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Characterization and biological evaluation of the isolated phenolic constituents of *Urginea maritima* (L.) Aerial parts

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Urginea maritima L. (Family *Liliaceae*) is an important plant used for its medicinal properties. The present study aims to investigate the phenolic contents as well as evaluation of cytotoxic activity of the alcoholic extract and the compounds isolated from *Urginea maritima* L. aerial parts, in addition to evaluation of hypoglycemic activity for the alcoholic extract under investigation. Ten phenolic compounds were isolated and purified by chromatographic techniques. Identification and structural elucidation of the isolated compounds were carried out using chemical investigation (mild and complete acid hydrolysis). The structures were established by interpretation of their spectral data, including 1D- and 2D-NMR (^{1}H, ^{13}C, DEPT, ^{1}H-^{1}H COSY, HMBC, HSQC and HMQC) and by comparison of the reported spectral data (UV, EI/MS and ESI/MS). The ten compounds were identified as kaempferol 3-O-*β*-(6''-O-*α*-rhamnopyranosyl)-glucopyranoside (1), quercetin 3-O-α-rhamnopyranosyl-7-O-*β*-glucopyranoside (2), kaempferol 3-O-β- (6''-E-*p*-coumaroyl)-glucopyranoside (3), kaempferol 3-O-*α*-arabinopyranoside (4), kaempferol 3-O-*β*-galactopyranoside (5), quercetin 3-O-*β*-glucopyranoside (6), kaempferol (7), quercetin (8), ferulic acid (9) and caffeic acid (10). Compounds 1, 2, 3, 5, 7, 9 and 10 were isolated for the first time from this plant. Furthermore, the alcoholic extract and the ten phenolic compounds were evaluated for their cytotoxic activity against human heptocellular carcinoma (HepG2), human prostatic small cell carcinoma (PC3) and human colorectal carcinoma (HCT116) cell lines, while, the hypoglycemic activity was evaluated for the alcoholic extract.

Keywords: *Urginea maritima*, Flavonoids, Phenolic acid, TPC, TFC, cytotoxic and hypoglycemic activity.

INTRODUCTION

People have used plants since ancient times for many purposes as food, treatment from certain diseases, also as complementary diet …, etc. Primitive men and women treated illness by using plants that were not part of their usual diet. Nowadays, chemists begin to produce synthetic pharmaceuticals from raw herbs. The chemical controls used in agriculture are one of the problem sources. Pesticides, used to increase the yields may create tolerance in harmful organisms, kill the natural predators, and thus cause pollution of the natural equilibrium. Many pesticides have high toxicity to human and environment. These pesticide residues can accumulate in humans and cause important health problems as cancer or genetic disorder (Mert and Betul, 2010; Madanlar et al., 2002; Ozmen and Sumer, 2004; Delen et

al., 2005). Because of the risks and damage from synthetic pesticides, in recent years there has been a great increase in the number of the studies carried out to examine the effects of bio pesticides in the agricultural context as an alternative to chemicals (Civelek and Weintraub, 2004). Plant extracts, especially terpenoids, alkaloids and phenolic compounds have been examined with respect to their effects on the growth and development of harmful insects (Erturk et al., 2004). *Urginea maritima* (L.) is one of the plant extracts used as medicinal plant and biopesticide. *Urginea maritima* has been used as a medicinal plant through centuries over the world. Bulb of Squill was used in case of heart failure, injury, haemorrhoids, warts (skin problems), cough, diuretic, chronic bronchitis and asthma. White squill contains glycosides known as bufadienolides. Scillaren A is the most important one, also, it contains the aglycone scillaridin A, in addition to other cardiac glycosides like glucoscillaren A and proscillaridin A (Kokate et al., 2006). Other constituents found in squill include flavonoids as sinistrin, anthocyanins, fatty acids and polysaccharides (Adamsa et al. 2009; Nawal et al., 2009; Kawa and Badr-Aldin, 2010). Scilliroside, the major toxic glycoside, occurs in all plant parts including the leaves, flowers, stalks, scales, and especially the roots and the core of bulbous part (Sharaf et al., 2006). Scilliroside is further used as a rodenticide and mouse repellent (Paolo et al., 2005). It was found that the bulb extract from *Urginea maritime* L. had a strong insecticide effect [Pascual-Villalobos and Fernandez, 1999]. Also, the direct exposure of the plant's tubers to the sun increases the activity (Maria et al., 2002). The aim of this study is to isolate and investigate the phenolic constituents from the aerial parts of *Urginea maritima* (L.) alcoholic extract as well as to evaluate the cytotoxic activities of the alcoholic extract and the ten compounds, in addition to evaluation of the hypoglycemic activity of the alcoholic extract.

MATERIALS AND METHODS

The aerial parts of *Urginea maritima* (L.) were collected from North Sianai (Rafah), Egypt in 2016. Authentication was performed at the National Research Centre (NRC) by Prof. Dr. Mona Mohamed Marzouk. A voucher specimen (M2729) was deposited in the Herbarium of NRC (Cairo, Egypt).

Chemicals and instruments:

NMR experiments were recorded on a Joel Ex-500 spectrometer: 500 MHz (^{1}H-NMR), 125 MHz (^{13}C-NMR). UV spectro photometer (Shimadzu UV-240). EI-MS was determined on a Finnigan MAT-SSQ 7000 instrument. ESI-MS were recorded on a Waters-Micromass Quattro Premier Triple Quadrupole mass spectrometer.R_f values were measured on Polygram SILF/UV254 sheets (Merck pre coated sheets). Column chromatography (CC) was performed using Polyamide 6S (Riedel, De Haen AG, SeelzeHaen AG, SeelzeHanver, Germany), Sephadex LH-20 (Pharmazia) using MeOH/H_2O as eluent, Cellulose (Merck), paper chromatography (PC): Whatman No.1 and preparative (PPC) on 3 MM paper using the following solvent systems: (1) BAW (*n*-BuOH/AcOH/H_2O, 4:1:5 upper layer); (2) H_2O; (3) 15% AcOH (AcOH: H_2O 15:85), (4) (benzene/ *n*-BuOH/ H_2O/pyridine 1:5:3:3, upper layer) and were visualized under UV light using aluminium chloride $AlCl_3$ and Naturstoff reagent A (NA) (Diphenyl boric acid-*β*-amino ethyl ester) as spraying reagents. Aniline hydrogen phthalate was used as specific reagent for sugar analysis. Complete acid hydrolysis (2N HCl, 2 h, 100ºC) was carried out and followed by paper co-chromatography with authentic samples to identify the aglycones and sugar moieties (Mabry et al., 1970; Markham 1982).

Methods:

Total Phenolic Content (TPC) Quantification:

The total phenolic content of the alcoholic extract of the aerial parts of *Urginea maritima* L. was spectrophotometrically quantified using Folin-Ciocalteau Reagent (FCR) (Montreau, 1972) and gallic acid as standard. 500 µL of Folin-Ciocalteau reagent and 0.45 ml of sodium carbonate (7.5 % w/v) were added to 1 ml of total volume sample. After the incubation at room temperature for 2 hrs, the absorbance at 765 nm of the sample was detected in UV-VIS spectrophotometer and the content of total phenolics of the extract was expressed as gallic acid equivalent (mg GAE)/g dried weight of plant extract, using a calibration curve of gallic acid as standard. The resulted values were expressed as mean of triplicate determination ± standard deviation [Singleton and Rossi, 1965].

Total Flavonoid (TF) Content Quantitation:

The alcoholic extract of the aerial parts of *Urginea maritima* (L.) is estimated for its total flavonoid content spectrophotometrically by aluminum chloride method, which is based on the

formation of yellow complex [Kumaran and Karunakaran, 2006]. The alcoholic extract of the plant was added to the solution of 5% (w/v) sodium nitrite ($NaNO_2$) and incubated for 5 minutes with the 10% (w/v) of aluminium chloride solution; addition of 0.5 ml of 1M sodium hydroxide (NaOH) after 5 minutes [Kim et al. 2003]. The developed yellow color intensity was measured at 510 nm with the UV-VIS spectrophotometer, using rutin as a reference (its absorption in ethanol was measured at the same conditions). The determination was done in triplicate. The following equation is used to estimate the amount of total flavonoids in *Urginea maritima* alcoholic extract in mg rutin equivalents (RE) / gram of plant extract:

$X = (A \cdot m_0) / (A_0 \cdot m)$

Where:

X: Flavonoid content was expressed in milligrams of rutin equivalents (RE) /milligrams of plant extract.

