

## Assessment of Environmental Fluctuations in Phytochemical Constituents of Some Xerophytes Inhabiting Wadi Sudr and their Antimicrobial Bioactivity

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TRADITIONAL medicinal plants are an important economic source of raw materials for the drug industry. Nowadays, searching for new sources of drug raw materials is an important issue to meet the ever increasing demands. Therefore, quantitative and qualitative phytochemical screening of the dominated xerophytes inhabiting Wadi Sudr, South Sinai, including *Retama raetam*, *Reaumaria hirtella*, *Tamarix nilotica* and *Zygophyllum dumosum*, was performed. The accumulation levels of some natural bioactive products including phenols, flavonoids and saponins were determined during spring and summer. The investigated species exhibited significant increases in total free amino acids during spring, while they tend to accumulate total soluble sugars, sucrose and secondary metabolites including phenols, tannins and saponins during the dry summer season. Such increments in these metabolites were concomitant with the greatly reducing power capacity. Moreover, the antimicrobial activities of extracts from these shrubs were evaluated against four pathogenic microorganisms (2 bacterial species; *Escherichia coli* and *Staphylococcus aureus* and 2 fungal species; *Candida albicans* and *Aspergillus fumigatus*). Of the tested alternatives, methanolic extract was the most active fraction, while *Tamarix nilotica* was the most active plant species against the four tested microorganisms, especially *Candida albicans*. Furthermore, The MIC of the methanolic fraction of *Tamarix nilotica* and its effect on ergosterol and leakage of intracellular components of cells of *Candida albicans* showed that this shrub displays its fungicidal effects by targeting the ergosterol biosynthesis in *Candida albicans* and disrupting the membrane integrity. In conclusion, these shrubs are promising new sources for potent antioxidants and useful antimicrobial agents.

**Keywords:** Wadi Sudr, Xerophytes, Metabolites, Antioxidant, Antimicrobial activity.

### Introduction

Wild medicinal plants emanate from their long use in folk medicines as well as their prophylactic properties, especially in developing countries. According to World Health Organization (WHO) more than 80% of the world's population relies on traditional medicine for their primary healthcare needs. Medicinal plants contain large varieties of chemical substances, which possess important therapeutic properties that could be utilized in the treatment of human diseases. The potential of higher plants as source of new drugs is still largely unexplored. Among the estimated 250,000–500,000 plant species, only a small percentage has been investigated phytochemically and the fraction submitted to biological or pharmacological screening is even smaller. Thus, any phytochemical investigation

of a given plant will reveal only a very narrow spectrum of its constituents. Historically, pharmacological screening of compounds of natural or synthetic origin has been the source of numerous therapeutic agents. Random screening as a tool in discovering new biologically active molecules has been most productive in the area of antibiotics (Kroschwitz & How e-Grant, 1992 and Mahesh & Satish, 2008).

Plants generally produce several secondary metabolites like phenols, flavonoids, quinones, tannins, alkaloids, saponins, and sterols which are important sources of biocides and many other pharmaceutical drugs (Naili et al., 2010). Moreover, a large number of wild plants has been investigated for their antioxidant properties. Natural antioxidants either in the form of raw extracts or their chemical constituents are very

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effective in neutralizing reactive oxygen species (ROS) and thereby, preventing the destructive processes caused by oxidative stress (Zengin et al., 2011). Considerable evidence has been gathered and indicated the key roles of ROS and other oxidants in causing numerous disorders and diseases. Meanwhile, the human body has an inherent antioxidative mechanism and many of the biological functions such as the antimutagenic, anti-carcinogenic and anti-aging responses originate from this property (Gocer & Gulcin, 2011 and Gulcin, 2012). Medicinal plants also represent a rich source of antimicrobial agents (Mutasa et al., 2015).

Wadi Sudr is one of the largest and most developed wadis in south west Sinai. W. Sudr is exposed to several environmental variables, which have impacted the ecosystem particularly the vegetation. Four xerophytes *Retama raetam* (True xerophyte), *Reaumaria hirtella* (Crynohalophyte), *Tamarix nilotica* (Crynohalophyte) and *Zygophyllum dumosum* (Succulent xerophyte) were chosen for exploiting the antioxidant level and bioactivity of secondary metabolites during spring and summer. The aim of this study was to evaluate *in vitro* antioxidant as well as the antimicrobial properties of different extracts of some wild plants collected from Wadi Sudr.

## **Materials and Methods**

### *Location of the study area*

Wadi Sudr is one of the most developed wadis of the southern section of the western coast of Sinai, delimited by latitudes 29°36'54"-29°51'54" N and longitudes 32°41'30"-33°09'07" E. It is bounded by Gebel El Raha (c. 600m) in the north and Sinn Bishr (c. 618m) in the south. The main trunk of the wadi extends roughly in a NE-SW direction for about 55 km and flows into the Suez Gulf at Ras Sudr town (C. 55km south of El-Shatt). The wadi originates in the hill.

