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# Bioactive constituents of *Atriplex halimus* plant

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## ABSTRACT

Chemical analysis of *Atriplex halimus* (Family: *Chenopodiaceae*) revealed that the plant at Wadi-Hof and Wadi-Sudr habitats contained eleven free amino acids with different range of concentrations, while glycine and phenylalanine were present only at Wadi-Sudr habitat, meanwhile lysine and cystine were present only at Wadi-Hof habitat. The plant also contained fifteen protein amino acids at the two habitats but with different concentrations. The percentage of proline was higher in plants of Wadi-Sudr than Wadi-Hof. GLC of the unsaponfiable matter revealed the presence of six hydrocarbon in each habitat and two sterols in Wadi-Sudr only, GLC of saponifiable matter revealed the presence of three and five fatty acids, beside three and four unsaturated fatty acids at Wadi-Hof and Wadi-Sudr respectively. Investigation of carbohydrates using HPLC showed that the plant contained six and seven free sugars at Wadi-Hof and Wadi-Sudr habitats, respectively beside seven combined sugars at the two habitats. Chromatographic investigation of ethyl acetae extract indicated that the plant at the two habitats contained, flavonol, flavanone, flavone glycosides while isoflavone glycosides and vicenineII were present only at Wadi-Sudr. Chromatographic investigation of alkaloids revealed the presence of three alkaloids at Wadi-Sudr habitat and two alkaloids at Wadi-Hof.

**Keywords:** *Atriplex halimus*; Carbohydrate; Alkaloids; Flavanone; Glycosides.

## INTRODUCTION

The aim of this study is to investigate the bioactive constituents of *Atriplex halimus* plant. *Atriplex* is highly tolerant to cold and heat stress and does not require irrigation above an annual rainfall of 250 mm. Atriplex species are acceptable to many ruminants (El-Shaer, et al., 1990). Twenty-three selected plants belonging to 12 families were collected and extracted with organic solvents. The extracts were tested for their antimicrobial activities against 14 Gram-positive and Gram-negative bacteria, results showed that the greatest activity was exhibited by the ethyl acetate extract of *Atriplex parvifolia*, the methanol extract of *Satureia nervosa* and hexane and acetone extracts of *Hypericum crispum* (Sassi, et al., 2007).

Flavonods are antioxidants, metal chelators and possess antiinflammatory, antiallergic, antiviral, anticarcinogenic and antithrombotic, activities (Tapas, et al.,

2008). A new acetylated flavonol glycoside:- patuletin-3-o-[5```-o-feruloyl-ß-D-apiofuransyl (1``→2``)-ß-D-glucopyranoside] together with a known patuletin-3-o-ß-D-glucopyranoside were isolated from the aerial part of *Atriplex littorallis* L. (Bylka, 2007). Two new flavonoids, quercetin-6,4`-dimethoxy-3-rhamnofructosyl and quercetin-4`-dimethoxy-7-rhamnofructosyl were isolated from *Atriplex lentiformis*, in addition to another five known compounds (Awaad, et al., 2007). Six compounds, I) isorhamnetin, tricin (II), querecetin-7-O-alpha-L-rhamnoside (III), isoorientin (IV), beta-sitosterol (V), beta-daucosterol (VI) were isolated and identified from the seeds of *Atriplex centralasiatica* (Zhang and Zhao, 2005). A number of medicinal conditions could be prevented or improved with the use of flavonoids. These include circulatory disorders, lung disorders, diabetes, eye disorders and macular degeneration (Ray Sahelian, 2005).

Lipids are essential component of biological membranes, free molecules and metabolic regulators that control cellular function and homeostatis (Chiang, 2005). Proline improved the growth of salt stressed to cell cultures and the improvement was attributed to the role of proline as an osmoprotectant for enzymes and membranes against salt inhibition rather than as a compatible (Okuma, et al., 2000). Berbamine, an alkaloid compound, posses arrhythmia and anti-inflammatory properties, (Guo and Fu, 2005), it exhibited an inhibitory effect on skin graft rejection (Ren, et al., 2008). Two new bisbenzylisoquinoline alkaloids, neothalfine (1) and thaliatrine (2), together with three known dimeric alkaloids, thalifaberine, thalistine, and thalirecebine from the root of *Thalictrum atriplex* Finet et Gagnep (Gao, et al., 2005). Some alkaloids prevent the paralysis of the muscles in the respiratory tract and the heart, other alkaloids used to unwind the muscles of the heart during open heart surgeries.

## MATERIALS AND METHODS

The whole well-grown *Atriplex halimus* plant was collected from Wadi Hof and Wadi-Sudr during the period of investigation. The plant was identified by Prof. Dr. Sayed Farag Khalifa (Prof. of Plant Taxonomy and Flora, Botany department, Faculty of Science Ain Shams University) and Prof. Dr. Adel Kamel Youssef (Prof. of Plant Ecology and Phytochemisty, Desert Research Center). The voucher number of the plant is 2).

**Quantitative determination of amino acids contents*:*** by the method of Pellet and Young, 1980. Amino acids contents of *Atriplex halimus* were accomplished by using amino acid analyzer (Beak man system 7300 high performance analyzer).

***Free amino acids:*** one gram of the defatted plant powder was extracted by boiling under reflux with 50ml of 50% ethanol for 3 times. The combined ethanolic solutions were filtered and treated with Trichloroacetic acid solution (10%) for clarification. The supernatant fluid was concentrated under reduced pressure to 5ml. The residue was washed with distilled water; the volume of the filtrate was adjusted to 100ml using distilled water. 5ml of diluted sample were dried at 70$^{o}$C, and then dissolved in 5ml loading buffer (0.2N sodium citrate buffer PH2). The sample was filterated through 0.45 micropore filter and injected in amino acid analyzer. Retintion times were measured and compared with a standard sample containing known amino acid, which were always run before and after each analysis to identify the separated amino acids.

***Protein hydrolysate:*** the defatted plant powder (0.1gm) was dissolved in 10ml of 6N HCl in a sealing tube. The mixture was hydrolyzed at 110$^{o}$C for 24 hours, filtered and the hydrolyzed protein-amino acids were obtained by evaporation of the hydrolysate to dryness. The residue was washed with distilled water; the volume of the filtrate was

adjusted to 100ml, using distilled water. The investigation of protein hydrolysate was completed as previously discussed for free amino acids.

**Calculations:**

$$\text{Amino acids (gm \%)} = \frac{\text{Conc./ml x dill. x 100}}{\text{1000x1000x wt. of sample (gm)}}$$

**Quantitative determination of unsaponifiable and saponifiable matters:** by the method of Farag et al., (1986). The determination of unsaponifiable matter (hydrocarbons & sterols) and saponifiable matter (fatty acids) were accomplished by using Gas-Liquid Chromatography (GLC) GCV Pye-Unicam.

