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Effects of Salinity on Some Compounds in
***Portulaca oleracea* L. and It`s Inhibitory Effect on**
Some Microorganisms

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By

Durgham Khaled Nasser Taj Al-Deen

B.Sc. general biology/College of Science

University of Al-Muthanna

Supervised by

Assist. Pro. Dr. Batool Mohammad Hassan

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سورة الانعام

وَهُوَ الَّذِي أَنْزَلَ مِنَ السَّمَاءِ مَاءً فَأَخْرَجْنَا بِهِ نَبَاتَ كُلِّ
شَيْءٍ فَأَخْرَجْنَا مِنْهُ خَضِرًا نُخْرِجُ مِنْهُ حَبًّا مُتَرَاكِبًا

صدق الله العلي العظيم

سورة الانعام آية (99)

Dedication

**I Dedicate This Modest Effort
For my master, my Imam and my leader**

Al-Imam Al-Mahdi

**Closer to you ... I hope for acceptance and
Mercy**

Researcher

Acknowledgment

Praise be to God for what He has bestowed upon Him, and to Him is gratitude for what He inspired... A great thank you that never ceases and his permanent and complete blessings be upon his Noble Messenger, Muhammad, and his good and pure family.

I begin by thanking God, His Messenger and the family of the Messenger for success.

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Researcher

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List of Abbreviations

No.	abbreviation	The meaning
1	O.D	Light intensity
2	GAE	Gallic acid equivalent
3	SPSS	Statistics is a powerful statistical software platform
4	HF	Hydrofluoric acid
5	GPT	Glutamate pyruvate transaminase
6	GOT	Glutamate oxaloacetate transaminase
7	MeOH	Methanol
8	EPA	U.S. Environmental Protection Agency
9	JNK	C-Jun N-terminal kinase
10	MAPK	Mitogen-activated protein kinase
11	hela	is an immortal cell line used in scientific research It is the oldest and most commonly used human cell line
12	HepG2	is a liver hepatocellular carcinoma from a 15 year old Caucasian male.
13	PPAR	Peroxisome proliferator-activated receptor
14	PERK	Protein kinase-like endoplasmic reticulum kinase
15	TNF- α -	Tumor necrosis factor alpha
16	HUVECs	Human umbilical vein endothelial cell
17	MCP	Monocyte chemo attractant protein
18	FkF1	Photoreceptor
19	BCG	Bromocresol Green

Summary

Summary

Summary

The current study aimed to find out the effect of salinity level on the content of *Portulaca olearacea L* of substances of nutritional and medical importance. The seeds of the plant were planted in plant pots and then irrigated with tap water. After 10 days, those plants were divided into four groups to be watered according to the needs of the plant. The plant was watered after dividing it into four flocks for 30 days from saline solutions whose concentration is (0,5,10,20 dsm^{-1}). until the plant reached the beginning of flowering stage, and at the end of that period, total chlorophyll, chlorophyll a,b, carotenoids, total protein, total sugars and antioxidants were measured. Proline, fatty acids, vitamin C, vitamin A, alkaloids and flavonoids.

The results showed that the values of total chlorophyll was (2.35 mg/g.f.w) in control and (1.37 mg/g.f.w) in (20 dsm^{-1}), chlorophyll a, b ranged between (1.39-0.83) and (0.96-0.54) (mg/g.f.w), respectively, proline recorded the highest concentration in the group irrigated with concentration (20 dsm^{-1}) and it was (2.12), vitamin C recorded the highest value (96.07) (mg/g.F.W) in the control group while it decreased in both (10 dsm^{-1}) and (20 dsm^{-1}) with the increase in salinity concentration, total soluble sugars was recorded highest value (127) in at control, less value was (67.53) $\mu\text{g/g.F.W}$ when the concentration (20 dsm^{-1}), Also, examinations showed the highest value total Protein was (31.29) in control, less value was (3.69) $\mu\text{g/g.F.W}$ in (10 dsm^{-1}), and the highest value of Proline was (2.12) in (20 dsm^{-1}), less value is (0.47) $\mu\text{mole/g.F.W}$ when the concentration (0 dsm^{-1}), Carotenoids was the highest value (1.24) control, less value is (0.39) (mg/g.F.W) in (5 dsm^{-1}), and the total anti-oxidant highest value was (16.27) in (20 dsm^{-1}), less value was (5.13) (mg AEE/g.F.W) when the concentration (5 dsm^{-1}).

Summary

The results of secondary metabolic compounds assays showed that the highest value of Vitamin A (883.73 IU) at control , the lowest value was (328.4 IU) at (20 dsm^{-1}), the highest value of total flavonoid was (0.84) (mg Rutin / gm) at (5 dsm^{-1}) and the lowest value was (0.27) at (20 dsm^{-1}), also the highest value of aotal alkaloids is (3.48%) at (5 dsm^{-1}) and the minimum value is (2.05%) at (20 dsm^{-1})

P. olearacea L content of essential fatty acids (a- linolenic acid, Ecosopentaenoic , Docosahexaenoic , Linolic acid , Palmatic acid , Oleic acid , Stearic acid) that from Omega-3 , Among this fatty acids , Linolic acid recorded a decrease In concretion with an Increase In the salinity using , In general, a significant decrease was recorded in all the fatty acids that were examined with an increase in salt concentration

When studying the effectiveness of the *P. olearacea L* plant as an inhibitor on some microorganisms, it differed according to the salt concentration to which the plant was exposed, as well as the type and concentration of the extract. Two concentrations were used (1000 mg/ml) and (600 mg/ml). The results of the study showed that the highest effect of the plant was When using the hot alcoholic extract, the percentage of inhibition on *Staph aureus* was (16.33 mm), and the same extract recorded a lower inhibition value on *E. Coli* bacteria, as the inhibition was (8.5 mm) and less on *C.albicans* yeast. (5.5 mm) in plants irrigated with the control group and at a concentration of (1000 mg/ml), while the effect of the cold aqueous extract was less effective on microorganisms compared to the hot alcohol and showed significant and clear differences at both concentrations and exposure to saline concentrations, according to what was recorded in the tables.

CHAPTER ONE

General Introduction

1.1 Introduction

In any component of the environment (natural or man-made environment) capable of having a direct effect on living organisms (Gilpin, 1996).

Ecology is the term which refers to study of the distribution and abundance of living organisms resulting from biotic and abiotic factors in nature. A plant's growth and success depend on the presence of many abiotic or non-living environmental factors. The relative importance of these factors and their interaction varies according to global and local scale, as well as according to a specific species. The long-run average distribution of relative abundance may reflect a dynamic equilibrium dependent on appropriate specialization of the constituent species.(Crowley 1997).

Salinity is the most serious abiotic factor of abiotic stresses and is one of the major environmental constraints limiting agricultural productivity and influencing the concentration of bioactive compounds of vegetables (Tang *et al* ,2020) .

Salinity is a major problem affecting crop production all over the world: 20% of cultivated land in the world, and 33% of irrigated land, are salt-affected and degraded. This process can be accentuated by climate change, excessive use of groundwater (mainly if close to the sea), increasing use of low-quality water in irrigation, and massive introduction of irrigation associated with intensive farming. Excessive soil salinity reduces the productivity of many agricultural crops, including most vegetables, which are particularly sensitive throughout the ontogeny of the plant. The salinity threshold (ECt) of the majority of vegetable crops is low (ranging from 1 to 2.5 dsm^{-1} in saturated soil extracts) (Almeida and Serralheiro, 2017)

Each species requires a specific nutrient content and chemical composition to best growth. The combination of these factors determines the

essential position of the organism, which is defined as the set of conditions and resources in which members of a species can persist Ricklefs (2001).

The boundaries of a fundamental niche are determined by the physiological tolerance of abiotic factors; Therefore, it is found that the abiotic factors are the determining factors for the growth of some plant species Karowe (2003). Thus, a plant's distribution and relative abundance within its environment is determined by a combination of abiotic factors, some of which are more important than others.

Salts affect plant growth due to increasing soil osmotic pressure and to interference with plant nutrition. a high salt concentration in soil solution reduces the ability of plants to acquire water, which is referred to as the osmotic or water-deficit effect of salinity. Damage occurs when the concentration is high enough to begin reducing crop growth. The osmotic effect of salinity induces metabolic changes in the plant identical to those caused by water stress-induced “wilting” (Munns *et al.*, 2002) and shows few genotype differences (Lauchli and Grattan, 2007). Moreover, salt stress reduces plant growth due to specific-ion toxicities and nutritional imbalances (Läuchli and Epstein ,1990)

Soil salinity affects many aspects of plant physiology, as it reduces its ability to absorb water as a primary effect, leading to a decrease in growth rate and the induction of many metabolic changes. (Sai *et al.* ,2009), the overall vegetative growth and the formation of flowers and fruits (Sairam and Tyagi, 2004), and The most obvious metabolic changes include both the regulation of ions and the induction of the construction of osmotic-reducing substances, the induction of antioxidant enzymes, the induction of the construction of plant hormones and the transformation of the photosynthesis pathway (Parida and Das, 2005) The inhibition of plant growth when soil salinity increases is attributed to both the effect of water deficiency or the osmotic effect, and the effect of the specific ion or its increase (Green and

Munns, 1980), and The increase of sodium and chloride ions in the soil can cause both the reduction of the surface area For the developing leaves by reducing the number and dimensions of the epidermal cells, and each of them contributes to an increase in the degree of succulent for some plants as a result of the increase in the difference in the German effort between the leaves and the surrounding environment,

1.2 Aim of study

The aim of the current study is to know the potential effects of salinity factor on some active secondary compounds, secondary metabolites and fatty acids in *Portulaca oleracea L.* and their effects on some microorganisms.

CHAPTER TWO
LITERATURES
REVIEW

4. Results and Discussion

It is well known that excess minerals in the plant is called salt stress and this phenomenon a major problem that negatively affects the physiological and metabolic processes in plant life, decreasing growth and ultimately yield. This editorial highlights the harmful effect of salt stress on the nutritional value of vegetables, including *Portulaca oleracea* L. (Prasad *et al.* , 2014) .

These inhibitory effects included stress on plant growth and biomass production which are well known (Hajer *et al.*, 2006, Alqurainy 2007, Long *et al.*, 2008, Ma *et al.*, 2013). In addition, salinity leads to dehydration and a decrease in the pressure potential (Munns 2002) .The present study showed that the NaCl stress effected all the studied growth traits as explained table (4-1) .

4.1: Soil characters

Soil is a highly dynamic and complex system that is a mixture of living and dead organisms, decaying and residual organic matter, minerals, air, and water. that support life together is difficult to understand the various physical, biological and chemical processes that occur in the soil; However, understanding soil properties helps in crop development and the influence of soil on plant content and growth (Schulte *et al.*, 2005) . *Portulaca oleracea* L. grows in full sun in almost any soil, from organic-rich clay to heavy clay, and tends to grow best in drier soils (Karkanis. 2017).

The soil texture used in the study is sandy mixture, and after conducting laboratory tests on it, the proportions of its components were (sand = 53.83%, salt = 29.84, clay = 16.33%) (Table 4-1 & Figure 4-1).

Table (4-1) properties of the soil used in the study

soil texture			Organic matter g/kg ⁻¹	EC dsm ⁻¹	PH
clay	silt	sand	7.6	4.22	8
16.33%	29.84%	53.83%			
sandy mixture					

Sandy soils have less charge of ions compared to clay soils, so they are less capable of absorbing ions, and thus the accumulation of salts in this soil decreases. Also, the higher the percentage of water in sandy soils, the higher the percentage of soil washing from ions, and the electrical conductivity values increase (Sonmez,2008) Soil texture influences soil slurry electrical conductivity (EC), anion and cation concentrations, and the retention of different soil-to-water ratios (Ozcan *et al.*, 2006) .

