniol B (5), one diterpene (18-Hydroxy-17-nor-3,16-aphidicolanedione (9), one triterpene; Cycloartanol (18), one tetraterpene; Trianthenol (21) and one alkaloid; 4'-O-Methylsceletenone (14) [Table 2 and Figure 1].

On the other hand, LC-HRMS profile of *A. canariense* showed the presence of four flavonoids; (Leptorumol [7], Isorhamnetin-7-(6-trans-feruloylglucoside) [8], 2',5-Dihydroxy-7-methoxy-6,8-dimethylflavone [16] and Sesuvioside F [25], three sterols; [stigmasta-5,22-diene-3,7,11-triol (1), Stigmasta-5,25-diene-3,7-diol-3-Ketone (3) and Stigmast-7-en-3-ol (26), one hydrocarbon; [1-Eicosanol (24), one glucide; 1,2,3-Butanetriol (4) one diterpene; 18-Hydroxy-17-nor-3,16-aphidicolanedione (9), one triterpene; Cycloartanol (18) and one nucleoside; Adenosine (11) Table 2 and Figure 1.

Table 1: IC_{50} (μg/mL) of the extracts of *Trianthema portulacastrum* Land *Aizoon canariense* L. against human hepatocellular carcinoma cell lines

Sample	IC_{50} (μg/mL)	
	T. portulacastrum L.	*A. canariense* L.
Total ethanol extract	84.6±7.9	50.9±6.3
n-Hexane	103±8.4	24.7±3.5
EtOAc	207±19.8	93.8±8.7
n-Butanol	>500	55.3±4.9

T. portulacastrum: Trianthema portulacastrum; A. canariense: Aizoon canariense; EtOAC: Ethyl acetate fraction

Structural elucidation of the isolated compounds

To isolate the main metabolites in the active fractions, different chromatographic procedures were adopted. Phytochemical investigation of hexane extract of *T. portulacastrum* L resulted in the isolation of four compounds (T2–T5), while hexane extract of *A. canariense* afforded three compounds (A8–A10). Meanwhile, the examination of EtOAc fraction of *T. portulacastrum* L. resulted in the isolation of compounds (T1, T6 and T7). TLC screening of EtOAc and *n*-butanol fractions of *A. canariense* showed the same spots. Accordingly, EtOAc and *n*-butanol fractions were pooled together and the pooled fractions of *A. canariense* yielded two compounds A11 and A12. The structure elucidation of the isolated compounds was performed, adopting different spectroscopic techniques. Among the isolated compounds; (T1) was isolated as a yellow powder with $[\alpha]_D^{20}$ +121 (*c* 0.01, $CHCl_3$). It displays UV spectrum similar to kaempferol-3-O-glycosides, UV $(MeOH)_{\lambda max}$ 327, 267, 213. HRMS spectrum of compound (T1) showed a molecular ion peak at m/z 786.6860 from which the molecular formula $C_{37}H_{38}O_{19}$ was deduced. ^{13}C NMR spectrum displayed 37 signals, among which 12 are attributable to two sugar moieties, 15 due to 3-*O*-glycosylated kaempferol and 10 due to feruloyl moiety [Table 3]. The spectrum displayed two anomeric carbons at δ_C 100 and 104.9, in addition to other signals characteristic for two glucopyranosyl units. ^1H-NMR spectrum displayed doublets at δ_H 4.76 and 5.05 attributable to two anomeric protons with coupling constant 7.6 Hz indicating β-configuration of both protons. Other signals at δ_H 6.77 (1 H, s), 6.66 (2 H, m), 6.04 (d, *J* = 16 Hz), 7.32 (d, *J* = 16 Hz) and 3.77 (3H, s) characteristic for feruloyl moiety. The coupling constant of the two doublets at δ_H 6.04 and 7.32 is calculated as 16 Hz, thus indicating trans configuration of feruloyl moiety [Table 2]. The important HMBC correlations are shown in Figure 2. HMBC spectrum displayed three important $^3J_{CH}$ correlations. First is the correlation between the anomeric proton at δ_H 5.05 (H-1″) and carbon signal at δ_C 133.7, indicating the attachment of the first glucose unit to the hydroxylated carbon (C-3) of the flavonoid moiety. Second is the correlation between δ_H 4.75 (H-1″) and carbon signal at δ_C 83.4 (C-2″) that proves the attachment of the terminal glucose unit to C2″ of the first glucose unit. This is further confirmed by the downfield shift of C2″ together with the upfield shift of C-1″. The third significant correlation between δ_H 4.47 (H-6″) and δ_C 167.7 confirms the attachment of feruloyl moiety to C-6″ of glucose unit.[24] Acid hydrolysis of T1 followed by derivatization was done according to Tewtrakul *et al.*[21] The sugar derivatives thus obtained are analyzed by GC. It shows a retention time of 22.26 min, identical with that of authentic D-glucose. Accordingly, (T1) is identified as kaempferol-3-*O*-(2"-O-β-D-glucopyranosyl)-6"-*O*-E-feruloyl-β-D-glucopyranosid.

