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MEASUREMENT OF SOME MEDICAL SUBSTANCES IN ROSELLA PLANT (*HIBISCUS SABDARIFFA* L.) EXTRACT USING HPLC TECHNIQUE UNDER THE INFLUENCE OF DIFFERENT TREATMENTS

Madeha H. Hussain[1] and Ali S. Hassoon[2]*

[1]Department of Pharmacy, Medical Institute Tech., Mansour, Middle Tech. University, Iraq.
[2]Department of Soil and Water Techniques, Al-Musaib Tech. College, Al-Furat Al-Awsat Tech., University, Iraq.

Abstract

The experiment was conducted in Al Saniya Township (located 15km north of Al-Qadisiyah) in the Summer Season 2016, to study the Measurement of some medical substances in Rosella plant (*Hibiscus sabdariffa* L.) extract using HPLC technique under the influence of different treatments. A factorial experiment was applied according to complete randomized blocks design (R.C.B.D), with three replicated. Three factors included three cultivars of Roselle plant are (red, white and lined), and The second factor is salicylic acid in three Concentration (0, 25, 50 mg.L^{-1}) divided into four sprayings and The third factor is seaweed extract in three concentration (0, 7.5, 15 ml.L^{-1}) divided into four sprayings. The results showed that the superiority of the red variety was significantly higher than that of Quercetin, Anthocyanin and Delphinidin-3-glucoside in the sepales, and the salicylic acid spray treatment was 50 mg/l. The seaweed extract was also sprayed at 15 ml, In most active substances. There were no significant differences in the concentration of vitamin C, Gossypetine, Kaempferol and Hibiscetine in the sepales.

Key words : Roselle, cultivars, salicylic acid, seaweed extract, HPLC.

Introduction

In recent years, medicinal plants have received great interest from interested researchers for the importance of pharmaceuticals that have contributed effectively to the development of the medical side in all its specialties. These plants are the Roselle (*Hibiscus sabdariffa* L.) of the Malvaceae family (Ajithadoss *et al*., 2006). The importance of Roselle plant lies in its many uses. It is considered an important food and industrial crop of high economic value in many countries of the world. It is a good source of income.The different parts of the plant are used in many food uses. Fresh and dried plant leaves are used in the preparation of hot and cold beverages. Also in the manufacture of sweets, ice cream, chocolate and ice mixes for adding flavor and color to the food. The plant extracts are also used in the manufacture of cosmetics such as lipstick, perfumes and others. Extracts are a source of natural colors and flavor in many food industries such as sweets and ice (Da-Costa-Rocha *et al*., 2014). It also has an important role in the treatment of many of the pathogens that confront human health. It was found that the extract of cassia leaves contribute to the treatment of blood pressure and reduce the proportion of cholesterol in the blood and also reduce the proportion of blood viscosity and found that the extract of dry cassow leaves effects important against cancer tumors because of containment (PCA), which is a pharmacologically effective agent in minimizing the carcinogenic effect of diethylntrosarline in the liver (Lin *et al*., 2007). Salicylic acid and its derivatives are one of the plant hormones produced naturally by the plant. It belongs to the group of phenolic acids. Salicylic acid plays an important role in the growth and development of the plant for the important physiological roles such as stimulating the flowering, absorbing the ions, transferring the nutrients, increasing the representation of CO_2, gaseous and protein production and raise the proportion of nuclear and amino acids and the accumulation of dry substance also accelerates the formation of different plant

***Author for correspondence :** E-mail : alisalealtaie2015@gmail.com

dyes and increase their levels such as chlorophyll and carotene and prevent the representation of ethylene gas and his work opposite ABA, which is responsible for leaf fall, also plays an important role in increasing cellular metabolism (Amin *et al*., 2011; Davies, 2004). The seaweed extract is one of the important organic sources used in increasing agricultural production. It improves the soil conditions and increases their fertility and exchange capacity by improving the upper layer of soil. It also encourages the growth and increase of the number of active bacteria growing in soils. This improves the absorption efficiency, stimulates root growth. It is usually used as fertilizer for soil and is given as fertilizer and herbicides. It is currently used around 15 million tons annually in the agricultural field in various countries of the world (Dhriti *et al*., 2015; Jensen, 2004).

