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Research Paper

Biological investigation of some wild *Aizoaceae* and *Chenopediaceae* species growing in Egypt

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ABSTRACT

Six plants belonging to *Aizoaceae* and *Chenopodiaceae* families from Egypt were investigated for their potential use as antimicrobials against several Gram positive, Gram negative bacteria and fungi. The *n*-hexane extract of the tested plants showed inhibition zone of 27-22mm against *Bacillus subtilis*, *Staphylococcu aureus*. None of the tested extracts showed any activity against *Pseudomonas aeruginosa* or *Botrytis cinerrea*. The polar extracts did not show any remarkable inhibition for the tested microorganisms. The nonpolar and polar extracts of *Atriplex lindleyi* Moq. susp. *inflata* Fam. *Chenopodiaceae* were further phytochemically screened for their secondary metabolites. Eight compounds were isolated from the whole plant. Compounds **2, 3, 4** and **8** are first reported from *Atriplex lindleyi*. The polar fractions of *Atriplex lindleyi* Moq. susp. *inflata* significantly decreased fasting blood glucose level to 93.33±10.43 and 94.60±8.55mg/dl as compared to the diabetic control value. The screening of crude extracts obtained from some *Aizoaceae* and *Chenopodiaceae* species growing in Egypt has shown that some of them were potentially rich sources for antifungal, antibacterial and antidiabetic agents.

Keywords: *Atriplex lindleyi*; *Mesembryanthemum*; *Aizoon*; *Anabasis*; *Trianthema*.

INTRODUCTION

Many terrestrial plants have been subjected to chemical and pharmacological screening in order to discover their potential for human medicinal use. Several studies have investigated many *Chenopodiaceae* and *Aizoaceae* species for their biological activities, such as the antioxidant and antiviral activity of *Mesembryanthemum edule* L. and *Mesembryanthemum crystallinum* L. and the antifungal activity of *Atriplex inflata* (Ben Sassi, et al., 2008; Boughalleb, et al., 2009; Falleh, et al., 2011).

However *Aizoaceae* is poorly studied in Egypt. Reports have shown that genus *Mesembryanthemum* has long been used as food and in traditional medicine for the treatment of liver diseases and diabetes (Van Wyk, 2008; Bouftira, et al., 2009; Al-Faris, et al., 2010; Falleh, et al., 2011). *Mesembryanthemum forsskaolii* Hochst. Ex. Boiss seeds (*Aizoaceae*) were found to be superior as a replacement of wheat flour (Mustafa, et al., 1995; Al-Qahiz, 2009). *Trianthema portulacastrum* L. (*Aizoaceae*)

was also found to possess antibacterial and antifungal activity against several human pathogens (Animesh, et al., 1998; Sukanya and Malay, 1998; Nawaz, et al., 2001; Gautam, et al., 2006; Kumar, et al., 2006).

The plants under investigation from Egypt were not explored for their antimicrobial activities except for the work of Aboutabl (1997) on the antimicrobial activities of the volatile constituents of *Anabasis setifera* Moq (*Chenopodiaceae*). *Aizoon* and *Mesembryanthemum* (*Aizoaceae*) and *Atriplex lindleyi* Moq. susp. *inflata* (F. Muell) P.G. Wilson. (*Chenopodiaceae*) were not chemically or pharmacologically investigated as medicinally useful none toxic plants from the Egyptian deserts.

Atriplex is a plant genus of 100-200 species, known by the common name of saltbush. The genus is quite variable and widely distributed. It includes many desert and seashore plants and halophytes, as well as plants of moist environments. Saltbushes are extremely tolerant of salt content in the ground. Their name derives from the fact that they retain salt in their leaves, which makes them of great use in areas affected by soil salination (Djerroudi, et al., 2011). Various species of *Atriplex* have been used for their important medicinal values. For example; *Atriplex semibacata* R. Br and *Atriplex vestita* Thunb. Aellen have been used as an antifungal agent and in the treatment of bronchitis (Boughalleb, et al., 2009). *Atriplex hortenisis* L. has been regarded as a source of vitamin A. *A. inflata* has been reported to have antifungal activity in the non-polar extract against number of plant fungal pathogens. *Atriplex inflata* Muell and *Atriplex parvifolia* Lowe extracts were found to be effective against Herpes simplex viral infection (Ben Sassi, et al., 2008). Several species have been evaluated for their antidiabetic effects, such as *A. halimus* (Rodriguez and Murray 2010).

Several species of *Atriplex* has been investigated for their chemical constituents. The presence of saponins (Shaker, et al., 2003; Jabrane, et al., 2011), proteins, flavonoids, terpenoids (Broegger and Omar 1985; Siddiqui, et al., 1994) and amino acids (Aman, et al., 2011; Tawfik, et al., 2011), has been reported in many species.