Based Upon ^1H and ^{13}C NMR, mass spectral data and comparison with previously reported literature, compounds were identified as cycloartanol (T2),[25] β-sitosterol (T3),[26] stigmasterol (T4),[26] β-sitosterol glucoside (T5),[22] kaempferol-3-*O*-β-glucopyranoside (T6),[27] 20-hydroxyecdyson (7),[28] 1-eicosanol (A8),[29] spinasterol (A9),[30] 7-stigmastenol (A10),[30] isorhamnetin 3-*O*-β-glucopyranoside (A11),[31] and adenosine (A12).[32]

Table 2: The liquid chromatography-high-resolution-ESIMS Dereplication results of *Trianthema portulacastrum* L. and *Aizoon canariense* different fractions (*n*-hexane, ethyl acetate, *n*-butanol)

n	Metabolites name	Source	MF	RT (min)	*m/z*	Polarity	*T. portulacastrum* L.			*A. canariense* L.			References
							H	Et	Bu	H	Et	Bu	
1	Stigmasta-5,22-diene-3,7,11-triol	*A. yunnanensis*	$C_{29}H_{48}O_3$	2.79	444.3603	P		+	+			+	[35]
2	2',3,3',4'-Tetrahydroxychalcone-3'-Me ether	*G. africana*	$C_{16}H_{14}O_5$	2.89	286.0841	P		+	+				[36]
3	Stigmasta-5,25-diene-3,7-diol-3-Ketone	*A. relicta*	$C_{29}H_{46}O_2$	2.94	426.3497	P		+	+			+	[37]
4	1,2,3-Butanetriol	*C. ajowan*	$C_4H_{10}O_3$	2.98	106.0629	N						+	[38]
5	Apteniol F	*A. cordifolia*	$C_{19}H_{20}O_6$	3.55	344.1259	N		+	+				[39]
6	Sesuvioside C.	*S. portulacastrum*	$C_{29}H_{34}O_{17}$	3.72	654.1796	N		+					[40]
7	Leptorumol	*L. miqueliana* and from *T. portulacastrum*	$C_{11}H_{10}O_4$	3.76	206.0579	N		+	+		+		[41]
8	Isorhamnetin 7-(6-trans-feruloylglucoside)	*S. aizoon*	$C_{31}H_{28}O_{14}$	3.85	624.2471	P					+	+	[42]
9	18-Hydroxy-17-nor-3,16-aphidicolanedione	*Aizoon canariens*	$C_{19}H_{28}O_3$	4.00	304.2038	N		+	+		+		[43]
10	7,8-Dimethoxyflavanone	*T. expansa*	$C_{17}H_{16}O_4$	4.03	284.1048	P		+	+				[44]
11	Adenosine	*C. ajowan*	$C_{10}H_{13}N_5O_4$	4.07	267.0967	N					+	+	[38]
12	2',5,7-Trihydroxyflavanone	*G. Africana*	$C_{15}H_{12}O_5$	4.26	272.0684	P		+					[36]
13	*β*-sitosterol glycoside	*T. portulacastrum*	$C_{35}H_{60}O_6$	4.29	576.855	N	+						[41]
14	4'-O-Methylsceletenone.	*A. cordifolia*	$C_{16}H_{19}NO_2$	4.55	257.1415	P	+	+					[45]
15	20-hydroxyecdysone	*T. portulacastrum*	$C_{27}H_{44}O_7$	4.72	480.3087	N		+	+				[46]
16	2',5-Dihydroxy-7-methoxy-6,8-dimethylflavone.	*T. portulacastrum*	$C_{18}H_{16}O_5$	4.77	312.0997	N		+	+		+		[39]
17	2',4'-Dihydroxychalcone	*G. africana*	$C_{15}H_{12}O_3$	5.02	240.0786	P		+					[34]
18	Cycloartanol	*P. vulgare*	$C_{30}H_{52}O$	5.03	428.4018	P	+			+			[25]
19	Ferulic acid	*T. portulacastrum*	$C_{10}H_{10}O_4$	5.54	194.0579	P		+					[47]
20	Tridecane	*S. portulacastrum*	$C_{13}H_{28}$	6.10	184.2191	P	+						[48]
21	Trianthenol	*T. portulacastrum*	$C_{40}H_{78}O$	6. 34	574.6052	P	+						[49]
22	1-O-(6-Deoxy-6-sulfoglucopyranosyl) glycerol-3-Tetradecanoyl	*T. expansa*	$C_{23}H_{44}O_{11}$	6.37	528.2604	P		+	+				[50]
23	Astragalin	*P. vulgaris*	$C_{21}H_{20}O_{11}$	6.39	448.1005	P		+					[27]
24	1-Eicosanol	*G. glabra*	$C_{20}H_{42}O$	6.40	298.3235	P	+			+			[51]
25	Sesuvioside F	*S. portulacastrum*	$C_{36}H_{46}O_{21}$	6.62	814.2531	N		+	+		+	+	[40]
26	Stigmast-7-en-3-ol	*P. vulgaris*	$C_{29}H_{50}O$	6.78	414.3861	P	+			+			[30]
27	B-sitosterol	*T. portulacastrum*					+			+			[41]