Objectives

Study aims to estimation of some effective medical substances in the sepals extract of several cultivars of Roselle plant with the effect of salicylic acid spraying and seaweed extract using HPLC technique.

Materials and Methods

The experiment was carried out in Al Saniya Township (located 15km north of Al-Qadisiyah) in the Summer Season 2016. To study the measurement of some medical substances in Rosella plant (*Hibiscus sabdariffa* L.) extract using HPLC technique under the influence of different treatments. The seeds of three cultivars of al-Gujarat were obtained from the development of Al-Gujarat tea project in Diwaniyah of Diwaniyah Agriculture Directorate. The soil of the experiment was plowed by the plow-bearing plow with two orthogonal plows and then was cleaned and settled and then divided into three blocks. Each blocks included 27 experimental units with an area of 3×4 m^2. The experimental unit included 4 lines with a length of 3 m and the distance between the line and the last 75 cm. The soil was fertilized with phosphate fertilizer in the form of super phosphate by 160 kg. h^{-1}, one batch before planting and then added 100 kg. $Hectar^{-1}$. Nitrogen fertilizer urea added the first two batches after the second and the second before flowering. The seed was grown on 1/4/2016 and then gave the field irrigation irrigation without immersion until the completion of germination and when they reach the height of (10-15 cm) were carried out the process of dimming and patching after the plants were sprayed as needed with all the operations of the service of the crop of Izzak and the weed and the fight against others. Experimental factorial is applied according to complete randomized blocks design (R.C.B.D). Included the first factor three cultivars (red, white and lined) its symbol (V1, V2, V3), respectively. The second factor is salicylic

Table 1 : Physical and chemical properties of soil.

Traits	pH	Ec	N(Mg.kg^{-1})	P(Mg.kg^{-1})	K(Mg.kg^{-1})	Organic material(%)	Soil separators			Texture
							Sand gm.kg^{-1} soil	Loamy gm.kg^{-1} soil	Clayg m.kg^{-1} soil	
Value	7.4	2.3	35	14.28	16.2	0.72	446	403	151	Sand loamy

Table 2 : Seaweed extractcontent from major and minor nutrients.

Element	N(%)	P_2O_5(%)	K_2O(%)	Mg(ppm)	Fe(ppm)	Mn(ppm)	Zn(ppm)	Cu(ppm)
Concentration	4	4	4	32	30	31	17.5	12.6

Table 3 : Chromatographic separation conditions for some active substances in the sepals using (HPLC).

Type (C-18), regeneration (250 * 4.6 mm Id), syringe size 5µm	Colum
70% CH3OH:30% Tetra Hydro Furan(THF)	M.Phase
1 ml/min	F.Rate
Uv-vis at 338 nm	Detector
20 µm	Volume of Injection

Table 4 : The retention time and the area of some antioxidants in the sepals of the Rosella.

Area	Retention time	Subject	Seq
8297245	5.458	Vitamin C	1
3980061	3.588	Anthocyanin	2
39817950	4.626	Hibiscetine	3
1965411	6.035	Gossypetine	4
31312350	7.007	Delphinidin-3-glucoside	5
8035000	3.846	Quercetin	6
1795016	6.329	Kaempferol	7

acid in three levels (0, 25 and 50 mg.lit^{-1}) its symbol (S0, S1, S2) respectively and The third factor is seaweed extract three levels (0, 7.5 and 15 ml. lit^{-1}) its symbol (A0, A1, A2) respectively. The least significant difference was measured under the 5% probability level using the statistical program Genstat (Al-Rawi and Khalafallah, 1980).