The lipids of *Atriplex halimus* were extracted with petroleum ether : diethl ether (1:1) using soxlet apparatus then filtered. Saponifield 5gm of lipids by refluxing with alcoholic potassium hydroxide (40g/100ml 95% ethanol) for three hours. The solution was then evaporated to two thirds of its volume. Excess water was added and the soap solution was shaken in a separating funnel for several times with diethyl ether until complete extraction was obtained. The combined ethereal extracts were washing with water until free from alkalinity, dried over anhydrous sodium sulphate then filtered. The non-saponifiable matter was obtained by removal of ether solvent using rotary evaporator. After the removal of the unsaponifiable fraction with ether; the soapy solution was acidified with 2.5% sulphuric acid then extracted with ether. The ether extract was washed several times with distilled water until free from acids to obtain the fatty acids. The ether extract was dried over anhydrous $Na_2SO_4$, then filtered and the ether was removed by rotary evaporator. Methylation was carried out by trimethyl silylation reaction. The hydrocarbons, sterols and fatty acids were subjected to Gas-Liquid Chromatography (GLC) analysis. The authentic samples were also injected under the same conditions and the relative retention times (RRT) were calculated. The results of Itoh et al., (1973) and Farag et al., (1986) were used as a guide to characterize some of the unknown compounds.

**Investigation of carbohydrates**

***Identification of free sugars:*** by the method of Chaplin and Kennedy, 1994.

***(a) Extraction of free sugars:*** 20gm of the defatted plant powder were extracted with 80% ethyl alcohol and filtered. The filtrate was clarified by Carrez reagent, filtered and then evaporated. The residue was dissolved in 3ml of 10% aqueous isopropanol for chromatographic investigation.

***(b) HPLC of the free sugars:*** by the method of Nagel, 1992.The sugars were determined by using high performance liquid chromatography (HPLC) 1050, whereas the extracted sugars were injected.

***Identification of combined sugars***

***(a) Hydrolysis of combined sugars:*** 20gm of defatted air-dried plant powder were boiled under reflux for 3hours with 25ml of 6N HCl, then cooled and filtered. The HCl was evaporated under vacuum at 45$^{o}$C and the residue was dissolved in 10 ml of 10% isopropanol for chromatographic investigation.

***(b) HPLC of the hydrolyzed-combined sugars:*** by the method of Nagel, 1992.

A few mgs of the dried sugar hydrolysate were dissolved in 10% aqueous isopropanol solution and subjected to HPLC.

Detector: Refractive index (RI) Shodex RI-71 (Japan).

**Chromatographic investigation of flavonol, flavanone, flavone and isoflavoneglycosides:** by the method of Markham, 1982. The plant material (2.5kg) was extracted with 90% MeOH, to the aqueous suspension of the MeOH extract

acetone was added to precipitate undesirable material and the aquous layer was concentrated and extracted with ethyl acetate.

***Paper chromatography*:** the concentrated ethyl acetate extract of each habitat was applied on two dimensional paper chromatography (Whatmann No.1) to investigate the flavonoid contents using two solvents systems: S1: N-Butanol: Acetic acid : water 4:1:5 (v/v/v) for first way and S2 : Acetic acid : water 15:85 (v/v) for the second way.

***Column chromatography:*** the separation of flavonoids compounds were carried out using adsorption column chromatography packing with polyamide 6S adsorbent.

The ethyl acetate extract was dissolved in minimum amount of water mixed with suitable amount of polyamide powder. The solvent was evaporated on water path with occasional stirring to form a free flowing dry powder and applied to the top of the column. The elution initiated with addition of water. Uneluted bands were observed in UV light the polarity was changed by increasing the percentage of ethanol gradually until 100% ethanol was employed for the final elution of the column. The eluted fractions were subjected to PC using ($S_1$) and ($S_2$). Similar fractions were pooled together and concentrated. Sub columns and PPC were performed for further isolation of flavonoid compounds. For the final purification of flavonoid compounds Sephadex LH-20 column was used and the solvent system was methanol: water.

***Identification of flavonoid compounds:*** by the method of Mabry et al., (1970).

***UV Analysis:*** the pure material was dissolved in pure methanol then subjected to UV spectrophotometric measurements using Schimadzu UV 240 spectrophotometer. In case of flavonoids, NaOMe (2.5gm/100ml methanol), NaOAc (anhydrous powder)/$H_3BO_3$ (anhydrous powder) and $AlCl_3$ (5gm/100ml methanol)/HCl (conc.) reagents were separately added to the solution, and UV measurements were then carried out

***$^1$H- and $^{13}$C-NMR Analysis:*** $^1$H-and $^{13}$C-NMR are the most efficient method for identification and elucidation of structure of various types of flavonoid. The NMR measurement was carried out on AJEOL EX-270 NMR spectrometer apparatus (270 MHz FOR $^1$H-NMR and 67.5 MHz for $^{13}$C-NMR).

***MS Analysis:*** the isolated chromatographically pure compounds were subjected in most cases to fast atom bombardment (positive and negative) mass spectrometric analysis (FAB-MS).

Some other compounds were subjected to electron impact and/or chemical ionization mass spectrometric analysis (El, Cl-MS). The spectra were conducted using mass spectrometer Varian Mat 711, Finnigan SSQ 7000 and MM 7070 E.

***Chemical reactions:*** by the method of Mabry et al., (1970).

***Controlled (mild) acid hydrolysis:*** a known weight of the flavonoid material under investigation was subjected to mild acid hydrolysis in 0.1N aqueous HCl at 100$^o$C for 15 minutes. The reaction mixture was examined at definite intervals by comparative paper chromatography for tracing any intermediate that might be released during the course of hydrolysis.

***Complete (normal) acid hydrolysis*:** a known weight of the flavonoids material under investigation was subjected to acid hydrolysis in either 2N HCl at 100$^o$C for 2hours or in 2N HCl in aqueous MeOH (1:1) in vaccuo. The obtained hydrolysate in both reactions was then extracted with ethyl acetae. The received AcOEt extract was washed with water twice, concentrated under vaccuo and subjected to (CoPC) using authentic reference markers. The remaining aqueous layer was then freed from acid through extraction witg 20% N-methyl dioctylamine in $CHCl_3$ and subjected to (COPC) using authentic sugars as reference markers.

***Enzymatic hydrolysis:*** 5mg of the compound were treated with corresponding enzyme in an acetate buffer (pH=5) and the mixture was worked up for 24 hours at 37-40$^{o}$C. The hydrolysate was then extracted with ethyl acetate and the extract was subjected to CoPC against flavonoid authentic markers. Also the sugar moieties were detected using authentic sugars markers.

***Quantitative determination of phenolic acids:*** by the method of Ben-Hammouda et al., (1995): the determination of phenolic acids of *Atriplex halimus* plant was accomplished by using high performance liquid chromatography (Hewlett-Packard HPLC, Model 1100).