### *Plant analysis*

#### *Plant collection*

The aerial parts of *Retama raetam*, *Reaumaria hirtella*, *Tamarix nilotica* and *Zygophyllum dumosum* shrubs of similar age and size were collected from different sites of Wadi Sudr located in South Sinai during spring and summer. The plants were identified by botany department, Ain Shams University. The fresh and air-dried aerial

parts of *Retama raetam*, *Reaumaria hirtella*, *Tamarix nilotica* and *Zygophyllum dumosum* were subjected to pigments and metabolic analysis, respectively as well as the antimicrobial activity.

#### *Analysis of primary metabolites*

##### *Determination of total soluble carbohydrates:*

Total soluble carbohydrates were extracted following the method of Homme et al. (1992) and determined using anthrone reagent according to the method described by Fairbairn (1953).

##### *Determination of sucrose:*

Sucrose content was estimated according to the method described by Hubbard & Pharr (1992). The concentration of sucrose was determined from the standard curve of sucrose and calculated as mg/g dry weight.

##### *Determination of total free amino acids:*

Free amino-acids were determined photometrically with ninhydrin according to the procedure described by Muting & Kaiser (1963). The concentration of amino acids was finally determined from the standard curve of glycine and calculated as mg/g dry weight.

#### *Analysis of secondary metabolites*

##### *Extraction of phytochemicals:*

After complete shade drying, the plant materials (100g) were grinded with a mechanical grinder and the powder was kept in tightly closed containers. Plant material (20g) was soaked for 6h in 100ml of distilled water in tightly sealed vessels at room temperature. The methanolic, hexan and petroleum ether extracts were prepared by extracting the dry leaves powder (20g) three times each with 100ml of solvent. All crude extracts were filtered through Whatman filter paper and kept at 4°C until tested.

##### *Determination of total polyphenol content and tannins:*

The polyphenol and tannin contents were determined according to the methods described by Makkar et al. (1993). The total phenolic content was expressed as mg tannic acid equivalents/g sample.

##### *Determination of total flavonoids content:*

To confirm the presence of flavonoids, the method of Woisky & Salatino (1998) was used. The total flavonoid content was calculated as  $\mu\text{g g}^{-1}$  dry weight from the standard curve of quercetin.

##### *Determination of saponins:*

The presence of saponins was demonstrated by the methods of

Makkar et al. (2007). The total Saponin content was calculated as mg g<sup>-1</sup> dry weight from the standard curve of diosgenin.

*Determination of free radical scavenging activity:* The free radical scavenging activity of the fractions was measured by 2, 2-diphenyl 1-1-picrylhydrazyl (DPPH) assay according to Bursal & Gulcin (2011). The scavenging activity was estimated based on the percentage of DPPH radical scavenged as the following equation:

$$\text{Scavenging effect \%} = \frac{(\text{Control absorbance}) - (\text{Sample absorbance})}{\text{Control absorbance}} \times 100$$

#### *Antimicrobial activity assay*

##### *Microbial species*

Two species of pathogenic bacteria; *Escherichia coli*, *Staphylococcus aureus* and two species of pathogenic fungi; *Candida albicans* and *Aspergillus fumigatus* were used as test organisms. All pathogenic organisms were obtained from Ain Shams University, Specialized Hospital. Bacterial strains were grown on nutrient agar slants for 24h at 37°C, *Candida albicans* and *Aspergillus fumigatus* were grown on potato dextrose agar at 28°C for 2-4 days. For bacterial and yeast strains, the culture was gently swabbed, and the suspension was transferred to a sterile tube, then adjusting the concentration using the 0.5 McFarland standard tubes. This will give a suspension of 10<sup>6</sup> spores/ml (CLSI M27-A3, 2008a). For fungal strain, spore suspensions were counted with a hemocytometer to a concentration of 0.5-4 x10<sup>4</sup> spores/ml (CLSI M38-A2, 2008b).

##### *Preparation of plant extracts*

Extracts of the four tested xerophytes collected during summer season were prepared at a concentration of 100mg/ml using different solvents viz. Methanol, ethyl acetate and water.

##### *Antibacterial and antifungal assessment of plant extracts*

The surface of Muller Hinton (MH) agar plates was streaked in 4 different directions (at 90-degree angles) to cover the entire surface and allowed to dry at 35°C. The agar was allowed to set and harden and the required numbers of holes were cut using a sterile cork borer, Agar plugs were removed. Amphotericin and Chloramphenicol (1mg/ml) were used as positive control for fungal and bacterial strains, respectively. About 200µl of different concentrations of plant solvent extracts were put into the wells and allowed to diffuse at

room temperature for 1h. The plates were incubated at 37°C for 18-24h for bacterial pathogens and 28°C for 2-4 days for fungal pathogens. The diameter of the inhibition zone (DIZ) was measured in mm and the activity index was also calculated. The experiment was repeated twice, where for each replicate. The diameters of the inhibitory zones were measured in three different fixed directions and the average values were recorded (Magaldi et al., 2004).