Active substances (vitamin C, Anthocyanin, Gossypetine, Hibiscetine, Delphinidin-3-glucoside, Quercetin and Kaempferol) were extracted according to the method mentioned in Obouyeba *et al*. (2014) and measured the content of the physical leaves of the active substances through the duration of their retention by a device (HPLC) High Performance Liquid Chromatography (Suarez *et al*., 2005). The concentration of the separated materials in the sample was calculated by the following equation:

$$\text{Concentration of sample } \mu g/ml = \frac{\text{Area of sample}}{\text{Area of standard}} \times \text{conc. of standard} \times \text{dilution factor}$$

Results and Discussion

Hibiscetine

Table 5 shows there is no significant differences in the experimental factors and their interactions in Hibiscetine concentration in the sepals.

Quercetin

Table 6 shows that the concentration of Quercetin in sepals differed according to the cultivars. V1 plants gave the highest averageof 0.343 mg.L^{-1} while the V2 plants gave an average of 0.245 mg.L^{-1}. The different cultivarsin the concentration of Quercetin may be due to genetic differences that distinguish the red cultivarfrom the other two cultivars and this is consistent with Majeed and Ali (2011). The concentration of Quercetin in the sepals was significantly affected by salicylic acid spraying. The treatment S2 gave the highest average (0.404 mg.L^{-1}), while the control treatment S0 gave an average of 0.187 mg.L^{-1}. The increase in the role of acid may be due to the increased efficiency of photosynthesis to form many compounds such as sugars, amino acids and secondary metabolites. The effect of seaweed extract with treatment A2 was significantly excelled by giving it the highest average of Quercetin concentration in the sepals of 0.507 mg.L^{-1}, while an average of 0.090 mg.L^{-1} was recorded with the control treatment A0 of the experiment sites in sequence. The result of the spraying of the extract may be due to the increase in the efficiency of the plant in carbon representation, which is accompanied by an increase in the production of secondary compounds in the sepals. The interaction of the cultivar with salicylic acid had a significant effect, the interaction treatment V1S2 gave the highest average (0.488 mg.L^{-1}), while the average was 0.160 mg.L^{-1} was given by the interaction treatment V2S0. A significant differences were found between the cultivar and seaweed extract. V1A2 gave the highest concentration of quercetin in sepals of 0.624 mg.L^{-1}. While the interaction treatment V2A0 gave the lowest average (0.078 mg.L^{-1}). Salicylic acid and seaweed extract have been shown to have a significant effect. The interaction treatment S2A2 gave the highest concentration of quercetin in sepals 0.657 mg.L^{-1}, while the control treatment S0A0 gave An average (0.035 mg.L^{-}

Table 5 : Effect of salicylic acid and seaweed extract and their interactions in Hibiscetine concentrate (mg.L^{-1}) in the l sepals of several cultivars of Roselle.

Varieties	Salicylic (mg.lit^{-1})	Seaweed extract (ml.lit^{-1})			Mean (V×S)
		A0	A1	A2	
V1	S0	0.135	0.219	0.260	0.204
	S1	0.181	0.340	0.353	0.291
	S2	0.188	0.355	0.380	0.307
V2	S0	0.125	0.208	0.241	0.191
	S1	0.160	0.313	0.353	0.275
	S2	0.167	0.344	0.359	0.290
V3	S0	0.130	0.213	0.258	0.200
	S1	0.173	0.336	0.354	0.287
	S2	0.176	0.349	0.377	0.300
L. S. D$_{(0.05)}$		n.s			n.s
Seaweed / Varieties		A0	A1	A2	Mean (V)
V1		0.168	0.304	0.331	0.267
V2		0.150	0.288	0.317	0.252
V3		0.159	0.299	0.329	0.262
L. S. D$_{(0.05)}$		n.s			n.s
Seaweed / Salicylic		A0	A1	A2	Mean (S)
S0		0.130	0.213	0.253	0.198
S1		0.171	0.329	0.353	0.284
S2		0.177	0.349	0.372	0.298
L. S. D$_{(0.05)}$		n.s			n.s
Mean A		0.159	0.297	0.326	
L. S. D$_{(0.05)}$		n.s			

Table 6 : Effect of salicylic acid and seaweed extract and their interactions in Quercetin concentrate (mg.L^{-1}) in the sepals of several cultivarsof Roselle.