***Chromatographic investigation of alkaloid constituents:*** the alcoholic extract of the plant was concentrated under vacuum till dryness. The dried extract was dissolved in 2N-hydrochloric acid on a water bath, shaken and filtered, the obtained filtrate was shacked with chloroform to remove undesirable matters.

The acidic aqueous layer was adjusted to alkaline pH with ammonia and the liberated alkaloid bases were extracted by chloroform till exhausted and then it was by Mayer's and Dragendorrf 's reagent.

***TLC analysis of alkaloids:*** each chloroformic residue of the investigated plant at the two habitats was dissolved in chloroform, then chromatographed on silica gel G TLC as adsorbent using the following solvent systems.

a- Chloroform : methanol (8:2 v/v).

b- Chloroform : methanol (9:1 v/v).

The developed chromtograms were air-dried and sprayed with Dragendorrf ' s reagent, systems c and d gave best separation.

***Isolation:*** the chloroformic alkaloids of *Atriplex halimus* was applied on the top of silica gel column . Elution was started with benzene then the polarity was gradually increased by gradual addition of chloroform and finally gradual addition of methanol, the elutes were collected in fractions each fraction was concentrated under reduced pressure to small volume, then chromatographically screened on silica gel G plates. Similar fractions were pooled together and concentrated.

***Preparative TLC:*** preparative TLC silica gel G plate was used for isolation and purification of compounds using system (b). The band corresponding to alkaloidal compound were scraped off and eluted with methanol, the eluted bands were freed from solvent under vacuum; and then it was re-purified by the same manner.

## RESULTS

The naturally growing *Atriplex halimus* plants were collected from Wadi-Hof and Wadi-Sudr. The plant is perennial. The habitat nature affected the synthesis of plant's metabolites. There were variations in temperature, rainfall and evaporation at the two habitats. The soil was sandy loam in texture at Wadi-Hof and loamy sand in Wadi-Sudr.

### Quantitative determination of amino acids

***Determination of free amino acids :*** the free amino acids of *Atriplex halimus* at the two studied habitats were present in table (1), which declared presence of aspartic acid, threonine, serine, glutamic acid, proline, methionine, isoleucine, leucine, tyrosine, histidine and arginine in the plants of the two habitats with different ranges of concentration. While glycine and phenyl alanine were present only in the plants of Wadi-Sudr habitat, on the other hand cystine and lysine were present only at Wadi-Hof habitat.

***Determination of protein amino acids:*** determination of hydrolyzed protein amino acids of *Atriplex halimus* at the two studied habitats were achieved using amino acid analyzer. The obtained data (Table 1) revealed that *Atriplex halimus* at the two

habitats contained fifteen amino acids with different ranges of concentration, where glutamic acid, aspartic acid and proline represent the highest concentrations of the separated protein amino acids.

**Table-1: Relative percentages of free and protein amino acids of *Atriplex halimus*.**

| Amino acids | Localities | | | |
|---|---|---|---|---|
| | Wadi-Hof | | Wadi-Sudr | |
| | Free amino acids % | Protein amino acids % | Free amino acids % | Protein amino acids % |
| Aspartic acid | 0.04 | 0.28 | 0.05 | 0.46 |
| Threonine | 0.03 | 0.13 | 0.06 | 0.21 |
| Serine | 0.05 | 0.10 | 0.07 | 0.26 |
| Glutamic acid | 0.07 | 0.57 | 0.09 | 0.65 |
| Proline | 0.11 | 0.27 | 0.12 | 0.45 |
| Glycine | - | 0.25 | 0.008 | 0.33 |
| Alanine | - | 0.13 | - | 0.24 |
| Cysteine | 0.02 | - | - | - |
| Valine | - | 0.12 | - | 0.24 |
| Methionine | 0.04 | - | 0.005 | - |
| Isoleucine | 0.05 | 0.09 | 0.009 | 0.19 |
| Leucine | 0.009 | 0.17 | 0.04 | 0.34 |
| Tyrosine | 0.004 | 0.07 | 0.01 | 0.15 |
| Phenyl alanine | - | 0.09 | 0.01 | 0.22 |
| Histidine | 0.01 | 0.08 | 0.025 | 0.10 |
| Lysine | 0.008 | 0.11 | - | 0.26 |
| Arginine | 0.006 | 0.09 | 0.009 | 0.20 |

## Quantitative determination of unsaponfiable and saponfiable matters

***Contents of unsaponfiable matter fraction by Gas-Liquid Chromatography (G.L.C.)*:** The unsaponfiable matter content of *Atriplex halimus* at the two habitats were determined using GLC technique, where the relative percentage of each component was calculated and tabulated in table (2) for Wadi-Hof and Wadi-Sudr habitats.

The obtained results revealed that *Atriplex halimus* at Wadi-Sudr and Wadi-Hof habitats contained n-eicosane, n-hexacosane, n-octacosane and squalene with higher concentration at Wadi-Hof habitat, while lipid at Wadi-Sudr contained also ndocosane, n-triacontane and two sterols, cholesterol and stigmasterol, meanwhile lipid at Wadi-Hof contained n-tricosane, n-pentacosane and no sterols.

**Table-2: G.L.C. of hydrocarbons and sterols of *Atriplex halimus* at both habitat.**

| Number of carbon atoms | Name | Locations | |
|---|---|---|---|
| | | Wadi-Hof | Wadi-Sudr |
| | | Percentage% | Percentage% |
| 20 | n-Eicosane | 7.550 | 4.200 |
| 22 | n-Docosane | - | 10.734 |
| 23 | n-Tricosane | 3.645 | - |
| 25 | n-Pentacosane | 4.599 | - |
| 26 | n-Hexacosane | 6.248 | 4.978 |
| 27 | Cholestrol | - | 14.519 |
| 27 | Stigmasterol | - | 17.0241 |
| 28 | n-Octacosane | 12.471 | 10.408 |
| 29 | Squalene | 20.827 | 11.512 |
| 30 | n-triacontane | - | 2.230 |
| - | Unknown | 12.496 | 3.785 |
| - | Unknown | 2.835 | 19.393 |
| - | Unknown | 14.839 | |
| - | Unknown | 23.490 | |

***Contents of saponifiable fractions of the lipids by Gas-Liquid Chromatography (G.L.C.):*** the fatty acid contents of the lipid of *Atriplex halimus* were determined using G.L.C. technique. The relative percentage of each component was calculated, tabulated in (Table 3). The obtained data revealed the presence of saturated fatty acids, myristic, palmitic, stearic acids in *Atriplex halimus* growing in Wadi-Hof and in Wadi-Sudr, beside the unsaturated fatty acids palitoleic, oleic and linoleic acids. While pelargonic and capric acids in *Atriplex halimus* growing in Wadi-Sudr only.