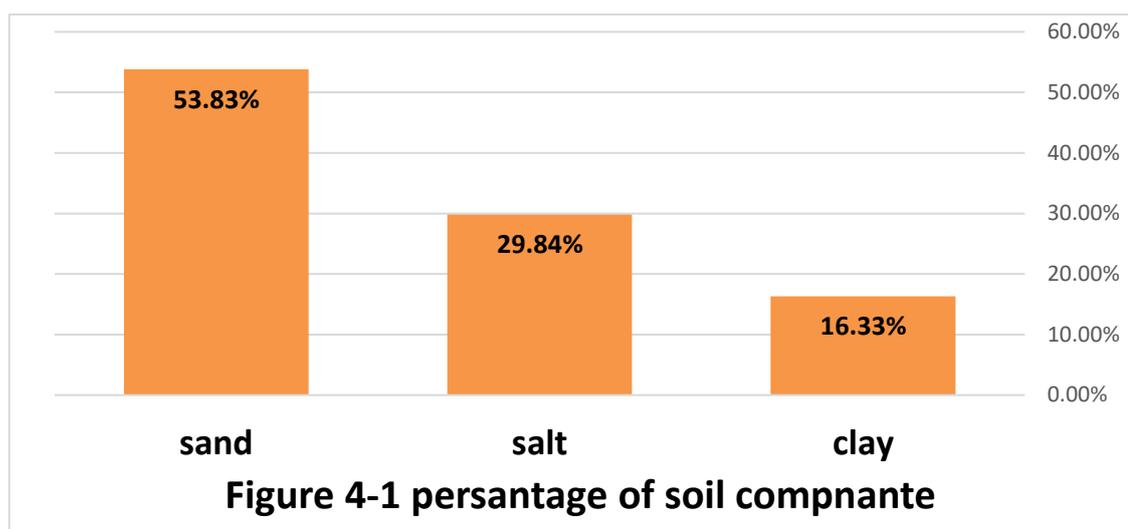
The results of laboratory analyzes of soil used in agriculture showed that it contains a high percentage of organic matter, and organic matter is a light substance with high porosity and has an important role in improving the composition of the soil and increasing its ability to conserve water in addition to its other effects on soil properties (Hagan *et al.*, 2013) ,The increase in the percentage of organic matter is accompanied by an increase in the value of the bulk density of the soil (Bonini *et al*, 2010) , As it is proven that with the increase in the bulk density, the percentage of total salts in the soil increases, as well as the percentage of exchanged sodium ions (Kadhim, 2015) , One of the causes of salinization is the poor physical and chemical properties resulting from the low content of soils of organic matter and thus turning the soil into saline, and that the more organic matter in the soil, the lower its salinity (Dweni, 2003) , This indicates that the soil used in agriculture is low in salt .

pH: In chemistry, denoting (potential of hydrogen) or (power of hydrogen) is a scale used to specify the acidity or basicity of an aqueous solution. Acidic solutions (solutions with higher concentrations of H⁺ ions) are measured to have lower pH values than basic or alkaline solutions (Jensen, 2004). The laboratory tests of the studied soil showed that the pH value is 8, and thus the soil is basic.

The addition of organic matter reduces the degree of soil reaction pH due to the role of organic matter in the production of CO₂ with water when it decomposes, forming carbonic acid and some organic acids resulting from decomposition such as humic and fulvic (Gascó and Lobo, 2007).

EC: Soil electrical conductivity, is a measure of the amount of dissolved salts in the soil (soil salinity). It affects crop yield, crop suitability, plant nutrient availability, and microorganism activity. Although EC does not provide a direct measurement of specific ions or salt compounds, it has been linked to nitrate, potassium, and sodium chloride concentrations, sulfates and ammonia. For some non-saline soils, determination of EC can be a convenient and economical way to estimate the amount of nitrogen (N) available for plant growth (Shakir, 2011).

the organic matter leads to the chemical properties of the soil, and the addition of the organic matter to the soil led to a decrease in the electrical conductivity (EC). The reason for the decrease is attributed to the fact that the organic matter contributes to increasing the water permeability in the soil, which leads to the washing of salts from the surface to depth (Amir, *et al.*, 2017). Organic matter increases the surface area of the soil by isolating soil particles from each other, which leads to an increase in water retention (Stock, 2008).



4.2 : Plant Biochemical responses

The table below shows us the extent of the effect of different salt concentrations compared to the control group on the plant's content of chemical response compounds and its effect on its general rates between high and low, as well as the value of LSD.

Table (4-2): Biochemical responses of *Portulaca oleracea* L. induced by treatment with NaCl

Treatments Parameters	Control (0 dsm ⁻¹)	Con1 (5 dsm ⁻¹)	Con2 (10 dsm ⁻¹)	Con3 (20 dsm ⁻¹)	LSD (0.05)
	Mean±S.D				
Total Chlorophyll (mg/g.F.W)	2.35±0.29	1.99±0.31	1.52±0.16	1.37±0.10	0.607
Chlorophyll a (mg/g.F.W)	1.39±0.16	1.13±0.18	0.97±0.33	0.83±0.10	0.326
Chlorophyll b (mg/g.F.W)	0.96±0.17	0.86±0.13	0.55±0.07	0.54±0.01	0.413
Carotenoids (mg/g.F.W)	1.24±0.22	0.39±0.12	0.55±0.14	0.40±0.03	0.230
Total Protein (mg/g.F.W)	31.29±3.88	9.07±2.35	3.69±0.73	4.43±2.03	3.820
Proline(μole/g.F.W)	0.47±0.19	0.93±0.10	0.86±0.08	2.12±0.06	0.588
Vitamin C (mg/g.F.W)	96.07±13.46	64.73±6.03	51.79±8.95	60.25±12.51	16.187
Total Antioxidant Activity (mg AEE/g.F.W)	10.50±0.76	5.13±0.62	13.49±1.13	16.27±1.31	2.078
Total Soluble Sugars (μg/g.F.W)	127.00±11.58	84.58±3.00	84.95±6.85	67.53±4.09	12.526

4.2.1 : Photosynthesis pigment

1- Total Chlorophyll

Reduction of chlorophyll contents can be as a result of either slow synthesis or fast breakdown, indicated that there was a photoprotective mechanism through reducing light absorbance by decreasing chlorophyll contents (Elsheery and Cao, 2008). The loss of chlorophyll under salt stress could be related to photoinhibition or ROS (reactive oxygen species) formation (Kato and Shimizu, 1985).

The study refers the value of total chlorophyll in the control group was 2.35 mg / g. The content of total chlorophyll decreased slightly with an increase in the concentration of NaCl salt, and noticed that there was a significant difference between other values.

The reduction in photosynthesis under salinity can also be attributed to a decrease in chlorophyll content. Salinity reduces the chlorophyll content in salt susceptible plants and increases it in salt tolerant plants salinity, level could be attributed to a reduction in leaf area expansion and hence to a lower light interception (Marcelis and Hooijdonk, 1999).

Salt stress affects chloroplast components of the main organs of plant photosynthesis, such as pigments, enzymes, proteins and membrane lipids, reduces plant photosynthesis and consequently reduces plant productivity. (Parida *et al.* 2003)

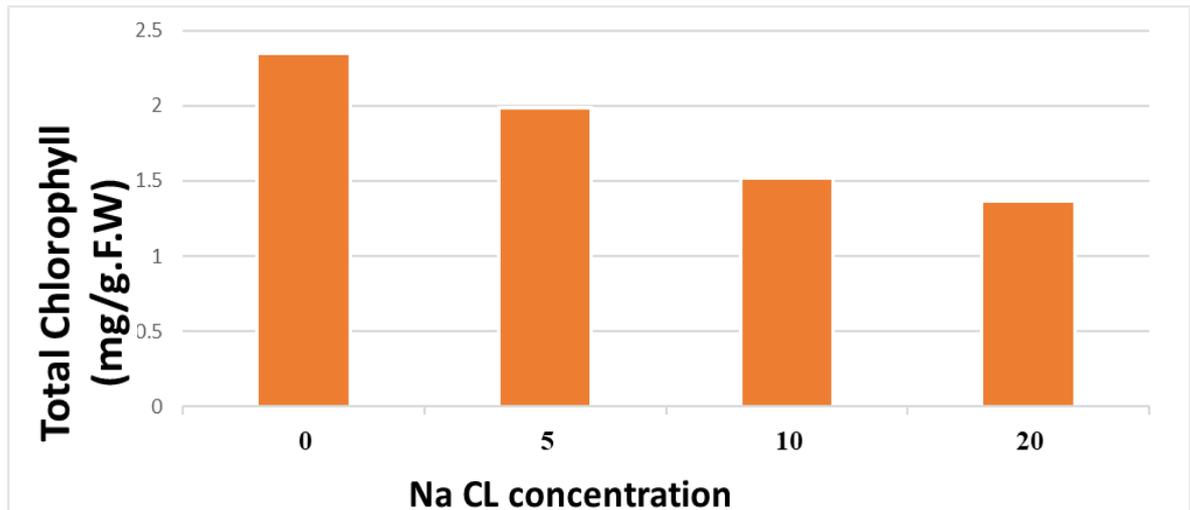


Figure (4-2) Effect of NaCl concentration on content of Total Chlorophyll (mg/g.f.w) in *P. oleracea* L

2- chlorophyll a and b

Higher plant is identified with their content of both chlorophyll a & b and chlorophyll. is used to as a bioindicator to explain the effect of environmental factor (Sitko,2017) plant growth and yield are directly related to photosynthetic performance. Chlorophyll is the main photosynthetic pigment and plays an important role in plant photosynthesis (Tang *et al.* 2020). Salt stress decreased chlorophyll synthesis and inhibited photosynthesis (Barhoumi *et al.* 2007) .

figure (4-2) explain the effect of NaCl on chlorophyll a and b of *Portulaca oleracea* L. respective. despite of purslane is a halophyte, but salinity decrease the chlorophyll a & b content, so the higher significant decrease wase in content ration.

The study refers the value of chlorophyll a and b in the control plant that was treated with distilled water was 1.39 mg / g for chlorophyll a, while the value of chlorophyll b was 0.96 mg / g. The content of chlorophyll decreased slightly with an increase in the concentration of NaCl salt, and we noticed that there was no significant difference.

Through our observation of each group that was irrigated with certain concentrations of NaCl, we noticed that with the increase of NaCl concentration in the irrigated water, this leads to a decrease in the proportions of both chlorophyll a and b generally. The maximum decrease was at 20 dsm^{-1} for NaCl, while the group irrigated with distilled water had vegetable chlorophyll a,b content with the highest value compared to the other three concentrations.

these results of chlorophyll a and chlorophyll b under salt stress which is in agreement with previous results of (Taïbi *et al.*, 2016) on *Phaseolus vulgaris L* and (Turan *et al.* 2007) and (Taffouo *et al.* 2010) on *Vigna subterranean L*.

Our results also agreed with the study (Heidari.2012), who concluded that the different levels of salinity and the interaction between salinity and different genotypes had a significant impact on the content of chlorophyll a, b and carotenoids. Also, by increase sing salinity levels, the three photosynthetic pigments are reduced.