Varieties	Salicylic (mg.lit^{-1})	Seaweed extract (ml.lit^{-1})			Mean (V×S)
		A0	A1	A2	
V1	S0	0.053	0.210	0.407	0.223
	S1	0.080	0.239	0.621	0.314
	S2	0.179	0.446	0.841	0.488
V2	S0	0.021	0.161	0.300	0.160
	S1	0.052	0.211	0.441	0.234
	S2	0.163	0.321	0.533	0.339
V3	S0	0.033	0.176	0.332	0.180
	S1	0.061	0.226	0.490	0.259
	S2	0.171	0.391	0.597	0.386
L. S. D $_{(0.05)}$		0.01677			0.00968
Seaweed / Varieties		A0	A1	A2	Mean (V)
V1		0.104	0.301	0.624	0.343
V2		0.078	0.231	0.424	0.245
V3		0.088	0.264	0.473	0.275
L. S. D $_{(0.05)}$		0.00968			0.00559
Seaweed / Salicylic		A0	A1	A2	Mean (S)
S0		0.035	0.182	0.346	0.187
S1		0.064	0.225	0.518	0.269
S2		0.071	0.386	0.657	0.404
L. S. D $_{(0.05)}$		0.00968			0.00559
Mean A		0.090	0.264	0.507	
L. S. D $_{(0.05)}$		0.00559			

Table 7 : Effect of salicylic acid and seaweed extract and their interactions in Kaempferol concentrate (mg.L^{-1}) in the sepals of several cultivarsof Roselle.

Varieties	Salicylic (mg.lit^{-1})	Seaweed extract (ml.lit^{-1})			Mean (V×S)
		A0	A1	A2	
V1	S0	0.058	0.168	0.176	0.134
	S1	0.121	0.204	0.348	0.224
	S2	0.130	0.342	0.479	0.317
V2	S0	0.025	0.130	0.136	0.097
	S1	0.109	0.215	0.335	0.219
	S2	0.121	0.286	0.351	0.252
V3	S0	0.042	0.157	0.172	0.123
	S1	0.119	0.271	0.341	0.243
	S2	0.127	0.275	0.401	0.265
L. S. D $_{(0.05)}$		n.s			n.s
Seaweed / Varieties		A0	A1	A2	Mean (V)
V1		0.103	0.238	0.334	0.225
V2		0.085	0.210	0.274	0.189
V3		0.096	0.234	0.304	0.221
L. S. D $_{(0.05)}$		n.s			n.s
Seaweed / Salicylic		A0	A1	A2	Mean (S)
S0		0.041	0.151	0.161	0.118
S1		0.116	0.230	0.341	0.229
S2		0.126	0.301	0.410	0.279
L. S. D $_{(0.05)}$		n.s			n.s
Mean A		0.094	0.227	0.304	
L. S. D $_{(0.05)}$		n.s			

^{1}). Tri-interaction among experimental factors had a significant on this trait. V1S2A2 gave the highest average 0.841 mg.L^{-1}, while the lowest average was 0.021 mg.L^{-1} for the control treatment V2S0A0.

Kaempferol

Table 7 shows there is no significant differences in the experimental factors and their interactions in Kaempferol concentration in the sepals.

Sabdaretine

Table 8 shows there is no significant differences in the experimental factors and their interactions in Sabdaretine concentration in the sepals.

Vitamin C

Table 9 shows there is no significant differences in the experimental factors and their interactions in vitamin C concentration in the sepals.

Anthocyanin

Table 10 shows that the anthocyanin concentration in sepals differ according to different cultivars, treatment V1 gave an average of 0.498 mg.L^{-1} while the lowest average was 0.179 mg.L^{-1} for treatment V2. The differences in the concentration of anthocyanin may be due to the difference in genetic factors between cultivars. Salicylic acid spraying was significantly increased. Plants

Table 8 : Effect of salicylic acid and seaweed extract and their Interactions in Sabdaretine concentrate (mg / l^{-1}) in the sepals of several cultivarsof Roselle.