However no phytochemical screening has been reported on the plant species *Atriplex lindleyi* Moq. susp. *inflata.* In this research work the *n*-hexane and ethyl acetate fractions of *Atriplex lindleyi* Moq. susp. *inflata* showed remarkable activity against *Bacillus subtilis, Staphylococcus aureus, Aspergillus fumigatus* in addition to three *Fusarium* species. We have isolated the major secondary metabolites in these fractions and tested them against the specified microbes. *Atriplex lindleyi* Moq. susp. *inflata* solvent fractions (*n*-hexane, $CHCl_3$, ethyl acetate, *n*-butanol) were examined for their antidiabetic and antioxidant activities.

MATERIALS AND METHODS

***Plant Material*:** Six plants belonging to the *Chenopediacea* and *Aizoaceae* families were collected and identified by Prof. AbdelHaleem AbdelMotagaly; Agriculture Museum, Giza, Egypt. The plants were collected (between March and August 2009-2010), at the flowering stage, from the way from Cairo to Suez Canal, located on the east desert of Egypt. The voucher specimen's numbers; R-Atri-09, R-Ana-09, R-Aizo-09, R-MC-10, R-MF-10 and R-Trian-10 were deposited at the Botanic Herbaium, Agriculture Meuseum, Dokey, Egypt. Scientific names, parts used and extract yields of the plants in this study are listed in Table-1.

***Preparation of plant extracts*:** Air-dried and finally ground samples (100g) of each plant material were successively extracted three times with ethanol (70%) (3 x

300ml). The alcoholic extracts were concentrated in vacuum using rotavapour at 40°C. The residue was dissolved in distilled water and was subjected to solvent partitioning using *n*-hexane (3 times x 200ml), $CHCl_3$ (3 times x 200ml), ethyl acetate (3 times x 200ml), and *n*-butanol (3 times x 200ml). All solvents were dried under vacuum, weighed and stored in sealed vials in a freezer at the temperature –20ºC, until antimicrobial testing. Scientific names, parts used and extract yields of the plants in this study are listed in Table-1.

Mesembryanthemum crystallinum L. and *Mesembryanthemum forsskaolii* Hochst. Ex. Boiss fruits (100g each) were collected and grounded in a blender while fresh and were dried under vacuum. The dried material was then subjected to solvent partitioning as described above.

***Extraction and Isolation of Atriplex lindleyi* Moq. susp. *inflata* secondary metabolites:** Materials used for chromatography study include Pre-coated silica TLC plates 60 F 254 (20x20 cm) (E. Merck), Silica gel (E. Merck) and Sephadex LH-20 (Pharmacia Fine Chemicals AB) for Column Chromatography. NP reagent and *p*-anisaldehyde reagents were used as spray reagents for flavonoids, triterpenoids and sterols. Air-dried and powdered plant *Atriplex lindleyi* Moq. susp. *inflata* (300g) were exhaustively and sequentially extracted with ethanol (70%). Each extract was concentrated in vacuum to obtain the dried alcoholic residue. The alcoholic extract (10g) was subjected to solvent partitioning using (*n*-hexane, $CHCl_3$, ethyl acetate and *n*-butanol). Each solvent fraction was dried under vacuum until dryness. The *n*-hexane fraction (4g) was subjected to silica gel column chromatography. Different sub-fractions were collected and combined using TLC to give white needles of β-sitosterol and stigma sterol (**5, 6**). The ethyl acetate fraction (1.2g) was chromatographed over silica gel using gradient elution technique of EtOAc:MeOH and successive sephadex LH20 columns using MeOH as eluent and afford one major flavonol quercetin (**1**, 100mg) and the 20-hydroxyecdysone (**8**, 10mg) in addition to β-sitosterol-3-*O*-glucoside (**7,** 20mg). The *n*-butanol fraction (2g) was screened by TLC, two major spots for flavonoid glycosides were visualized using NP reagent. The *n*-butanol compounds were purified using successive chromatographic columns of polyamide and Sephadex LH20 two major flavonoid glycosides were purified and identified as; quercetin 3-*O*-β-glucopyranoside (**2,** 7mg) and isorhamnetin 3-*O*-β-glucoside (**3,** 5mg) in addition to the 20-hydroxyecdysone (**8**, 7 mg)

Biological activity: The different fractions of the six plants were tested for their antibacterial and antifungal activities against several human and plant pathogens according to (Jigna and Sumitra, 2007). The results are presented in Table-2A and 2B.

Different fractions of *Atriplex lindleyi* Moq. susp. *inflata* were found to be of the highest yield and were tested for their antidiabetic and antioxidants activities. The results are presented in Tables-3 and 4.