A: Absorption of plant extracts solution.

A_0: Absorption of standard rutin solution.

m: Weight of the plant extract in mg.

m_0: Weight of standard rutin solution in mg.

Extraction and fractionation:

The fresh aerial parts (1.700 Kg) were exhaustively extracted with 70% ethanol/H_2O, the ethanolic extract was dried under vacuum (giving 280 gm) and examined by both $AlCl_3$ and Shinoda's test which indicate the presence of compounds of strong phenolic and flavonoid nature. Its TDPC, using the solvent systems BAW and 15% AcOH, respectively, revealed the presence of many compounds of flavonoid nature. The extract (280 gm) was defatted with petroleum ether (40-60ºC), the residue (250 gm) was slurred with water, mixed with a small amount of polyamide and subjected to a polyamide CC (6 x 130 cm). Starting with water as an eluent then decreasing the polarity by increasing the methanol concentration up to 100%. 51 fractions (200 ml each), were obtained, and grouped based on their PC properties using BAW, H_2O and 15% AcOH as eluents. PC was carried out for isolation of the flavonoid compounds, using glass chromatography tanks, applying the paper descending technique. The developed chromatograms were air-dried and examined in both visible and UV light. The chromatograms were exposed to ammonia vapors, then immediately re-examined to observe changes in colors or fluorescence under UV light. Five main fractions (F1-F5) were collected, dried and subjected to repeated purification on columns using sephadex LH-20 column (40 x 3 cm) eluted with 80% methanol/ H_2O and preparative paper chromatography (PPC) (Whatmann 3MM) using BAW 4:1:5 to give the isolated compounds (compounds 1-10). Identification of the isolated compounds were carried out through R_f values, color reactions, chemical investigations (complete and mild acid hydrolysis), physical investigations (UV, NMR, ESI-MS, EI/MS) and by comparing the spectral data with those previously published (Linard et al., 1982; Zhang et al., 2009; Chen et al., 2010; Oliveira et al. 2013; Moo-Puc et al., 2014; He et al., 2015; Kim et al., 2016; Hussein et al., 2017).

Compounds 1 (20 mg) and 2 (24 mg) were isolated from fraction F1, which was eluted with 20% MeOH: H_2O, while, compound 3 (18 mg) was isolated from F2, eluted with 40% MeOH: H_2O. F3 was eluted with 60% MeOH: H_2O yielded three compounds, compound 4 (18 mg), 5 (22 mg) and 6 (25 mg), on the other hand, elution of F4 with 80% MeOH: H_2O resulted in the separation of compounds 7 (31 mg) and 8 (28 mg). Finally, two compounds, 9 (16 mg) and 10 (19 mg) were isolated from F5 eluted with 100% MeOH.

Complete acid hydrolysis:

About 3 mg of the compound was dissolved in 10 ml methanol mixed with 10% HCl refluxed on a boiling water bath for 2 hrs. The solution was diluted with distilled water and extracted with ethyl acetate (3 x 50 ml). The ethyl acetate extract was washed with distilled water and evaporated in vacuo at 45ºC till dryness; the obtained residue was chromatographed on PC with authentic aglycone sample. The aqueous acidic solution after separation of the aglycone was neutralized with barium carbonate, filtered and evaporated till dryness. The residue was dissolved in isopropanol and subjected to PC using ethyl acetate: pyridine: water 12: 5: 4 and benzene: n-butanol: pyridine: water 1: 5: 3: 3 as developing solvents with authentic references from different sugars. The chromatograms were visualized by spraying with aniline phthalate (Swanton-Flatt et al., 1990; Partridge, 1949) and heated at 105ºC for few minutes, their data were identical to those previously reported (Al-Wakeel et al., 1988; Saleh et al., 1990).

Biological evaluation:

In vitro cytotoxic bioassay on human tumor cell lines

All cell lines were taken as a gift from Professor Doctor Stig Linder, Department of Oncology-Pathology, Karolinska Institute, Sweden. All media were purchased from Lonza (Belgium), serum from Gibco (Thermo Fisher Scientific), trypsin and MTT from Biobasic (Canada).

Cell culture—

Human hepatocellular carcinoma HepG2 (ATCC number HB-8065) cell line was maintained in Eagle's Minimum Essential Medium (EMEM), both human prostatic small cell carcinoma PC3 (ATCC number CRL-1435) and human colorectal carcinoma HCT116 (ATCC number CCL-247) cell lines were maintained in Dulbecco's Modified Eagle Medium: Nutrient Mixture F-12 (DMEM-F12) with l-glutamine, 10% foetal bovine serum at 37°C in 5% CO_2 and 95% humidity. Cells were sub-cultured using trypsin versene (EDTA) 0.15%.

Viability test—

After about 24h of seeding 20000 cells per well (in 96-well plates), when cells have reached 70-80% confluence, the media was adjusted to 5% serum containing a final concentration of the test samples of 100 ppm in triplicates. The cells were treated for 72h. Doxorubicin was used as a positive control and medium with 5% serum was used as a negative control (Osman et al., 2015, Ismail et al., 2016).

Cell viability was determined using the MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] assay as described by Mosmann, 1983.

The equation used for calculation of percentage cytotoxicity: (1-(av(x)/(av(NC)))*100

Where: Av: average, X: absorbance of sample well measured at 595nm with reference 690nm, NC: absorbance of negative control measured at 595nm with reference 690nm

IC_{50} calculation and statistical analysis—

Exactly the same procedure in the viability test was done. Only in this case the test samples which gave 50% cytotoxicity or more on the cells were chosen. Four concentrations of those test samples were tested (in triplicates) on the cell lines. The results obtained were analyzed statistically using the graphpad PRISM version 6.01 performing non-linear regression analysis to obtain the IC_{50} values.

In-vitro hypoglycemic activity

***α*-Amylase inhibitory activity**

α-amylase from porcine pancreas (SIGMA A3176) of concentration 4 U/ml was prepared in phosphate buffer saline (pH 6.8). 15 µl of sample at varying concentrations (7 to 55 ppm in the final volume) were mixed with 60 µl of α- amylase and incubated for 15 min at 37°C in a 96 well plate. 60 µl of 0.2% soluble starch solution (dissolved in buffer by heating in a microwave and then filtered) were added and incubated at 37°C for 10 min. The reaction was terminated by the addition of 30 µl of 1M HCl. 150 µl KI/I2 aqueous solution were added. α-amylase activity was determined spectrophotometrically at 595 nm by measuring the quantity of blue color released. The negative control had 15 µl of buffer solution in place of the test entity while acarbose (SIGMA-ALDRICH PHR1253) was used as a positive control. The results obtained were analyzed statistically using the graphpad PRISM version 6.01 performing non-linear regression analysis to obtain the IC_{50} values (Xiao et al., 2006).

***α*-Glucosidase inhibitory activity**

α-glucosidase from saccharomyces cerevisiae (SIGMA G5003-100UN) of concentration 0.2 U/ml was prepared in phosphate buffer saline (pH 6.8). 10 µl of sample at varying concentrations (3 to 23 ppm in the final volume) were mixed with 60 µl of 0.2 U/ml *α*-Glucosidase and incubated for 20 min at 37˚C in a 96 well plate. The 150 µl of 1.25 mM p-nitrophenyl *α*-D-glucopyranoside (SIGMA N1377) (p-NPG) were added and incubated at 37˚C for 20 min. The reaction was terminated by the addition of 50 µl of 2 g/L NaOH. α-glucosidase activity was determined spectrophotometrically at 405 nm by measuring the quantity of bright yellow p-nitrophenol released from the colourless p-NPG. The negative control had 10 µl of buffer solution in place of the test entity while acarbose was used as a positive control. For blank p-nitrophenyl *α*-D-glucopyranoside with buffer solution was added instead of the enzyme (Elya et al., 2012; Qaisar et al., 2014).