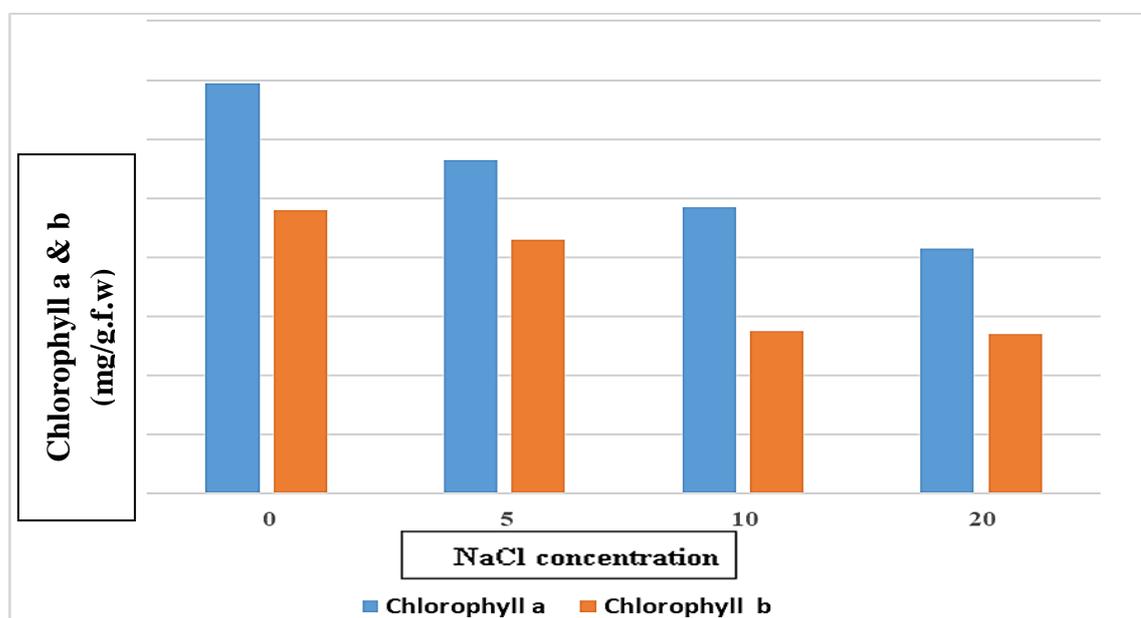


Figure (4-3) Effect of NaCl concentration on content of Chlorophyll a & b (mg/g.f.w) of *P. oleracea L*

4.2.2: Carotenoids

Carotenoids are pigments with several functions in plants, besides their direct role in photosynthesis, they are involved in the oxidative stress defence mechanisms (Gill and Tuteja, 2010) .

The data related to the carotene content are presented in (figure 4-4). The statistical analysis of the data showed that the salinity levels induced significant variator among group of the plant. Compared with the control value, the higher the salt stress on the plant, the lower the carotene value .

The most decreases wase (0.0230 mg/g) in the group concentration (20 dsm^{-1}) It found that the carotene content of the plant increased slightly in the second group compared to the first group and returned to decrease in the third group.

Similarly, to other studies, It is well known that carotenoids, being antioxidants, have the ability to detoxify plants from the effects of reactive oxygen species (Verma & Mishra, 2005). Salinity caused a decrease in carotenoid contents in all groups in which the concentration of NaCl was increased. These results are consistent with those found by (Jadallah ,1999) in *P. vulgaris* L. and (Singh *et al.* 2008) in the genetic structures of maize and wheat. Carotenoids They are known to act as light energy accumulators for photosynthesis and as dampers for trichotrophy and O_2 .

The decrease of carotenoid contents indicates that the protection by carotene was not one of the most important mechanisms under salt stress. Ali & Ismail, 2014) found that the use of 100 mm NaCl in the nutrient solution resulted in a significant decrease in tomato fruit carotenoids. In addition, salinity levels higher than 4.4 dsm^{-1} decreased carotenoid content (Pascale *et al* .,2001). This result could be due to the negative effects of saline stress on

carotenoid biosynthesis and the inhibition of lycopene accumulation (Riggi *et al* 2008)

The results of the mean values of the data showing the decrease in carotenoids under salinity stress thus it agree with the results obtained (Heidari ,2012) on *Ocimum basilicum* L .

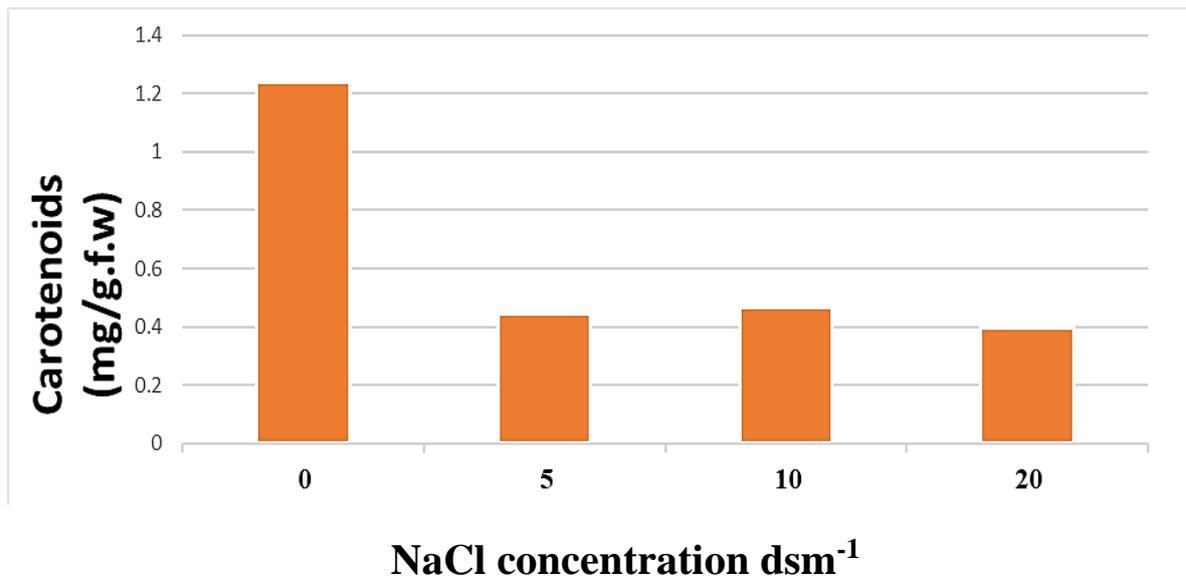


Figure (4-4) Effect of NaCl concentration on content of Carotenoids (mg/g.f.w) in *P. oleracea* L.

4.2.3: Total Proteins

Proteins are considered to be the building blocks of life and, in addition to their nutritional role, have other functions in living organisms.

All vital activities of plants are carried out through the interference and influence of proteins, and any interaction with external and internal factors depends on whether the plant has a mechanism for the production of supporting proteins or not (Musidlak , 2017) . This feature varies with different plants and their varieties in different regions. Therefore, these plant, climatic or

environmental differences that affect protein synthesis clearly indicate that the amount of some proteins, they can be an indicator of resistance or sensitivity to an external or internal factor and how resistant a plant is to environmental stresses (Hasanuzzaman, 2013).

The data related to the protein content was presented in (figure 4-5) protein content decreased significantly with the increase in the concentration of sodium chloride salt in the rest of the other three groups, and we noticed a significant difference, that it's decreased from (31.29 mg / g) in control group to (9.07 , 3.69 and 4.43 mg/g) in concentration (5 , 10 , 20 dsm⁻¹) respectively.

Also, when comparing the value of the third group, which was watered with the third concentration (10 dsm⁻¹) of NaCl salt and its value (3.69mg/g) with the fourth concentration (20 dsm⁻¹) of NaCl salt and its value (4.43 mg/g), the difference between them is (0.74 mg/g) which is lower than the LSD value of (3.820) and therefore no significant difference

Several salt-induced proteins have been identified in plant species and classified into two categories proteins, which accumulate only due to salt stress, and stress associated proteins, which also accumulate in response to heat, cold, drought, water, salinity and high and low mineral nutrients. Studies of Farghaly *et al.*, (2013), and Mansour *et al.*, (2000) revealed as salinity stress induced insignificant change in the soluble proteins of the tolerant and sensitive wheat cultivars. A higher content of soluble proteins has been observed in salt tolerant than in salt sensitive cultivars of sunflower Ashraf *et al.*, (1995), finger millet (Uma *et al.*, 1995) and rice (Pareek *et al.*, 1997)

In lentil Ashraf and Waheed , (1993) reported that leaf soluble proteins decreased due to salt stress in all lines, irrespective of their salt tolerance. (Ashraf and Harris ,2004) reported that the proteins produced under salt stress

are not always associated with salt tolerance, thus using proteins as salt tolerant indicators depending on the nature of the plant species or cultivar. The protein degradation under saline environment has been attributed to the decrease in protein synthesis, accelerated proteolysis, decrease in availability of amino acids and denaturation of enzymes involved in protein synthesis (Jaleel *et al.*, 2007 ; Lakhdar *et al.*, 2008).

It is note from the above values that the protein content in the third group returned slightly, and this indicates one of the plant's ways of resisting salinity, and this agrees with the study of (Gomathi. *et al.*, 2013) concluded that increasing protein content under salt stress involves activating transcription and translation processes for specific stress tolerance genes.

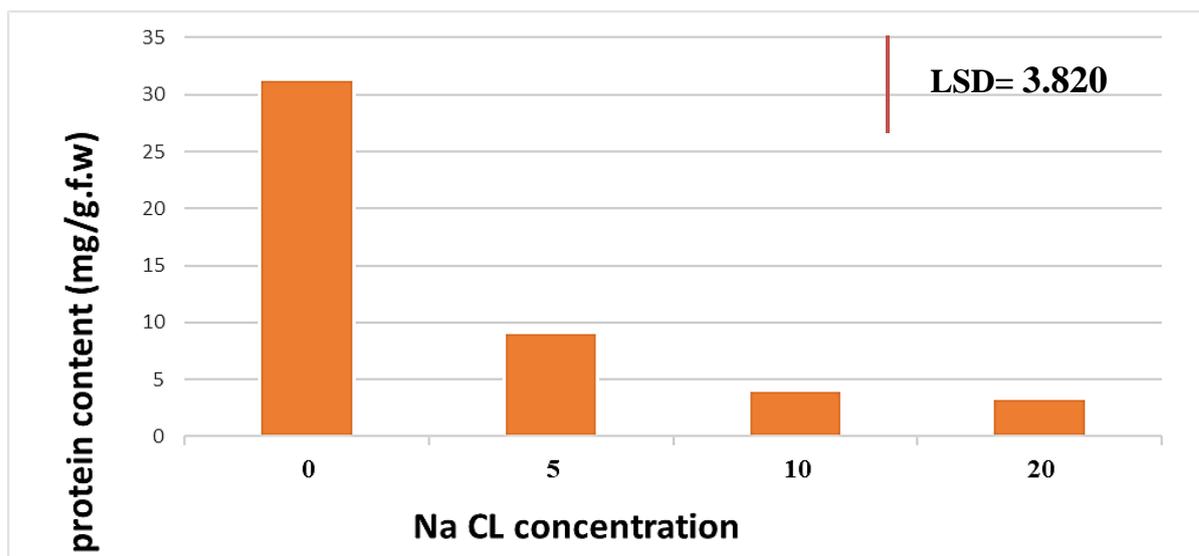


Figure (4-5) Effect of NaCl concentration on content of Protein (mg/g.f.w) in *P. oleracea* L.