Antibacterial activity of the tested plants extracts: Antibacterial activities were investigated using agar well diffusion method. The activity of the tested samples was studied against *Staphylococcus aureus* (RCMB 010028) and *Bacillus subtilis* (RCMB 010067) as Gram positive bacteria, and *Pseudomonas aeruginosa* (RCMB 010043), *Salmonella typhimurium* (RCMB 010072), *Escherichia coli* (RCMB 010052) as Gram negative bacteria. The solution of 5mg/ml of each sample in dimethyl sulfoxide (DMSO) was prepared for testing against bacteria. Centrifuged pellets of bacteria from a 24 h old culture containing approximately 104-106 CFU (colony forming unit) per ml were spread on the surface of Nutrient agar (typtone 1%, yeast extract 0.5%, NaCl 0.5%, agar 1%, 1l distilled water, pH 7.0) which was autoclaved at 121ºC for at

least 20 min. Wells were created in the medium with the help of sterile metallic bores and then cooled down to 45ºC. The activity was determined by measuring the diameter of the inhibition zone (in mm). All samples were prepared in DMSO which was loaded as control. The plates were kept for incubation at 37ºC for 24 h then the plates were examined for the formation of zone of inhibition. Each inhibition zone was measured three times by caliper to get an average value. The test was performed three times for each bacterium culture. Ampicillin and gentamicin were used as antibacterial standard.

Antifungal activity of the tested plants extracts: Tested samples were screened separately *in vitro* for their antifungal activity against various fungi viz. *Candida albicans* (RCMB 05031)**,** *Aspergillus fumigatus* (RCMB 02568)**,** *Penicillium italicum* (RCMB 03924)**,** *Fusarium* oxysporum (RCMB 08056)**,** *Fusarium s. cucurbitae* (RCMB 08042)**,** *Fusarium niveum* (RCMB 08037)**,** and *Botrytis cinerrea* (RCMB 07324)**.** The culture of fungi was purified by single spore isolation technique. The antifungal activity was achieved by agar well diffusion method by the following procedure:

Sabourad dextrose agar plates: A homogeneous mixture of glucose-peptone-agar (40-10-15) was sterilized by autoclaving at 121ºC for 20 min the sterilized solution (25ml) was poured in each sterilized Petri dish in laminar flow and left for 20 min to form the solidified sabourad dextrose agar plates. These plates were inverted and kept at 30ºC in incubator to remove the moisture and to check for any contamination.

Antifungal assay: fungal strain was grown in 5ml sabourad dextrose broth (glucose: peptone; 40:10) for 3-4 days to achieve 105 CFU/ml cells. The fungal culture (0.1ml) was spread out uniformly on the sabourad dextrose agar plates by sterilized triangle folded glass rod. Plates were left for 5-10 min so the culture is properly adsorbed on the surface of the sabourad dextrose agar plates. Now small wells of size (4mm x 2mm) were cut into the plates with the help of well cutter and bottom of the wells were sealed with 0.8% soft agar to prevent the flow of test sample at the bottom of the well. 100µl of the tested samples (10 mg/ml) were loaded into the wells of the plates. All samples were prepared in DMSO, which was loaded as control. The plates were kept for incubation at 30ºC for 3-4 days and then the plates were examined for the formation of zone of inhibition. Each inhibition zone was measured three times by caliper to get an average value. The test was performed three times for each fungus. Amphotericin B was used as antifungal standard drug.

Antibacterial and Antifungal activity of the purified compounds (1-8): Serial dilution method was performed for determination of minimum inhibitory concentration (MIC) of the isolated compounds **1-8**: sets of test tubes, each containing 2ml of nutrient broth were prepared and sterilized. one mg of the tested compound dissolved in one ml DMSO were aseptically transferred to the first tube and after thorough shaking, one ml of the mixture was aseptically introduced into a second tube. Such process was repeated from the second till the last tube of the series. Each tube was then inoculated with one drop of freshly prepared spore suspension of tested organism and the whole series was incubated for 24 h for bacteria and 48 h for fungi at 30ºC. The tubes showing no growth of the tested organism (no turbidity) were counted and the minimum dilution of the extract, which caused the inhibition of the tested organism, was calculated.

Antidiabetic activity: Table-3 is showing the fasting blood glucose of non diabetic, diabetic, diabetic treated rats with oral hypoglycemic (positive control) and diabetic administered different fractions of *Atriplex lindleyi* Moq. susp. *inflata* Day 0 was the

start of the experiment. The oral hypoglycemic agent and the fractions were administered for the first time after the baseline blood glucose concentration was measured.

The normal control value of fasting blood glucose level was 90.32±5.51mg/dl. Streptozotocin significantly increased fasting blood glucose level to 353.80±39.42mg/dl as compared to normal control value.

Glimiperide (positive control) significantly decreased fasting blood glucose level to 95.08 ± 5.83 mg/dl as compared to the diabetic control value.

*Atriplex lindley*i Moq. susp. *inflata n*-butanol and ethyl acetate fractions were significantly decreased fasting blood glucose level to 93.33±10.43 and 94.60±8.55mg/dl as compared to the diabetic control value.

It is to be noted that both *n*-hexane and chloroform fractions of *Atriplex lindley*i did not significantly alter fasting blood glucose level of the diabetic rats.

Estimation of GSH and lipid Peroxidation (TBARS) levels: Table-4 is showing the GSH and MDA levels of non diabetic, diabetic, diabetic treated with oral hypoglycemic (positive control) and diabetic administered rats' different fractions of *Atriplex lindly*.