RESULTS AND DISCUSSION

Identification of the isolated compounds

The current study deals with the isolation of ten phenolic compounds using chromatographic

methods (Mabry et al., 1970) (Fig. 1).

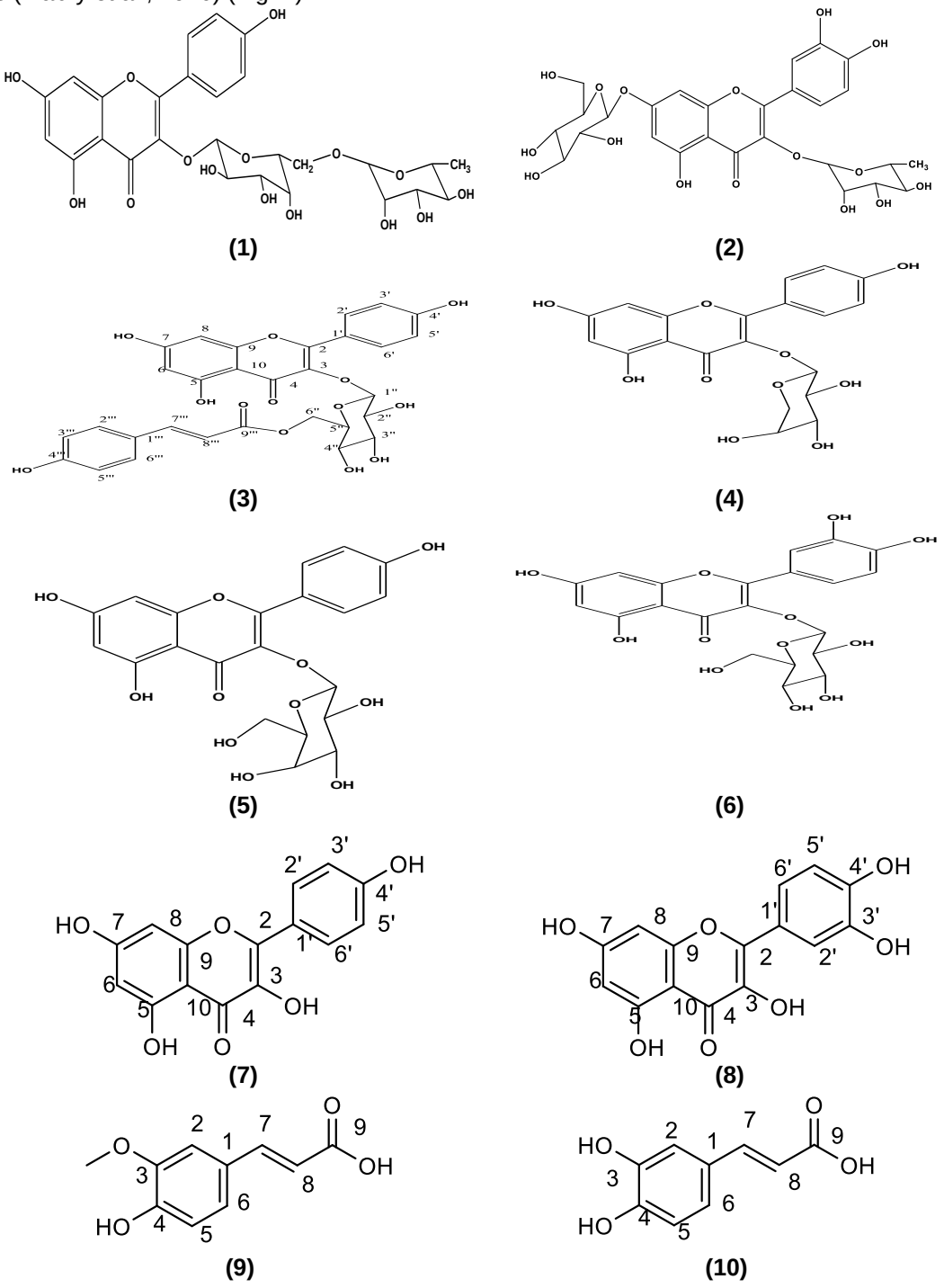

Figure (1): Structure of the isolated compounds (1-10) from the aerial parts of *Urgenia maretima* L.

Their structure elucidation was carried out through color reactions, R_f values, chemical investigations (mild and complete acid hydrolysis) and physical investigations (EI/Ms, ESI/Ms, UV and NMR) (Markham 1982, Marzouk et al., 2009; Pauli 2000).

Compound 1:

Pale-yellow needles (20 mg); m.p. 188-190 ºC; R_f BAW: 0.28, 15% AcOH/ H_2O :0.19; UV λ_{max} (nm) (MeOH): 268, 298 sh, 302, 351; +NaOMe 278, 327, 406; +$AlCl_3$ 275, 307, 353, 399; +$AlCl_3$/HCl 277, 304 sh, 349, 398; +NaOAc 276, 327, 363; +NaOAc/H_3BO_3 268, 305 sh, 352 nm. (+)-ESI-HR-MS of the $[M+H]^+$ at *m/z* 595. 3241 (calculated for C_{27} H_{30} O_{15}). ^{1}H-NMR (500 MHz, DMSO-d_6), δppm: 12.57 (1H, brs, H-HO-5), 7.99 (2H, d, *J* = 8.5 Hz, H-2' and H-6'), 6.88 (2H, d, *J* = 8.8 Hz, H-3' and H-5'), 6.41 (1H, d, *J* = 2.0 Hz, H-8), 6.18 (1H, d, *J* = 2.0 Hz, H-6), 5.31 (1H, d, *J* = 7.1 Hz, H-1''), 4.37 (1H, d, *J* =2.0 Hz, H-1'''), 3.01-3.51 (10H, m, sugar protons overlapped with –OH proton signals), 0.78 (3H, d, *J* = 6.0 Hz, H-6'''); ^{13}C-NMR (125 MHz, DMSO-d_6), δppm: 156.46, 134.19, 177.35, 160.19 [C (2, 3, 4, 5)], 98.53, 164.28, 93.88, 156.82, 104.0 [C (6, 7, 8, 9, 10)], 120.79 (C-1'), 130.88 (C-2' and 6'), 115.10 (C-3' and 5'), 158.89 (C-4'), 101.41, 74.16, 77.41, 69.89, 76.72, 67.41 [C (1'', 2'', 3'', 4'', 5'', 6'')], 100.81, 70.32, 70.55, 71.88, 68.11, 17.81 [C (1''', 2''', 3''', 4''', 5''', 6''')]. Acid hydrolysis of compound 1 yields the aglycone and the two sugars, which their R_f values were identical with kaempferol (aglycone) and the standards, glucose and rhamnose (sugars). So, from the previous results, compound 1 is established as kaempferol 3-O-*β*-(6''-O-*α*-rhamnopyranosyl)-glucopyranoside (nicotiflorin), (Cardoso et al., 2013).

Compound 2:

Yellow amorphous powder (24 mg), m.p.242-244 ºC, R_f 0.43 (BAW), 0.19 (15%AcOH/ H_2O). UV spectral data λ_{max}(nm): MeOH 256.78, 294 sh, 355; +NaOMe 265, 404; +$AlCl_3$ 272, 299 sh, 339 sh, 425; +$AlCl_3$/HCl 268, 298 sh, 357, 400; +NaOAc 258, 395 sh, 366, 415; +NaOAc/H_3BO_3 260, 294 sh, 374. (+)-ESI-HR-MS of the $[M+H]^+$ at *m/z* 611.3726 (calculated for C_{27} H_{30} O_{16}) ^{1}H-NMR (500 MHz, DMSO-d_6), δppm: 7.54 (1H, d, *J* = 2.5 Hz , H-2'), 7.65 (1H, dd, *J* = 2.5 Hz, 8 Hz, H-6'), 6.76 (1H, d, *J* = 8.5 Hz, H-5'), 6.2 (1H, d, *J* = 2.5 Hz, H-8), 6.42 (1H, d, *J* = 2.5 Hz, H-6), 5.24 (1H, d, *J* = 2.0 Hz, H-1''), 5.98 (1H, d, *J* = 7.5 Hz, H-1'''), 3.01-3.5 (m, sugar protons overlapped with –OH proton signals), 0.78 (3H, d, *J* = 6.0, CH_3 of rhamnose moiety). ^{13}C-NMR (125 MHz, DMSO-d_6), δppm: 157.69, 134.87, 177.98, 161.79 [C (2, 3, 4, 5)], 98.87, 165.14, 93.89, 157.64, 103.86 [C (6, 7, 8, 9, 10)], 122.14, 115.26, 145.65, 149.34, 116.97, 122.56 [C (1', 2', 3', 4', 5', 6')], 102.63, 70.86 , 71.43, 72.12, 70.86, 18.32 [C (1'', 2'', 3'', 4'', 5'', 6'')], 101.46, 72.87, 75.68, 71.23, 77.86, 61.98 [C (1''', 2''', 3''', 4''', 5''', 6''')]. Acid hydrolysis of compound 2 yields the aglycone and the two sugars, which their R_f values were identical with Quercetin (aglycone) and the standards, rhamnose and glucose (sugars). So, from the previous results, compound 2 is established as quercetin 3-O-*α*-rhamnopyranosyl-7-O-*β*-glucopyranoside (Filippo, 2008).