##### *Determination of minimum inhibitory concentration (MIC)*

The minimum inhibitory concentration (MIC) was determined according to the Clinical Laboratory Standards Institute M27-A3 microdilution method (CLS, 2002) using 96-wells microtiter plates. 100µL of two-fold diluted fractions and reference drugs in RPMI 1640 (Sigma Aldrich) were added in the wells of the microtiter plate followed by addition of 100µL of *C. albicans* inoculum standardized at 2.5×10<sup>3</sup> cells/ml. The plate was incubated at 30°C for 24h. The MIC was determined spectrophotometrically with a microplate reader at 595nm. MIC was defined as the lowest concentration that inhibited visible fungal growth.

##### *Determination of ergosterol content*

Ergosterol content in the plasma membrane was measured according to Tian et al. (2012). An amount of 100ml containing 10<sup>7</sup> spores/ml (the spore population was counted using a hemocytometer) of *C. albicans* spore suspension was inoculated in a Potato Dextrose Broth (PDB) medium containing sub inhibitory concentrations of methanolic extract of *Tamarix nilotica*. Samples without any oil treatment were considered as controls. After incubation, cells were harvested and washed twice with distilled water. The net wet weight of the cell pellet was determined. Five milliliters of 25% alcoholic potassium hydroxide solution were added to each sample and vortex mixed for 2min, followed by incubation at 85°C for 4h. Sterols were extracted from each sample by adding a mixture of 2ml sterile distilled water and 5ml n-heptane. The mixture was then sufficiently mixed by vortex for 2min allowing the layers to separate for 1h at room temperature. The n-heptane layer was analyzed using scanned spectrophotometry between 230 and 300nm. The presence of ergosterol (at 282nm) and the late sterol intermediate 24(28) dehydroergosterol (at 230 and 282nm) in the n-heptane layer led to a characteristic curve. Ergosterol amount was

calculated as a percentage of the wet weight of the cells and was based on the absorbance and wet weight of the initial pellet. The calculated formula of the ergosterol amount is as follows:

$$\% \text{ total ergosterol} + \% \text{ 24(28) dehydroergosterol} = (A_{282}/290)/\text{pellet weight}$$

$$\% \text{ 24(28) dehydroergosterol} = (A_{230}/518)/\text{pellet weight}$$

$$\% \text{ ergosterol} = [(\% \text{ total ergosterol} + \% \text{ 24(28) dehydroergosterol}) - (\% \text{ 24(28) dehydroergosterol})]$$

where 290 and 518 are the E values (in percentages per cm) determined for crystalline total ergosterol and 24(28) dehydroergosterol, respectively and pellet weight is the net wet weight (g).

#### *Effect on cell membrane integrity*

This assay was performed to determine the ability of the plant extracts to alter the fungal cell membrane integrity causing leakage of intercellular molecules (Cordeiro et al., 2014). *Candida albicans* was exposed to MIC/2 of the methanolic extract of *Tamarix nilotica*. Growth control (without extract) and blank solution (culture medium only) were also included in the test. One millimeter from the tube containing the fungal inoculum and methanolic extract of plant was transferred to sterile microcentrifuge tubes and centrifuged for 15min. (13,400 x g). Of the supernatant, 70µl were removed from each tube and diluted 1:10 with sterile distilled water and the absorbance of the content was measured spectrophotometrically at 260 and 280nm for nucleic acid and proteins, respectively.

#### *Statistical analysis*

In plant analysis, three replicates were used. The results were statistically analyzed using the SPSS (Statistical Package for Social Scientists) software, version 17. Data was statistically analyzed using Duncan's multiple range test ( $P > 0.05$ ) between mean values for the results of spring and summer seasons as described by Snedecor & Cochran (1980).

## **Results and Discussion**

#### *Plant analysis*

The spatial and seasonal variations in the succulence, pigments levels and some metabolites were investigated in four dominant and codominant

xerophytes including *Zygophyllum dumosum* (succulent xerophyte), *Retama raetam* (non-succulent "true" xerophyte) and *Tamarix nilotica* and *Reaumuria hirtella* (crynohalophytes) inhabiting Wadi Sudr (South Sinai).

#### *Analysis of primary metabolites*

The physiological and biochemical processes are altered by stress such as water relation (Silva et al., 2015), gas exchange, photosynthesis (Pagter et al., 2005) and the metabolism of carbohydrates, protein, amino acids and other organic compounds (Šircelj et al., 2005).