Varieties	Salicylic ($mg.lit^{-1}$)	Seaweed extract ($ml.lit^{-1}$)			Mean (V×S)
		A0	A1	A2	
V1	S0	0.133	0.168	0.191	0.164
	S1	0.141	0.187	0.259	0.195
	S2	0.149	0.235	0.293	0.225
V2	S0	0.118	0.159	0.171	0.149
	S1	0.129	0.164	0.197	0.163
	S2	0.136	0.186	0.208	0.176
V3	S0	0.128	0.161	0.174	0.154
	S1	0.136	0.180	0.219	0.178
	S2	0.142	0.202	0.269	0.204
L. S. $D_{(0.05)}$		n.s			n.s
Seaweed / Varieties		A0	A1	A2	Mean (V)
V1		0.141	0.196	0.247	0.195
V2		0.127	0.169	0.192	0.163
V3		0.135	0.181	0.220	0.179
L. S. $D_{(0.05)}$		n.s			n.s
Seaweed / Salicylic		A0	A1	A2	Mean (S)
S0		0.126	0.162	0.178	0.155
S1		0.135	0.177	0.225	0.179
S2		0.142	0.207	0.256	0.202
L. S. $D_{(0.05)}$		n.s			n.s
Mean A		0.134	0.182	0.220	
L. S. $D_{(0.05)}$		n.s.			

Table 9 : Effect of salicylic acid and seaweed extract and their interactions in vitamin C concentrate (mg/l^{-1}) in the sepals of several cultivars of Roselle.

Varieties	Salicylic ($mg.lit^{-1}$)	Seaweed extract ($ml.lit^{-1}$)			Mean (V×S)
		A0	A1	A2	
V1	S0	2.990	9.490	10.090	7.523
	S1	5.650	11.610	19.346	12.202
	S2	8.970	13.920	25.110	16.000
V2	S0	1.010	3.930	6.080	3.673
	S1	3.120	8.600	13.820	8.513
	S2	4.220	10.870	18.250	11.113
V3	S0	1.810	7.110	7.450	5.456
	S1	3.990	9.880	15.920	9.930
	S2	6.480	12.510	21.690	13.560
L. S. $D_{(0.05)}$		n.s			n.s
Seaweed / Varieties		A0	A1	A2	Mean (V)
V1		5.870	11.673	18.182	11.908
V2		2.783	7.800	12.716	7.766
V3		4.093	9.833	15.020	9.648
L. S. $D_{(0.05)}$		n.s			n.s
Seaweed / Salicylic		A0	A1	A2	Mean (S)
S0		1.936	6.843	7.873	5.551
S1		4.2533	10.030	16.362	10.215
S2		6.556	12.433	21.683	13.557
L. S. $D_{(0.05)}$		n.s			n.s
Mean A		4.248	9.768	15.306	
L. S. $D_{(0.05)}$		n.s			

that sprayed with concentration S2 were significantly excelled by giving it the highest average of 0.383 $mg.L^{-1}$, whereas the S0 treatment gave an average of 0.264 $mg.L^{-1}$. The increase in anthocyanin due to using of salicylic acid may be due to its effect on the activity of physiological activities such as absorption and the process of building carbohydrates and proteins and thus increase the activity of enzymes and the composition of many compounds such as sugars and amino acids and fats while stimulating the transfer of nutrients from the source (leaves) and thus increase leaves content from the active substances, this result was confirmed by Najafi *et al.* (2014). The seaweed extract showed a significant increase in anthocyanin concentration of 0.430 $mg.L^{-1}$ for plants sprayed with high concentration A2, while non-sprayed plants (A0) showed an average of 0.230 $mg.L^{-1}$. This increase can be attributed to the role of foliar fertilization in increasing the vital activities in the plant, as well as its effect on the energy-rich compounds needed by the plant to build its various compounds and increase the efficiency of photosynthesis process, which is reflected in the increase in the production of carbohydrates and sugars that contribute to the activation of multiple activities and thus increase the concentration of secondary compounds produced by the plant, including leaves content of Anthocyanin. This is consistent with

Table 10 : Effect of salicylic acid and seaweed extract and their interactions in anthocyanin concentrate (mg.L^{-1}) in the sepals of several cultivarsof Roselle.