The normal control value of serum GSH and MDA levels were 73.50±5.37mg% and 1.42±0.15nmol/ml respectively. Streptozotocin significantly decreased serum GSH level to 42.77±3.27mg% and significantly increased serum MDA level to 4.38±0.51nmol/mL compared to the normal control group.

Glimiperide (positive control) significantly increased serum GSH level to 70.34±4.60mg% and significantly decreased serum MDA level to 1.63±0.27nmol/ml compared to the diabetic control value.

Both *n*-butanol and ethyl acetate fractions of *Atriplex lindly* significantly increased serum GSH levels to 64.12±4.20 and 65.35±3.35mg/dl respectively as compared to the diabetic control value.

Both *n*-butanol and ethyl acetate fractions of *Atriplex lindly* significantly decreased serum MDA levels to 1.55±0.38 and 1.74±0.35nmol/ml respectively as compared to the diabetic control value.

It is to be noted that both *n*-hexane and chloroform fractions of *Atriplex lindly* did not significantly alter GSH and MDA levels of the diabetic rats.

***Identification of the isolated compounds*:** Compounds **1-8** (Fig. 1) were established conclusively by ^{1}H NMR, DEPT-135 and ^{13}C NMR spectral analysis, MS and comparison with spectral data reported in the literature.

Compound **1: Quercetin**: $C_{15}H_{10}O_7$ negative ion ESI-MS m/z 301 $[M-H]^-$; ^{1}H NMR (DMSO, 400 MHz): δ 6.16 (1H, *d*, *J* = 2.0 Hz, H-6), δ 6.38 (1H, *d*, *J* = 2.0 Hz, H-8), δ 6.87 (1H, *d*, *J* = 8.4 Hz, H-5′), δ 7.52 (1H, *dd*, *J* = 2.4, 8.4 Hz, H-6′), δ 7.65 (1H, *d*, *J* = 2.0 Hz, H-2′); ^{13}C NMR, DEPT 135 and 2D NMR (HMQC, HMBC, COSY), (DMSO, 125 MHz): compared with the reported data in literature (Guyenalp and Demirezer, 2005).

Compound **2: Quercetin 3-O-*β*-glucopyranoside**: $C_{21}H_{20}O_{12}$; negative ion ESI-MS m/z 463 $[M-H]^-$;^{1}H NMR (DMSO, 400 MHz): δ 6.15 (1H, *d*, *J* = 1.84 Hz, H-6), δ 6.36 (1H, *d*, *J* = 2.0 Hz, H-8), δ 6.82 (1H, *d*, *J* = 9.16 Hz, H-5′), δ 7.56 (1H, *dd*, *J* = 1.84, 9.0 Hz, H-6′), δ 7.57 (1H, *d*, *J* = 2.0 Hz, H-2′), δ 5.44 (1H, *d*, *J* = 7.2 Hz, H-1″), δ 3.05-3.53 (6H, *m*, H-2″, H-3″, H-4″, H-5″, H-6″); ^{13}C NMR and DEPT 135 (DMSO, 125 MHz): compared with the reported data in literature (Guyenalp and Demirezer, 2005).

Compound **3: Isorhamnetin 3-O-*β*-glucopyranoside**: $C_{22}H_{22}O_{12}$; negative ion ESI-MS m/z 477 $[M-H]^-$;^{1}H NMR (DMSO, 400 MHz): δ 6.08 (1H, *d*, *J* = 1.8 Hz, H-6), δ 6.29 (1H, *d*, *J* = 2.0 Hz, H-8), δ 6.80 (1H, *d*, *J* = 8.24 Hz, H-5′), δ 7.50 (1H, *dd*, *J* = 1.84, 9.0 Hz, H-6′), δ 7.53 (1H, *d*, *J* = 2.0 Hz, H-2′), δ 5.45 (1H, *d*, *J* = 7.32 Hz, H-1″), δ 3.01-3.68 (6H, *m*, H-2″, H-3″, H-4″, H-5″, H-6″), δ 3.86 (3H, s, OCH_3); ^{13}C NMR and DEPT 135 (DMSO, 125 MHz): compared with the reported data in literature (Hassanean and Desoky, 1992).

Compound **4: 3β,23-dihydroxy betulin**: $C_{30}H_{50}O_2$; positive ion ESI-MS m/z 441 $[M-H]^-$; ^{1}H NMR and ^{13}C NMR and DEPT 135 ($CDCl_3$, 400 and 125 MHz) were compared to the data reported in literature (Tinto, et al., 1992; Mahato and Kundu, 1994).

Compound **8: 20-hydroxyecdysone:** $C_{27}H_{44}O_7$; positive ion ESI-MS m/z 481 $[M+H]^+$; ^{1}H NMR and ^{13}C NMR, DEPT 135 and 2D NMR (HMQC, HMBC, COSY, NOESY), (Pyridine, 400 and 125 MHz) were compared to the data reported in literature (Giiwjlt and Lafont, 1988).