It is obvious from (Table 3) that stearic acid represented the higher percentage of fatty acids (37.42%) followed by palitolic acid (35.99%) and the lowest one was myristic acid with percentage (0.44%) at Wadi-Hof habitat. Meanwhile the palitoleic acid represented the highest percentage of fatty acids followed by linolenic acid percentage (38.78% and 49.08%) and the lowest one was pelargonic acid (0.45%) at Wadi-Susr habitat.

**Table-3: G.L.C. of fatty acids of *Atriplex halimus* at at Wadi-Hof and Wadi-Sudr habitat.**

| Number of carbon atoms | Name | Locations | |
|---|---|---|---|
| | | Wadi-Hof | Wadi-Sudr |
| | | Percentage% | Percentage% |
| 9 | Pelargonic acid | - | 0.45 |
| 10 | Capric acid | - | 2.30 |
| 14 | Myristic acid | 0.44 | 3.35 |
| 16 | Palmitic acid | 3.07 | 3.47 |
| 16:1 | Palitoleic | 35.99 | 49.08 |
| 18:0 | Stearic acid | 37.42 | 5.74 |
| 18:1 | Oleic acid | 14.61 | 12.07 |
| 18:2 | Linoleic acid | 3.65 | 7.26 |
| 18:3 | Linolenic | - | 6.47 |
| - | Unknown | 1.07 | 1.56 |
| - | Unknown | 1.39 | 8.27 |
| - | Unknown | 2.42 | |

## Investigation of carbohydrates

***Determination of free sugars:*** investigation of the free sugars of *Atriplex halimus* using HPLC (Table 4), revealed the presence of rhamnose, arabinose, ribose, glucose and raffinose at Wadi-Hof and Wadi-Sudr habitats, while fructose was detected only in Wadi-Hof and sucrose and galactose were detected only at Wadi-Sudr habitat. The maximum percentage of free sugars (Table 4) was raffinose (36.6%) followed by fructose (31.5%) at Wadi-Hof, while at Wadi-Sudr glucose (28.6%) followed by galactose (19.4%) were found. Meanwhile the minimum percentage was the ribose at the two habitats (4.6% ,2.4%) for Wadi-Hof and Wadi-Sudr respectively.

**Table-4: Free and combined sugars of *Atriplex halimus* in the two studied habitats.**

| R.T. | Sugar name | Locations | | | |
|---|---|---|---|---|---|
| | | Wadi-Hof | | Wadi-Sudr | |
| | | Free sugars % | Combined sugars % | Free sugars % | Combined sugars % |
| 2.35 | Rhamnose | 8.3 | - | 9.2 | 11.5 |
| 2.43 | Xylose | - | - | - | - |
| 2.65 | Arabinose | 10.9 | 15.2 | 16.8 | 10.6 |
| 2.75 | Ribose | 4.61 | 8.5 | 2.49 | 6.3 |
| 3.4 | Fructose | 31.5 | 7.8 | - | - |
| 3.65 | Glucose | 22.1 | 20.5 | 28.6 | 17.5 |
| 3.7 | Galactose | - | 5.2 | 19.4 | 11.8 |
| 6.9 | Sucrose | - | 11.2 | 2.9 | 16.1 |
| 8.0 | Unknown | - | 5.2 | - | - |
| 10.35 | Raffinose | 36.6 | 16.5 | 16.9 | 8.2 |

***Combined sugars:*** investigation of the hydrolyzed combined sugars of *Atriplex halimus* using HPLC, revealed the presence of arabinose, glucose, galactose, raffinose, ribose and sucrose at Wadi-Hof and Wadi-Sudr, while rhamnose was detected only in Wadi-Sudr plants and fructose was detected only in Wadi-Hof plants.It was noticed that, the maximum percentage of hydrolyzed sugars was glucose with values of 20.5 and 17.5% at Wadi-Hof and Wadi-Sudr habitats, respectively (Table 4).

**Chromatographic investigation of flavonol, flavone, flavanone and isoflavone glycosides:** fractionation of ethyl acetat extract of *Atriplex halimus* on polyamide column and purification of the obtained fractions using preparative paper chromatography and sephadex LH-20 column yield flavonol glycosides, flavanone glycosides, flavone glycosides and isoflavone glycosides.

***Identification of flavonol glycosides:*** the flavonol glycosides compounds were characterized as isorhamnetin 3-o-glucopyranoside (1), rutin-4`, 7-dimethyl ether (2) and kaempferol-3,7-dirhaminoside-4`-methyloxide (3). Acid hydrolysis of compounds (1,2) using 2N HCl yielded isorhamntein as aglycone and glucose as sugar moiety for compound (1) while compound (2) yielded quercetin-4`, 7-dimethyl ether and the sugars glucose and rhamnose on the other hand compound (3) yielded kaempferol-4`-methyl ether and sugar rhamnose.

UV spectral date showed absorption maxima in methanol, band I (355, 352 and 350nm) for compounds (1,2,3) indicating that these compound are flavonol with 3-OH substitution, addition of NaOAc indicated the presence of free OH group at C-7 in compound (1) only, while addition of boric acid indicated the absence of 3`,4` dihydroxy groups in the three compounds. Addition of NaOMe cause a bathochromic shift in compound (1) only indicating the presence of free OH at C-4` in this compound (Harborne, 1984; Liu, et al., 1989).

$^1$H-NMR spectrum showed signals similar to those of isorhamnetin, rutin and kaempferol for compound (1,2,3) respectively, appearance of a doublet at δ 4.8, 4.9 indicated the presence of a glucose in (1,2) and δ 5.1, 5.2 due to the presence of rhamnose in compound (2,3) and signal at δ 3.92 (S, $OCH_3$) in the three compounds. Also $^1$H-NMR spectrum indicate that the linkage between rhamnose and glucose is (1-6) in compound (2) (Harborne and Mabry, 1982).

***Identification of flavone glycosides:*** the isolated flavone glycosides were characterized as vicenine-II (4) and isoorientin (5). $R_f$-values of compound (4) $R_f$, 0.33 (S1) and 0.71 (S2) indicated that compound (4) was diglycosides and compound (5) was monoglycoside, $R_f$, 0.39 (S1) and 0.32 (S2). Both compounds (4,5) resisted acid hydrolysis, indicating that these compounds were C-glycosides. Enzymatic hydrolysis using glucosidaze gave apigenin and glucose for compound (4), and luteolin and glucose for compound (5).

UV spectral data (Table 5) indicated that compounds (4,5) were flavone glycosides. Addition of NaOAc gave a bathochromic shift in band II indicating the presence of free OH at C-7. Addition of boric acid gave no shift in compound (4) indicating the presence of 3`, 4` hydroxyl groups in compound (5) only, while addition of NaOMe gave a bathochromic shift band I, indicating the presence of free OH at C-4`. Addition of $Alcl_3$ revealed the presence of free OH at C-5.