4.2.4: Proline

A huge studies and data indicate a positive relationship between proline accumulation and plant stress. Proline, an amino acid, plays a very beneficial role in plants exposed to various stress conditions. In addition to being an excellent osmolality (Meena , 2019).

In response to various environmental stresses, many plant species synthesize proline in the cytosol and accumulates in the chloroplasts. Proline accumulation in plants is a well-recognized physiological reaction to osmotic stress prompted by salinity, drought and other abiotic stresses. Proline plays several protective functions such as osmoprotective, stabilizing cellular structures, enzymes, and scavenging ROS and keeps up redox balance in adverse situations. In addition, ample-studied osmoprotective capacity, proline has been also ensnared in the regulation of plant improvement, including flowering, pollen, embryo, and leaf enlargement (Meena *et al.*, 2009), Proline comprises less than 5% of the total pool of the free amino acids in plants under regular conditions (Shahbaz *et al.*, 2013).

After calculating the protein values of purslane in groups that were exposed to certain salt concentrations and comparing them with the control group, it noticed that they are clear significant differences and an increase in the value of proline with an increase in the concentration of sodium chloride, as it was noted that the value of proline in the control group is (0.47 $\mu\text{mole/g.F.W}$) while in the third group (2.12 $\mu\text{mole/gFW}$) in which the salt concentration is (20 dsm^{-1}) sodium chloride, and the difference between them is (1.65) which is greater than the LSD value which is (0.588), as there is a significant difference, as well as For the second and third group, as shown in in figure (4-6) .

Proline protects the plants from various stresses and also helps plants to recover from stress more rapidly. When applied exogenously to plants exposed to stress, Proline results in increased growth and other physiological characteristics of plants (Hayat. *et al*, 2012). The results of this study agreed with the study of Parvanehet *al.*, (2012) on *P. oleracea L*. An increase in the amount of leaf proline with an increase in sodium chloride, this increases half plant to reduce osmatic presser iv response to increased salinity (Ueda. *et al*, 2007).

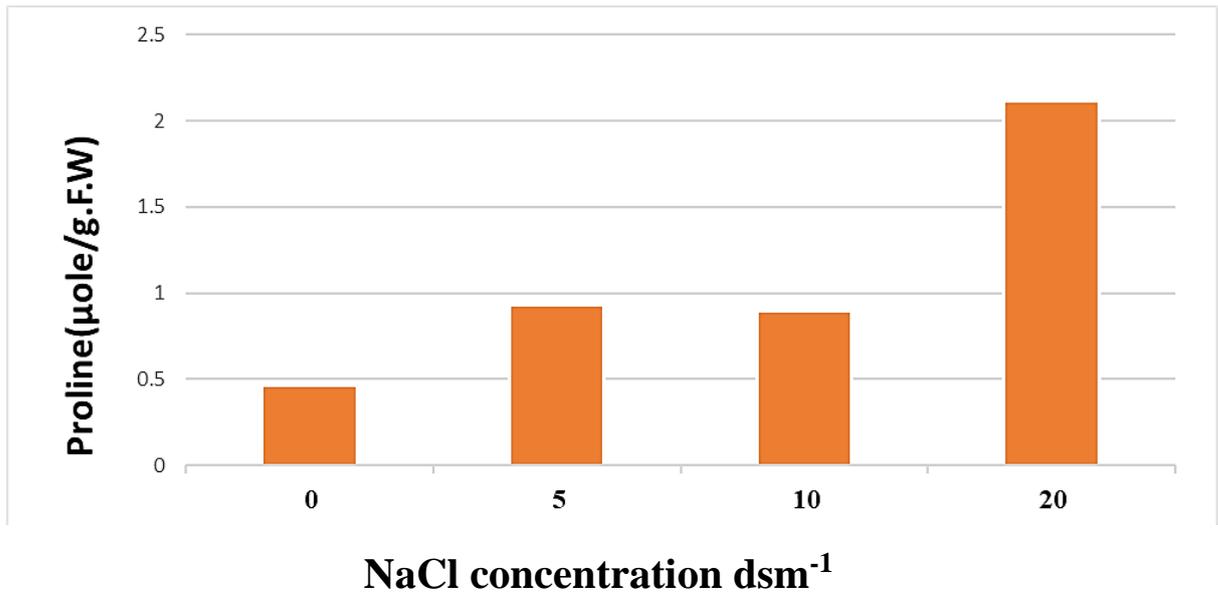


Figure (4-6) Effect of NaCl concentration on content of Proline (mg/g.f.w) in *P. oleracea* L

3.1.5 : Vitamin C

Salinity causes oxidative stress in plants by enhancing production of reactive oxygen species, so that an efficient antioxidant system, of which Vitamin C (ascorbic acid) is a key component, is an essential requirement of tolerance. Ascorbic acid (vitamin C) is an abundant component of plants. It reaches a concentration of over 20 mM in chloroplasts and occurs in all cell compartments, including the cell wall. It has proposed functions in photosynthesis as an enzyme cofactor (including synthesis of ethylene, gibberellins and anthocyanins) and in control of cell growth. Vitamin C (ascorbic acid) is very popular for its antioxidant properties (Arrigoni and De Tullio, 2002).

In the current study, the value of vitamin C decreases as the salt concentration increases, as its value recorded in the control group is (96.07 mg/gFW) while its value was (64.73, 51.79 and 60.25) mg/gFW respectively (figure 3-7) and, in the three treatment groups, we note a that high value of vitamin C in the third group, which was exposed to the highest value of sodium

chloride, and this indicates the adaptation of the plant to resist salinity stress. And through the statistical analysis, the value of LSD was (16.187) and from noting the difference between the control group and the first group, the difference between them is (64.73 mg/gFW) which means a significant.

In fact, several studies have shown that ascorbic acid plays an important role in improving plant tolerance to abiotic stress and intensifying its osmotic adaptability and growth activities (cell division and expansion) (Azzedine *et al* ,.2011). The results are also in agreement with the study conducted by Parida *et al*., (2004) who studied the effect of salt stress on the concentrations of ascorbic acid and Glutathione decreased in halophytic plants *Bruguiera parviflora*. The value of ascorbic acid during exposure to salt stress in the plant and this indicates a decrease in the levels of glutathione and ascorbic acid was due to consumption. One of these antioxidants in ascorbate is the oxidized glutathione .

Ascorbic acid (Vitamin C) plays a key role in salt tolerance of many halophytes (Jithesh *et al*. 2006), It also recycles the lipid-soluble antioxidant α -tocopherol (Lushchak and Semchuk 2012). It may also contribute to maintaining photosynthesis, cell-cycle progression, cell wall expansion, gene expression, synthesis of many hormones, anthocyanin and flavonoids (Smirnoff & Wheeler 2000).

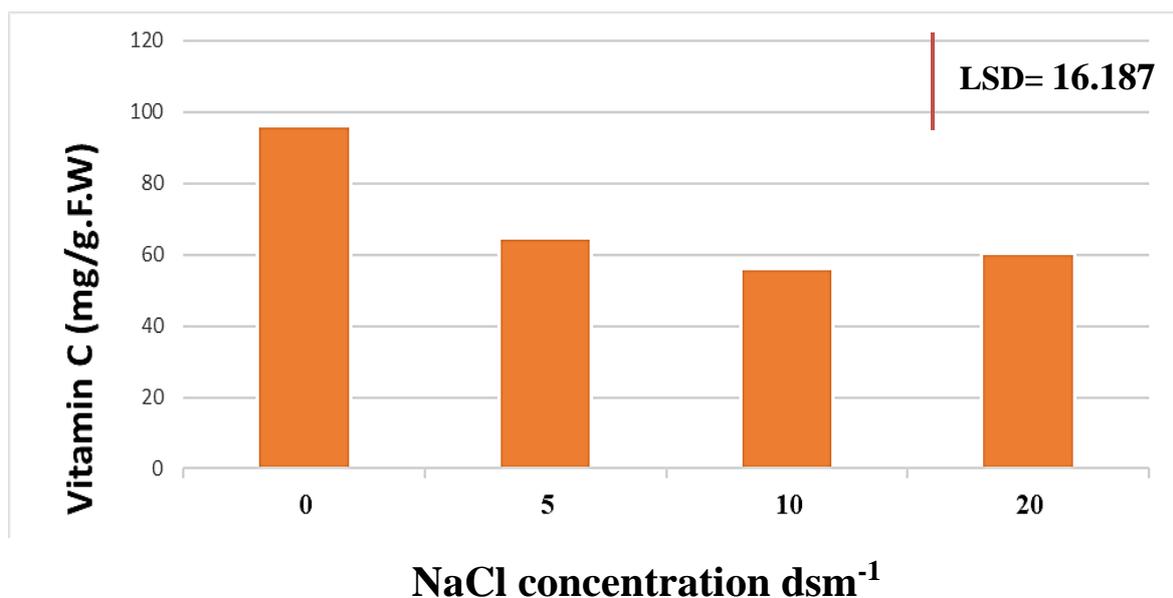


Figure (4-7) Effect of NaCl concentration on content of Vitamin C (mg/g.f.w) in *P.oleracea L*

4.2.6 : Total Antioxidant Activity (TAO)

Purslane is reported as an excellent source of the antioxidant vitamins α -tocopherol, ascorbic acid and β carotene, as well as glutathione (Dkhil *et al* ,. 2011) Salinity not only influences the nutrients of vegetable crops but also degrades the antioxidant property (Saira *et al* ,. 2012)

The results of the study showed that the amount of antioxidants in the purslane is in the control group is (10.50 mg AEE/gFW) and its quantity in the rest of the three groups that were irrigated with sodium chloride salt is (5.13, 13.49 and 16.27) mg AEE/gFW respectively,we note from During the statistical analysis, explain clear significant differences between the values of the four groups (Figure 4-8)

The plant contents of antioxidants increased in the third group, which was exposed to (20 dsm⁻¹) of NaCl salt and a clear increase from the values of the previous groups. This result indicates that the increase in antioxidants is one of the ways of plant adaptation and its resistance to Salt stresses. It agrees

with the result of the study Tang. *et al.*, (2020) in which they were concluded that Salt stress, which can provide a technical reference for the further improvement of saline and alkaline soils and the development of salinity-resistant plant germplasm resources. The role of antioxidant enzymes as components of key tolerance mechanisms developed in response to various environmental extremes has already been elucidated. Several studies have shown a relationship between resistance to environmental stresses and the efficiency of the antioxidant system (Demiral and Türkan, 2005) A study on green bean showed that the antioxidant enzyme activities, there were noticeable differences in the antioxidant enzyme activities between two green bean cultivars with an increase in NaCl concentration and the APX (Antioxidant enzymes activities) ability increased with an increase in NaCl concentration in both cultivars (Yasar *et al.*, 2008).

The results of the study also agree with the study conducted by Carrasco-Ríos & Pinto. (2014). on the corn plant under saline stress, it contains an enzymatic antioxidant system that is more active, and therefore this property can be considered as a defense mechanism that contributes to bearing the oxidative effect of salinity, and therefore the oxidative damage is less.