Compound **5** and Compound **6:** obtained as **sterol mixture**, ESI-MS, ^{1}H NMR, ^{13}C NMR and DEPT 135 ($CDCl_3$, 400 and 125 MHz) were compared to the data reported in literature (Kamboj and Saluja, 2011), and with data of authentic samples.

Compound **7**: ESI-MS, ^{1}H NMR, ^{13}C NMR and DEPT 135 (CD_3OD, 400 and 125 MHz) were compared to the data reported in literature (Mizanur Rahman, et al., 2009) and with data of authentic sample

RESULTS AND DISCUSSION

Our research aimed at identifying natural products from Egyptian desert plants with new biological activity. The 24 nonpolar and polar solvent extracts of these plants were evaluated for their antimicrobial activities. Table-1 shows the botanical names, families, plant parts used and extract yield in grams. The antimicrobial activities of the investigated extracts are shown in Table 2-A and 2B, the positive standards used for the tested microbes are listed in Table 2C. The plant extract is considered significantly active when it shows an inhibition zone of ≥ 14 mm. The nonpolar fraction of *Atriplex lindleyi* Moq. susp. *inflata, Anabasis setifera* Moq, *Mesembryanthemum crystallinum L.* and *Mesembryanthemum forsskaolii* Hochst. Ex. Boiss showed remarkable activity against the gram positive *Bacillus subtilis* and *Staphylococcus aureus* strains with inhibition zones ranged from 27-22 mm. The nonpolar fraction of *Atriplex lindleyi* Moq. susp. *inflata* showed remarkable activity against the tested fungal strains (except *Botrytis cinerrea*) with inhibition zones ranging from 23-17 mm. The nonpolar fractions of *Mesembryanthemum crystallinum* L., *Mesembryanthemum forsskaolii* Hochst whole plant extracts were remarkably active against the tested organisms compared with their fruit extracts. None of the tested extracts showed any activity against *Pseudomonas aeruginosa* or *Botrytis cinerrea*. Results with the inhibition zone of each extract against the twelve tested microorganisms are listed in Table-2A and 2B.

The polar and nonpolar extracts of *Atriplex lindleyi* Moq. susp. *inflata* showed the highest activity against the tested microbes as well as the highest extract yield and was further screened for purification of the major secondary metabolites. Compounds **1-8** (Fig. 1) were purified from the polar and nonpolar fractions of *Atriplex lindleyi* Moq. susp. *inflata* and were tested against the twelve microbes. Compound **4** was active against *Fusarium oxysporum* and *Fusarium s. cucurbitae* with MIC of 0.24 and

0.6µg/ml respectively. Compound **4** and **8** were active against *Staphylococcus aureus* and *Bacillus subtilis* with MIC of 0.98, 1.95 and 7.8, 9 µg/ml respectively.

Both the *n*-butanol and ethyl acetate fractions of *Atriplex lindleyi Moq.* susp. *inflata* significantly decreased fasting blood glucose level to 93.33±10.43 and 94.60±8.55 mg/dl as compared to the diabetic control value. It is to be noted that both *n*-hexane and chloroform fractions of *Atriplex lindley*i did not significantly alter fasting blood glucose level of the diabetic rats.

Both *n*-butanol and ethyl acetate fractions of *Atriplex lindly* significantly decreased serum MDA levels to 1.55±0.38 and 1.74±0.35 nmol/ml respectively as compared to the diabetic control value. Results of the antidiabetic and antioxidant activities are reported and illustrated in Tables 3-4. The alcoholic extracts of the six plants did not show any cytotoxic activity against SK-MEL, KB, BT-549, SK-OV-3 cell lines.

Our results showed that the extracts obtained from the six plants in this study which grow in Egypt are efficient against several human and plant pathogens. Ethyl acetate and *n*-butanol which are the polar solvents used for fractionation were not or marginally active against all the tested microbes. The *n*-hexane extract which is the less polar solvent used for fractionation was more active against the tested microbes (inhibitory zone of 27mm, compared with inhibitory zone of ampicillin Table-2C). These results support the fact that the antagonistic material is concentrated in the nonpolar extract (Boughalleb, et al., 2009).

The bioassay guided isolation of the active metabolites from the extract showed the highest inhibitory activity (*Atriplex lindleyi*) leads to the identification of compounds **4** and **8.** The two compounds belonging to the chemical classes triterpenoids and sterols and have shown remarkable activity against *Fusarium oxysporum, Fusarium s. cucurbitae*, *Staphylococcus aureus* and *Bacillus subtilis*. These compounds probably inhibited the multiplication of these microbial pathogens. The two classes of metabolites are mostly common in the nonpolar fractions which may explain the remarkable activity of the *n*-hexane extracts of the other plants.

The polar extracts of *Atriplex lindleyi* were evaluated for their antidiabetic and antioxidant activity. The phytochemical screening of these extracts leads to the identification of three major flavonoids and flavonoid glycosides (compounds **1-3**) which are previously known for their antidiabetic and antioxidant activities (Mahesh and Menon, 2004) .