Varieties	Salicylic (mg.lit^{-1})	Seaweed extract (ml.lit^{-1})			Mean (V×S)
		A0	A1	A2	
V1	S0	0.331	0.465	0.476	0.424
	S1	0.348	0.477	0.672	0.499
	S2	0.360	0.591	0.768	0.573
V2	S0	0.103	0.134	0.149	0.128
	S1	0.117	0.156	0.281	0.184
	S2	0.127	0.241	0.304	0.224
V3	S0	0.219	0.248	0.251	0.228
	S1	0.228	0.280	0.447	0.318
	S2	0.237	0.350	0.475	0.354
L. S. D $_{(0.05)}$		0.004			0.002
Seaweed / Varieties		A0	A1	A2	Mean (V)
V1		0.346	0.511	0.638	0.498
V2		0.115	0.177	0.244	0.179
V3		0.228	0.292	0.391	0.304
L. S. D $_{(0.05)}$		0.002			0.001
Seaweed / Salicylic		A0	A1	A2	Mean (S)
S0		0.217	0.282	0.292	0.264
S1		0.231	0.304	0.466	0.334
S2		0.241	0.394	0.515	0.383
L. S. D $_{(0.05)}$		0.002			0.001
Mean A		0.230	0.327	0.424	
L. S. D $_{(0.05)}$		0.001			

Table 11 : Effect of salicylic acid and seaweed extract and their interactions in Gossypetine concentrate (mg.L^{-1}) in the sepals of several cultivarsof Roselle.

Varieties	Salicylic (mg.lit^{-1})	Seaweed extract (ml.lit^{-1})			Mean (V×S)
		A0	A1	A2	
V1	S0	0.335	0.857	0.971	0.721
	S1	0.503	1.068	1.098	0.889
	S2	0.729	1.033	1.129	0.963
V2	S0	0.233	0.839	0.897	0.656
	S1	0.486	1.044	1.063	0.864
	S2	0.694	1.011	1.116	0.940
V3	S0	0.329	0.852	0.955	0.712
	S1	0.497	1.057	1.079	0.877
	S2	0.723	1.023	1.121	0.955
L. S. D $_{(0.05)}$		n.s			n.s
Seaweed / Varieties		A0	A1	A2	Mean (V)
V1		0.522	0.986	1.066	0.858
V2		0.471	0.964	1.025	0.820
V3		0.516	0.977	1.051	0.848
L. S. D $_{(0.05)}$		n.s			n.s
Seaweed / Salicylic		A0	A1	A2	Mean (S)
S0		0.299	0.849	0.941	0.696
S1		0.495	1.056	1.080	0.877
S2		0.715	1.022	1.122	0.953
L. S. D $_{(0.05)}$		n.s			n.s
Mean A		0.503	0.976	1.047	
L. S. D $_{(0.05)}$		n.s			

Taiz and Ziger (2006), Mounir *et al*. (2015). Interactions between cultivar and Salicylic acid showed significant differences in Anthocyanin concentration, V1S2 treatment gave the highest average (0.573 mg.L^{-1}), while V2S0 treatment gave the lowest average (0.128 mg.L^{-1}). As a result of the interaction of cultivars with seaweed extract, the V1A2 interaction treatment were significantly on the other interaction treatments. The highest concentrations of Anthocyanin were given in the sepals (0.638 mg.L^{-1}), while the interaction treatment V2A0 gave the lowest average (0.115 mg.L^{-1}). The effect of salicylic acid and seaweed extract is significantly excelled in this trait. S2A2 treatment was excelled by giving it the highest average (0.515 mg.L^{-1}), while the S0A0 treatment gave an average (0.217 mg.L^{-1}). Tri-interaction of the study factors showed significant differences between the interactions. The V1S2A2 interaction treatment were excelled on the other interaction treatments by giving it the highest concentration of anthocyanin 0.768 mg.L^{-1} while the concentration decreased to a lowest value of 0.103 mg.L^{-1} to the V2S0A0 control treatment.