$^1$H-NMR spectrum showed the absence of H-6 in both compound and the absence of H-8 in compound (4) only, H-3 appear as singlet at δ 6.77 and the sugar protons at δ 4.8 and 3.3-3.8 (m) (Ibrahim, et al., 2008).

**Table-5: UV-spectral data (λmax,nm) of isolated flavonoids.**

| Compounds | Reagents | | | | | |
|---|---|---|---|---|---|---|
| | **MeOH** | **NaOMe** | **NaOAc** | **NaOAc + $H_3BO_3$** | **$AlCl_3$** | **$AlCl_3$ + HCl** |
| Isorhamnetin-3-glucopyranose | 256,269sh, 305sh,355 | 274,325, 416 | 275,318, 388 | 257,268sh, 306,359 | 267,297sh ,405 | 266,296sh , 355,400 |
| Rutin-4`,7-dimethyl ether | 257sh,267, 298,352 | 272,315, 396 | 268,300,354 | 268,299, 354 | 272,302, 335,400 | 272,302, 340,400 |
| Kaempferol-3,7-dirhamnoside-4`-methoxide | 256sh,267, 318sh,350 | 254sh,267, 297sh,380 | 266,295 sh, 318sh, 350 | 266,295sh, 319sh,350 | 275,302sh , 352,400 | 275,302sh , 350,400 |
| VicenineII | 272,325 | 282,332sh, 398 | 281,303,360 | 283,320, 344 | 279,302, 343,382 | 279,302, 343,382 |
| Isoorientin | 254,268sh, 303sh,324sh,372 | 270,326, 433 | 259sh, 275, 318,390 | 256,270sh, 305sh,324sh,376 | 265,302sh , 360sh, 430 | 263,270sh , 301sh, 355,426 |
| 4`-methoxy-7-glucoside-5-hydroxyisoflavone | 261,324sh | 264,365 | 260,322 | 260,320 | 272,305sh , 380 | 272,304sh ,378 |
| Naringenin-4`-o-rhamnopyranoside | 288,327sh | 246,325 | 282sh, 325 | 289,330sh | 315,374 | 314,372 |
| Hesperidin | 281,327 | 243,288, 351 | 282,329 | 282,327 | 306,381 | 304,378 |

***Identification of isoflavone glycosides:*** the isolated isoflavone glycoside was characterized as 4`-methoxy-7-glucoside-5-hydroxyisoflavone (6). Compound (6) appear on paper chromatography as light blue spot, $R_f$ values, 0.54 ($S_1$) and 0.63 ($S_2$). Acid hydrolysis of compound (6) gave 4`-methoxy-5,7-dihydroxy isoflavone and glucose as sugar.

UV spectral analysis in methanol (Table 5) showed that compound (6) was isoflavone, addition of NaOMe indicated the absence of free OH at C-4` while addition of NaoAc indicate the absence of free OH at C-7. $AlCl_3$ addition indicated the presence of OH at C-5.

$^1$H-NMR spectrum of compound (6) showed characteristic signals for H-2 of isoflavone at δ7.82 (S), at 7.45 (2H,d,J=8Hz H-2`), 6.99 (2H, d, J=8Hz), 5-OH at 13.16 and $OCH_3$ at 3.8 (S,3H) (Abdel-Kader, et al., 2006).

Mass spectrum of compound (6) revealed the presence of a molecular peak $M^+$ at m/e446 (El-Masry, et al., 2002).

***Identification of flavanone glycosides:*** the isolated flavanone glycosides were characterized as naringenin-4`-o-rhamnopyranoside (7) and hesperidin (8). Complete acid hydrolysis using 2N HCl, compound (7) gave the aglycone naringenin and the sugar rhamnose while compound (8) gave hisperitine as aglycone and the sugars glucose and rhamnos.

UV spectral analysis indicated that compounds (7,8) are flavanone glycosides. Addition of NaOMe showed the absence of free OH at C-4`in the compounds (7,8) while addition of NaoAc indicate the presence of free OH at C-7 in compound (7) only. Addition of $AlCl_3$ indicated the presence of free OH at C-5 in the two compounds. $^1$H-NMR spectrum revealed the presence of signals at δ 5.3 (S, H-2) and δ 6.8 (S, H-3) conferming that compounds (7,8) were flavanone. While presence of signal at δ 3.8 (S, $OCH_3$) in compound (8) only. Presence of glucose and rhamnose in compound (8) (2 anomeric protons at δ 5.01, 4.5) and 1.08, S, $CH_3$ rhamnose and the presence of rhamnose only in compound (7).

Enzymatic hydrolysis using glucosidase indicated that the glucose moiety directly was attached to the aglycone incompound (8). Also $^{1}$H-NMR spectrum showed that the linkage between rhamnose and glucose was (1-6) in compound (8).

Chromatographic investigation of flavonol, flavanone, flavone and isoflavone glycosides declared the presence of isoflavone glycosides and vicenin II in Wadi-Sudr habitat only.

