Chemical and biological investigation of the aerial parts of *Aizoon canariense* L

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Abstract:

Purpose: Herbal plants contain a great diversity of bioactive compounds with multiple targets and mechanisms of action for treating different types of cancers. So, the aim of this study to investigate antimicrobial, antitumor potential and chemical composition of the aerial parts of *Aizoon canariens*e. **Methods**: Chemical profiling of methanol extract was determined by LC-ESI-TOF/MS technique. Isolation chemical constituents of ethyl acetate extract was carried out by chromatographic methods, identification and structure elucidation of these compounds was proven by spectroscopic methods. In-vitro antimicrobial activity was determined by diffusion agar technique according to CLSI, while cytotoxic effects were evaluated by the MTT assay method. **Results**: Sixty-one compounds included stilbene derivative, phenolic acids, flavonoids, and alkaloids were identified from methanol extract, in addition to six compounds were isolated and identified from ethyl acetate extract named cholic acid, syringetin, gossypetin, okanin-4'-glucosides, myrictrin, and datiscin. *In vitro* pharmacological studies showed the potency of ethyl acetate extract as antimicrobial agent against all tested gram-negative bacteria, and as antitumor agent against all tested carcinoma cell lines (hepatic, colon, and prostate) with safety influence on normal cell line. **Conclusion**: the results of this study and literature promote the efficiency of *Aizoon canariense* as potent antimicrobial and antitumor agent.

Keywords: Phytochemical constituents, *Aizoon canariense,* LC-Ms, Antimicrobial activity, Antitumor activity.

INTRODUCTION:

There is no doubt that, natural products are important sources for new pharmaceutical compounds, where terrestrial plants have been subjected to chemical and pharmacological screening to evaluate their potential as drugs in medicine. Plant kingdom involved wide families which have nemours therapeutic activity as anti-inflammatory, anti-hypertensive, anti-diabetic, anti-tumor, anti-viral and antimicrobial activity …...etc. Aizoaceae family comprises 143 genera with about 2300 species (Heywood, 1993). *Aizoon canariense* L is prostrate, thick-stemmed annual or perennial herb, with stems up to 40 cm long. Leaves subcircular to obovate, entire, decurrent at base, pilose. Flowers are solitary and sessile, perianth segments yellowish inside, greenish or reddish and pilose outside. Fruits are red or pink, star-shaped, depressed in the middle (Boulos, 1999). It was traditionally used to treat gastrointestinal problems and as a hypotensive (Al-Laith et al., 2019). In addition, it had antitumor activity against liver carcinoma (Abuzaid et al., 2020) and moderate antioxidant and antibacterial activities (El-Amier et al., 2016; Al-Laith et al., 2019). Currently, the prevalence of cancer is estimated at 12.7 million people in 2008 and is expected to rise to 21.4 million by 2030 (Ferlay et al., 2010). As we interest with the evaluation of biological and chemical properties of the phytomedicine and discovering new alternative drug extracted from the herbal plants with more activity, low cost, and no side effects (El-Desouky et al., 2019; Atta et al., 2019; Abdelgawad et al., 2021; Abdelgawad and El-Bassossy, 2021; El-Bassossy, 2022). Moreover, the. Limitation of chemical and biological studies performed on *A. canariense* especially, limitation data of phenolic constituents and their biological role in combating some types of diseases like cancers. So, the current study aimed to look at the chemical constituents of *A. canariense* plant, as well as assess their biological potential as antimicrobial and anticancer agents.

MATERIAL AND METHODS:

Plant material:

Aerial parts of *A. canariense* (Family: Aizoaceae) were collected from their wild habitat in Wadi Dai'eb, Gebel Elba region, Halieb, Egypt in May 2019. The plant samples were identified and

authenticated by Dr. Omran Ghaly, Desert Research Center. A voucher herbarium sample was deposited in the herbarium of Desert Research Center (CAIH) with Code Number: CAIH-1022-R.

Preparation of Extracts:

The air-dried powdered of the *A. canariense* (1 kg) were extracted by soaking in 80% methanol for 72 h then, filtered off, the marc lifted was re-extracted by the same way several times (6×3L) till exhaustion, then the filtrate was concentrated in vacuum at 40 °C to afford a sticky dry extract 140 g, then the crude extract was dissolved in little amount of water (500 ml) and fractionated successively by separating funnel with increasing polarity using n-hexane, ethyl acetate and methanol to afford finally (34g, 18g, 29g), respectively. The crude methanolic extract was subjected to liquid chromatography–mass spectrometry (LC–ESI-TOF–MS) analysis. The ethyl acetate (EA) fraction was applied to silica gel (60-120 mesh) column chromatography.

LC–ESI-TOF–MS Analysis:

The phytochemical investigation of flavonoids, alkaloids, phenolic and other compounds for *A. canariense* aerial parts was not previously studied. So, in this study, chemical profiling was achieved by LC-ESI-TOF/MS technique, which supplied us with valuable data about this plant. The non-targeting small molecule comprehensive analysis of large-scale plant metabolomics that depend on liquid chromatography coupled with electrospray ionization tandem mass spectrometry (LC-ESI-TOF/MS) is considered a ubiquitous technique for recognition of different natural products in plants as it can provide us with information about molecular mass, molecular fragmentation, molecular formula, the relative abundance of different components, as well as determining the type of glycosides and position of substituents.