Gossypetine

Table 11 shows that there is no significant differences in the experimental factors and their interactions in Gossypetine concentration in the sepals.

Table 12 : Effect of salicylic acid and seaweed extract and their interactions in Delphinidin-3- glucosid concentrate ($mg.L^{-1}$) in the sepals of several cultivarsof Roselle.

Varieties	Salicylic ($mg.lit^{-1}$)	Seaweed extract ($ml.lit^{-1}$)			Mean (V×S)
		A0	A1	A2	
V1	S0	0.091	0.101	0.105	0.099
	S1	0.094	0.111	0.125	0.110
	S2	0.099	0.118	0.138	0.118
V2	S0	0.076	0.098	0.103	0.092
	S1	0.083	0.104	0.118	0.101
	S2	0.089	0.113	0.128	0.110
V3	S0	0.082	0.100	0.105	0.095
	S1	0.085	0.108	0.121	0.104
	S2	0.092	0.119	0.135	0.115
L. S. $D_{(0.05)}$		n.s			0.004206
Seaweed / Varieties		A0	A1	A2	Mean (V)
V1		0.094	0.110	0.122	0.108
V2		0.082	0.105	0.116	0.101
V3		0.086	0.109	0.120	0.105
L. S. $D_{(0.05)}$		0.004206			0.002428
Seaweed / Salicylic		A0	A1	A2	Mean (S)
S0		0.085	0.099	0.104	0.096
S1		0.087	0.107	0.121	0.105
S2		0.093	0.116	0.133	0.114
L. S. $D_{(0.05)}$		0.004206			0.002428
Mean A		0.088	0.108	0.119	
L. S. $D_{(0.05)}$		0.002428			

Delphinidin-3- glucosid

Table 12 shows the cultivars differences in the concentration of Delphinidin-3-glucosid in the sepals. The V1 treatment was excelled on the other cultivars by giving it the highest average (0.108 $mg.L^{-1}$). Different cultivars may be due to the genetic differences among them, which distinguish the plants of the red cultivar from the other two cultivars and this agree with Suliman *et al*. (2011). The increased in salicylic acid levels significantly led increased the concentration of Delphinidin-3 glucosid in the sepals. The effect was increased by increasing the spraying concentration to an average of 0.114 $mg.L^{-1}$ at concentration S2 and the lowest mean (0.096 $mg.L^{-1}$) S0. This may be due to the fact that salicylic acid is a plant hormone that contributes to the increased efficiency of photosynthesis to form many compounds such as sugars, amino acids, fatty acids and nuclei. These are the primary metabolites that are used as feedstock for the production of secondary metabolites (Turkyilmaz *et al*., 2005). Also, the seaweed extract, which increased the concentration of Delphinidin-3 glucosid in the sepals by increasing the concentration of the spray. The plants that sprayed with high concentration A2 gave the highest averages of 0.119 $mg.L^{-1}$. while the A0 treatment gave lowest average was (0.088 $mg.L^{-1}$). The interaction between the cultivars and seaweed extract had a significant effect on the increased concentration of Delphinidin-3-glucosid. The interaction treatment V1A2 gave the highest average of 0.122 $mg.L^{-1}$, while V2A0 treatment gave the lowest average (0.082 $mg.L^{-1}$). The results of the table showed that the increase in salicylic acid spray concentrations and seaweed extracts significantly increased the concentration of Delphinidin-3 glucosid in the sepals. The S2A2 treatment gave the highest average of 0.133 $mg.L^{-1}$ compared to control treatment S0A0, which gave an average of 0.085 $mg.L^{-1}$.

Conclusion

Through the obtained results, we can conclude that the red cultivar in the concentration of most medical materials effective in the sepals, spraying salicylic acid and high concentrations of seaweed extract gave the best results of the studied traits.