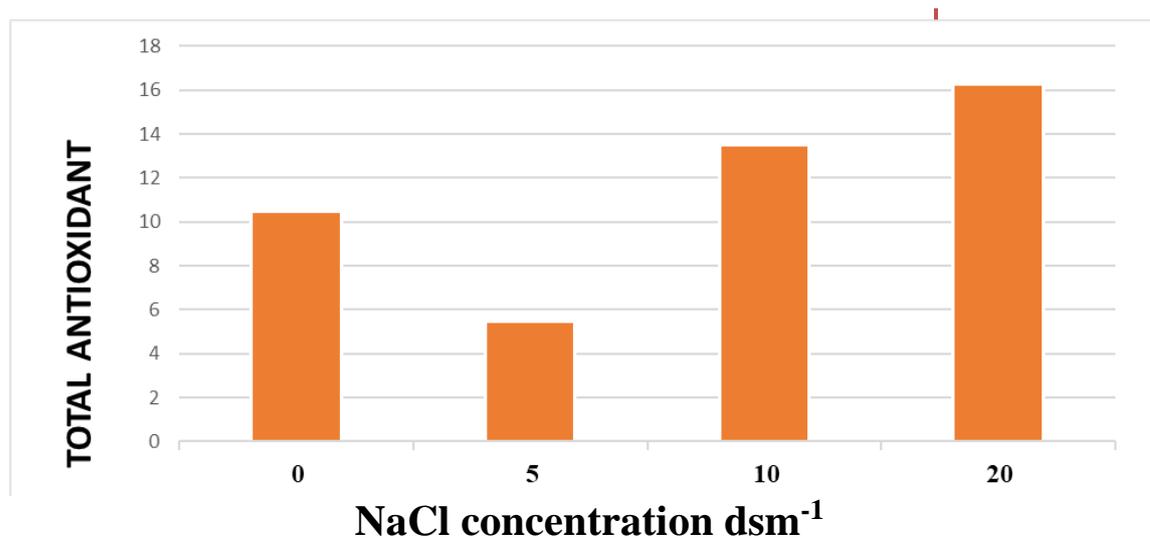


Figure (4-8) Effect of NaCl concentration on content of Total antioxidant activity ((mg AEE/g.F.W)) in *P. oleracea* L

4.2.7 : Total Soluble Sugars (TSS)

It has been known for a long time that soluble sugars are among the main metabolites that sustain plant growth and development (Irigoyen *et al.*,1992)Soluble sugars do not only function as metabolic resources and structural constituents of cells, they also act as signals regulating various processes associated with plant growth and development (Loreti *et al* ,.2001) Differences in environmental factors, such as salinity and others, lead to a significant decrease in the efficiency of photosynthesis in plant tissues, thus reducing the supply of soluble sugars to flood the tissues. Under conditions of sugar deprivation, significant physiological and biochemical changes occur to maintain respiration and other metabolic processes in the life cycle of plants (Yu, 1999).

the results of laboratory tests to measure the plant's total sugar content show that the value of sugars is (127.00 $\mu\text{g/gFW}$) in the control group, which is the highest value for the rest of the three groups, which were treated with different concentrations of NaCl salt, and its value was (84.58, 84.95 and 67.53) $\mu\text{g/gFW}$, respectively, and the LSD value was (12.526), these there was clear significant differences between the values and that the rate of sugars decreased with the increase in the plant's exposure to salt stress .

Different studies on this species explained value of sugars decreased with salinity stress increasing (Kazeroni *et al.*, 2005) . Some results indicated a decrease in the salinity stored sugars, Increasing of sugar content under condition of salinity stress in such plants as tomato Amini and Ehsanpour, (2005) and barley Bagheri and Sadeghipour, (2009) was reported increasing of sugar under environmental stress was recognized as a result of starch degradation, sugar synthesis by non-photosynthesis pathways, non-converting of these components to other productions and decreasing of transporting from

leaves . Sugar is the main molecule symptoms which influence on different physiological response in regulated genes in photosynthesis, metabolism and defensive Reponses (Crowe. *et al.*, 2002). The low level of sugars in the purslane plant is one of the ways of adapting the plant to the stresses, as the accumulation of sugar particles in the staplast causes osmotic pressure (Bartels and Ramanjulu, 2005 ; Khavarinejad. *et al.*,2002). The concentrations of sugar solution of two types of plants have led to an increase in the severity of drought due to preventing plasma dissolution and modifying osmosis (Hosseini, 2019).

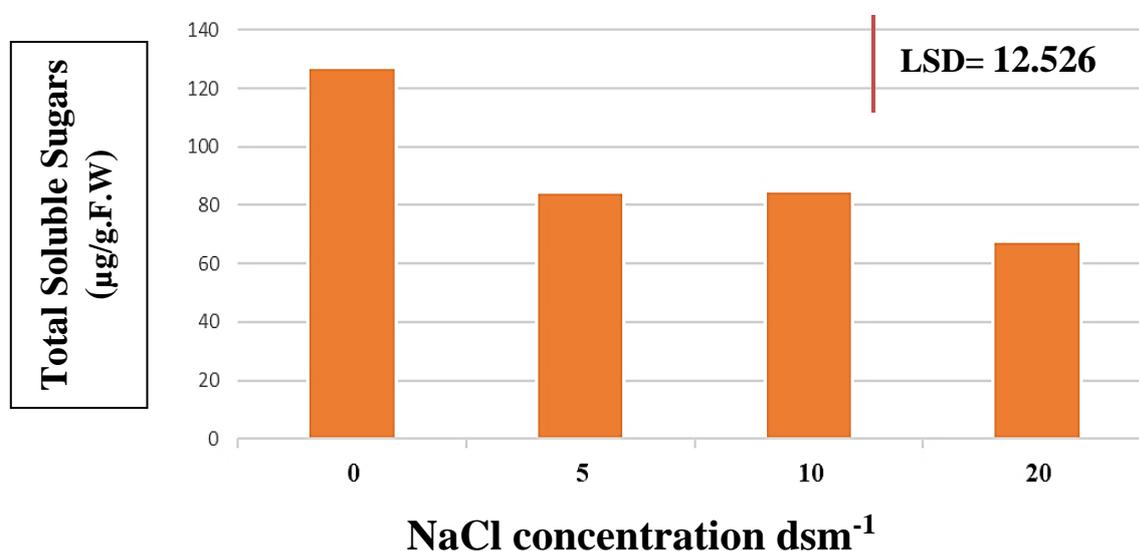


Figure (4-9) Effect of NaCl concentration on content of Total Soluble Sugars (µg/g.F.W) in *P. oleracea* L

4.3 : Secondary Metabolite Product of *Portulaca oleracea* L.

The results shown below show the effect of different salt concentrations, combined with the control group, on the amount of the plant's content of secondary metabolites, as we note the high and low values of alkaloids, flavonoids and vitamin A.

Table (4-3): Secondary Metabolite Product *P. oleracea* induced by Salinity

Treatments Parameters	Total Alkaloids content (%)	Total flavonoid content (mg Rutin / gm)	Vitamin A (IU)
	Mean±S.D		
Control	2.92±0.08	0.58±0.03	651.07±3.60
5 dsm ⁻¹	2.47±0.07	0.37±0.02	430.07±4.14
10 dsm ⁻¹	2.05±0.04	0.27±0.02	328.4±8.16
20 dsm ⁻¹	3.48±0.13	0.84±0.04	883.73±24.03
LSD (0.05)	0.135	0.048	19.719

4.3.1 : Total Alkaloid

Alkaloids are alkaline nitrogenous organic compounds, which extracted from this plant. These compounds have antibacterial, anti-inflammatory, antitumor, anti-hypertensive and antidepressant effects (Di Chen *et al.*,2019) ,Alkaloids are final or intermediate results of primary metabolic processes and are part of a group of natural products in plants and are called secondary compounds (Graf *et al* ,. 2007) Amino acids are the basic units of many alkaloid compounds (Nahar& Sarker, 2019).

The results of the study showed that exposure of purslane to saline concentrations led to a decrease in the plant's total alkaloid content, and as shown in Table No. (3) that the value of alkaloids in the control group was (3.48%), while the alkaloids were evaluated in the other three groups that were irrigated with NaCl salt It is (2.92%, 2.47%, 2.05%), respectively, and the results of the statistical analysis showed that there is a significant difference between the four groups and that the LSD value is (0.135).

The environmental factors affecting the metabolism of photosynthesis affect the production and quantity of alkaloids in plants (Saad Al-Din *et al.*, 2005). Because of the saline pressures, therefore, we notice a decrease in the plant's content of total alkaloids as the salt concentrations increase in a proportional relationship, and the decrease in the medicinal content of the plant under the influence of salinity is due to chemical and physiological fluctuations and the manufacture of these compounds.

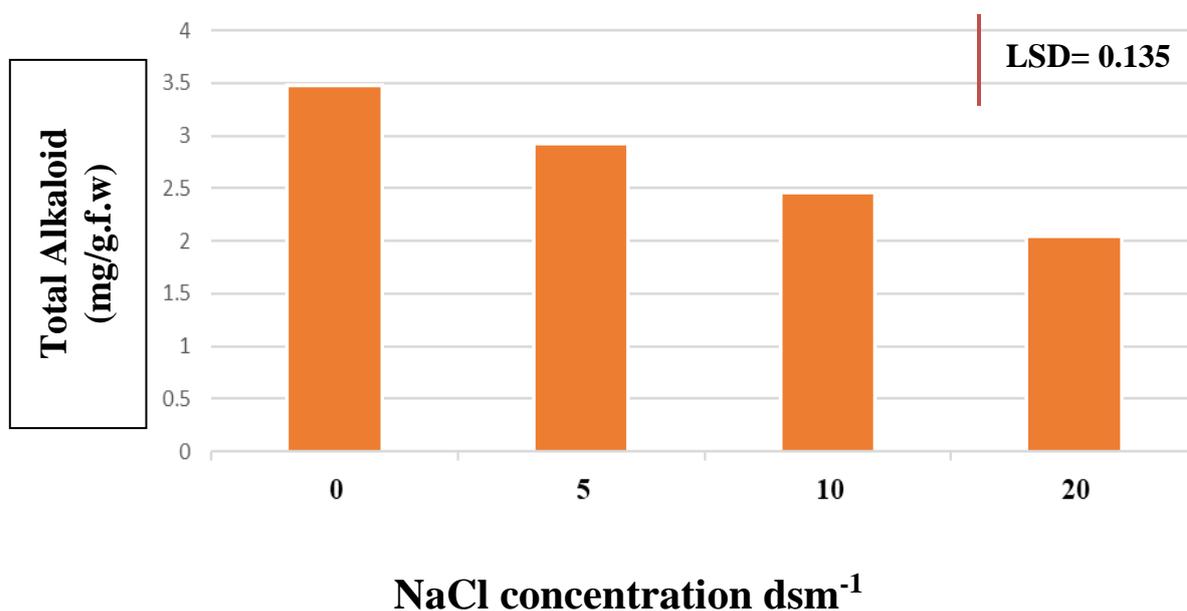


Figure (4-10) Effect of NaCl concentration on content of Total Alkaloid (mg/g.f.w) in *P. oleracea* L

4.3.2 : Total flavonoid

Flavonoids are structurally diverse secondary metabolites in plants, with a multitude of functions. These span from functions in regulating plant development, pigmentation, and UV protection, to an array of roles in defence and signaling between plants and microorganisms. Because of their prevalence in the human diet, many flavonoids constitute important components of medicinal plants and are used in the control of inflammation and cancer prevention (Mathesius. 2018). Flavonoids (flavonols, flavones, flavan-3-ols,

anthocyanidins, flavanones, isoflavones and others) they are commonly found conjugated to sugars and organic acids , Several works reported the presence of flavonoids as main bioactive purslane constituents (Erkan , 2012).