#### *Total soluble sugars*

Soluble sugars might play a central role in photochemical apparatus protection and ROS scavenging in stressed plants. Total soluble sugars content increased significantly during summer in all the four-studied species (Table 1). Total soluble sugars are negatively related to the pigment content in the all investigated species. The increments in sugars under water scarcity resulted from the decrease of photosynthesis, which was associated with an increase in respiration rate and led to the reduction in carbohydrates concentration in plant and hence the breakdown of starch into free soluble sugars needed for osmoregulation (Rosa et al., 2009). Carbohydrates act as a nutrient and also works as signaling molecules, modulating the expression of large number of genes (Osuna et al., 2007) and they are also involved in the response to abiotic stresses. Drought generally reduces the biochemical capacity for carbon assimilation and utilization. The adaptation of plants to heat stress induced accumulation of metabolites that serve as compatible solutes in chloroplasts, which were related to enhance thermostability (Hasegawa & Bressan, 2000). Similar results have been reached by Zhou et al. (2013) on *Tamarix ramosissima*.

#### *Sucrose*

Sucrose is the dominant form of carbohydrate transported to developing plant organs and is one of the stored sugars in higher plants (Khayat & Zieslin, 1987). Sucrose also serves as an osmotic solute (Rekikia et al., 1998). The early increase in sucrose content may be an adaptive response to water deficiency, as it may induce a shift in the partitioning of carbon in favor of sucrose synthesis (Castrillo, 1992). It is of interest to notice that *Tamarix nilotica* showed the highest significant value of sucrose content among all the studied plants during summer, while it attained the lowest value

during spring (Table 1). Higher sucrose levels in the leaves of water-stressed plants might contribute to improved drought tolerance by decreasing osmotic potential in leaves in response to prolonged periods of water stress (Fu et al., 2010). The data implies that increase in sucrose content might be a possible explanation to the increase in production of total flavonoids and phenolics. The result agreed with the finding of Guo et al. (2011), who proposed that the increase in production of plant secondary metabolites that were observed in their studies on broccoli was due to increased production of sucrose.

In plants, carbohydrates produced by photosynthesis play vital roles as sources of energy and carbon skeletons for organic compounds and storage components as well as antioxidants and secondary metabolites. Carbohydrates contributed in ROS production and scavenging in plants exposed to abiotic stresses (Keunen et al., 2013). Moreover, carbohydrates play potential key roles in plant immunity as an essential element for plant defenses against microbes and participate in the regulation of defense genes (Bolouri-Moghaddam & Van Den Ende, 2013 and Trouvelot et al., 2014). So, carbohydrates could be helpful in controlling plant diseases in field conditions (Delaunois et al., 2014).

#### Total free amino acids

The total free amino acids accumulation in

the studied species displayed remarkable changes according to seasons and location variations. There is a tendency in accumulation of free amino acids during spring (Table 2). The reduction in free amino acids accumulation during summer might be due the decline in soil moisture content which reduce the uptake and transport of nitrogen, thereby amino acid biosynthesis and utilization in protein synthesis which might participate in root growth and fruit development. The reduction in the total free amino acids during summer may be also, due to the increase of the ability of the studied species to divert photoassimilates and changes the partitioning of carbon allocated to growth and secondary metabolism including phenols, lignin (Lavinsky et al., 2015).

#### Analysis of secondary metabolites (phytochemicals)

It was suggested that when plants are stressed, a trade-off occurs between allocating carbon to biomass production or the formation of defensive secondary compounds (Herms & Mattson, 1992). The synthesis of secondary metabolites including phenols, flavonoids, saponins, tannins and cardiac glycosides was stimulated under environmental stresses on the expense of some primary metabolites to contribute in the adaptive strategy and leading to tolerance of abiotic stresses. Meanwhile, most of the secondary metabolites have significant ecological functions, including protection against microbial or insect attack and significant economic and medicinal purposes.

**TABLE 1. Seasonal fluctuations in total soluble sugar (mg.g<sup>-1</sup> DW) and sucrose (mg.g<sup>-1</sup> DW) contents of the studied species inhabiting Wadi Sudr.**

Plant species	Total soluble sugar (mg.g <sup>-1</sup> dry wt.)		Sucrose (mg .g <sup>-1</sup> dry wt.)	
	Spring	Summer	Spring	Summer
<i>Retama raetam</i>	1.65±0.04 <sup>a</sup>	1.86±0.14 <sup>b</sup>	0.34±0.03 <sup>b</sup>	0.43±0.08 <sup>a</sup>
<i>Reaumuria hirtella</i>	0.92±0.07 <sup>a</sup>	1.46±0.12 <sup>c</sup>	0.18±0.01 <sup>a</sup>	0.21±0.04 <sup>ab</sup>
<i>Tamarix nilotica</i>	2.91±0.14 <sup>b</sup>	3.00±0.17 <sup>b</sup>	0.55±0.09 <sup>b</sup>	0.16±0.01 <sup>a</sup>
<i>Zygophyllum dumosum</i>	2.88±0.21 <sup>a</sup>	4.16±0.21 <sup>c</sup>	0.62±0.07 <sup>b</sup>	0.43±0.05 <sup>d</sup>