References

Ajithadoss, K., T. Pandian, S. Rathinkumar, R. Edwin, T. Sekar, P. Sakar and S. Munusamy (2006). *Botany Higher Secondary Second Year*. 1st Edition. Government of Tamil Nadu Textbook Corporation College Road, Chennai.

Al-Rawi, K. M. and A. A. M. Khalafallah (1980). Design and analysis of agricultural experiments (University of Dar Al Kutub for printing and publishing.

Amin, A. A., A. A. A. El-Kader, M. A. F. Shalaby, F. A. E. Gharib, E. S. M. Rashad and J. A. T. da Silva (2011). Physiological effects of salicylic acid and thiourea on growth and productivity of maize plants in sandy soil. *Commun. Soil Sci. Plant Anal*., **44** : 1141–1155.

Da-Costa-Rocha, B. Bonnlaender, H. Sievers, I. Pischel and M. Heinrich (2014). *Hibiscus sabdariffa* L.–A phytochemical and pharmacological review. *Food Chem*., **165** : 424-443.

Davies, P. J. (2004). The plant hormones : their nature, occurrence and function. In : Davies, P. J. (Ed.), Plant Hormones : Biosynthesis, Signal Transduction, Action. 3rd ed. Kluwer Academic Publishers, Dordrecht, pp. 1 – 15.

Dhriti, B., M. Z. Babgohari, P. Rathor and P. Balakrishnan (2015). Seaweed extracts as biostimulants in horticulture. *Jour. Sci. Hort*., **9(1)** : 1 – 9. Canada.

Jensen, E. (2004). Seaweed ; Fact or Fancy. From the Organic Broadcaster, Published by mosses the Midwest Organic and Sustainable Education . From the *Broadcaster*, **12(3)** : 164 - 170.

Lin, T., H. Lin and C. Chen (2007). *Hibiscus sabdariffa* L. extract reduces serum cholesterol in men and women. *Nutr Res*., **27** : 140 – 145. Taiwan.

Majeed, K. A. and A. S. Ali (2011). Effect of foliar application of NPK on Some growth characters of two cultivars of Roselle (*Hibiscus sabdariffa* L.). *Amer J. Plan. Physic*., **6(4)** : 220 – 227.

Mounir, M., H. Chernane, S. Latique, A. Benaliat, D. Hsissou and E. Mimou (2015). Effect of seaweed extract (*Ulva rigida*) on the water deficit tolerance of *Salvia officinalis* L. *Jour. Apll. Phycol*. Cadi Ayyad University, Springer. **671(1)** : 1 – 9. Morocco.

Najafi, S., R. Jamei and N. Farnad (2014). Effect of silver nanoparticles and magnetic field on the yield and chemical composition of (*Triticum aestivum* L.) seedlings. *Bulletin of Environment, Pharma. Life Sci*., **3 (2)** : 263 – 268.

Obouayeba Abba Pacomel and DjyhNazaireBernardl (2014). Phytochemical and antioxidant activity of Roselle *Hibiscus Sabdariffa* L. petal extracts. *Reseach Journal of Pharmaceutical Biological and Chemical Sciences*, **4(5)** : 1454.

Suárez, B., N. Palacios, N. Fraga and R. Rodríguez (2005). Liquid chromatographic method for quantifying polyphenols in ciders by direct injection. *Journal of Chromatography A*, **1066(1-2)** : 105-110.

Suliman, A. M., O. A. Ali, E. A. A. Idriss - Sharaf and M. A. Abdualrahman (2011). A comparative study on red and white karkade (*Hibiscus sabdariffa* L.) calyces, extracts and their products. *Pakistan Journal of Nutrition*, **10(7)** : 680 - 683. Sudan.

Taiz, L. and E. Ziger (2006). *Plant Physiology*. 4rd Sinecure Associates, Inc. Published Sunderland, Massachusetts.

Turkyilmaz, B., L. Y. Aktas and A. Guven (2005). Salicylic acid induced some biochemical and physiological changes in *Phaseolus vulgaris* L. *Science and Engineering. J. of Al-Firat Univ*., **17(2)** : 319 – 326.