Phytochemical Isolation:

The EA fraction (18 g) was subjected to column chromatography (CC) on silica gel (60-120 mesh) stationary phase by wet method packing and eluted with *n*-hexane: EA with gradually increasing polarity till 100% MeOH to afford 43 sub-fractions (E1-E43), each fraction 200 ml. Similar fractions were collected according to paper chromatography (PC) manner using system (BAW 4:1:

5). Sub-fractions (E1-E6) by eluting system (n-hexane: EA 8: 2) were collected and applied to preparative thin layer chromatography (PTLC) with dissolving system (n-hexane: EA 8.5: 1.5), then subjected to Sephadex CC to give one major compound **1** (27 mg). The combined sub-fractions (E9-E14) were subjected to (PPC) using system (BAW 4:1:5 upper layer) showed two major flavonoid spots, which purified on column Sephadex LH-20 CC using methanol as eluting system to afford compounds **2** and **3** (38 and 29 mg), respectively. Also, fractions (E17-E23) were collected and applied to column (EA/MeOH 7:3) which afford sub-fraction, applied to (PPC) (BAW 6:1:1) showed one major chalcon spot which applied to further purification on Sephadex column to give compound **4**. Further isolation of combined (E26-E43) fractions by sub-column polyamide with an eluting system (EA: MeOH: H_2O 30:5:4) to afford sub-sub-fractions ($E27_{1a}$-$E27_{5a}$), then applied to PPC 6% AcOH to show containing one major compound, further purification was performed to finally give compounds **5** (33 mg). Collected sub-sub-fractions ($E38_{1b}$- $E38_{7b}$), applied to polyamide sub-column (MeOH: H_2O 7:3) give one major spot on TLC (MeCOMe-butanone-HCOOH 10:7:1) to show containing one major flavonoid di-glycosides, compound **6** (27 mg). Identification and structure elucidation of the purified compounds were proven by R_f values in PC, spectral data UV, Ms, ^{1}H-NMR and ^{13}C-NMR. The sugar moieties were identified by partial and complete acid hydrolysis using PC with authentic samples and HPLC. TLC analysis was carried out using silica gel 60 F_{254} plates (Merck). For column chromatography was performed using silica gel (60-120 mesh), polyamide and Sephadex column.

Antimicrobial activity

Antimicrobial activity was determined by diffusion agar technique at RCMB according to CLSI (CLSI, 2004; 2012). The gram-positive bacteria [*Staphylococcus aureus* ATCC 25923 and *Bacillus subtilis* RCMB 015 (1) NRRL B-543] while, the gram-negative bacteria [*Proteus vulgaris* RCMB 004 (1) ATCC 13315 and *Escherichia coli* ATCC 25922]. The fungal strains: [*Candida albicans* RCMB 005003 (1) ATCC 10231and *Aspergillus fumigatus* RCMB 002008] were obtained from the bacteria stock present at RCMB. The evaluation of antimicrobial activity of hexane, ethyl

acetate and methanol extracts of *A. canariense* were based on the measurement of the diameter of the inhibition zone formed around the well according to method described in (CLSI, 2004; 2012). The positive control used for fungi was ketoconazole with MIC 100 mg/ml, while positive control used for bacteria strains was gentamycin with MIC 4 mg/ml.

Antitumor activity (Viability assay)

The human hepatic (HepG2), colon (HCT-116), prostate (PC-3) carcinoma cells and human lung cell (WI-38) were obtained in a frozen state under liquid nitrogen (-180°C) from the American Type Culture Collection. The tumor cell lines were conserved by serial sub-culturing in RCMB. The antitumor effect of hexane, EA and MeOH extracts of *A. canariense* were evaluated in the (RCMB) according to the MTT assay method (Mosmann, 1983). Briefly, cells were seeded in 96 well plates at a density of 5000 cells/well in the 100 µl culture medium. Following 24 h incubation, cells were treated with various concentrations of chloroform and ethyl acetate extracts and then incubated for 24 h at 37°C with 5% CO_2. After incubation, the medium was replaced with 100 µL of MTT solution prepared fresh as 0.5 mg/ml in Dulbeccos Modified Eagles medium (DMEM), filtered through 2 µm filter, then it was added to each well, and the plates were incubated in the dark for 4 h at 37 °C. Then, the media were removed and 200 µl of dimethylsulphoxide (DMSO) was added to each well and absorbance was measured at 570 nm using a microplate reader. The results are expressed as the percentage of cell viability in comparison with the control cells (Cells without extracts). The cell viability of the control group without exposure to the extracts was defined as 100%.

RESULTS AND DISCUSSION

Identification of chemical compounds of MeOH extract using LC-Ms

Negative and positive modes of ESI masses were used in the identification of main 61 compounds in *A. canariense* methanolic extract. The identified compounds included phenolic carboxylic acids (8), sterols (1), terpens (2), flavonoids (33), quinolin and coumarine derivatives (2), stilbene derivative (1), alkaloids (8), flavins (1) and anthocyanin compounds (5). The presented data

indicated that flavonoid compounds represented the major abundance followed by alkaloid, phenolic acids, and anthocyanin compounds. Trigonelline, Peonidin-3,5-*O*-di-beta-glucopyranoside, Apigenin-8-C-glucoside represented the highest percentage for alkaloids, anthocyanin and flavonoid compounds, respectively about 9.13, 7.86 and 7.43 %. The outlined findings arranged according to their RT. The MS-DIAL 3.70 open-source software (Tsugawa et al, 2015) was used in the identification of products where ReSpect positive (2737 records) or ReSpect negative (1573 records) databases were used as the reference database. The relative percentages of the identified compounds in the plant extracts were calculated based on the total peak area in the chromatogram recorded in Table 1.