MF: Molecular formula; RT: Retention time, H: *n*-hexane fraction; EtOAC: Ethyl acetate fraction; B: *n*-butanol fraction, +: Present. *A. yunnanensis*: *Amoora yunnanensis*; *G. africana*: *Galenia Africana*; *A. relicta*: *Ajuga relicta*; *C. ajowan*: *Carum ajowan*; *A. cordifolia*: *Aptenia cordifolia*; *S. portulacastrum*: *Sesuvium portulacastrum*; *L. miqueliana*: *Leptorumohra miqueliana*; *T. portulacastrum*: *Trianthema portulacastrum*; *S. aizoon*: *Sedum aizoon*; *T. expansa*: *Tetragonia expansa*; *C. ajowan*: *Carum ajowan*; *P. vulgare*: *Polypodium vulgare*; *S. portulacastrum*: *Sesuvium portulacastrum*; *T. expansa*: *Tetragonia expansa*; *P. vulgaris*: *Phaseolus vulgaris*; *G. glabra*: *Glycyrrhiza glabra*; *P. vulgaris*: *Prunelia vulgaris*

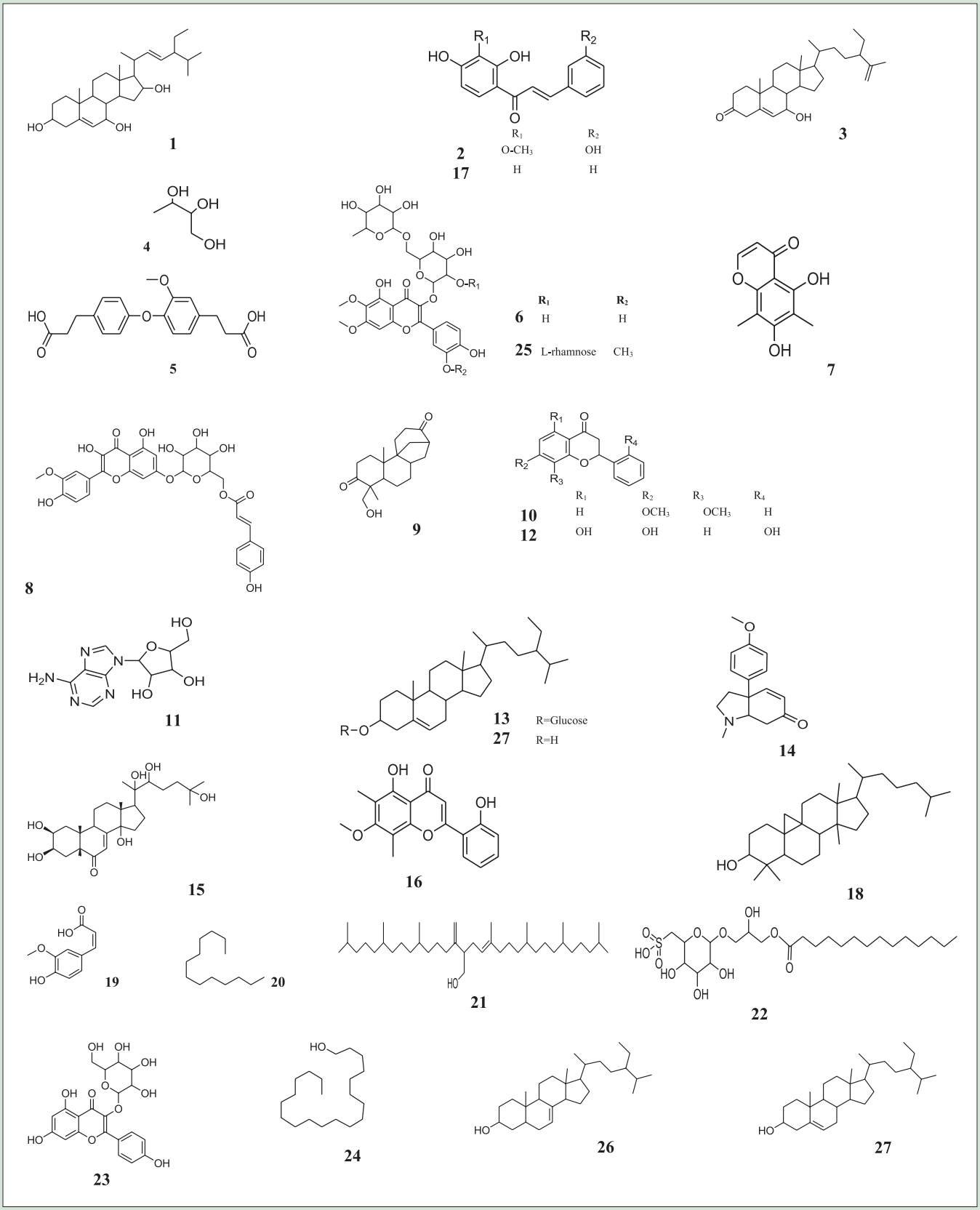