Compound **2, 3, 4** and **8** are first reported from *Atriplex lindleyi*. However compounds **1, 5, 6** and **7** have been reported from several *Atriplex* sp. These metabolites belonged to three different chemical classes; sterol, triterpenes and flavonoids and the isolation of these metabolites could be a useful contribution to the taxonomic studies of *Atriplex* species from Egypt.

We conclude that the nonpolar extract of *Atriplex lindleyi* Moq. susp. *inflata* could be medically used as antibacterial agent while its polar extract which is rich in flavonoids and flavonoid glycosides is medicinally useful as antidiabetic and antioxidant drug. The six plants under study are common in the Egyptian deserts as food for the human or their animals. The goal of this study is to investigate the potential use of these edible plants as medicine. Our ongoing research aimed to further investigate *Mesembryanthemum crystallinum* WP, *Mesembryanthemum forsskaolii* WP and *Aizoon canariense* L., to isolate the active metabolites in their *n*-hexane fractions which were responsible for their antimicrobial activity.

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The authors report no declarations of interest.

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Table- 1: Scientific names, plant parts used and extract yields of the plants used in this study.

Scientific name	Plant part used (100g, dried powder)	Extract weight (g)			
		***n*-hexane**	**$CHCl_3$**	**EtOAc**	***n*-butanol**
Atriplex lindleyi Moq. susp. *inflata* (F. Muell) P.G. Wilson. *(Chenopodiaceae)*	Whole plant	2.8	1.2	0.9	1.8
Anabasis setifera Moq (*Chenopodiaceae*)	Whole plant	1.0	0.5	0.3	1.0
Aizoon canariense L. (*Aizoaceae*)	Whole plant	0.9	0.7	0.2	0.6
Mesembryanthemum crystallinum L. (*Aizoaceae*)	Fruits	1.0	0.7	0.1	0.6
Mesembryanthemum crystallinum L. (Aizoaceae)	Whole plant	2.1	1.0	0.2	0.6
Mesembryanthemum forsskaolii Hochst. Ex. Boiss (*Aizoaceae*)	Fruits	1.0	0.7	0.1	0.6
Mesembryanthemum forsskaolii Hochst. Ex. Boiss (*Aizoaceae*)	Whole plant	2.0	1.0	0.2	0.6
Trianthema portulacastrum L. (*Aizoaceae*)	Whole plant	0.7	0.2	0.05	0.8

Table- 2A: Antimicrobial activity of plant extracts in agar diffusion assay.

Plant	Solvent extract	*Bacillus subtilis*	*Staphylococcus aureus*	*Escherichia coli*	*Pseudomonas aeruginosa*	*Salmonella typhimurium*	*Candida albicans*
Atriplex lindleyi Moq. susp. *inflata* (F. Muell) P.G. Wilson.	n-Hex $CHCl_3$ EtOAc n-Butanol	27 ±0.36 - 15.8±0.1 9.4±0.2	25.8±0.22 - - -	7.4±0.23 - 7.8±0.29 -	- - - -	10.2±0.2 - - -	17.4±0.23 - - -
Anabasis setifera Moq	n-Hex $CHCl_3$ EtOAc n-Butanol	20.9±0.3 - 13.1 ±.17 -	19.7±0.1 8±0.6 - -	18.2±0.1 - - -	- - - -	19.9±0.1 - - -	19.8±0.3 - - -
Aizoon canariense L.	n-Hex $CHCl_3$ EtOAc n-Butanol	15.9±0.3 10.2±0.3 - -	13.1±0.17 - 11.2 ±2.5 -	10.4±0.17 - - -	- - - -	12.9±0.1 - - -	10.3±0.3 - - -
Mesembryanthemum crystallinum L. **Fruits**	n-Hex $CHCl_3$ EtOAc n-Butanol	12.1 ±1.3 - - -	10.2 ±0.2 - - -	- 9.4 ±0.5 - -	- - - -	10.7 ±0.13 - - -	- - - -
Mesembryanthemum crystallinum L.	n-Hex $CHCl_3$ EtOAc n-Butanol	25.4±0.1 - - -	22 ±0.16 - - -	15,6 ±0.27 - - -	- - - -	18.3±0.23	15 ±0.1 - - -
Mesembryanthemum forsskaolii Hochst. Ex. Boiss **Fruits**	n-Hex $CHCl_3$ EtOAc n-Butanol	19.4 ±0.2 - - -	16.2±0.17 - - -	16.2 ±0.33 - - -	- - - -	10..3 ±0.23 - - -	12.4±0.3 - - -
Mesembryanthemum forsskaolii Hochst. Ex. Boiss	n-Hex $CHCl_3$ EtOAc n-Butanol	25.4 ±0.1 - - -	22±0.16 - - -	15.6 ±0.27 - - -	- - - -	17.6 ±0.23 - - -	18.3 ±.3 - - -
Trianthema portulacastrum L.	n-Hex $CHCl_3$ EtOAc n-Butanol	19.8±0.2 - - -	17.8±0.1 - - -	- - - -	- - - -	- - - -	15.2±0.1 - - -

- The test was done using diffusion agar technique, well diameter 6mm, 100ul was tested, data was expressed in the form of mean ±SD, - means Not Active

Table- 2B: Antimicrobial activity of plant extracts in agar diffusion assay.