Compound 3:

Pale yellow needles (18 mg), m.p. 261-264 ºC, R_f BAw: 0.26, 15% AcOH/ H_2O:0.17, UV spectral data λ_{max} (nm) MeOH 266, 302 sh, 315, 360 sh; +NaOMe 276, 313 sh, 371; +$AlCl_3$ 276, 309, 323 sh, 399; +$AlCl_3$/HCl 277, 308, 323 sh, 398; +NaOAc 277, 299 sh, 314, 371; +NaOAc/H_3BO_3 269, 303 sh, 317, 361nm. (+)-ESI-HR-MS of the $[M+H]^+$ at *m/z* 595.4232 (calculated for C_{30} H_{26} O_{13}). ^{1}H-NMR (500 MHz, DMSO-d_6), δppm: 12.59 (1H, s, OH-5), 7.99 (2H, d, *J* = 8.5 Hz, H-2',6'), 7.41 (2H, d, *J* = 6.9 Hz, H-2''', 6'''), 7.44 (1H, d, *J* = 16 Hz, H-7'''-β), 6.79 (2H, d, *J* = 8.5 Hz, H-3', 5'), 6.85 (2H, d, *J* = 8.9 Hz, H-3''', 5'''), 6.40 (1H, d, *J* = 2.0 Hz, H-8), 6.17 (1H, d, *J* = 2.0 Hz, H-6), 6.13 (1H, d, *J* = 15.9 Hz, H-8'''-α), 5.54 (1H, d, *J* =7.5 Hz, H-1''). ^{13}C-NMR (125 MHz, DMSO-d_6) δppm: 155.74, 134.67, 176.89, 160.91 [C (2, 3, 4, 5)], 97.96, 163.87, 93.77, 157.89, 104.10 [C (6, 7, 8, 9, 10)], 121.87 (C-1'), 129.92 (C-2', 6'), 114.21 (C-3', 5'), 156.21 (C-4'), 99.87, 73.52, 75.54, 70.27, 73.41, 61.86 [C (1'', 2'', 3'', 4'', 5'', 6'')], 125.89 (C-1'''), 129.21 (C-2''', 6'''), 114.89 (C-3''', 5'''), 156.13 (C-4'''), 143.75, 113.84, 165.11 [C (7'''- β, 8'''-α, 9''')]. The R_f values resulted after acid hydrolysis of compound 3 were identical with kaempferol (aglycone), the standard glucose (sugar), and *p*-coumaric acid. Compound 3 is established as kaempferol 3-O-*β*-(6''-E-*p*-coumaroyl)-glucopyranoside (trans-tilliroside) (Lokadi et al., 2016).

Compound 4:

Yellow amorphous powder (18 mg), m.p.198-201ºC, R_f 0.37 (BAW), 0.18 (15%AcOH/ H_2O). UV spectral data λ_{max} (nm) MeOH 264, 349; +NaOMe 273, (324), 403; +$AlCl_3$ 271, 302 sh, 348, 400; +$AlCl_3$/HCl 272, 303 sh, 346, 400; +NaOAc 273, 301 sh, 368; +NaOAc/H_3BO_3 267, 293 sh, 320 sh,

347 nm. (+)-ESI-HR-MS of the $[M+H]^+$ at *m/z* 419.2145 (calculated for C_{20} H_{18} O_{10}). ^{1}H-NMR (500 MHz, DMSO-d_6), δppm: 12.53 (1H, s, OH-5), 7.91 (2H, d, *J* = 8.5 Hz, H-2', H-6'), 6.78 (2H, d, *J* = 8.5 Hz, H-3', H-5'), 6.35 (1H, d, *J* = 2.0 Hz, H-8), 6.13 (1H, d, *J* = 2.0 Hz, H-6), 5.21 (1H, d, *J* = 5.1 Hz, H-1''), 3.67-3.14 (m, sugar protons overlapped with –OH proton signals). ^{13}C-NMR (125 MHz, DMSO-d_6): 155.53, 134.45, 176.65, 160.43 [C (2, 3, 4, 5)], 97.67, 163.54, 93.66, 155.84, 103.78 [C (6, 7, 8, 9, 10)], 121.27 (C-1'), 129.21 (C-2', 6'), 114.23 (C-3', 5'), 159 10 (C-4'), 100.52, 71.76, 70.58, 65.46, 63 83 [C (1'', 2'', 3'', 4'', 5'')]. Acid hydrolysis of compound 4 yields the aglycone and the sugar, which their R_f values were identical with kaempferol (aglycone) and the standard, arabinose. Compound 4 is established as kaempferol 3-O-*α*-arabinopyranoside (Nguyen, 2017).

Compound 5:

Pale yellow powder (22 mg), m.p. 254-256ºC. R_f BAW:0.29, 15%AcOH/ H_2O: 0.18; UV λ_{max} (nm) MeOH 264, (294), (320), 350; +NaOMe 271, 326 sh, 406; +$AlCl_3$ 273, 304 sh, 346, 401; +$AlCl_3$/HCl 273, 302 sh, 344, 401; +NaOAc 271, 301 sh, 371; +NaOAc/H_3BO_3 266, 292 sh, 321 sh, 351 nm. (+)-ESI-HR-MS of the $[M+H]^+$ at *m/z* 449.1658 (calculated for C_{21} H_{20} O_{11}). ^{1}H-NMR (500 MHz, DMSO-d_6), δ ppm: 12.52 (1H, s, OH-5), 8.03 (2H, d, *J* = 8.5 Hz, H-2', H-6'), 6.67 (2H, d, *J* = 8.9 Hz, H-3', H-5'), 6.34 (1H, d, *J* = 2.5 Hz, H-8), 6.15 (1H, d, *J* = 2.5 Hz, H-6), 5.32 (1H, d, *J* = 7.6 Hz, H-1''), 3.55-3.14 (m, sugar protons overlapped with –OH proton signals). ^{13}C-NMR (125 MHz, DMSO-d_6), δppm: 156.12, 134.43, 177.26, 161.52 [C (2, 3, 4, 5)], 99.81, 167.14, 94.23, 157.42, 103.98 [C (6, 7, 8, 9, 10)], 121.73 (C-1'), 129.42 (C-2', 6'), 115.63 (C-3', 5'), 160.17 (C-4'), 102.62, 71.54, 73.65, 67.95, 75.89, 61.14 [C (1'', 2'', 3'', 4'', 5'', 6'')]. After acid hydrolysis of compound 5, R_f values of the aglycone and the sugar were identical with kaempferol (aglycone) and the standard, galactose. Compound 5 is established as kaempferol 3-O-*β*-galactopyranoside (trifolin) (Pacome et al., 2015).