The results of the study showed (Figure 4-11)) that the total flavonoids in the purslane plant in the control group was (0.84mg Rutin / gm), and that its value in the rest of the groups that were irrigated with NaCl salt was (0.58, 0.37 and 0.27) mg Rutin / gm, and as shown to us The decrease in the plant's content of flavonoids with the increase in the amount of salinity to which the plant was exposed, the results of the statistical analysis showed that the LSD value is (0.048), and from calculating the differences between the value of the control and the values of the other groups together, it is clear that there are significant differences between them.

Compared with previous studies, it was concluded (Yuan *et al.*, 2010) that the total phenol content of 5–7-day old radish sprouts was significantly decreased due to moderate salinity compared to the control group, Rezazadeh *et al.*, (2012) showed in his study on *Cynara scolymus L* that the amount of flavonoids in plant leaves decreased as the concentration of NaCl salt increased, and an increase in the response of phenolic compounds to salinity in extracts of different tissues of some other plants was also reported (Agastian *et al.*, 2000) .

Salt stress limits plant growth more than photosynthesis; As a result, the plant converts carbohydrate synthesis to produce secondary metabolites (Coley.1986). In contrast, at a high salt concentration, the uptake of phosphorous and potassium, which are the main material for secondary metabolites such as total flavonoids, decreases (Waring and Pitman.1985). Also, due to the disturbance of enzymatic activities at high salinity,

photosynthesis decreases. Therefore, the production of flavonoids will decrease (Wong.*et al.*, 2006).

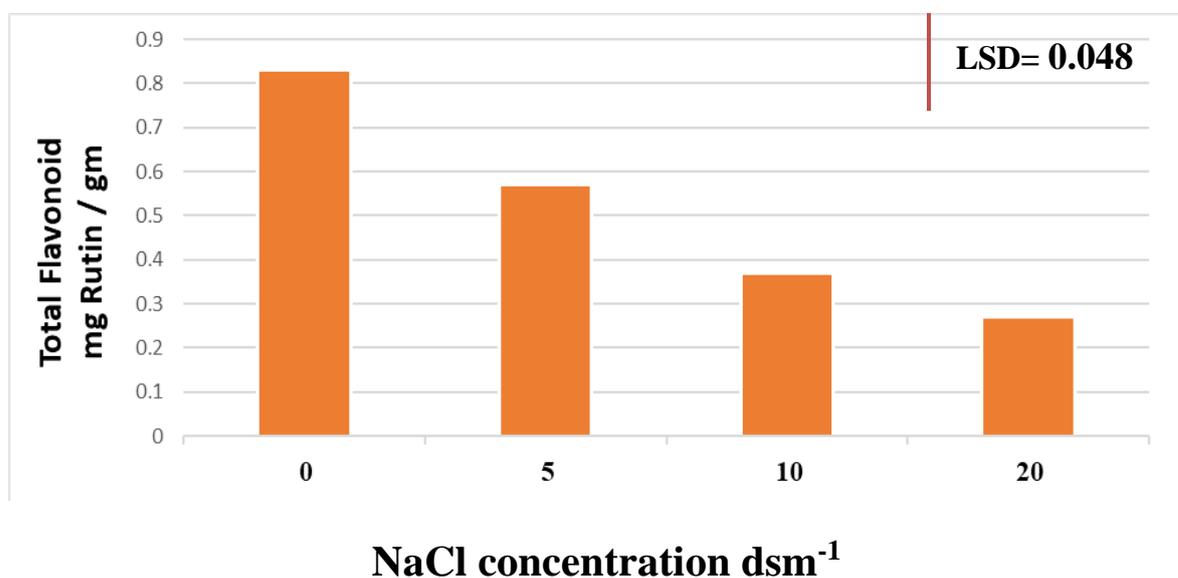


Figure (4-11) Effect of NaCl concentration on content of Total flavonoid (mg/g.f.w) in *P.oleracea L*

4.3.3 : Vitamin A

P.oleracea L Rich in vitamin A, a natural antioxidant, it can play a role in the health of the mucous membranes of vision and to protect against lung and oral cavity cancer, purslane contains the highest percentage of vitamin A among leafy vegetables (Uddin *et al.*, 2014) The content of vitamins in purslane varies with genotype, origin and environmental conditions (Petropoulos *et al.*, 2015).

From (Figure 4-12), the increase in the concentration of NaCl led to a decrease in the content of *P. oleracea L* of vitamin A, where the percentage of vitamin A in the control group was (883.73 IU), while its percentage in the rest of the groups was (651.07, 430.07 and 328.4) IU, we conclude that increase in salinity led to a significant decrease in the vitamin content. From the results of the statistical analysis, there is a clear significant difference.

Studies of salt stress effect on vitamin A content of vegetables are scarce. Carotene concentrations are commonly determined as these vegetable yellow pigments are converted to vitamin A in the human body and are accordingly refer to as provitamin A (Ratnakar and Rai, 2013), In the current study, the content of vitamin A in *Portulaca oleracea* significantly decreased with the increase in the concentration of NaCl in the growing medium, WouYou *et al.*, (2017) study on *Amaranthus cruentus* showed a decrease in the amount of vitamin A at 30 mm of NaCl , It was also observed in *Trigonella foenum-graecum* that the content of vitamin A decreased under salt stress (Rapala-Kozik *et al.*, 2008) .

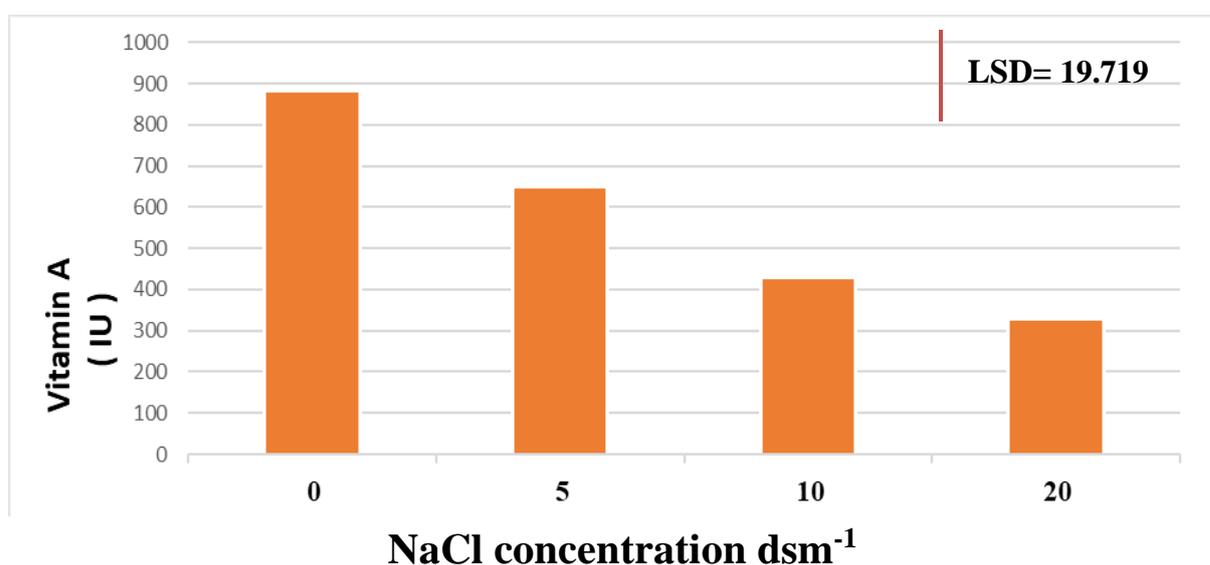


Figure (4-12) Effect of NaCl concentration on content of Vitamin A (mg/g.f.w) in *P. oleracea* L

4.4 : Fatty acids

The table below shows the values of the seven important fatty acids that were isolated and their values measured in the plant, and the values of the extent of the decreasing amount of these acids in the plant appear with the increase in the salt concentrations to which the plant was exposed, according to what is shown in front of each of them.

Table (4-4): Salinity Effect on Fatty acids content in *P. oleracea* L

Treatments	Control	5 dsm ⁻¹	10 dsm ⁻¹	20 dsm ⁻¹	LSD (0.05)
Parameters (%)	Mean±S.D				
a- linolenic acid	8.33±0.80	6.93±1.05	5.57±0.60	4.77±0.65	1.573
Ecosopentaenoic	7.80±0.80	6.63±0.90	5.33±0.65	4.40±0.55	1.467
Docosahexaenoic	9.17±0.85	7.90±0.95	6.50±0.55	5.77±0.65	1.501
Linolic acid	10.77±0.70	9.47±1.01	7.87±0.70	7.43±0.55	1.502
Palmatic acid	4.47±0.65	3.50±0.72	2.53±0.51	1.97±0.51	1.134
Oleic acid	10.47±1.06	8.87±0.85	7.77±0.65	7.07±0.70	1.530
Stearic acid	4.90±0.85	3.83±0.70	2.80±0.50	2.27±0.45	1.215

P. oleracea L is one of the richest plant sources of polyunsaturated fatty acids, the most important of which is the omega-3 linolenic acid (Yan *et al.*, 2009) , Environmental conditions affect the content and compositions of fatty acids in plants (Yeilaghi, *et al.*, 2012) , The findings on the effect of salt stress on fatty acid content are contradictory in purslane. (Carvalho *et al.*, 2009)

Table (4-4) Shows the fatty acid values of *P. oleracea* L by exposure to saline concentrations, From the above data, The results of data analysis of fatty acids in the seven fatty acids that were detected in *Portulaca oleracea* L under control conditions and saline pressure, as it appears that the greater the plant's exposure to salt, the lower the value of fatty acids, and the results of the statistical analysis showed a significant difference and the value of LSD is as shown in front all kind of acids .

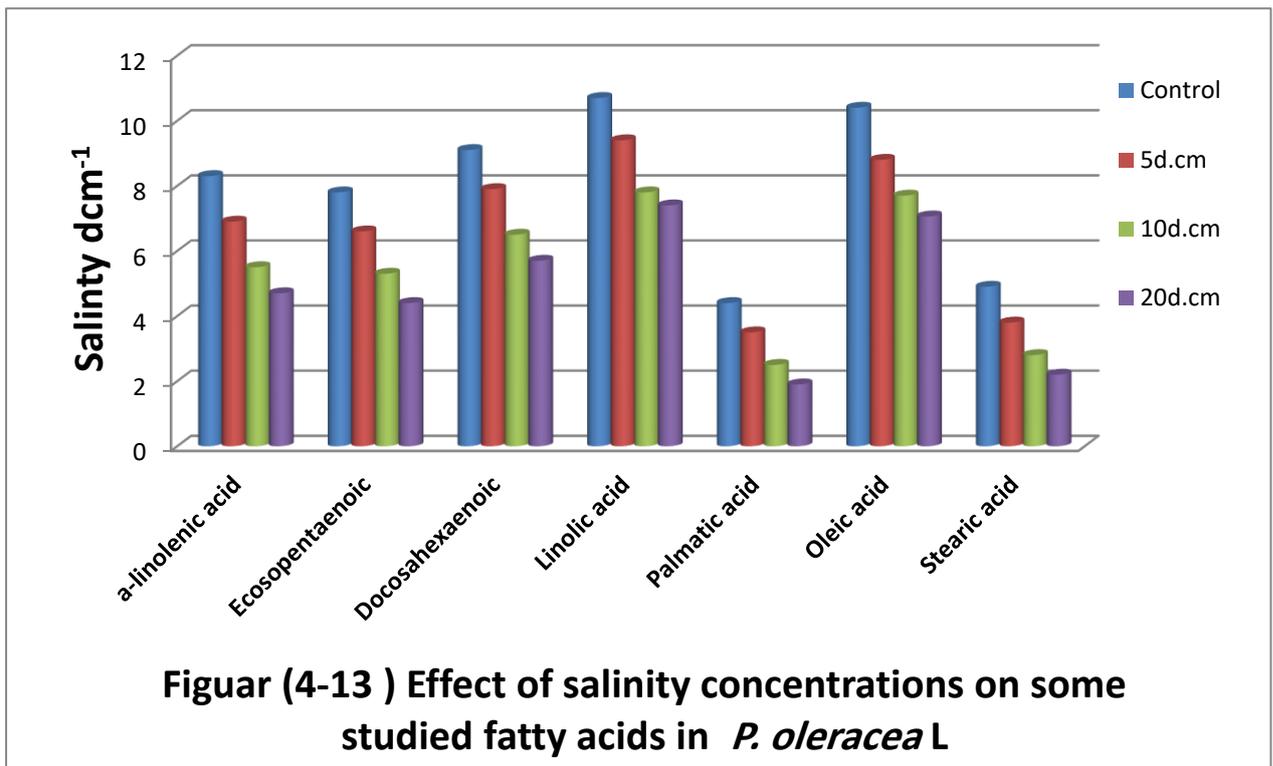
From previous studies, salinity changes the composition of fatty acids and is very important in stress tolerance for plants (Malkit *et al.*, 2002), Salt