Plant	Solvent extract	*Aspergillus fumigatus*	*Penicillium italicum*	*Fusarium oxysporum*	*Fusarium s. cucurbitae*	*Fusarium niveum*	*Botrytis cinerrea*
Atriplex lindleyi Moq. susp. *inflata* (F. Muell) P.G. Wilson.	n-Hex $CHCl_3$ EtOAc n-Butanol	20.6±0.22 - 10.4±0.11 10.4±0.11	19.9±0.15 - - -	23.6±0.18 - - -	22.4±0.15 - - -	20.3±0.24 - - -	- - - -
Anabasis setifera Moq	n-Hex $CHCl_3$ EtOAc n-Butanol	17.3±0.26 - - -	19.3±0.23 - - - -	19.9±0.2 - - -	17.7±0.13 - - -	14.2±0.34 - - -	- - - -
Aizoon canariense L.	n-Hex $CHCl_3$ EtOAc n-Butanol	11.4±0.21 - - -	12.4±0.5 - - -	18.2±0.18 - - -	14.9±0.23 - - -	17.8±0.19 - - -	- - - -
Mesembryanthemum crystallinum L. **Fruits**	n-Hex $CHCl_3$ EtOAc n-Butanol	- - - -	- - - -	12.7±0.31 - - -	10.7 ±0.28 - - -	9.8 ±0.15 - - -	- - - -
Mesembryanthemum crystallinum L.	n-Hex $CHCl_3$ EtOAc n-Butanol	20.2 ±0.2 - - -	17.9 ±0.05 - - -	21.4±0.2 - - -	20.7 ±0.34 - - -	18.9 ±0.19 - - -	- - - -
Mesembryanthemum forsskaolii Hochst. Ex. Boiss **Fruits**	n-Hex $CHCl_3$ EtOAc n-Butanol	- - - -	- - - -	- - - -	- - - -	- - - -	- - - -
Mesembryanthemum forsskaolii Hochst. Ex. Boiss	n-Hex $CHCl_3$ EtOAc n-Butanol	20.5±0.22 - - -	18.3±0.26 - - -	15.7±0.15 - - -	20.9±0.18 - - -	19.8±0.22 - - -	- - - -
Trianthema portulacastrum L.	n-Hex $CHCl_3$ EtOAc n-Butanol	18.7±0.11 - - -	19.3±0.1 - - -	15.2±0.27 - - -	17.7±0.14 - - -	142±0.34 - - -	- - - -

- Footnotes are the same as in Table-2A.

Table- 2C: Antibiotic used as positive controls for the tested microorganisms.

Tested Organism	**Standard**
Fungi	**Amphotericin B**
Aspergillus fumigatus	23.7±0.10
Penicillium italicum	21.9±0.12
Candida albicans	19.8±0.20
Fusarium oxysporum	25.4±0.16
Fusarium s. cucurbitae	26.7±0.13
Fusarium niveum	24.3±0.14
Botrytis cinerrea	21.9±0.18
Gram positive bacteria	**Ampicillin**
Staphylococcus aureus	27.4±0.18
Bacillus subtilis	32.4±0.3
Gram negative bacteria	**Gentamicin**
Pseudomonas aeruginosa	17.3±0.15
Salmonella typhimurium	28.8±0.24
Escherichia coli	22.3±0.18

Table- 3: Effect of 300 mg/kg of different fractions of *Atriplex lindly* on fasting blood glucose concentration in streptozotocin-induced diabetic rats.

Treatment Groups	**Fasting blood glucose levels (mg/dl)**	
	0 day	**7th day**
Group I :Normal Control	89.09±8.93	90.32±5.51
Group II: Diabetic Control	362.90±29.42 *	353.80±39.42 *
Group III: Positive Control	325.30±36.86 *	95.08±5.83 [a]
Group IV: *n*-butanol Fraction	329.50±36.25 *	93.33±10.43 [a]
Group V: ethyl acetate Fraction	337.80±36.88 *	94.60±8.55 [a]
Group VI: *n*-hexane Fraction	343.10±39.33 *	339.80±33.54 *
Group VII: Chloroform Fraction	336.10±40.74 *	331.90±31.40 *

- Each values is a mean ± SD (n = 6 in each group). Values are statistically significant at $p < 0.05$. Statistical analysis was carried out using one way analysis of variance (ANOVA) followed by Tukey-Kramer multiple comparisons test.
- *: Significantly different from the normal control group.
- a: Significantly different from the diabetic control group.

Table- 4: Effect of 7 days daily dose administration of 300 mg/kg of different fractions of *Atriplex lindly* on levels of GSH and TBARS in streptozotocin- induced diabetic rats.