Table 1: Chemical compounds identified from methanol extract of *A. canariense* using LC-ESI-TOF-MS

No	RT (min)	%	Adduct ion	MS fragmentation *m/z*	Formula	Identification
1	1.02	0.12	$[M-H]^-$	154.9, 111.04	$C_5H_4N_2O_4$	Orotic acid
2	1.04	0.22	$[M-H]^-$	285.1, 239.04	$C_{15}H_{10}O_6$	Luteolin
3	1.07	0.09	$[M-H]^-$	178.9, 163.0, 145.01, 135.1, 107.1	$C_9H_8O_4$	Caffeic acid
4	1.08	0.20	$[M-H]^-$	300.9, 282.4, 232.9, 167.03, 118.06	$C_{15}H_{10}O_7$	Quercetin
5	1.10	4.78	$[M-H]^-$	117.0, 99.0, 73.03	$C_4H_6O_4$	succinic acid
6	1.10	0.27	$[M+H]^+$	319.1, 301.1, 229.1, 214.0, 139.0, 137.0	$C_{18}H_{22}O_5$	Zearalenone
7	1.16	0.29	$[M-H]^-$	317.05, 281.09, 279.10, 249.00, 191.05	$C_{15}H_{10}O_8$	Myricetin
8	1.19	0.62	$[M-H]^-$	115.08, 71.017	$C_4H_6O_5$	D-(+)-Malic acid
9	1.21	1.66	$[M-H]^-$	153.01, 109.02, 94.9, 92.92, 80.97	$C_7H_6O_4$	3,4-dihydroxybenzoic acid
10	1.33	9.13	$[M+H]^+$	138.05, 94.06, 93.05, 78.05, 65.04	$C_7H_7NO_2$	Trigonelline
11	1.38	0.35	$[M+H]^+$	81.02, 53.03	$C_4H_4N_2$	Pyrimidine
12	1.40	1.66	$[M+H]^+$	215.05, 156.0, 137.1, 128.0, 116.06, 70.06	$C_{13}H_{14}N_2O$	Harmaline
13	1.48	0.28	$[M+H]^+$	85.02	$C_2H_4N_4$	3-Amino-1,2,4-triazole
14	1.71	2.08	$[M+H]^+$	124.07, 80.04, 78.03, 53.03	$C_6H_5NO_2$	Nicotinic acid
15	1.73	1.05	$[M-H]^-$	419.15, 376.19, 257.14, 239.09, 202.88	$C_{21}H_{24}O_9$	E-Isorhapontin
16	1.85	0.46	$[M+H]^+$	179.11, 163.1, 151.08, 133.0, 119.05, 85.03	$C_9H_6O_4$	Daphnetin
17	2.32	0.20	$[M-H]^-$	188.03, 144.04, 118.02	$C_{10}H_7NO_3$	Kynurenic acid
18	2.52	1.86	$[M-H]^-$	407.1, 345.1, 305.1 259.1, 263.14, 125.02, 99.04, 57.03	$C_{24}H_{40}O5$	Cholic acid
19	3.01	5.45	$[M-H]^-$	137.01, 93.03, 65.0	$C_7H_6O_3$	P-Hydroxybenzoic acid
20	3.86	0.35	$[M-H]^-$	359.17, 313.1, 228.9, 223.1, 151.0, 119.01	$C_{18}H_{16}O_8$	rosmarinic acid
21	4.54	0.81	$[M+H]^+$	283.09, 179,10, 171.09, 146.95, 135.07, 73.03	$C_{16}H_{12}O_5$	Acacetin
22	4.66	0.21	$[M-H]^-$	135.09, 95.02	$C_{10}H_{16}$	Gamma-terpinene
23	5.01	1.21	$[M+H]^+$	481.1, 462.07, 449.07, 389.05, 359.03, 317.02, 163.06, 73.02, 3.01	$C_{21}H_{20}O_{13}$	Gossypin
24	5.07	1.01	$[M-H]^-$	339.1, 207.07, 186.02, 145.02, 119.05	$C_{15}H_{16}O_9$	Esculin
25	5.12	6.03	$[M-H]^-$	315.1, 269.1, 257.1, 255.03, 246.89, 169.08	$C_{16}H_{12}O_7$	3'-methoxy-4',5,7-trihydroxyflavonol
26	5.19	0.54	$[M+H]^+$	271.09, 167.02, 152.01, 131.04, 124.01	$C_{15}H_{10}O_5$	Genistein
27	5.58	0.38	$[M-H]^-$	593.18, 570.67, 547.15, 487.2, 447.1, 392.9	$C_{27}H_{30}O_{15}$	Kaempferol-7-neohesperidoside