Compound 6:

Yellow powder (25 mg), m.p.220-223ºC; R_f BAW: 0.28, 15%AcOH/ H_2O: 0.17. UV spectral data λ_{max}(nm): MeOH 254, 267, 299, 357; +NaOMe 269, 324, 409; +$AlCl_3$ 269, 306, 329, 437; +$AlCl_3$/HCl 264, 299, 357, 401; +NaOAc 270, 321, 383; +NaOAc/H_3BO_3 259, 297, 381. (+)-ESI-HR-MS of the $[M+H]^+$ at *m/z* 465.2865 (calculated for C_{21} H_{20} O_{12}). ^{1}H-NMR (500 MHz, DMSO-d_6), δppm: 12.98 (1H, s, OH-5), 7.68 (1H, dd, *J* = 2.1 Hz, 8.1 Hz, H-6'), 7.95 (1H, d, *J* = 2.1 Hz, H-2'), 6.96 (1H, d, *J* = 8.5 Hz, H-5'), 6.84 (1H, d, *J* = 2.5 Hz, H-8), 6.84 (1H, d, *J* = 2.5 Hz, H-6), 6.12 (1H, d, *J* = 7.5 Hz, H-1''), 3.15-3.54 (m, sugar protons overlapped with –OH proton signals). ^{13}C NMR (125 MHz, DMSO-d_6, , δppm: 159.4, 136.5, 179.9, 162.6 [C (2, 3, 4, 5)], 98.8, 165.7, 94.7, 159.4, 104.8 [C (6, 7, 8, 9, 10)], 123.0, 117.1, 146.4, 151.0, 117.6, 123.3 [C (1', 2', 3', 4', 5', 6')], 101.3, 75.4, 78.6, 70.98, 77.0, 62.2 [C (1'', 2'', 3'', 4'', 5'', 6'')]. Acid hydrolysis gave quercetin and glucose by comparison with authentic references. Compound 6 is established as quercetin 3-O-*β*-glucopyranoside (Isoquercetin) (Maria and Irena, 2008).

Compound 7:

Yellow amorphous powder (31 mg), m.p. 265-267 ºC, R_f 0.58 (BAW), 0.09 (15%AcOH/ H_2O). UV spectral data λ_{max} (nm): MeOH 265, 291 sh, 318 sh, 365; +NaOMe 275, 319 sh, 410; + $AlCl_3$ 268, 304, 349, 422; +$AlCl_3$/HCl 266, 304, 349, 423; +NaOAc 273, 305, 377; +NaOAc/H_3BO_3 264, 293, 318, 368. EI/MS *m/z* 286. ^{1}H-NMR (500 MHz, DMSO-d_6), δ ppm: 7.98 (2H, d, *J* = 8.5, H-2',6'); 6.91 (2H, d, *J* = 8.5, H-3',5'); 6.38 (1H, d, *J* = 2.0, H-8); 6.13 (1H, d, *J* = 2.0, H-6). ^{13}C-NMR (125 MHz, DMSO-d_6), δ ppm: 155.62, 133.24, 176.76, 160.87 [C (2, 3, 4, 5)], 99.7, 166.51, 94.32, 156.81, 103.45 [C (6, 7, 8, 9, 10)], 120.91 (C-1'), 129.14 (C-2', 6'), 114.98 (C-3', 5'), 159.89 (C-4'). Compound 7 is identified as kaempferol (Cardoso et al., 2013).

Compound 8:

Yellow amorphous powder (28 mg); m.p. 312-314ºC; R_f BAW: 0.64, 15% AcOH/H_2O:0.08. EI/MS *m/z* 302. UV spectral data λ_{max} (nm): MeOH 255, 368, 370; +NaOMe 252, 320; + $AlCl_3$ 270, 360, 458; +$AlCl_3$/HCl 258, 301, 400; +NaOAc 254, 276, 390; +NaOAc/H_3BO_3 273, 388. ^{1}H-NMR (500 MHz, DMSO-d_6), δppm: 7.64 (1H, d, *J* = 2.5 Hz, H-2'), 7.52 (1H, dd, *J* = 2.5, 8.5 Hz, H-6'), 6.87 (1H, d, *J* = 8.5 Hz, H-5'), 6.44 (1H, d, *J* = 2.5 Hz, H-8), 6.18 (1H, d, *J* = 2.5 Hz, H-6); ^{13}C-NMR (125 MHz, DMSO-d_6, 125 MHz, δppm: 146.8, 134.7, 175.11, 160.12 [C (2, 3, 4, 5)], 97.7, 163.8, 94.22, 155.7, 104.5 [C (6, 7, 8, 9, 10)], 121.81, 114.6, 144.9, 146.11, 114.9, 120.6 [C (1', 2', 3', 4', 5', 6')]. The structure of compound 8 is confirmed as quercetin by comparison with authentic reference (Hao et al., 2010).

Compound 9:

Colorless powder (16 mg), m.p. 168-172ºC. R_f 0.48 (BAW), 0.8 (15% AcOH/ H_2O). EI/MS *m/z* 194. ^{1}H-NMR (500 MHz, CD_3OD), δppm: 7.67 (1H, d, *J* = 2.0 Hz, H-2), 9.12 OH, s, H-4), 6.42 (1H, d, *J* = 8.0 Hz, H-5), 6.65 (1H, dd, *J* = 8.0, 2.0 Hz, H-6), 6.98 (1H, d, *J* = 16.0 Hz, H-7), 6.12 (1H, d, *J* = 16.0 Hz, H-8), 3.60 (3H, s, O-Me). ^{13}C-NMR (125 MHz, CD_3OD), δ ppm: 127.1, 110.8, 148.7, 150.0, 115.7

[C (1, 2, 3, 4, 5)], 123.5, 146.2, 116.1, 170.8 [C (6, 7, 8, 9)], 56.2 (O-Me). Compound 9 is confirmed by comparison with literature data which is established as ferulic acid (Subramani et al., 2016).

Compound 10:

Yellowish brown powder (19 mg), m.p. 223-225 ºC. R_f 0.72 (BAW), 0.55 (15% AcOH/ H_2O). EI/MS *m/z* 180. ^{1}H-NMR (500 MHz, CD_3OD), δppm: 7.65 (1H, d, *J* = 2.0 Hz, H-2), 6.24 (1H, d, *J* = 9.2 Hz, H-5), 6.38 (1H, dd, *J* = 8.2, 2.0 Hz, H-6), 7.21 (1H, d, *J* = 15.9 Hz, H-7), 6.12 (1H, d, *J* = 15.9 Hz, H-8). ^{13}C-NMR (125 MHz, CD_3OD), δppm: 127.87, 115.12, 146.89, 149.45, 116.79 [C (1, 2, 3, 4, 5)], 123.12, 147.23, 115.0, 171.32 [C (6, 7, 8, 9)]. Compound 10 is confirmed by comparison with literature data which is established as caffeic acid (Mohamed et al., 2015).

Further authentication was carried out by comparing their spectroscopic data with their values in literature. The chemical structures of the known compounds were determined by complete acid hydrolysis, UV, one-dimensional, two-dimensional (1D, and 2D) NMR and mass spectrometry techniques and confirmed by comparing their spectral data with those from the literature. Compounds 1, 3, 4, 5 & 6 have sugar linkage at carbon number 3, and free hydroxyl groups at C-5, 7 and 4', while, compound 2 has sugar linkage at carbon number 3 & 7, and free hydroxyl groups at C-5 & 4' as indicated by their UV spectra (see experimental data) with addition of specific shift reagent (Mabry et al., 1970).

Anomeric configurations, linkage sites and sequence of sugars in the flavonol glycosides can be determined using ^{1}H and ^{13}C-NMR chemical shifts (Agrawal, 1989).

^{1}H and ^{13}C-NMR, spectral data showed that compounds 1, 3, 4, and 5 are kaempferol 3-dervatives. It was observed from the ^{1}H-NMR spectrum of compound 1 two signals at δppm 5.31 and 4.37 which indicated the presence of two sugar moieties, and doublet at δ 0.78 (d, *J*= 6.00) indicted the presence of rhamnose methyl protons. Also, comparison of the ^{1}H and ^{13}C-NMR spectra of compound 1 with the literature, confirmed the presence of glucose and rhamnose moieties in the molecule. In the ^{13}C-NMR spectrum of compound 1, the presence of the signal at δppm 134.19 agreed with glycosylation at C-3. The two anomeric carbon signals were observed at δ 101.41 and 100.81. The glucose moiety as *β* glucose by distinct anomeric proton at δppm 5.31 (d, *J*= 7.1 Hz) correlating with C-3 of aglycone. The signal for C-6 of glucose was at δ 67.41, we observed a downfield shift due to interglycosidic linkage between C-6 of glucose and C-1 of rhamnose. The (+)-ESI-HR-MS of compound 1 exhibited a molecular ion peak $[M+H]^+$ at *m/z* 595. Therefore, from the above spectral data, acid hydrolysis and literature, compound 1 was confirmed as kaempferol 3-O-*β*-(6''-O-*α*-rhamnopyranosyl)-glucopyranoside (nicotiflorin).