**TABLE 2. Seasonal fluctuations in total amino acid (mg.g<sup>-1</sup> DW) contents of the studied species inhabiting Wadi Sudr.**

Plant species	Total amino acid (mg.g <sup>-1</sup> dry wt.)	
	Spring	Summer
<i>Retama raetam</i>	6.21±1.41 <sup>ab</sup>	4.30±1.75 <sup>ab</sup>
<i>Reaumuria hirtella</i>	2.21±0.04 <sup>b</sup>	1.00±0.15 <sup>a</sup>
<i>Tamarix nilotica</i>	0.90±0.09 <sup>b</sup>	1.02±0.14 <sup>b</sup>
<i>Zygophyllum dumosum</i>	3.94±0.18 <sup>c</sup>	2.82±0.28 <sup>b</sup>



### Phenols

All tested species showed an increase in phenolic content during summer, except *Retama raetam* which showed a reverse response. *Tamarix nilotica* showed the greatest value of phenols during summer. Phenol synthesis and accumulation is generally stimulated in response to biotic or abiotic stresses (Muthukumarasamy et al., 2000). The accumulation of phenolic compounds may be attributed to disrupted metabolism caused by high radiation levels in arid regions thereby, the imbalance between phenolic compound synthesis and its use in the cell wall synthesis (Kefeli & Kalevitch, 2003). It was also postulated that plants that grow under unfavorable environmental conditions, contain relatively large amounts of phenolic compounds (Sezai et al., 2008 and Patel & Patel, 2014) which play a role in cell acclimation against stress (Lee et al., 2003). In addition, phenolic compounds exhibit antioxidant activity by buffering lipid free radicals or by preventing hydroperoxides into free radicals (Pokorny et al., 2001). Water stress can stimulate the accumulation of phenolic compounds in desert plants during summer by hydrolyzing the glycosides (Gehlert et al., 2011). Consequently, the phenolics are powerful antioxidants in plant tissues under stress (Silva et al., 2015 and Kljusurić et al., 2016). They are chemically heterogeneous compounds and include flavonoids, lignins and tannins. They play various roles as they can act as antiherbivore and antipathogens, lend mechanical support, attract pollinators, absorb high energy radiations and reduce the growth of nearby competing plants (Harborne & Williams 2000 and Taiz & Zeiger, 2002).

### Tannins

The studied plant species showed an increase in the tannins content during summer except *Retama raetam* which showed no significant difference between the two seasons and the different locations (Table 3). The combined stress of drought, high temperature and excessive light as well as salinity characteristic for desert habitat increased the production of polyphenolic compounds such as tannins (Ramakrishna & Ravishankar, 2011; Ibrahim, 2012 and Abdolzadeh et al., 2014). The occurrence of large amount of tannins can confer protection and acclimation against biotic and abiotic stresses. Tannins can neutralize reactive oxygen species (Bekerecioglu et al., 1998). They are generally antitoxins that can reduce the growth and survival of many herbivores and serve as

defenses against microorganisms (War et al., 2012 and Redondo et al., 2014).

### Flavonoids

Chaves et al. (1993) reported that the flavonoid content of plants is known to vary quantitatively and qualitatively depending on the growth stage, degree of senescence, season and geographical location. Flavonoids have been shown to be highly effective scavengers of most oxidizing molecules (Havsteen, 2002 and Tahara, 2007) and implicated to confer protection to plants grown under stresses (Winkel-Shirley, 2002 and Gould, 2004). *Retama raetam* and *Reaumuria hirtella* showed an increase in flavonoid content in spring, while *Tamarix nilotica* and *Zygophyllum dumosum* showed a reverse effect during summer. Similarly, the phenolic content of *Tamarix nilotica* recorded the highest mean value (2 folds) of total flavonoids content during summer (Table 3). The increments in the total flavonoids in the dry hot season particularly may be associated to the gradient of environmental conditions imposed by high temperature, relative humidity and solar radiation. Similar results have been obtained by Ahmed et al. (2014) and Patel & Patel (2014) on *Vernonia schimperi*, *Tecomella undulate* and Khodaie et al. (2012) on *Pedicularis L.*, respectively.