28	5.73	0.64	$[M-H]^-$	447.02, 429.07, 402.86, 357.05, 327.04, 394.9, 247.08, 189.03, 119.03	$C_{21}H_{20}O_{11}$	Luteolin-8-C-glucoside
29	5.77	2.19	$[M-H]^-$	417.13, 371.16, 285.92, 209.07	$C_{20}H_{18}O_{10}$	Kaempferol-3-O-alpha-L-arabinoside
30	6.05	1.23	$[M+H]^+$	449.1, 431.0, 395.08, 353.0, 329.07, 299.05, 95.08	$C_{21}H_{20}O_{11}$	Luteolin-6-C-glucoside
31	6.21	0.99	$[M+H]^+$	377.12, 359.1, 257.0, 243.0, 212.0, 172.0, 155.0, 115.0, 43.0	$C_{17}H_{20}N_4O_6$	(-)-Riboflavin
32	6.25	0.83	$[M-H]^-$	461.1, 435.1, 400.8, 392.9, 324.9, 256.9	$C_{21}H_{18}O_{12}$	Kaempferol-3-Glucuronide
33	6.30	2.21	$[M+H]^+$	447.16, 429.07, 312.12, 269.04, 177.05, 145.02, 90.03, 60.02, 29.0	$C_{21}H_{18}O_{11}$	Baicalein-7-O-glucuronide
34	6.41	0.77	$[M+H]^+$	287.1, 255.1, 273.1, 211.1, 193.1, 147.1, 121.1, 91.05	$C_{16}H_{14}O_5$	4',5-dihydroxy-7-methoxyflavone
35	6.44	1.98	$[M-H]^-$	593.1, 447.0, 284.0	$C_{27}H_{30}O_{15}$	Datiscin
36	6.54	0.30	$[M]^+$	595.2, 4449.1, 433.1, 287.0	$C_{27}H_{31}O_{15}$	cyanidin-3-O-rutinoside
37	6.60	1.60	$[M+H]^+$	579.2, 433.1, 415.04, 397.1, 337.07, 313.06, 283.05	$C_{27}H_{30}O_{14}$	Vitexin-2''-O-rhamnoside
38	6.92	3.98	$[M-H]^-$	577.15, 430.09, 431.09, 285.03	$C_{27}H_{30}O_{14}$	Kaempferol-3,7-O-bis-alpha-L-rhamnoside
39	7.06	2.61	$[M-H]^-$	415.1, 319.12, 269.1, 248.9,	$C_{21}H_{20}O_9$	Daidzein-8-C-glucoside
40	7.16	2.04	$[M-H]^-$	447.07, 402.90, 383.23, 365.14, 285.03, 255.02	$C_{21}H_{20}O_{11}$	Luteolin-7-O-glucoside
41	7.22	0.18	$[M+H]^+$	465.1, 448.1, 303.1, 163.0, 134.05, 102.03, 83.08, 31.01	$C_{21}H_{20}O_{12}$	Hyperoside
42	7.50	0.30	$[M-H]^-$	477.09, 431.22, 364.82, 331.04, 315.04, 300.04	$C_{22}H_{22}O_{12}$	Isorhamnetin-3-O-glucoside
43	7.61	2.93	$[M+H]^+$	609.1, 463.1, 301.04, 286.0, 177.0, 147.06, 117.05, 43.01	$C_{28}H_{32}O_{15}$	Diosmin
44	7.68	0.26	$[M]^+$	449.2, 287.05, 269.1, 234.2, 192.1, 137.05, 85.02	$C_{21}H_{21}O_{11}$	Cyanidin-3-glucoside
45	7.70	0.50	$[M-H]^-$	430.0, 431.09, 362.88, 294.89	$C_{21}H_{20}O_{10}$	Kaempferol-3-O-alpha-L-rhamnoside
46	7.72	2.14	$[M+H]^+$	301.1, 223.07, 164.05, 153.00, 123.07	$C_{16}H_{12}O_6$	3 5 7-trihydroxy-4'-methoxyflavone
47	7.76	1.11	$[M+H]^+$	130.06, 112.07, 85.06, 71.06, 42.03	$C_4H_{11}N_5$	Metformin
48	7.80	0.21	$[M]^+$	479.1, 462.1, 417.08, 387.07, 316.05, 88.01, 59.01, 29.00	$C_{22}H_{23}O_{12}$	Petunidin-3-O-beta-glucopyranoside
49	8.13	7.43	$[M+H]^+$	433.1, 283.1, 161.02, 117.03,	$C_{21}H_{20}O_{10}$	Apigenin 8-C-glucoside
50	8.20	0.27	$[M]^+$	493.1, 475.14, 331.0, 329.07, 326.06	$C_{23}H_{25}O_{12}$	Malvidin-3-galactoside
51	8.66	0.73	$[M+H]^+$	146.06, 118.06, 91.05, 77.03	$C_9H_7N_O$	3-FORMYLINDOLE
52	8.28	0.41	$[M-H]^-$	577.28, 508.8, 470.9, 313.06	$C_{30}H_{26}O_{12}$	Procyanidin B2
53	9.01	0.14	$[M+H]^+$	593.1, 575.3, 447.13, 285.07, 270.0	$C_{28}H_{32}O_{14}$	Acacetin-7-O-rutinoside
54	9.49	0.37	$[M+H]^+$	509.14, 347.1, 298.04, 239.0	$C_{23}H_{24}O_{13}$	Syringetin-3-O-galactoside
55	11.03	1.47	$[M-H]^-$	595.14, 549.29, 501.1, 433.14, 417.2, 298.1, 296.1	$C_{27}H_{32}O_{15}$	Eriodictyol-7-O-neohesperidoside
56	11.36	7.86	[M-	623.23, 460.17, 297.11	$C_{28}H_{33}O_{16}$	Peonidin-3,5-O-di-beta-glucopyranoside