tolerant species have the ability to adjust membrane lipid fluidity by increasing the proportion of unsaturated fatty acid (Anastácio and Carvalho, 2013) Free alpha-linolenic acid is itself a salt stress signal (Ivanova *et al.*, 2016) At higher levels of plant salt exposure decreased lipid synthesis in purslane leaves (Teixeira and Carvalho 2019), (Szalai *et al.*, 2010) indicated the effect of fatty acid values on purslane leaves with the variety and their exposure to sunlight, also (Zaman *et al.*, 2019) found that at 200 mM of NaCl stress, the percentage of ω -6 and ω -3 acids decreased significantly in the leaves of the purslane plant.

The lipid peroxidation of leaves was suppressed at 70 and 140 mM NaCl by increasing the ability to scavenge reactive oxygen species. Therefore, the ability of purslane to withstand the effects of salinity stress in leaves depends on a balance between the accumulation of total fatty acids and chemical degradation. Salt concentrations up to 120 mM NaCl also affected the total fat content (Anastácio & Carvalho. 2013)

a study Yeilaghi *et al.*, (2012) on *Carthamus tinctorius* L showed that all fatty acids in the plant were affected Significantly, under pressure and increase in salinity, and also under stress conditions, the oil content in olives decreased and the fatty acid composition also changed. Generally, salinity stress induces inactivation of PSI and PSII, Unsaturated fatty acids in membrane lipids shelter PSI (first photovoltaic system) and PSII (second photovoltaic system) inactivation as one of effective protective strategy. Where it affects dually; alleviating the salinity induced damage to PSI and PSII and improving the healing of injury (Allakhverdiev *et al.*, 2011) These high amounts of Linolic acid present in the leaves of *P. oleracea* of plants of the control group are unusual. Previous studies have reported 17% (Liu *et al.*, 2000), 18% (Paéz *et al.* 2007) and 24% (Palaniswamy *et al.* 2001) of this percentage in the total fatty acids detected.

This study specifically identified salt stress as an important factor affecting both the concentrations of fatty acids in the purslane plant and thus its effect on the nutritional value. Severe salt stress reduces the concentration of monounsaturated fatty acids, present results expanded our knowledge of the composition and proportions of fatty acids in purslane under salt stress, and the extent of the effect of salt on the nutritional value of purslane rich in fatty acids.



4.5: Effect of aqueous and alcoholic extracts of *Portulaca oleracea* L on some types of bacteria and yeast

A study of the effect of cold aqueous extracts and warm alcoholic extracts of *Portulaca oleracea* plant on some types of bacteria *E.coli*, *Staph.aurues* and yeast, which included *C.albicans*,

The effect of different concentrations of cold and hot aqueous and alcoholic extracts, as well as extracts from the salt groups that were previously

studied, was studied on the tested microorganisms. Table (4-5) and when the values of the inhibition areas for the cold aqueous extract for the control group and the rest of the three groups reached (12.44 , 4.5 and 0 , 0) mm, respectively, at the concentration (600) mg / ml to reach (13.44, 6.5 and 1.5,0) mm Respectively at a concentration of (1000) mg / ml, The hot alcoholic extract was significantly more effective compared to the cold aqueous extract when the inhibition zone reached (17.33) ml at a concentration of 1000 mg/ml compared to (13.44) for the cold aqueous extract at the same concentration. It does not dissolve well in water, it dissolves in the presence of organic solvents such as ethanol and methanol (Abo-Shanab *et al* ,. 2004), While the decrease in the inhibitory activity of the types of extracts with the increase in salt concentrations can be explained by the decrease of the active substances in the plant such as alkaloids, flavonoids and terpenes, and as we indicated for the results of this study , Previously, all of these compounds were reduced with an increase in salt concentrations, thus reducing the content of the extract and reducing its inhibitory ability. The hot alcoholic extract was significantly more effective compared to the cold aqueous extract when the inhibition area reached (17.33) ml at the concentration of 1000 mg / ml in the control group compared to (13.44) for the cold German extract in the control group and respectively at the same concentration, the reason may be due In addition, many effective plant compounds that do not dissolve well in water dissolve in the presence of organic solvents such as ethanol and methanol (Abo-Shanab *et al*,.2004)

This is consistent with what Al-Dhahab , (1998) found that ethyl alcohol has a high ability to withdraw active compounds from the plant sample due to its high polarity. It also agrees with Tepe *et al*,.(2004) that the alcoholic ethanolic extract of sage had a high inhibition effect against *Staphylococcus aureus* and *C. albicans* yeast were less affected than bacteria of the alcoholic

extract and it could explain the inhibitory activity of the *Portulaca oleracea* plant because it contains some active compounds such as alkaloids, terpenes, tannins, flavonoids, resins and soaps, as shown in Table (4-6). (Hassan *et al* ,.2000) indicated that soaps reduce sugar inside the bacteria which leads to cell death.

The results showed that the toxic effect of the plant depends on the method of extraction, the concentration of the extract and the type of bacteria, as shown by the statistical analysis. (Augustit ,1996) indicated that the alcoholic extract of the plant *Portulaca oleracea* has high activity against *Staphylococcus* positive bacteria and activity against one negative bacteria *E.coli*.

Also (Parkh and Chanda. 2016) indicated that the reason for the discrepancy in the inhibitory activity of the extracts is due to the variation in the method of extraction, the type of solvent and the nature of the membranes of the microorganisms. The plant was exposed to it.

Table (4-5) Effect of type of the extract (1000 mg / ml) of *P.oleracea L* on some microorganism

Extract 1000 mg / ml		Isolation type											
		<i>Staph aureus</i>				<i>E.coli</i>				<i>C. albicans</i>			
		Salinity dsm^{-1}											
		0	5	10	20	0	5	10	20	0	5	10	20
Inhibition	cold aqueous	12.5	4.5	0	0	9.33	1.5	0.00	0.00	0.00	0.00	0.00	0.00
	warm alcoholic	16.33	10.5	2.5	0	8.5	3.02	0.00	0.00	11.26	5.5	0.00	0.00

LSD(0.05) for extract*bacteria.*treatment=0.719

Table (4-6) Effect of type of the extract (600mg / ml) of *P.oleracea L* on some microorganism

Extract 1000 mg / ml		Isolation type											
		<i>Staph aureus</i>				<i>E.coli</i>				<i>C. albicans</i>			
		Salinity dsm^{-1}											
		0	5	10	20	0	5	10	20	0	5	10	20
Inhibition	cold aqueous	13.20	4.5	0	0	6.5	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	warm alcoholic	17.06	9.5	2.5	0	8.91	2.5	0.00	0.00	9.83	6.5	0.00	0.00

LSD(0.05) for extract*bacteria*treatment=0.358

The above results are in agreement with it in the response of bacteria *Staphylococcus aureus* (Gram-positive) to the effect of the extract more than *E.coli*. This may be due to the structural structure of the bacterial wall, as Gram-positive bacteria lack an outer membrane layer that makes the permeability of materials to the interior of the cell greater compared to Gram-negative bacteria Hassouni & Liqaa. (2013)

In order to reach the most efficient plant extract, the results were compared. The results of the statistical analysis showed that there were significant differences at the probability level (0.5) in the effect of the cold water extract and the hot alcoholic extract of paraben on the tested organisms, and as shown in Table (4-6), it was The hot alcoholic extract had the most effect on *Staph aureus* and *E.coli* and in the control group when the diameter of inhibition was (17.06) mm on *Staph aureus*, at a concentration of (600) mg/ml, and also, the diameter of inhibition was (9.33) on *E.coli* bacteria, while the was hot alcohol extract at a concentration (1000) mg/ml on the control group has

the highest activity on *C. albicans* yeast when the inhibition diameter is (11.26) mm.

CHAPTER THREE
MATERIALS AND
METHODS

3. MATERIALS AND METHODS

3.1. Sample preparation

Sandy agricultural soil type has been determined for planting the seeds of the *Portulaca oleracea* L plant .

The pH, EC, PPS, tissue and total organic matter of these soils were studied according to standard methods used to know soil investigations (ICARDA, 2002).

The soil was distributed in similar-sized pots, each with a capacity of (3 kg) of soil, then these pots were divided into five groups, each group consisting of five pots.

The *P. oleracea* L seeds were grown evenly as possible in these pots, then they were watered with tap water, and after (10 days) after the seeds germinated, each group of them was watered with concentrations of NaCl salt, which are as follows:

- 1) 0 dcm⁻¹
- 2) 5 dcm⁻¹
- 3) 10 dcm⁻¹
- 4) 20 dcm⁻¹

Then watering the plants continued for a month (30 days), when it was observed that the plant had reached a part of vegetation that could be picked (the pre-flowering stage), after which all of the basic compounds were measured:

3.2: Soil character

3.2.1 : Estimation of pH

Estimation according to (McKeague, 1978 and McLearl, 1982).

As follow:

- 1- Weigh 50 g of aerated soil (less than 2 mm) into a 100 ml beaker.
2. 50ml of distilled water was added using a 50ml graduated cylinder

3. The suspension was mixed well with a glass rod, and then the suspension was left for 30 minutes. Stir the suspension every 10 minutes during this period.
4. After an hour, stir the suspension well and place the built-in electrode directly into the suspension (about 3 cm deep). Take the reading after 30 seconds.
5. Remove the common electrode from the suspension, wash it well with distilled water in another beaker, then carefully wipe off the excess water with a soft tissue.

3.2.2: Estimation of EC (Soil electrical conductivity)

Estimation according to (Richards.1954) was used to measure electrical conductivity

First, a suspension was prepared in a ratio of 1: 1 (soil: water), as when estimating the soil pH, and then the suspension was filtered using a vacuum pump, Whatman No 42 in a Buchner funnel. Then the filter paper was moistened with distilled water and made sure that it adhered to the base of the funnel so that it covered all the holes.

We then turned on the vacuum pump and opened the pump spout, then added the suspension to the Buchner funnel and continued filtration until the soil in the funnel began to crack.

The clear filter was transferred to a 50ml beaker, then the conduction cell was immersed in the solution, the reading was taken and the cell removed from the filter, then washed thoroughly with distilled water, and then the excess water was carefully dried with a soft tissue.