Figure 1: Dereplicated metabolites from LC-HR-ESIMS analysis of *Trianthema portulacastrum* and *Aizoon canariens* different fractions

Table 3: ^{1}HNMR and ^{13}CNMR (CD_3OD) spectral data of compound (1)

Carbon number	Carbon	Proton HSQC
2	157.3	-
3	133.8	-
4	178.5	-
5	161.4	-
6	98.6	6.15
7	164.3	-
8	93.5	6. 18
9	156.7	-
10	104.3	-
1'	121.1	-
2'	131.3	8.03 (d, *J*=8.4Hz)
3'	114.96	6.91 (d, *J*=8.4Hz)
4'	160.2	-
5'	114.96	6.91 (d, *J*=8.4Hz)
6'	131.3	8.03 (d, *J*=8.4Hz)
1''	100.0	5.05 (d, *J*=7.6 Hz)
2''	83.4	3.72
3''	74.7	3.56
4''	69.3	3.4
5''	76.2	3.5
6''	63.1	4.47, 5.43
1'''	104.9	4.76 (d, *J*=7.6 Hz)
2'''	74.3	3.66
3'''	76.3	3.49
4'''	70.4	3.67
5'''	76.7	3.11
6'''	60.6	3.58, 3.7
1''''	125.8	-
2''''	109.7	6.77 (1 H, s)
3''''	147.6	-
4''''	148.95	-
5''''	114.87	6.66 (2 H, m)*
6''''	122.5	6.66 (2 H, m)*
7''''	145.5	7.32 (d, *J*=16 Hz)
8''''	113.4	6.04 (d, *J*=16 Hz)
9''''	167.7	-
OCH3	54.8	3.77 (3H, s)