### Saponins

Saponins are glycosides accumulated in many plants. They display hemolytic, expectorative, anti-inflammatory and immune-stimulating activity and antimicrobial properties particularly against fungi and additionally against bacteria and protozoa (Westendarp, 2005). The studied species showed an increase in saponin content during summer except for *Retama raetam* (Table 3). The increments in saponins level in summer might be due to the differential synthesis and/or accumulation of saponins and their aglycones which depend on species or genotype, age and environmental conditions (Inderjit & Foy, 1999 and Golawska et al., 2006). The accumulation of saponins could be related to its protective role against oxidative stress (Kim et al., 2012 and Szakiel et al., 2009). Our results were in line with De Costa et al. (2013) and Odjegba & Alokolaro (2013) on *Quillaja brasiliensis* and *Acalypha wilkesiana*, respectively. Moreover, the combined influence of agro-climatic conditions was shown to affect the quantity of saponins (Szakiel et al., 2011 and Moses et al., 2014). In contrast, the reduction in saponins in *R. raetam* was also reported by Soli'z-Guerrero et al. (2002) in water stressed *Chenopodium quinoa*.

**TABLE 3. Seasonal fluctuations in total phenols, tannins, flavonoids and saponins contents (mg.g<sup>-1</sup> DW) of the studied species inhabiting Wadi Sudr.**

Plant species	Total phenols		Total tannins		Total flavonoids		Total saponins	
	(mg.g <sup>-1</sup> dry wt.)		(µg.g <sup>-1</sup> dry wt.)		(µg.g <sup>-1</sup> dry wt.)		(mg.g <sup>-1</sup> dry wt.)	
	Spring	Summer	Spring	Summer	Spring	Summer	Spring	Summer
<i>Retama raetam</i>	2.68±0.23 <sup>b</sup>	1.12±0.58 <sup>b</sup>	2.98±0.2 <sup>a</sup>	2.68±0.96 <sup>a</sup>	3.27±0.42 <sup>a</sup>	3.45±0.79 <sup>a</sup>	4.18±0.31 <sup>a</sup>	4.05±0.18 <sup>a</sup>
<i>Reaumuria hirtella</i>	2.28±0.12 <sup>a</sup>	2.53±0.14 <sup>b</sup>	1.34±0.01 <sup>a</sup>	1.91±0.06 <sup>b</sup>	5.49±0.41 <sup>c</sup>	3.58±0.3 <sup>a</sup>	0.35±0.01 <sup>c</sup>	0.53±0.04 <sup>d</sup>
<i>Tamarix nilotica</i>	3.09±0.28 <sup>b</sup>	3.68±0.24 <sup>c</sup>	1.43±0.29 <sup>a</sup>	2.80±0.17 <sup>c</sup>	5.63±0.20 <sup>b</sup>	10.48±0.6 <sup>d</sup>	0.68±0.11 <sup>a</sup>	2.41±0.17 <sup>c</sup>
<i>Zygophyllum dumosum</i>	1.58±0.2 <sup>b</sup>	1.90±0.16 <sup>c</sup>	1.47±0.08 <sup>a</sup>	2.44±0.19 <sup>b</sup>	2.49±0.44 <sup>a</sup>	5.46±0.12 <sup>b</sup>	1.87±0.14 <sup>a</sup>	2.84±0.04 <sup>c</sup>

*Total antioxidant capacity*

All studied plant extracts exhibited antioxidant activity. The greatest reducing power was measured during hot summer season (Table 4). Similar results have been reached by Ahmed et al. (2014) who observed increments in the total antioxidant capacity of *Melilotus indicus* methanolic extract collected during summer. This was concomitant with the increments in the magnitudes of antioxidants such as phenols, flavonoids and tannins which may suggest the involvement of antioxidants in the main strategy of adaptation of the studied species to arid environment. Moreover, phenolic acids and flavonoids seem to be the major contributors for the increments in the total antioxidant activity and thereby the highly effective scavengers of most oxidizing molecules (Nunes et al., 2012 and López-Marín et al., 2013). The results of the present investigation showed that the increase in sucrose content was concomitant with production of secondary metabolites. Such results are in accordance with these reported by Briskin & Gawienowski (2001) who observed that sucrose might have influence on the up regulation of secondary metabolites.

*Antimicrobial activity*

Antimicrobial activity of aqueous, methanolic

and ethyl acetate extracts of the four plants (*Retama raetam*, *Reaumuria hirtella*, *Tamarix nilotica* and *Zygophyllum dumosum*) showed variable results against the four microbial strains (*Staphylococcus aureus*, *Echerichia coli*, *Candida albicans* and *Aspergillus fumigatus*). The methanolic extract of *Tamarix nilotica* showed the highest antimicrobial activity against all tested microorganisms followed by the aqueous extract of the same plant. This might be related to the greater accumulation of secondary metabolites especially phenols and flavonoids accumulated in *T. nilotica*. It could also be related to the fact that *T. nilotica* being a crynhalophyte which accumulates high amount of minerals in its tissue which had an inhibitory effect on the microorganisms. On the other hand, the extracts (aqueous, methanolic and ethyle acetate) of *Retama raetam*, *Reaumuria hirtella* lack the antimicrobial activity and *Zygophyllum dumosum* showed very weak activity (Table 5). Alghazeer et al. (2012) also mentioned that the antibacterial potential was increased notably using the methanol plant extracts than the activity of water extracts against both G +ve and G -ve bacteria. *Candida albicans* was the most susceptible strain to the methanolic extract of *Tamarix nilotica* followed by *Aspergillus fumigatus*, *Staphylococcus aureus* and finally *Echerichia coli* (Table 5 and Fig. 1).