**Table-6: $^{1}$H-NMR spectral data (δppm) of the isolated flavonoids.**

| Compounds | $^{1}$H-NMR spectral data (δppm) |
|---|---|
| Isorhamnetin-3-o-glucopyranoside | δ7.8(1H,d,J=8.5Hz,H-2`),7.61(1Hdd,J=8.5Hz,H-6`),6.92(1H,d,J=8.5Hz,H-5`),6.74(1H,d,J=2.5Hz,H-8),6.3(1H,d,J=2.5Hz, H-6),4.8(1H,d,J=7HzH1``glucose),3.9(3H,S,$OCH_3$),3.2-3.9(m). |
| Rutin-4`,7-dimethylether | δ7.9(1H,d,J=8.5Hz,H-2`),7.8(1H,dd,J=8.5Hz,H-6`),6.88(1H,d,J=8.5Hz,H-5`),6.4(1H,d,J=2.5Hz,H-6),6.3(1H,d,J=2.5Hz,H-8),5.1(1H,d,J=2.5HzH-1`` rhamnose),4.9(1H,d,J=8Hz,H1```glucose),3.1-3.8(m),3.9(S,OCH3), 1.1(3H,d,j=6Hz,CH3). |
| Kaempferol-3,7-dirhamnoside-4`-methoxide | δ7.8(2H,d,J=8.5,H-2`,6`),6.9 (2H,d,J=8.5,H-3`,5`),6.8 (1H,d,J=2.5,H-8), 6.4 (1H,d,J=2.5,H-6), 5.2(1H,d,J=2.5,H1``rhamnose), 5.3(1H,d,J=2.5Hz,H1```rhamnose),3-3.8(m),1.1 (3H,d,J=6,$CH_3$ rhamnose), 3.9 (3H,d,J=6, $CH_3$). |
| VicenineII | δ8.0(d,J=8Hz,H-2`,6`),6.8(d,J=8Hz,H-3`,5`),6.75(S,H-3),4.8 (d,J=8Hz, anomeric sugar proton),3.3-3.8(m). |
| Isoorietin | δ7.9(1H,d,J=8.5Hz,H-2`),7.5 (1H,d,J=8.5 Hz,H-6`),6.8 (1H,d,J=8.5 Hz,H-5`),6.7(S,H-3),6.4(1H,S,H-8),4.8(1H,d,J=8Hz,H-1``glucose),3.1-3.9(m). |
| 4`-methoxy-7-glucoside-5-hydroxyisoflavone | δ8.3(1H,S,H-2),7.5(2H,dd,J=8.5,2.3Hz,H-2`,H-6`),6.9(2H,dd,J=8.5,2.3Hz,H-3`,H-5`),6.4(1H,d,J=2Hz,H-6),6.1(1H,d,J=2Hz,H-8). |
| Naringenin-4`-O-rhamnopyranoside | δ7.3(2H,dd,J=8.5Hz,H-2`,H-6`),6.8(2H,dd,J=8.5,2.3Hz,H3`,H-5`),6(1H,d,J=2.5Hz,H-8),5.8(1H,d,J=2.5Hz,H-6),5.2(cisand trans2H,d,J=17Hz,H-3),5.1(1H,d,J=2.5H1``rhamnose),1.1(3H,d,J=6Hz,$CH_3$ rhamnose). |
| Hesperidine | δ7.5(1H,d,J=8.5Hz,H-2`),7.4 (1H,d,J=3.6Hz,H-6`),7.2 (1H,d,J=9Hz,H-5`), 6.9(1H,S,H-3), 6.7(1H,d,J=3.4Hz,H-8),6.6(1H,d,J=3.4Hz,H-6),5.3(1H,S,H-2),5.1(1H,d,J=2.5Hz,H1``rhamnose),4.9(1H,d,J=8Hz,H-1```glucose), 3.9(S,$OCH_3$),3.2-3.8(m),1.1(3H,d,J=6Hz,$CH_3$rhamnose). |

**Quantitative determination of phenolic acids:** the obtained results (Table 7) declared the presence of ρ-hydroxy benzoic acid, chlorogenic acid, cinnamic acid, ferulic acid and salicylic acid in the *Atriplex halimus* plant at the two habitats, while gallic acid and ρ-coumaric acid were present only at Wadi-Sudr habitat.

**Tabe-7: Concentration of phenolic acids of *Atriplex halimus* in both the habitats using HPLC.**

| Phenolic acids | Concentration (µg/gm) | |
|---|---|---|
| | Wadi-Hof | Wadi-Sudr |
| P-hydroxy benzoic acid | 0.09 | 0.014 |
| Pyrogallic acid | - | - |
| Gallic acid | - | 3.61 |
| Chlorogenic acid | 0.17 | 0.24 |
| Cinnamic acid | 0.02 | 0.25 |
| P-Coumaric acid | - | 0.27 |
| Ferulic acid | 4.42 | 8.7 |
| Salicylic acid | 5.7 | 7.8 |
| o-Coumaric acid | - | - |

**Chromatographic investigation of alkaloid constituents:** when each chloroformic extract of *Atriplex halimus* at the two habitats was chromatographed on silica gel G TLC using the solvent systems:-

- Chloroform : methanol (8:2 v/v) $C_1$.
- Chloroform : methanol (9:1 v/v)., $C_2$.
- Then the developed chromatograms were air-dried and sprayed with Dragendorff´s reagent, system $C_2$ gave the best resolution and showed that the presence of the alkaloids, piperineand ephedrine, in Wadi-Hof habitate while the alkaloids, piperine, berberine and palmatine were detected at Wadi-Sudr habitat.

***Identification of the isolated alkaloids***

***Identification of compound ($D_1$):*** compound ($D_1$) was obtained as white yellow crystals, soluble in chloroform and methanol, m.p 130-131°C.

Mass spectrum:- mass spectrum of compound ($D_1$) revealed the presence of molecular ion peak [$M^+$] at m/e 285 and other important fragments.

$^1$H-NMR spectrum:-$^1$H-NMR spectrum showed signals at δ 1.75 (m) and δ 3.7 (t) for four symmetric protons adjacent to nitrogen, δ 6 (2H, methylene group), δ 6.3 (d, J=15Hz ethylenic protons), δ 7.4 (d, d J=15, J=8 Hz protons in the aromatic ring).

$^{13}$C-NMR Spectrum:- $^{13}$C-NMR Spectrum showed signals at δ 165 ppm (C=O), five carbons 24.3-46.2 in cyclic ring and ten carbons in conjugation 119-147.9.

Comparison of these spectral data with those reported in the literature, compound $D_1$ was identified as piperine (Shamma and Hindlniang, 1979).

***Identification of compound ($D_2$):*** Mass spectrum:- mass spectrum of compound ($D_2$) showed a molecular ion peak [$M^+$] at m/e 165 and other important fragments, at m/e 107, 105, 58.

$^1$H-NMR spectrum:- $^1$H-NMR spectrum of compound ($D_2$) showed signals at δ 7.13 (5H, S, monosubstituted benzene ring), 4.6 (1H,d), 4.37 (2H,S, NH,OH), 2.4 (1H, m) and 2.18 (6H, S, 2$CH_3$).

IR spectrum:- IR spectrum of compound $D_2$ showed absorption bands at 710 and 770 $cm^{-1}$ (monosubstituted benzene ring), 3500 $cm^{-1}$ (broad) (OH), 300 $cm^{-1}$ (NH), 1600, 1510, 1470 (C=C aromatic).

From the previous data, compound ($D_2$) was identified as ephedrine.

***Identification of compound ($D_3$ ):*** Mass spectroscopy:- mass spectroscopy technique has been used for the structural characterization and identification of alkaloids (Wang et al., 2004). Mass spectrum indicated the presence of a molecular ion peak at ($M^+$) at m/e 336 (62%), 321 (85%), 320 (100%), 306 (30%), 304 (18%) and 292 (85%).

UV spectral data:- UV spectrum of compound ($D_3$) in methanol showed absorption maxim at, λmax, nm: 228, 236, 267, 275, 344, 349.

$^1$H-NMR spectrum:- nuclear magnetic resonance spectroscopy is a power method used for investigation of the structure of natural products (Reynols and Enriquez, 2002). $^1$H-NMR spectrum of compound ($D_3$) showed signals at δ 3.26 (2H,t, J=6.4Hz, $H_5$), 3.9 (3H,S,$OCH_3$), 4.1 (3H,S,$OCH_3$), 4.81 (2H,t,J=6.4Hz,H-6), 6.0 (2H,S,O-$CH_2$-O), 6.72 (1H,S,H-4), 7.54 (1H,S, H-1), 7.9 (1H,d,J=8.8Hz,H-12), 8.1 (1H,d,J=8.8Hz,H-11), 8.5 (1H,S,H-13), 9.65 (1H,S,H-8).