The ^{1}H and ^{13}C-NMR spectra of compound 2 showed the presence of quercetin as aglycone, glucose and rhamnose as two sugar moieties. Glycosylation of the aglycone (quercetin) at C-3 and C-7 was observed. The glucose moiety at δ 5.98 (d, *J*=7.5Hz) correlating with C-7 at δ ppm 165.14 of aglycone and rhamnose sugar at δ 5.24(d, *J*=2.00 Hz) correlating with C-3 at δ ppm 134.87. The (+)-ESI-HR-MS of compound 2 exhibited a molecular ion peak $[M+H]^+$ at *m/z* 611, from the UV spectra (see experimental data), the above spectral data and complete acid hydrolysis, thus compound 2 was identified as quercetin 3-O-*α*-rhamnopyranosyl-7-O-*β*-glucopyranoside.

Compound 3 was confirmed from UV, ^{1}H and ^{13}C NMR spectra, (+) ESI and acid hydrolysis, which were in accordance with kaempferol 3-O-*β*-(6''-E-*p*-coumaroyl)-glucopyranoside. The ^{1}H-NMR spectrum showed a doublet for the anomeric proton of glucose with a large coupling constant, which revealed that glucose was *β*-linked. Two doublets at 7.44 and 6.13 ppm with the coupling constant *J*=16 Hz which was an indication for trans configuration of coumaric acid (see experimental data). The UV spectral data and NMR spectra of compound 4 revealed kaempferol as aglycone and arabinose as sugar unit, in addition, the anomeric proton at δ 5.21 (d, *J*=5.1Hz) correlating with C-3 (δppm 134.45) indicated that glycosylation of arabinose with kaempferol is at C-3. The (+)-ESI-HR-MS of compound 4 exhibited a molecular ion peak $[M+H]^+$ at *m/z* 419. Thus, the above data and acid hydrolysis showed that compound 4 was identified

as kaempferol 3-O-*α*-arabinopyranoside. Compound 5 was confirmed from spectral data (UV, NMR, (+) ESI, see experimental data) and complete acid hydrolysis as kaempferol 3-O-*β*-galactopyranoside (Trifolin). Compound 6 (as a yellow powder), was confirmed from UV, NMR, (+) ESI (see experimental data), and acid hydrolysis, as quercetin 3-O-*β*-glucopyranoside (Isoquercetin). From the above data, compounds 7 and 8 (as yellow amorphous powders) were confirmed as kaempferol and quercetin, respectively (see experimental data). Finally, from the above data for compounds 9 (as colorless powder) and 10 (as yellowish brown powder) with their ^{1}H and ^{13}C-NMR spectra (see experimental data) and EI/MS spectra at *m/z* 194 and 180 respectively, in addition to their corresponding molecular formula $C_{10}H_{10}O_4$ and $C_9H_8O_4$, respectively, also, through comparison of their data with the literature, it was found that compound 9 is identified as ferulic acid, while, compound 10 is caffieic acid

Total Phenolic (TPC) and Total Flavonoid Contents (TFC) for *Urginea maritima* L. alcoholic extract:

The alcoholic extract of the aerial parts of *Urginea maritima* L. was chemically estimated. The content of total phenolics in the extract was measured using FCR assay and is expressed in gallic acid equivalent (GAE), while, total flavonoid content in it was estimated using aluminum chloride method (expressed as rutin equivalent, RE). Table (1) showed that TPC/gm plant extract is 292.98 ± 2.92 and that of TFC/gm plant extract is 109.87 ± 13.27.

Biological activities:

In vitro cytotoxic bioassay on human tumor cell lines (HCT 116, PC3 and HepG2)

Using MTT assay, the cytotoxic effect of the alcoholic extract and the isolated compounds was assessed. The data summarized in table (2), and illustrated in Fig (2), proved that, comparing with doxorubicin as standard, compound 4 (kaempferol 3-O-α-arabinopyranoside) showed strong cytotoxic effect against HCT 116 (human colorectal carcinoma, 98.00±0.98%), PC3 (human prostatic small cell carcinoma, 99.00±0.26%) and HepG2 (human hepatocellular carcinoma, 91.95±0.50%) with IC_{50} 21.29±1.3 ppm, 9.54±0.96 ppm and 31.67±3.13 ppm, respectively, as shown in table (3). Also, the results revealed that compound 8 (quercetin) has significant cytotoxic effects against PC3 and HepG2 (86.24±0.25% and 60.57±2.85%, respectively), in addition, compound 2 (quercetin 3-O-*α*-rhamnopyranosyl-7-O-*β*-glucopyranoside) showed a high activity against PC3 (83.87±1.85%) and moderate activity against HepG2 (59.07±7.86%), while it has no effect against HCT116 (34.27±7.71%). On the other hand, compound 1 (kaempferol 3-O-*β*-(6''-O-*α*-rhamnopyranosyl)-glucopyranoside) has a moderate activity against PC3 (76.17±9.33%), low activity against HCT116 (40.89±6.80%) and no activity against HepG2 (18.77±7.17%). IC_{50} of compounds 1, 2, 4 and 8 which gave 50% or more at 100ppm illustrated in table (3). The results in table (2) showed that the alcoholic extract has no effect against the three investigated cell lines, as well as, compounds 3 (kaempferol 3-O-*β*-(6''-E-*p*-coumaroyl)-glucopyranoside), 5 (kaempferol 3-O-*β*-galactopyranoside), 6 (quercetin 3-O-β-glucopyranoside), 7 (kaempferol), 9 (ferulic acid) and 10 (caffeic acid) showed very low to moderate activities against the three investigated cell lines, as illustrated in Fig (2).

In vitro hypoglycemic activity

Alcoholic extract of *Urginea maritima* L. aerial parts has shown no hypoglycemic activity (table 4) when assayed by two assay models including in vitro α-Amylase and *α*-Glucosidase inhibition studies, compared with acarbose as a standard.

Table (1): Estimation of Total Phenolic (TPC) and Total Flavonoid Contents (TFC) for *Urginea maritima* L. alcoholic extract:

Extract	TPC (mg gallic acid equivalent / gm plant extract)	TFC (mg rutin equivalent / gm plant extract)
Alcoholic extract	292.98 ± 2.92	109.87 ± 13.27

Table (2): Cytotoxic effect (percentage cytotoxicity) at 100 ppm of the alcoholic extract and the isolated compounds from *Urginea maritima* L. aerial parts on colorectal carcinoma (HCT116), prostate small cell carcinoma (PC3) and hepatocellular carcinoma (HepG2) human tumor cell lines.