**TABLE 4. Percentage of DPPH free radical scavenging activity for all studied species.**

Plant species	% Free radical scavenging activity	
	Spring	Summer
<i>Retama raetam</i>	28.56±1.36 <sup>a</sup>	85.65±0.83 <sup>c</sup>
<i>Reaumuria hirtella</i>	18.80±0.50 <sup>a</sup>	32.13±1.66 <sup>b</sup>
<i>Tamarix nilotica</i>	14.53±1.51 <sup>a</sup>	42.61±1.28 <sup>b</sup>
<i>Zygophyllum dumosum</i>	35.40±0.26 <sup>a</sup>	59.95±2.25 <sup>c</sup>

TABLE 5. *In vitro* antimicrobial activity of extracts from the four studied plant species by well diffusion method.

Microbial species	Inhibition zone diameter (mm)												Amp. B	Ch
	<i>Reaumuria hirtella</i>			<i>Retama raetam</i>			<i>Tamarix nilotica</i>			<i>Zygophyllum dumosum</i>				
	Aq	Me	Et	Aq	Me	Et	Aq	Me	Et	Aq	Me	Et		
<i>Staphylococcus aureus</i>	ND	ND	ND	ND	ND	ND	12	18	12	7	10	8	NA	29
<i>Escherichia coli</i>	ND	ND	ND	ND	ND	ND	14	17	12	9	12	6	NA	20
<i>Candida albicans</i>	ND	ND	ND	ND	ND	ND	14	22	10	10	12	8	24	NA
<i>Aspergillus fumigatus</i>	ND	ND	ND	ND	ND	ND	12	20	10	8	10	7	25	NA

Aq, Aqueous; Me, Methanolic; Et, Ethyle acetate; Amp.B, Amphotericin B; Ch, Chloramphenicol; NA, not applicable; ND, not detected.

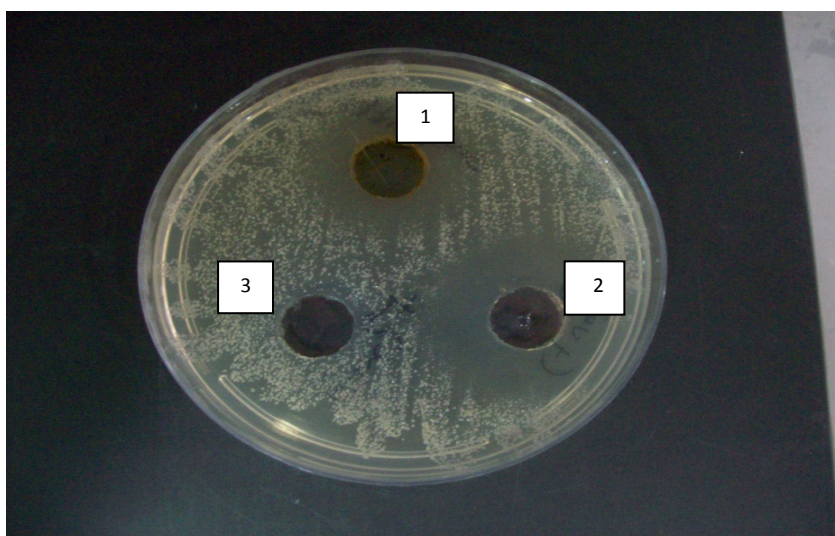


Fig. 1. *In vitro* anti-candida activity of methanolic extract of *Tamarix nilotica*.

#### Determination of the minimum inhibitory concentration (MIC)

The extent of inhibition on the fungal growth depends on the concentration used. The MIC of the most active plant extract (methanolic extract of *Tamarix nilotica*) was determined against most sensitive pathogenic microorganisms (*Candida albicans*). Results showed that the methanolic extract of *Tamarix nilotica* effectively inhibited the growth of *Candida albicans* at a low concentration (MIC = 0.56mg/ml) when compared to amphotericin B (MIC=0.70mg/ml) used as control. This result is similar to that reported by Omoruyi et al. (2014) who found that the essential oil of *Mesembryanthemum edule* extract tested against the pathogenic fungi, inhibited *Candida albicans*, *Candida krusei*, *Candida rugosa*, *Candida glabrata* and *Cryptococcus neoformans* with MICs range of 0.02-0.31mg/ml. The activity of the

essential oil was found comparable with nystatin and amphotericin B used as control.