*Overlapped signals

Cytotoxic activity testing of the isolated compounds

Testing the isolated compounds for the cytotoxic activity against HepG2 cell lines indicated significant activity of the new compound (T1) (IC_{50} = 7.19 ± 0.27 µg/mL). Moderate activity was observed for Isorhamnetin-3-*O*-glucopyranoside (A11), Astragalin (T6) and 20-hydroxyecdysone (T7) (IC_{50} = 28.1 ± 2.7, 30.7 ± 2.3, and 76.5 ± 4.9 µg/mL, respectively). Isorhamnetin-3-*O*-glucopyranoside was previously reported to exhibit cytotoxic activity against MCF-7 breast cancer cell lines with IC_{50} = 28.1 µg/mL,[33], while, Astragalin was stated to suppress the proliferation of HCC cells.[34] In addition, weak activity is observed for β-sitosterol-3-*O*-glucoside (5) (IC_{50} = 251 µg/mL) and no activity is observed with cycloartanol (T2), β-sitosterol + stigmasterol (3 + 4) mixture, 1-octadecanol (8), spinasterol + 7-sigmastenol (9 + 10) mixture and adenosine (12) against HepG2 cell lines [Table 4]. It is noteworthy that this is the first report for the isolation and evaluation of the cytotoxic potential of kaempferol-3-*O*-(2”-*O*-β-D-glucopyranosyl)-6”-*O*-E-feruloyl-β-D-glucopyranosid (T1).

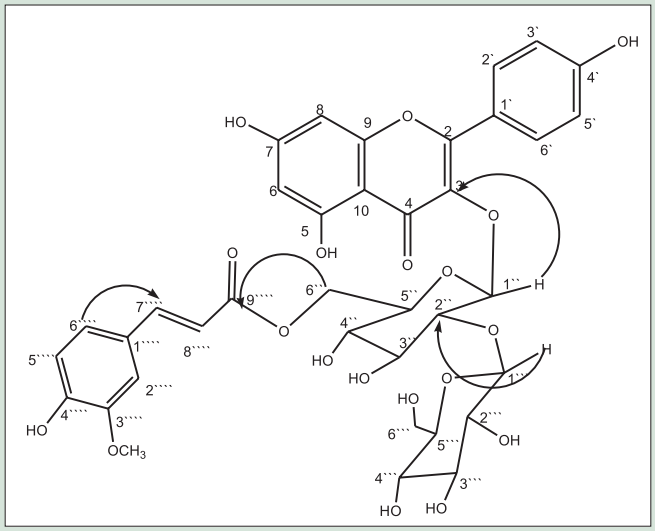

Figure 2: Important HMBC correlations of compound T1

Table 4: IC_{50} (µg/mL) of isolated compounds of *Trianthema portulacastrum* Land *A. canariense* L. against human hepatocellular carcinoma cell lines

Compound	IC_{50} (µg/mL)
kaempferol-3-O-(2”-O-β-D-glucopyranosyl)-6”-O-E-feruloyl-β-D-glucopyranosid. (T1)	7.19±0.27
Cycloartanol (T2)	>500
β-sitosterol+stigmasterol (T3+T4)	>500
β-sitosterol-3-O-glucoside (T5)	251
Astragalin (T6)	30.7±2.3
20-hydroxyecdysone (T7)	76.5±4.9
1-Eicosanol (A8)	>500
Spinasterol+7-sigmastenol (A9+A10)	>500
Isorhamnetin3-O-glucopyranoside (A11)	28.1±2.7
Adenosine (A12)	>500

CONCLUSION

The present study reports the cytotoxic activity of two plants belonging to family Aizoaceae against HCC HepG2 cell lines. Results reveal the higher activity of the ethanol, n-hexane, EtOAc and n-butanol extracts of *A. canariense* L, when compared to similar extracts of *T. portulacastrum* L. Metabolomic analysis of successive fractions of both plants shows the presence of several chemical classes, among them flavonoids are the most prominent in both plants. Interestingly, testing the isolated compounds for the cytotoxic activity indicates the significant activity of the new compound kaempferol-3-*O*-(2"-*O*-β-D-glucopyranosyl)-6"-*O*-E-feruloyl-β-D-glucopyranosid.

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Conflicts of interest

There are no conflicts of interest.

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