Treatment Groups	GSH mg%	TBARS (MDA) nmol/ml
Group I :Normal Control	73.50±5.37	1.42±0.15
Group II: Diabetic Control	42.77±3.27 *	4.38±0.51 *
Group III: Positive Control	70.34±4.60 [a]	1.63±0.27 [a]
Group IV: *n*-butanol Fraction	64.12±4.20 [a]	1.55±0.38 [a]
Group V: ethyl acetate Fraction	65.35±3.35 [a]	1.74±0.35 [a]
Group VI: *n*-hexane Fraction	44.75±6.02 *	4.84±0.34 *
Group VII: Chloroform Fraction	45.89±5.18 *	5.06±0.60 *

- Footnotes are same as shown in table-3.

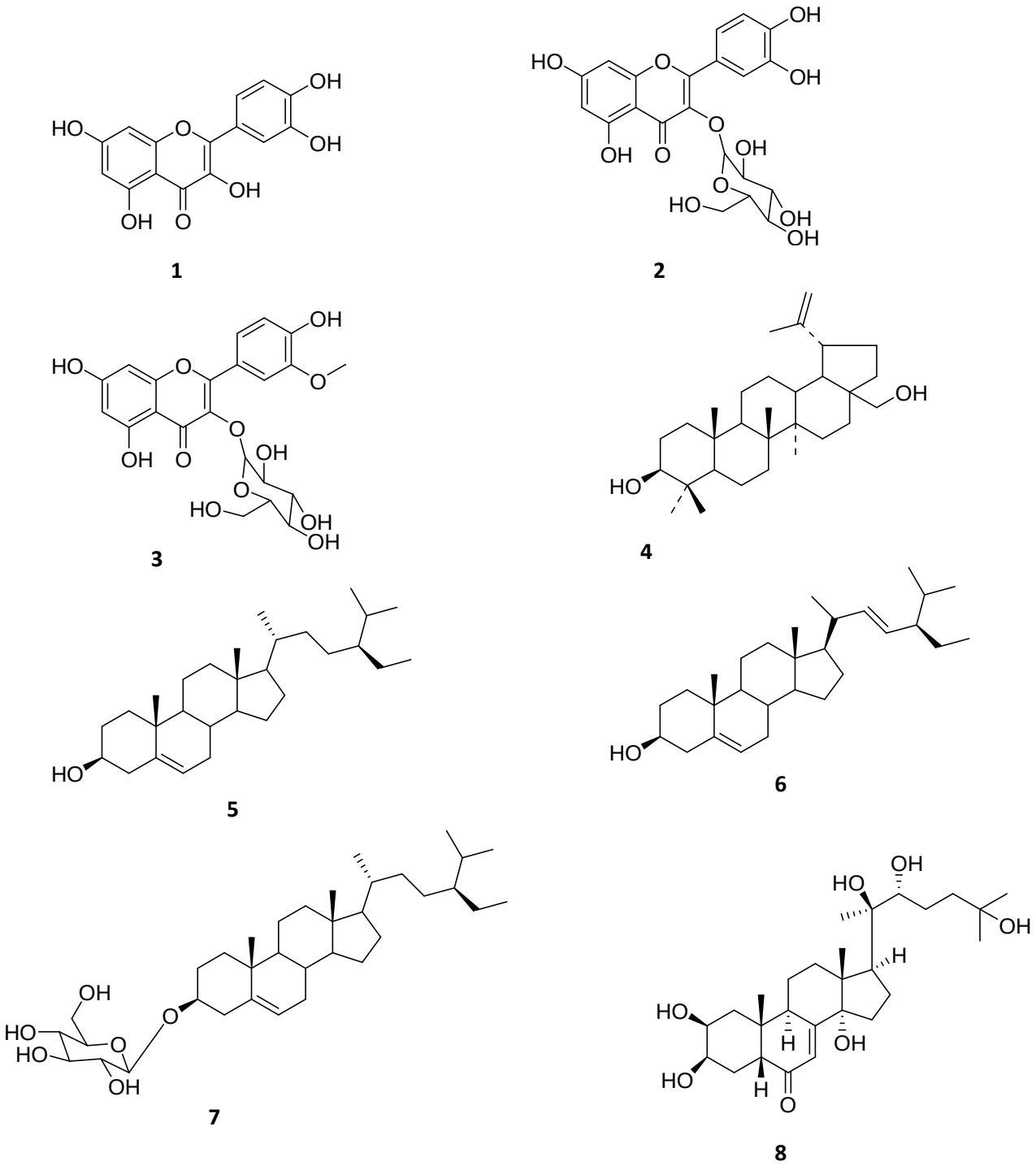

Figure -1: The identified compounds from *Atriplex lindleyi Moq.* susp. *Inflate.*

- 1= Quercetin; 2= Quercetin 3-O-*β*-glucopyranoside; 3= Isorhamnetin 3-O-*β*-glucopyranoside; 4=3β,23-dihydroxy betulin; 5= β-sitosterol; 6= stigma sterol; 7= β-sitosterol-3-*O*-glucoside; 8=20-hydroxyecdysone