Sample	HCT116 (%± SD)	PC3 (%±SD)	HepG2 (%±SD)
Alcoholic ext.	03.65±3.89	33.97±2.38	01.65±1.50
Compound 1	40.89±6.80	76.17±9.33	18.77±7.17
Compound 2	34.27±7.71	83.87±1.85	59.07±7.86
Compound 3	04.92±5.22	07.66±9.51	30.83±3.88
Compound 4	98.00±0.98	99.00±0.26	91.95±0.50
Compound 5	16.52±1.58	36.29±4.64	11.79±2.35
Compound 6	26.17±1.76	43.40±7.92	21.62±2.43
Compound 7	25.80±0.95	43.50±2.55	06.74±1.65
Compound 8	42.73±8.10	86.24±0.25	60.57±2.85
Compound 9	07.19±1.46	37.02±7.80	05.58±5.06
Compound 10	22.00±1.74	30.03±6.71	13.70±6.93
Doxorubicin	94.00±6.05	100	95±2.10

* Results are presented as mean percentage cytotoxicity ± Standard Deviation

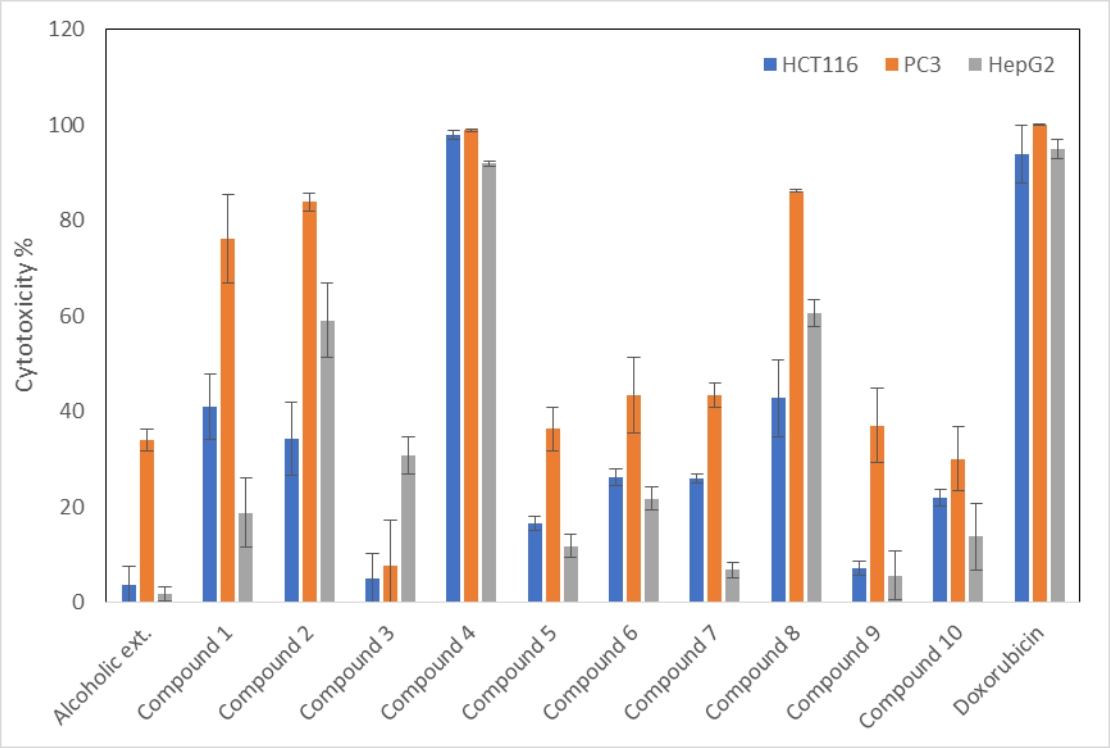

Figure (2): Percentage cytotoxicity at 100ppm of the alcoholic extract and the isolated compounds from *Urginea maritima* L. aerial parts on colorectal carcinoma (HCT116), prostate small cell carcinoma (PC3) and hepatocellular carcinoma (HepG2) human tumor cell lines

Table (3): IC_{50} (ppm) of compounds which gave 50% or more at 100 ppm

Sample	HCT116	PC3	HepG2
	IC_{50} (ppm)	IC_{50} (ppm)	IC_{50} (ppm)
Compound 1	-	75.61± 4.5, r^2= 0.98	-
Compound 2	-	44.58± 7.27, r^2= 0.93	67.63±11.98, r^2= 0.94
Compound 4	21.29±1.3, r^2= 0.99	9.54± 0.96, r^2= 0.98	31.67± 3.13, r^2= 0.97
Compound 8	-	38.42± 4.88, r^2= 0.96	76.67± 6.94, r^2= 0.98
Dox.	2.03±0.3, r^2= 0.91	6.8±1.2, r^2= 0.92	0.6±0.1, r^2= 0.96

IC_{50} value is the concentration required to give 50% cytotoxicity.
Values are expressed as Mean ± Standard Deviation (n=3).
r^2: goodness of fit

Table (4): The percentage inhibition of the tested *Urginea maritima* aerial parts alcoholic extract on alpha-Amylase at 55 ppm and on alpha-Glucosidase at 25 ppm

Sample	Percentage inhibition	
	α-Amylase (at 55 ppm)	**α-Glucosidase** (at 25 ppm)
Alcoholic extract	09.76±0.28	02.0±0.01
Acarbose	84.10±1.42	40±2.02

Values are expressed as Mean ± Standard Deviation (n=3).

CONCLUSION

The present study shows that ten phenolic compounds were isolated and purified by chromatographic techniques from the aerial parts of *Urginea maritima* L. (Family *Liliaceae*). Identification and structural elucidation of the isolated compounds were carried out using chemical investigation, and their structures were established by interpretation of their spectral data. The ten compounds isolated for the first time from this plant except compounds 4, 6 and 8. Also, in this work, cytotoxic activity of the alcoholic extract of *Urginea maritima* L. aerial parts and the isolated compounds was studied against three tested human tumor cell lines (HCT 116, PC3 and HepG2). The results revealed that the cytotoxic effects of the isolated compounds range from significantly high to moderate and mild, while, the alcoholic extract of *Urginea maritima* L. aerial parts has no effect against the three investigated cell lines and also has no hypoglycemic activity when assayed by two assay models including in vitro *α*-Amylase and *α*-Glucosidase inhibition studies.

CONFLICT OF INTEREST

The authors declared that present study was performed in absence of any conflict of interest.

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AUTHOR CONTRIBUTIONS

Mahmoud Ibrahim Nassar shared in plant organs extraction, isolation and in reviewing the manuscript. Ali Mohamed El-Hagrassi putting the idea, designed the experiments, plant extraction, separating pure compounds, collected results and reviewed the manuscript. Abeer Fouad Osman shared in putting the idea, collection the plant organs, separating pure compounds, determined TPC, TFC, writing the research and reviewed the manuscript. May Aly EL-Manawaty evaluated the cytotoxic and hypoglycemic activities of the extract and the isolated compounds. Dina Mahfouz Eskander shared in plant organs extraction, making the laboratory, storing experiments and shared in writing the research. All authors read and approved the final version.

REFERENCES

Adamsa M., Bersata C., Kesslerb M. and Hamburgera M. 2009. Medicinal herbs for the treatment of rheumatic disorders- A survey of European herbals from the 16th and 17th century. Journal of Ethnopharmacology, 121: 343-359.

Agrawal P. K. 1989. Carbon-13 NMR of Flavonoids, Elsevier Science Publishers B. V.

Al-Wakeel S.A.M., El-Garf, I.A.and Saleh, N.A.M.

1988. Biochem. Syst. Ecol., 16: 57 58.

Cardoso C. A. L., Coelho R. G., Honda N. K, Pott A., Pavan F.R. and Leite C.Q.F. Oct./dec. 2013. Brazilian Journal of Pharmaceutical Sciences, 49 (4).

Chen G.Y., Dai C.Y., Wang T.S., Jiang C.W., Han C.R. and Song X.P. 2010. A new flavonol from the stem-bark of Premna fulva. Arkivoc II.

Civelek H.S. and Weintraub P.G. 2004. Effects of two plant extracts on larval leafminer Liriomyza trifolii (Diptera: Agromyzidae) in tomatoes. J. Econ. Entomol., 97 (5): 1581-1586.

Delen N., Durmusoglu E.., Guncan A., Gungor N., Turgut C. and Burcak A. 2005. Turkiye'de pestisit kullanum, kalintt ve organizmalarda duyarlilik azalisi sorunlart. Turkiye Ziraat Muhendisligi 6, Teknik Kongresi, 629-648.

Elya B., Basah K., Mun'im A., Yuliastuti W., Bangun A. and Septiana E.K., 2012**.** Screening of α-Glucosidase Inhibitory Activity from Some Plants of Apocynaceae, Clusiaceae, Euphorbiaceae, and Rubiaceae. Journal of biomedicine and biotechnology, 1-6.

Erturk O., Kara O., Sezer E. and San G. 2004. Toxicity effect of some plant extracts on development of larvae of Plutella xylostella (L) (Lepidoptera; Pulutellidae). Ekoloji, 13 (50): 18-22.