			$2H]^-$			
57	11.93	2.04	$[M+H]^+$	269.08, 254.05, 226.05, 152.0, 124.01	$C_{16}H_{12}O_4$	Formononetin
58	12.25	0.56	$[M-H]^-$	269.0, 254.04, 106.0	$C_{15}H_{10}O_5$	Apigenin
59	20.07s	3.72	$[M+H]^+$	317.12, 286.04, 258.05, 197.04, 168.04, 138.0, 94.04	$C_{16}H_{12}O_7$	3 3' 4' 5-tetrahydroxy-7-methoxyflavone
60	23.16	3.08	$[M-H]^-$	449.1, 342.9, 287.0, 276.9, 151.0, 135.04, 117.03	$C_{21}H_{22}O_{11}$	Okanin-4'-O-glucoside
61	23.58	1.60	$[M-H]^-$	463.1, 403.15, 317.10, 301.09, 208.08, 194.03, 150.05	$C_{21}H_{20}O_{12}$	Myricitrin

Isolation and Identification of chemical constituents using column chromatography:

Cholic acid 1: Isolated as colorless amorphous powder, exhibited $[M+H]^+$ at *m/z*= 409; R_f: 0.21 BAW, 0.3 6% AcOH; Uv λ_{max} MeOH nm: 274.

Table 2: ^{1}H-NMR (CD_3OD, 400 MHz) and ^{13}C-NMR (CD_3OD, 100 MHz) assignments of cholic acid

C atom	^{1}H-NMR α	^{1}H-NMR β	^{13}C-NMR	C atom	^{1}H-NMR α	^{1}H-NMR β	^{13}C-NMR
1	1.91	1.01	36.69	13	----	----	49.22
2	1.25	1.66	32.15	14	1.82	-----	45.33
3	----	3.48 (1H, tt, *J*= 4.5, 12 Hz)	74.51	15	1.71	1.14	25.76
4	2.07	1.71	42.18	16	1.95	1.35	30.04
5	----	1.34	44.12	17	1.72	----	49.39
6	1.65	1.89	37.02	18	0.90 (3H, s)		15.03
7	----	3.89 (1H, d, *J*=2.5 Hz)	71.12	19	0.91 (3H, s)		25.12
8	----	1.57	42.08	20	1.39 (1H, dt, *J*= 2.0, 6.5 Hz)		27.98
9	2.21	----	29.17	21	1.02 (3H, d, *J*= 7.5 Hz)		20.21
10	----	----	37.24	22	1.74 (1H, dt, *J*= 2.0, 6.5 Hz)		35.82
11	1.59	1.59	31.02	23	2.24 (2H, t, *J*= 2.5		37.61
12	-----	3.84 (1H, t, *J*= 4.5 Hz)	76.01	24	-----		178.24

Syringetin 2: Was isolated as a yellow amorphous powder, exhibited $[M+H]^+$ at *m/z*= 347; R_f : 0.73 BAW, 0.4 15% AcOH; UV λ_{max} MeOH nm: 253, 263, 306, 370; +NaOAc: 255, 263, 324, 375; +NaOAc+H_3BO_3: 252, 270, 377; +$AlCl_3$: 264, 305, 354, 423; ^{1}H-NMR (CD_3OD, 400 MHz) δ 6.02 (1H, d, J=2.1 Hz, H-6), 6.37 (1H, d, J=2.1 Hz, H-8), 7.1 (2H, s, H-2` and H-6`), 3.79 (6H, s, OCH_3); ^{13}C-NMR (CD_3OD, 100 MHz) δ 151.1 (C-2), 136.5 (C-3), 175.7 (C-4), 161.8 (C-5), 97.3 (C-6), 166.7 (C-7), 93.5 (C-8), 158.8 (C-9), 105.5 (C-10), 123.7 (C-1` ), 103.2 (C-2`), 148.6 (C-3` ), 136.4 (C-4`), 148.6 (C-5` ), 103.6 (C-6`), 56.1 (C- OCH_3).

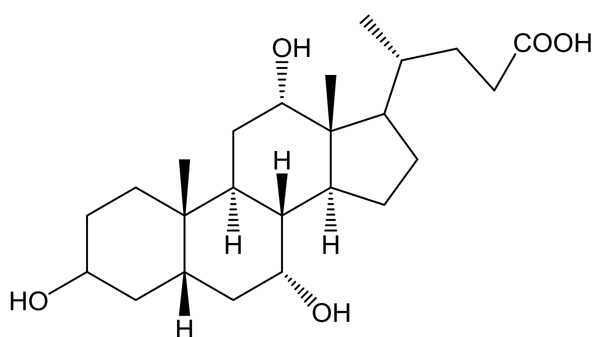
Gossypetin 3: Amorphous yellow powder, it showed $[M-H]^-$ at *m/z*= 319; R_f : 0.78 BAW, 0.4 15% AcOH; UV λ_{max} MeOH nm: 260, 275, 306, 335; + NaOMe: 250, 286, 364; +$AlCl_3$: 289, 326,

402, 490; +NaOAc: 280, 364; +NaOAc+H_3BO_3: 274, 280, 315, 360, 403; ^{1}H-NMR (CD_3OD, 400 MHz); δ 6.3 (1H, s, H-6), 7.3 (1H, d, J=2.4 Hz, H-2`), 6.9 (1H, d, J=7.5 Hz, H-5`), 7.8 (1H, dd, J= 2.4, 7.5 Hz, H-6`); $^{13}$C-NMR ($CD_3OD$, 100 MHz) δ 146.9 (C-2), 136.5 (C-3), 176.1 (C-4), 154.4 (C-5), 98.7 (C-6), 152.3 (C-7), 127.4 (C-8), 147.8 (C-9), 105.2 (C-10), 122.8 (C-1`), 116.2 (C-2`), 146.7 (C-3`), 147.3 (C-4`), 116.5 (C-5`), 122.4 (C-6`).