$^{13}$C-NMR spectrum:- $^{13}$C-NMR Spectrum of compound ($D_3$) showed signals at δ28.1 (C-5), 56.9 (C-6), 57.5 (C-10, $OCH_3$), 62.33 (C-9-$OCH_3$), 103.47 (-O-$CH_2$-O), 106.42 (C-1), 108.95 (C-4), 121.6 (C-1a), 121.72 (C-13), 123.23 (C-8a), 124.61 (C-12), 127.87 (C-11), 131.69 B (C-4a), 135.13 (C-12a), 139.52 (C-13a), 145.43 (C-8), 145.98 (C-9), 149.71 (C-2), 151.78 (C-10).

Compound ($D_3$) was identified as berberine by comparison of its spectral data with those reported in literatures (Kim et al., 2000).

***Identification of compound ($D_4$):*** Compound (D4) was obtained as yellow crystals, m.p. 208$^{o}$C.

UV spectral data:- UV spectrum of compound ($D_4$) in methanol revealed the presence of absorption maxima at λmax, nm: 225, 236, 263, 274, 332, 347.

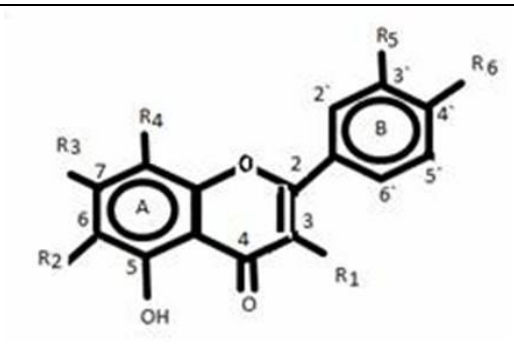
Mass spectrum:- mass spectrum of compound ($D_4$) showed a molecular ion peak ($M^+$) at m/e 352 and other fragments at m/e 337, 322 and 308.

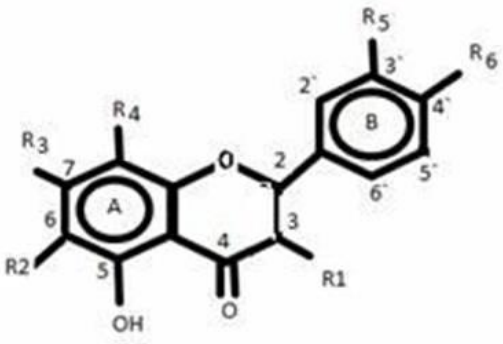
$^1$H-NMR spectrum:-$^1$H-NMR spectrum of compound ($D_4$) indicated the presence of signals similar to those of berberine except the presence of 2$OCH_3$ at $C_2$,$C_3$, at δ3.85 (3H,S,3-$OCH_3$) and 4.0 (3H,S,2-$OCH_3$).

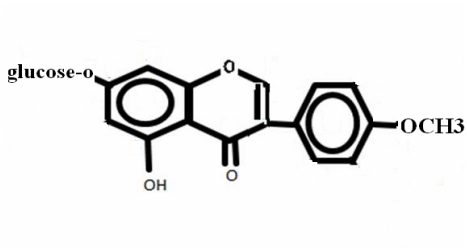
I.R. spectrum: I.R. spectrum showed absorption bands at 1686 cm$^{-1}$, (C=N), 1606 (aromatic C=C), 1197 (C=O).

Compound ($D_4$) was identified as palmatine by comparison of its spectral data with those reported in literatures (Kim, et al., 2000).

**Table-8: Isolated of flavonoid compounds.**

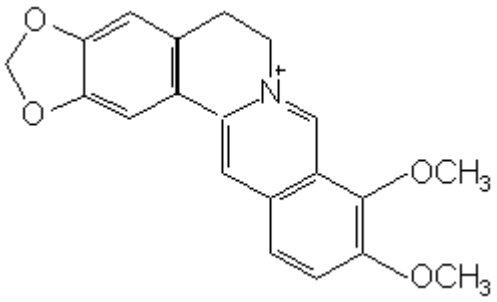
| **Compounds** | Structures<br>  <br>**Base structure** | | | | | |
|---|---|---|---|---|---|---|
| | R1 | R2 | R3 | R4 | R5 | R6 |
| Isorhamnetin-3-o-glucopyranoside | o-glucos | H | OH | H | $OCH_3$ | OH |
| Rutin-4`,7-dimethylether | o-rhamno glucosyl | H | | $OCH_3$ | OH | $OCH_3$ |
| Kaempferol-3,7-dirhamnoside-4`-methoxide | o-rhamnose | | | o-rhamnose | H | $OCH_3$ |
| VicenineII | H | C-glucose | OH | c-glucose | H | OH |
| Isoorietin | H | C-glucose | OH | H | OH | OH |

| **Compounds** | Structures<br>  <br>**Base structure** | | | | | |
|---|---|---|---|---|---|---|
| | R1 | R2 | R3 | R4 | R5 | R6 |
| Naringenin-4`-orhamnopyranoside | H | H | OH | H | H | o-rhamnose |
| Hesperidine | H | H | o-rhamno glycosyl | H | OH | $OCH_3$ |

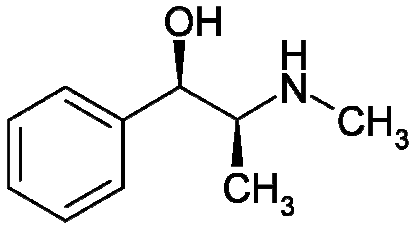



**4`-methoxy-7-glucoside-5-hydroxyisoflavone**

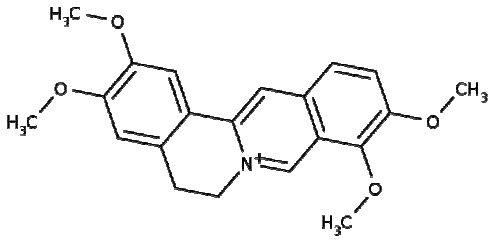
**Isolated alkaloids**



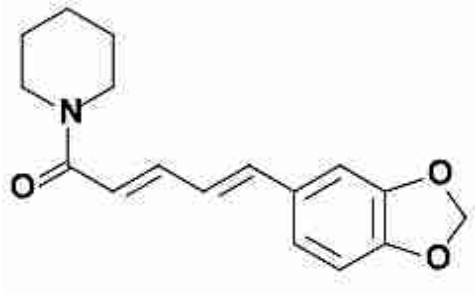

**Berberine**




**Ephedrine**




**Balmatine**




**Piperine**

## DISCUSSION

Environmental factors affect the production of biologically active constituents by plants at different habitats. Quantitative determination of free amino acids of *Atriplex halimus* at Wadi-Hof and Wadi-Sudr habitats revealed the presence of eleven amino acids in the two habitats with different ranges of concentration, while glycine and phenyl alanine were present only at Wadi-Sudr habitat, meanwhile lysine and cystine were present only at Wadi-Hof habitat. Determination of hydrolyzed protein amino acids of *Atriplex halimus* revealed that the two habitats contained fifteen protein amino acids with different ranges of concentration. The obtained results indicated that the percentage of proline was higher in plants of Wadi-Sudr than that in plants of Wadi-Hof, indicating the sever conditions of salinity and drought at Wadi-Sudr. Accumulation of cellular proline due to increased synthesis and decreased degradation under stress conditions such as salt and drought has been documented in many plant species (Kavi Kishor, et al., 2005). Proline has diverse roles under osmotic stress such as stabilization of proteins membranes and protecting subcellular structure (Vanrensburg, et al., 1993). Ali and Sawaf, (1992) stated that the content of free amino acids in the organs of *Datura innoxia* plant increased with the rise of