#### Determination of ergosterol content

Ergosterol is specific to fungi and is the major sterol component of the fungal cell membrane. It is also responsible for maintaining cell function and integrity (Rodriguez et al., 1985). The effect of the most active plant extract (methanolic extract of *Tamarix nilotica*) on ergosterol content of the plasma membrane of the most sensitive pathogenic microorganisms (*Candida albicans*) was determined. Results revealed that *Tamarix nilotica* can induce a considerable impairment of the ergosterol biosynthesis (55% compared to control). This was consistent with some previous studies that have exhibited that natural and synthetic drugs can cause a considerable reduction in the quantity of ergosterol (Arthington-Skaggs et al., 2000; Pinto



et al., 2006 and Pinto et al., 2009). These results indicate that the plasma membrane is an important antifungal target of plant extracts.

#### *Effect on plasma membrane integrity*

Leakage of intercellular molecules of fungal cells is an indicator of effect of the extract on permeability of cell membrane. The effect of the most active plant extract (methanolic extract of *Tamarix nilotica*) on the integrity of cell membrane of the most sensitive pathogenic microorganisms (*Candida albicans*) was determined. The results showed that plant extract at a concentration of MIC/2 caused the leakage of nucleic acid (0.05) and protein (0.07) with a significant increase in absorbance higher than those obtained by control (0.01) at (260 and 280nm, respectively). These results were similar to those of Brilhante et al. (2016) who reported that tyrosol caused leakage of protein and nucleic acid, emphasizing the effect of tyrosol on both cellular and nuclear membrane.

#### **Conclusion**

Antioxidative and antimicrobial properties of wild plants are proven to be of great interest in both health and industry. In this respect, the present study was designed to evaluate the *in vitro* antioxidant properties and antimicrobial activity of the four investigated plant extracts against various microorganisms. The results presented here give insight about the seasonal changes of the antioxidant and antimicrobial properties of those plants that have traditional uses in Egypt. *Tamarix nilotica* might be useful as an excellent antioxidant, antibacterial and antifungal agent. Additional studies are required on the mode of action of the active ingredients on pathogenic microorganisms.

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### تقييم التذبذبات البيئية في المكونات الكيميائية النباتية لبعض النباتات الجفافية التي تقطن وادي السدرة ونشاطها الحيوي المضاد للميكروبات

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النباتات الطبية التقليدية هي مصدر اقتصادي مهم من المواد الخام لصناعة الأدوية. في الوقت الحاضر، يعد البحث عن مصادر جديدة للمواد الخام للأدوية مسألة مهمة لتلبية المطالب المتزايدة. لذلك، تم إجراء مسح كمي وكيفي للمكونات الكيميائية النباتية بالنباتات الجفافية - الزيروفيتس - المستوطنة في وادي سدر، جنوب سيناء، بما في ذلك ريتاما ريتام، ريوميريا هيرتيلا، تاماريكس نيلوتيكا وزيجوفيللم ديوموزم. تم تحديد مستويات تراكم بعض المنتجات الطبيعية النشطة حيوياً بما في ذلك الفينولات والفلافونويدات والصابونين خلال فصل الربيع والصيف. أظهرت الأنواع المختبرة زيادة معنوية في إجمالي الأحماض الأمينية الحرة خلال فصل الربيع، في حين أنها تميل إلى تراكم كل السكريات القابلة للذوبان، السكروز ونواتج الأيض الثانوية بما في ذلك الفينولات والتانينات والصابونين خلال موسم الصيف الجاف. هذه الزيادات في نواتج الأيض كانت متزامنة مع زيادة قدرة الإختزال. وعلاوة على ذلك، فقد تم تقييم الأنشطة المضادة للميكروبات لمستخلصات من هذه الشجيرات ضد أربعة كائنات ميكروبية ممرضة (نوعان من البكتريا؛ إيشيريشيا كولاي وستافيلوكوكس أوريس، ونوعان من الفطريات؛ الكانديدا أليكانز و اسبيراجيلس فيوميجاتس). من بين البدائل التي تم اختبارها، كان مستخلص الميثانول الأكثر نشاطاً، بينما كان تاماريكس نيلوتيكا أكثر الأنواع النباتية نشاطاً ضد الكائنات الدقيقة الأربعة المختبرة، خاصة الكانديدا أليكانز. وعلاوة على ذلك، أظهرت نتائج الـ MIC الخاص بمستخلص الميثانول من تاماريكس نيلوتيكا وتأثيرها على الإرجوستيرول وتسرب المكونات الداخلية لخلايا الكانديدا أليكانز أن هذا الشجيرة تظهر آثارها المضادة للفطريات عن طريق استهداف التخليق الحيوي للإرجوستيرول في الكانديدا أليكانز وتعطيل تماسك الغشاء الخلوي. في المجمل، تعتبر هذه الشجيرات مصادر جديدة لمضادات أكسدة قوية وعوامل مفيدة مضادة للميكروبات.