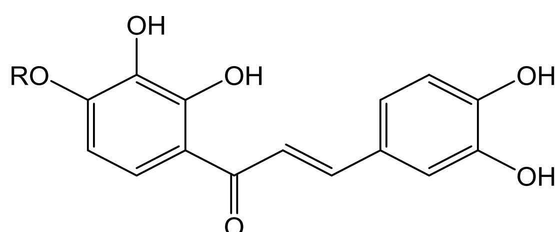
Okanin-4'-O-glucopyranosid 4: A pale yellow powder, exhibited $[M+H]^+$ at *m/z*= 449; *Rf*: 0.49 6% AcOH, 0.10 BAW; UV λ_{max} MeOH nm: 248,266,321, 380; +NaOMe: 252, 283, 347, 44; +$AlCl_3$,: 255, 306, 320, 490; + NaOAC: 258, 281, 350,397; +NaOAc/H_3BO_3 283, 330, 415, 490; ^{1}H-NMR (CD_3OD, 400 MHz); δ 7.18 (1H, d, *J*=2.2 Hz, H-2), 6.88 (1H, d, *J*=8.0 Hz, H-5), 6.62 (1H, dd, *J*=2.2, 8.0 Hz, H-6), 6.75 (1H, d, *J*=8.5 Hz, H-5`),6.55 (1H, d, *J*=8.5 Hz, H-6`), 5.22 (1H, d, *J*=8.5 Hz, H-1``), 3.97 (2H, dd, *J*= 12.5, 2.5 Hz, H-6``), 7.56 (1H, d, *J*=12.5 Hz, α), 8.12 (1H, d, *J*=8.5 Hz, β) ; ^{13}C-NMR (CD_3OD, 100 MHz) δ 129.21 (C-1), 116.4 (C-2), 147.90 (C-3), 151.2 (C-4), 118.2 (C-5), 123.10 (C-6), 117.4 (C-1'), 153.5 (C-2'), 133.8 (C-3'), 153.7 (C-4'), 108.6 (C-5'), 123.4 (C-6'), 102.5 (C-1"), 74.8 (C-2"), 76.6 (C-3"), 70.5 (C-4"), 77.7 (C-5"), 63.4 (C-6"), 191.8 (C=O), 119.1 (α), 145.0 (β).

Myrictrin 5: A yellow powder, exhibited $[M+H]^+$ at *m/z*= 465; UV λ_{max} nm MeOH: 257, 303, 356; +$AlCl_3$: 271, 332, 420; +$AlCl_3$/HCl: 271, 332, 416; +NaOMe: 276, 323, 398 (dec); +NaOAc: 271, 309, 362; +NaOAc/H_3BO_3: 262, 299,364; ^{1}H-NMR (CD_3OD, 400 MHz); δ 5.98 (1H, d, *J*=2.5Hz, H-6), 6.38 (1H, d, *J*=2.5Hz, H-8), 5.30 (1H, d, *J*=8.5Hz, H-1'), 6.96 (1H, d, *J*=2.5 Hz, H-2'), 6.96 (1H, d, *J*=2.5 Hz, H-6'), 4.92 (1H, d, *J*=8.5 Hz, H-1"), 3.34 -3.72 (5H, m, H-2" and 3"), .91 (3H, d, *J*=8.0 Hz, H-6"); ^{13}C-NMR (CD_3OD, 100 MHz) δ 156.8 (C-2), 133.8 (C-3), 178.3 (C-4), 160.8 (C-5), 98.2 (C-6), 165.3 (C-7), 92.7 (C-8), 156.6 (C-9), 104.5 (C-10), 121.5 (C-1'), 107.6 (C-2'), 145.4 (C-3'), 135.4 (C-4'), 145.4 (C-5'), 107.6 (C-6'), 102.2 (C-1"), 70.5 (C-2"), 71.7 (C-3"), 71.9 (C-4"), 70.8 (C-5"), 16.3 (C-6").