Filippo Imperato. Oct 2008. American Fern Journal, 98 (4): 251-253.

Hao Liu, Yan Mou, Jianglin Zhao, Jihua Wang, Ligang Zhou, Mingan Wang, Daoquan Wang, Jianguo Han, Zhu Yu and Fuyu Yang. 2010. Molecule, 15: 7933-7945.

He J., Yin T., Chen Y., Cai L., Tai Z., Li Z., Liu C., Wang Y. and Ding Z. 2015. Phenolic compounds and antioxidant activities of edible flowers of Pyrus pashia. J Funct Foods, 17: 371-379.

Hussein S.R., Kawashty S.A., Tantawy M.E., El-Magly U.I.A. and Saleh N.A.M.2017. Phenolics of selected species of Persicaria and Polygonum (Polygonaceae) in Egypt. Arab J Chem, 10:76-81.

Ismail N.S., George R.F., Serya R.A., Baselious F.N., El-Manawaty M., Shalaby, E.M. and Girgis A.S. 2016. Rational design, synthesis and 2D-QSAR studies of antiproliferative tropane-based compounds. RSC Advances, 6 (104): 101911-101923.

Kawa D., and Badr-Aldin K.H. 2010. Cardiovascular studies of White Squill (Urginea matitima) Extract. Zanco J. Med. Sci., 14 (1).

Kim D.O., Chun O.K., Kim Y.J., Moon H.Y. and Lee C.Y. 2003. Quantification of polyphenolics and their antioxidant capacity in fresh plums. J Agric Food Chem, 516: 509-6515.

Kim M.K., Yun K.J., Lim D.H., Kim J. and Jang Y.P. 2016. Anti-inflammatory properties of flavone di-C-Glycosides as active principles of Camellia Mistletoe, Korthalsella japonica. Biomol Ther (Seoul), 24: 630-637.

Kokate C.K., Purohit A.P., and Gokhale S.B. 2006. Pharmacognosy, (Thirty Fifth Edition), Nirali Parkashan., 8: 205.

Kumaran A. and Karunakaran J. 2006. In vitro antioxidant activities of methanol extracts of five Phyllanthus species From India. LWT, 40: 344-352.

Linard A., Delaveau P., Paris R.R., Dellamonica G. and Chopin J. 1982. Isocarlinoside, a di-C-glycosylflavone from Lespedeza capitata. Phytochemistry, 21: 797-799.

Lokadi Pierre Luhata, Namboole Moses Munkombwe and Hanzooma Hatwiko. 2016. Isolation and ^{1}H-NMR identification of a tiliroside from Odontonema strictum (Acanthaceae) Journal of Pharmacognosy and Phytochemistry, 5 (1): 206-210.

Mabry T.J., Markham K.R. and Thomas M.B. 1970. The systematic identification of flavonoids. Springer, New York.

Madanlar N., Yoldas Z. and Durmusoglu E. 2002. Izmir'de sebze seralarnda zararhlara karst dogal pestisitlerle savas olanaklan.Turk Entomol Derg, 26 (3): 181-195.

Maria C.B., Maria J.P. and Benjamin R. A. 2002. New 24-Nor-Oleanane Triterpenoid from Salvia Carduacea, J. of Nat. Prod., 65: 1513.

Maria S. and Irena M. 2008. Acta Poloniae Pharmaceutical, Drug Research, 65 (4): 467-471.

Markham K.R. Techniques of flavonoid identification. Academic Press. 1982. London.

Marzouk M.M, Kawashty S.A, Saleh N.A.M. and Al-Nowaihi A.S.M. 2009 Chem Nat Comp., 45 (4): 483-486.

Mert M. and Betul B. 2010. Effects of the High Doses of Urgenea maritima (L.) Baker Extract on Chromosomes. Caryologia,, 63 (4): 367-375.

Mohamed R. Elgindi, Abdel Nasser Singab, Ibrahim M. Mahmoud and Sherouk H. Abdullah. 2015. Journal of Pharmacognosy and Phytochemistry, 4 (1): 72-78.

Montreau F.R. 1972. On the analysis of total phenolic compounds in wines by the Folin-Cioacalteau method. Connaissance Vigne Vin, 24: 397-404.

Moo-Puc J.A, Martin-Quintal Z., Miron-Lopez G., Moo-Puc R.E., Quijano L. and Mena-Rjon G.J. 2014. Isolation and antitrichomonal activity of the chemical constituents of the leaves of Maytenus Phyllanthodes Brnth. (Celastraceae).Quim Nova, 37: 85-88.

Mosmann T. 1983. Use of MTT colorimetric assay to

measure cell activation. J. Immunol Methods, 65: 55-63.

Nawal M., Benbacer L., Amzazi S., Morjani H. and El Mzibri M. 2009. Cytotoxic effect of some Moroccan medicinal plant extracts on human cervical cell lines. Journal of Medicinal Plants Research, 3 (12): 1045-1050.

Nguyen Thi Mai. September 2017. Journal of Multidisciplinary Engineering Science and Technology (JMEST), 4 (9).

Oliveira D.M., Siqueira E.P., Nunes Y.R.F. and Cota B.B. 2013. Flavonoids from leaves of Mauritia flexuosa. Rev Bras Farmacogn, 23: 614-620.

Osman M.E., Eid M.M., Khattab O.H., El-Hallouty S.M., El-Manawaty M. and Mahmoud D.A. 2015. In vitro cytotoxicity of biosynthesized Ag/CS NP against MCf7, PC3 and A549 cancer cell lines. International Journal of Pharm Tech Research, 8 (5): 1011-1017.

Ozmen A. and Sumer S. 2004. Cytogenetic effects of kernel extracts fhrom Melia azedarach L. Caryologia, 57: 290-293.

Pacôme Sientzoff, Jane Hubert, Coralie Janin, Laurence Voutquenne-Nazabadioko, Jean-Hugues Renault, Jean-Marc Nuzillard, Do minique Harakat and Abdulmagid Alabdul Magid, 2015. Molecules, 20: 14970-14984.

Paolo M., Guarrera G.S. and Giulia C. 2005. Folk phytotherpeutical plants from Maratea area (Basilicata, Italy). Journal of Ethnopharmacology, 99: 367-378.

Partridge, S. M. 1949. Nature, 164: 443-447.

Pascual-Villalobos M.J. and Fernandez M. 1999. Insecticidal activity of ethanolic extracts of Urgenea maritime (L) Baker bulbs. Industrial Crops and Products,10: 115-120.

Pauli G.F. 2000. Journal Nat Prod., 63 (6) : 834-838.

Qaisar M.N., Chaudhary B.A., Sajid M.U. and Hussain N. 2014. Evaluation of α- glucosidase inhibitory activity of dichloromethane and methanol extracts of Croton bonplandianum Baill. Tropical Journal of Pharmaceutical Research, 13 (11): 1833-1836.

Saleh N.A.M., El-Hadidi, M.N. and Al-Wakeel S.A.M. 1990. Biochem. Syst. Ecol., 18: 49-52.

Sharaf A.T., Sawidis T., Diannelidis B.E., and Delivopoulos S. 2006. Anatomical studies on the adventitious roots of the geophyte Urginea maritime (L) Baker. Journal of Biological Research, 5: 61-70.

Singleton V.L. and Rossi J.A. 1965. Colorimetry of total phenolics with phosphomolybdic-phosphotungstic acid reagents. Am J Enol Viticult, (16):144-158.

Subramani K., Mohan S. and Anbarasan P.M. 2016. Natural Products and Indian Journal, 12 (2): 57-61.

Swanton-Flatt S.K., Daay C., Baily C.I. and Flatt P.R. 1990. Traditional plant treatments for diabetes; studies in normal and streptozotocin diabetic mice. Diabetologia, 33: 642.

Xiao Z., Storms R. and Tsang A**.**A. 2006. Quantitative starch–iodine method for measuring alpha-amylase and glucoamylase activities. Analytical biochemistry, 351(1): 146-148.

Zhang J., Wang Y., Zhang X.Q., Zhang Q.W. and Ye W.C. 2009. Chemical constituents from the leaves of Lophatherum gracile. Chin J Nat Med, 7: 428-431.