salinization level. Accumulation of proline and amino acids in sugar beet plants was linked to osmotic, water and salt stress (Gzilk, 1996).

Determination of the unsaponifiable matter content of *Atriplex halimus* at the two habitats were determined using GLC technique revealed that the plants at the two habitats contained n-eicosane, n-hexacosane, n-octasosane and squalene. While lipid at Wadi-Sudr contained also n-docosane, n-triacontane and two sterols, cholesterol and stigmasterol. Meanwhile lipid at Wadi-Hof contained n-tricosane, n-pentacosane and no sterols. Radwan et al., (2000) reported that GLC analysis of the hydrocarbons and sterols of *Saueda vermiculata* (Chenopodiaceae) showed that the presence of hydrocarbons, n-$C_{21}$, n-$C_{22}$, n-$C_{23}$, n-$C_{25}$ and n-$C_{30}$ and the sterols, cholesterol, stigmasterol and β-sitosterol. Gas liquid chromatography of fatty acids content of the lipids of *Atriplex halimus* at the two habitats revealed the presence of myristic, palmitic, stearic, palitoleic, oleic and linoleic, while pelargonic and capric acids in *Atriplex halimus* growing in Wad-Sudr only. Goss (1973) stated that most abundant fatty acids of desert plants were palmitic, stearic and linoleic acids. Tikhomirova et al., (2008) reported that analysis of fatty acids composition of *Salicornia europea* L. (Chenopodiaceae) indicated the presence of palmitic, linoleic, alpha-linolenic and oleic acids. The obtained data declared the presence of linoleic and linolenic acids, these essential fatty acids are used to form eicosanoic fatty acids which give rise to the prostaglandins, thromboxanes and leukotrienes (Murray, et al., 2003).

Investigation of the free sugars using HPLC revealed the presence of rhamnose, arabinose, ribose, glucose and raffinose at Wadi-Hof and Wadi-Sudr habitats while sucrose and galactose were detected only at Wadi-Sudr habitat. Meanwhile fructose was present only at Wadi-Hof habitat. For combined sugars of *Atriplex halimus,* the obtained results declared the presence of arabinose, glucose, raffinose, ribose and sucrose at Wadi-Hof and Wadi-Sudr habitats while rhamnose and galactose were detected only in Wadi-Sudr, Meanwhile fructose was detected only in Wadi-Hof habitat. Using paper chromatography, glucouronoc acid, glucose, arabinose and rhamnose were identified in *Suaeda vermiculat* (Chenopodiaceae) (Radwan et al., 2000). Accumulation of soluble carbohydrates may act as non-toxic in various salt tolerant plants (Marcum and Murdoch, 1992).

Chromatographic investigation of flavonol, flavanone, flavone and isoflavone glycoside declared the presence of isoflavone glycoside and vicenine II in the plants at Wadi-Sudr habitat only. Flavonoids of important biological function in plants. Some flavonoids act as flower pigments attracting pollinating birds and insects, controlling of plant growth, inhibiting and activating enzymes, having a role in sex biochemistry, some having fungicide properties and protect the plant against attack by parasites (Balbaa, 1981). Flavonoid compounds protect against cancer induction in human tissues. Chemo prevention the potential to be a major component of colon, lung, prostate and bladder cancer control (Thomass, et al., 2007). Certain flavonoids have protective effect on liver due its antioxidant properties and useful in the treatment of liver damage (Khalid, et al., 2002). Benhammou et al., (2009) reported that *Atriplex halimus* leaves and stems were characterized by the presence of the flavonoids, the tannins, the alkaloids and the saponins where the leaves exhibited the higher yields. These molecules were known to show medicinal activity as well as exhibiting physiological activity. Yang et al., (2008) reported that narigin and naringenin 7-O-glucoside are very common in species of Chenopodiaceae. Moreover, the flavonol class forms the major chemical compounds of *Atriplex* species. Salinity, water deficit and edaphic factors enhance the phenolic metabolism as a response to oxidative stress (Ksouri, et al., 2008). Quantitative determination of phenolic acids

using HPLC revealed the presence of five phenolic acids in *Atriplex halimus* at the two habitats, while gallic acid and ρ-coumaric acid were present only at Wadi-Sudr habitat, indicating the stress at Wadi-Sudr.

Quantitative and qualitative difference in the alkaloids content of the plant tissues affected by climatic factors, environment and soil composition **(**Grycova´, et al., 2007). Chromatographic investigation of alkaloids of *Atriplex halimus* at the habitats revealed the presence of piperine, berberine and palmatine in the plants growing at Wadi-Sudr habitat while ephedrine and piperine were isolated from the plants at Wadi-Hof habitat. Berberine and related compounds exhibit biological activities, antimicrobial (Slobodnikova, et al., 2004), anti-inflammatory (Ivanovska and Philipov, 1996), antimalaria (Mazzini, et al., 2003) and cytotoxic (Iwasa, et al., 2001). A rapid non-aqueous capillary electrophoresis method has been developed for the separation and determination of eight isoquinoline alkaloid (berberine, palmatine, jatrorrhizine, tetrandrine, berbamine, thalifaricine, northalfine and thalistine) from *Thalictrum atriplex* Finet (Su, et al., 2006). Piperine and its derivatives are effective anticonvulsant drugs, antiepileptic drug in treat of epilepsy and have sedative-hypnotic and muscle-relaxing action (Pei, 1983).

## CONCLUSION

Chromatographic investigation of bioactive constituents of *Atriplex halimus* plant revealed the presence of flavonol, flavanone, flavone and isoflavone glycosides beside the presence of alkaloid compounds, fatty acids, sterols, amino acids and combined sugars which possesses medicinal and physiological activity.

Quantitative and qualitative differences in the bioactive constituents of *Atriplex halimus* plant at the two habitats reflect the stress at Wadi-Sudr habitat due to the difference in the environmental conditions.

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