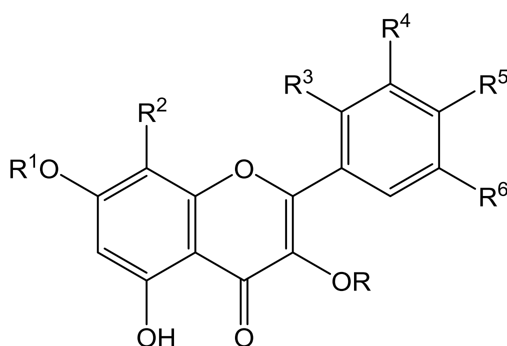
Datiscin 6: Pale yellow crystals, showed $[M+H]^+$ at *m/z*= 595; UV λmax nm MeOH: 261, 305, 335; +NaOMe: 266, 322, 366; $AlCl_3$ + HCl: 257, 320, 390; NaOAc: 257, 352; NaOAc +H_3BO_3: 261, 303, 335; ^{1}H-NMR (CD_3OD, 400 MHz); δ 6.12 (1H, d, *J*=2.4 Hz, H-6), 6.46 (1H, d, *J*=2.4 Hz, H-8), 6.81 (1H, d, *J*=9.5 Hz, H-3'), 7.32 (1H, dd, *J*= 9.5,2.5 Hz, H-4'), 7.11 (1H, dd, J= 9.5,2.5 Hz, H-5), 7.64 (1H, dd, J= 9.5,2.5 Hz, H-6'), 5.22 (1H, d, *J*= 9.5, Hz, H-1''), 3.34 -3.72 (6H, m, H-3''), 4.52 (1H, d, *J*= 9.5 Hz, H-1'''), 3.2-3.74 (5H, m, overlapped protons), 1.09 (3H, d, *J* = 8.5 Hz, H-6'''); ; ^{13}C-NMR (CD_3OD, 100 MHz) δ 156.6 (C-2), 135.2 (C-3), 179.2 (C-4), 161.8 (C-5), 99.1 (C-6), 167.4 (C-7), 95.6 (C-8), 157.8 (C-9), 104.5 (C-10), 119.5 (C-1'), 156.7 (C-2'), 117.6 (C-3'), 130.1 (C-4'), 123.5 (C-5'), 128.4 (C-6'), 109.6 (C-1''), 75.1 (C-2''), 76.9 (C-3''), 71.8 (C-4''), 80.8 (C-5''), 68.6 (C-6''), 112.0 (C-1'''), 73.8 (C-2'''), 72.4 (C-3'''), 73.7 (C-4'''), 74.2 (C-5'''), 16.4 (C-6''').



1 Cholic acid



4 Okanin-4'-glucoside R= glucopyranoside



2 Syringetin R= R^1= R^2= R^3 = H R^4= R^6=OCH_3 R^5= OH

3 Gossypetin R= R^1= R^3 = R^4=H R^2= OH

5 myrictrin R= rhamnose R^1= R^2=R^3= H R^4= R^5=R^6= OH

6 Datiscin R= Rutinoside R_1= R_2= R^4= R^5=R^6= H R^3=OH

Fig. 1 Chemical structures of the isolated compounds from *A. canariense* ethyl acetate extract

Antimicrobial activity

Natural antibacterial agents have been extensively explored, and have become new antibacterial drug discovery, where pharmaceutical development has historically depended on natural products to supply biological antimicrobial active compounds (Brown et al., 2014; Ahmed and El-Bassossy, 2020).

The antibacterial and antifungal activity of the different extracts of *A. canariense* were carried out by diffusion agar technique. Mean zone of inhibition in millimeter produced on a range of pathogenic microorganisms was measured and the results were recorded in Table 3.

The maximum inhibitory responses were indicated after the treatment of all tested bacteria and fungi strain with the concentration of 5 mg/ml of hexane, EA and MeOH extracts, where the only EA extract showed response against all tested strains where, the maximum response of Gram-positive bacteria was methicillin-resistant *Staphylococcus aureus* with inhibition zone 21 mm with activity about 87.5 % as compared to gentamycin control also, it showed potent activity against Gram Negatvie *Proteus vulgaris* and *Escherichia coli* with inhibition 22, 25 mm respectively with activity (88, 70%) as compared with Gentamycin reference drug. The maximum response of fungal strain was to *Candida albicans* with inhibition zone 11 mm with activity 55.5% as compared to Ketoconazole reference drug. The relative activity of EA extract against some of bacteria and fungi strains may be due to their flavonoid constituents which cause damage of cell membrane of bacteria and fungi leading to the inhibition of macromolecular synthesis, depolarization of membrane and inhibition of DNA, RNA, and proteins synthesis of microbe which effect on its growth then its death (Dzoyem et al., 2013). Also, the activity preference of EA extract may be due to two reasons. Firstly, the bioactive constituents such as saponins, tannins, alkaloids, anthraquinones, anthocyanin showed in the LC-Ms analysis of the crude methanolic extract may be

enhanced in the presence of ethyl acetate and methanol. Secondly, the stronger extraction capacity of ethyl acetate may be responsible, such that more active ingredients may be present in the polar extracts. Some of the detected compounds in the EA extract may be responsible for the antibacterial activity observed and thus, justifying their traditional use as medicinal plants for the treatment of bacterial gastroenteritis (Akinyemi et al., 2006; Shahzad et al., 2018).

Table 3: The antimicrobial activity of *A. canariense* extracts against different bacterial and fungal strains

Tested microorganisms	Hexane	EA	MeOH	Control
FUNGI		**Aizoon**		***Ketoconazole***
Aspergillus fumigatus (RCMB 002008)	NA	4	NA	17
Candida albicans RCMB 005003 (1) ATCC 10231	NA	11	NA	20
Gram Positive Bacteria:				***Gentamycin***
Staphylococcus aureus ATCC 25923	12	21	9	24
Bacillus subtilis RCMB 015 (1) NRRL B-543	NA	8	NA	26
Gram Negatvie Bacteria:				***Gentamycin***
Escherichia coli ATCC 25922	NA	21	6	30
Proteus vulgaris RCMB 004 (1) ATCC 13315	13	22	4	25

NA: No activity; Positive control for fungi: Ketoconazole (MIC) 100 mg/ml. Positive control for bacteria: Gentamicin (MIC) 4 mg/ml; RCMB: Regional Center for Mycology and Biotechnology in Cairo, Egypt; ATCC: American Type Culture Collection

Antitumor activity

In recent decades, natural compounds have made significant progress in the treatment of cancers through chemotherapy. This encouraged researchers to keep looking for anticancer drugs in natural components (Ahmed and El-Bassossy, 2020; Duthie et al., 2000).

The antitumor activity of n-hexane, EA and MeOH extracts of *A. canariense* were performed on *in vitro* models against HEPG-2, HCT-116 and PC-3 moreover, testing the safety of the promising potent extract against WI-38 human lung normal cell where, the outlined results showed IC_{50} of all tested extracts were recorded in Table 4**.**

Our results indicated that only EA extract exhibited potency as Antitumor activity against all tested strains furthermore, its safety on the normal cell where, EA extract revealed potent antitumor activity against HepG-2, HCT-116 and PC-3 with IC_{50} 2.62, 4.53and 7.19 µg/ml, respectively. By comparison, the potency IC_{50} of tested EA extract with Cisplatin as standard reference drug, it was found that EA extract showed eminent activity of HepG-2 more than Cisplatin with IC_{50} 3.41µg/ml. In the other side, Cisplatin recorded IC_{50} 2.55 and 5.09µg/ml against HCT-116 and PC-3, respectively. Consequently, the activity of the EA extract against HCT-116 and PC-3 less than Cisplatin but it can be near to its activity with percent about 56.2, and 70.7 %, respectively.

The high abundance of different species of phytochemical components especially, flavonoid, phenolic and alkaloid compounds may be contributing to its high antitumor activity where the sufficient OH groups found in their compounds play a great role in the attraction of free radicals which are considered one of the main causes of tumors where they can safety neutralize and get rid of radicals and tumors (Kanadaswami et al., 2005; Martinez-Perez et al., 2014).

The Potency antitumor activity of EA extract was largely linked to their relatively isolated flavonoid simple structure where, the presence of conjugated electron systems and aromatic rings make them stable and reactive, whereas their overall structure allows them to act as substrates, inhibitors or agonists for numerous enzymes or molecules involved in the development and progression of cancer where, they effect on estrogen production and signaling pathways has been linked to their role as aromatase inhibitors and their interaction with estrogen receptor and estrogen-metabolizing enzymes. They also act as anti-cancer resistant protein inhibitors and interact with cytochrome P_{450} both as inhibitors and substrates. Their ability to induce apoptosis and cell cycle arrest and to alter numerous signaling pathways involved in cancer-related phenomena such as inflammation and proliferation (Aggarwal and Shishodia, 2006; Ren et al., 2003; Panche et al., 2016).

Table 4: Antitumor activity of the successive extracts of *A. canariense* against hepatic (HepG2), colon (HCT-116), prostate (PC-3), and normal (MI-38) cell lines

Conc. µg/ml	Viability % of Cisplatin			Viability % of Hexane extract				Viability % of EA extract			Viability % of MeOH extract		
	HepG2	HCT-116	PC-3	HepG2	HCT-116	PC-3	WI-38	HepG2	HCT-116	PC-3	HepG2	HCT-116	PC-3
500	2.69	3.27	3.75	6.51	7.41	8.75	2.76	0.76	1.23	0.98	19.48	22.51	27.63
250	4.28	4.96	6.04	13.84	15.64	18.62	6.54	1.84	2.96	3.08	57.21	58.84	61.64
125	6.91	7.54	11.28	31.76	35.26	39.50	13.97	4.92	5.81	6.75	78.93	79.46	83.56
62.50	11.26	13.68	16.79	49.27	54.77	60.83	24.63	9.87	11.04	11.38	92.64	94.27	97.18
31.25	19.43	21.75	25.64	62.34	71.14	79.54	35.12	14.52	17.98	21.46	99.75	100	100
15.62	27.88	27.08	31.72	78.62	82.52	88.23	54.08	19.41	25.19	32.78	100	100	100
7.81	35.49	34.95	40.56	90.71	93.3	97.02	67.31	28.75	36.70	47.21	100	100	100
3.90	46.23	42.83	54.17	98.54	100	100	80.92	40.63	52.58	65.04	100	100	100
2	58.70	52.71	63.98	100	100	100	89.46	54.17	63.94	79.28	100	100	100
1	65.26	61.47	69.84	100	100	100	98.73	62.95	78.03	91.42	100	100	100
0	100	100	100	100	100	100	100	100	100	100	100	100	100
IC_{50}	3.4	2.55	5.09	60.8	73.8	94.2	19.2	2.62	4.53	7.19	298	315	336

CONCLUSION

Aerial parts of *A. canariense* showed potent activity of EA extract as anticancer agent, so specific and deep *in-vivo* studies required to know its effectiveness as safe source for treatment different cancer types rather than radiation and chemotherapy which have negative effects on the beside normal cells and causing death. On the other hand, EA extract revealed high inhibitory responses against all tested bacteria and fungi strain at concentration of 5 mg/ml.

Conflict of Interest: No conflict of interest associated with this work

Contribution of Authors:

All authors contributed in conceptualization, methodology, validation, data curation, writing-original draft, review the final manuscript and followed up publication process.

LIST OF ABBREVIATIONS

RCMB: Regional Center for Mycology and Biotechnology Al-Azhar University, Cairo, Egypt; **ATCC**: American Type Culture Collection; HCT-116; human colon cancer cell line; **HepG-2**:

human hepatocellular cancer cell line; **MTT**: 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide; **BAW**: n-Butanol: Acetic Acid; Water; **EA**: ethyl acetate; **MeOH**: Methanol; **CAIH**: Desert Research Center Herbarium; ***A. canariense***: *Aizon canariense*; **CC**: column chromatography; **PTLC:** preparative thin layer chromatography; **TLC**: Thin layer chromatography.

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