3.2.3: Estimation of Organic matter

Estimation according to oxidative method (Black.1965) was dependent to estimate organic material contain in the use soil by:

First, 1 g of air-dry soil (0.15 mm) was weighed into a 500 ml beaker, then add 10 mL of 1 N potassium dichromate solution using a pipette, add 20 mL of

concentrated sulfuric acid using a graduated cylinder, and stir the flask well to mix the suspension and then leave the suspension for 30 minutes.

Then about 200 ml of distilled water was added, then 10 ml of concentrated phosphorous acid was added using a dispenser, and the mixture was allowed to cool and then add 10-15 drops of Diphenylamine guide, add a magnetic rod, then put the cup on the magnetic stirrer and titration with a 0.5 molar solution of ferrous ammonium sulfate, until the color changes from blue-violet to green, and then two blank witnesses were prepared, containing all solutions except soil, and followed the same method that you followed with soil suspensions.

3.2.4: Estimation of Soil Texture

The soil texture used in the study was estimated by Beaux-scale (g/l) (Astm 15H) and based on the method shown by (Rayn *et al.*, 2003) and then compare it with soil texture triangle.

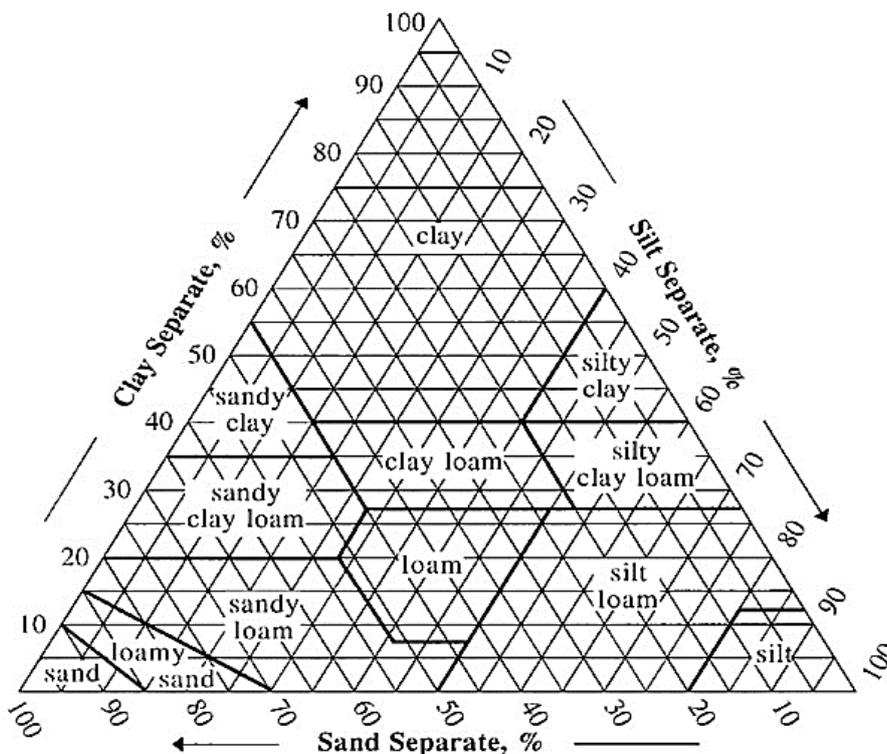


Figure (3-1) soil texture triangle

3.3: *Portulaca oleracea* L

3.3.1: Scientific classification

Kingdom:	<u>Plantae</u>
Clade:	<u>Tracheophytes</u>
Clade:	<u>Angiosperms</u>
Clade:	<u>Eudicots</u>
Order:	<u>Caryophyllales</u>
Family:	<u>Portulacaceae</u>
Genus:	<u>Portulaca</u>
Species:	<i>P. oleracea</i>



Figure (3-2) picture of purslane

3.4. Estimation of plant biochemical response :

3.4.1: Photosynthetic pigments estimation.

250 mg of *Portulaca oleracea* L fresh leaves was homogenized with 85% acetone for about 5 min. The sample was mixed using a magnetic stirrer; centrifuged and the absorbance taken at 663, 646, and 470 nm. The

concentrations of the pigments were estimated and expressed as mg.g⁻¹ fresh weight.

- Chlorophyll a: $C_a = 12.25 A_{663.2} - 2.79 A_{646.8}$ (μg per ml solution)
- Chlorophyll b: $C_b = 21.50 A_{646.8} - 5.10 A_{663.2}$ (μg per ml solution)
- Total carotenoids: $C_{x+c} = (1000 A_{470} - 1.82 C_a - 85.02 C_b) / 198$ (μg per ml solution) (Lichtenthaler and Wellburn 1983.)

3.4.2: Estimation of protein

500 mg of sample was ground well with 15 ml of phosphate buffer (0.2M, pH7.2). The extract was centrifuged for 10 minutes at 3000rpm. The lower layer was discarded and the supernatant was taken. To the supernatant, an equal amount of cold 5% TCA was added. It was left for 30 minutes in an ice bath. The precipitated protein was taken and again centrifuged. The supernatant was discarded and the pellet was dissolved in 25 ml of 0.2N NaOH. From this 1 ml was taken and mixed with 4 ml of alkaline copper reagent. It was shaken well and was allowed to stand for 10 minutes at room temperature. Then 0.1 ml of diluted Folin phenol reagent was added and mixed well. After 20 minutes, the O.D was read at 650 nm using UV-VIS Spectrophotometer. Bovine Serum Albumin was used as the standard (Lowry *et al.*, 1951) .

3.4.3: Estimation of proline

Proline was estimated according to the method of (Bates. *et al.*,1973). Briefly, a sample of 0.5 g fresh leaf tissue was homogenized in 10 mL of 3% sulfosalicylic acid and the homogenate was centrifuged at 13000 rpm for 10 min at 4 °C. Then 2 mL of the supernatant was mixed with 2 mL of acid ninhydrin and 2 mL of glacial acetic acid. This mixture was incubated at 100 °C for 1 h and then cooled at room temperature. Finally, 4 mL of toluene was added. Proline was extracted from the toluene layer and its absorbance was noted at 520

nm using toluene as a blank or reference. The proline concentration was determined from a standard curve .

3.4.4: Estimation of Vitamin C

3 g of fresh sample was ground with 25 ml 4% oxalic acid and filtered. 2 ml aliquots of the above extract were pipetted into each of the different test tubes and the volume was made up to 3 ml with distilled water. 1 ml of 2% DNPH (2, 4–dinitro phenyl hydrazine) reagent and 1 or 2 drops of 10% thiourea were added to each test tube. The contents of the test tubes were mixed thoroughly and incubated at 37°C for 3 hours. After incubation, 7 ml of 80% sulphuric acid was added to each test tube to dissolve the orange red oxazole crystals and the absorbance was measured at 540 nm against a reagent blank. The ascorbic acid content present in the sample was calculated by referring to a standard graph of ascorbic acid (Sadasivam and Manickam, 1992).

3.4.5: Estimation of Total Soluble Sugars (TSS)

100 mg of the fresh Leaves was crushed in 4 ml of ethanol at 80%. After incubation in water bath through agitation during 30 min at 80°C, the extract was centrifuged at 4500 rpm during (10 min). Total soluble sugars are then proportioned in means of the anthrone (150 mg of the anthrone /100 ml of sulphuric acid). The absorption was determined by spectrophotometry at 625 nm. The range of calibration was prepared by glucose (Dubois *et al.*,1956)

3.4.6: Determination Total Antioxidant Activity

The total antioxidant activity was determined by phosphomolybdenum method, it based on the reduction of MO (VI) to MO(V) by the sample and subsequence formation of a green Phosphate/ MO(V) complex at acidic pH. The absorbance is measured at 695nm using an UV/Vis spectrophotometrically. The

antioxidant capacity was expressed as Ascorbic acid equivalent (AAE) by using the standard Ascorbic acid.

Standard solution was prepared as: 50mg of Ascorbic acid was dissolved in 50ml standard flask using distilled water. (conc., 1mg/ml)

Extract solution was prepared by dissolving 50mg of methanolic dried extract in 50ml standard flask using distilled water (conc., 1mg/ml), then the following steps dependent to determine total antioxidant activity concentration:

(50-250 µg) of the buffer was prepared and extracted, 0.3 ml of each sample was taken in succession and 3.0 ml of phosphomolybdenum reagent was added to all tubes and 0.3 ml of water and 3.0 ml of reagent alone was used empty and all tubes were incubated at 97 °C for 90 min, then cooled and the absorbance measured at 695 nm using a UV/Vis spectrophotometer against vacuum. The antioxidant capacity was expressed as equivalent to ascorbic acid using standard ascorbic acid.

3.5: Estimation of secondary products of *P.oleracea* L:

3.5.1 : Total alkaloid content :

Extraction: The 20 gm of each plant material was ground and then extracted with methanol for 24 hours in a continuous extraction (Soxhlet) apparatus. The extract was filtered and methanol was evaporated on a rotary evaporator under vacuum at a temperature of 45°C to dryness than estimated by (Trease and Evans , 2002).

3.5.2 : Determination of total phenolic compounds.

The total amount of phenolic compounds was determined in the ethanolic extract with a standard Folin -Ciocalteu reagent . The reaction mixture contained 100 µl of the extract, and 500 µl of the Folin-Ciocalteu reagent (Merck, Germany) and 1.5 ml of 20% sodium carbonate. The sample was then mixed on

a vortex mixer and diluted with distilled water to the final volume of 10 ml. After 2 h reaction, the absorbance at 765 nm was determined and used to estimate the phenolic content using the calibration curve made with gallic acid (Sigma-Aldrich, Germany). The total amount of phenolic compounds was expressed in mg gallic acid equivalent (GAE) per g dry weight (Laouini and Ouahrani,2017).

3.5.3 : Determination of Vitamin A.

samples for the determination of vitamins were taken and prepared according to regulations contents were triturated in a porcelain mortar until a homogeneous mixture was obtained. For the determination of fat-soluble vitamins, 5 mL of and 0.1% solution of butyl hydroxytoluene in methanol was added to 0.05–0.1 g of the sample, held in the dark for 2 h in a tightly closed vessel, and next stirred for 20 min at room temperature. Samples were centrifuged before injection into the column and analyzed within 1 h,(Kozhanova *et al.*, 2002) .

3.5.4 : Determination of fatty acids :

The lipids were estimated based on the (AOAC. 1995) method using a fat extraction device (Soxholet).

3.6 Medicinal efficacy of *P. oleracea* L on some microorganisms (Bactria and Yest)

3.6.1: The culture media that was used in current study

In the study, the Muller-Hinton Agar medium and Neutron-Agar medium were used. These media were prepared according to the manufacturer's instructions and fixed on the package, and sterilized by autoclave under a temperature of 121 - and at a pressure of 15 pounds / inch for 15 minutes.

3.6.1: Cold aqueous extract

followed the method (Parekh and Chanda. 2007) with a weight of 10 gm of vegetable powder It was placed in a clean glass flask to which 100 ml of distilled water was added, then placed in a shaking incubator for 24 hours at a temperature of 37 °C, then the mixture was filtered by gauze into glass tubes and the tubes were discarded in a centrifuge at a speed of 500,000 rpm for 10 Minutes, then filter the sludge by means of filter papers, then evaporate the filtrate in the oven to obtain the dry powder of the extract, which was placed in a sealed and opaque tube, and kept in the refrigerator at a temperature of 4 ° C until use, the process was repeated several times for the purpose of obtaining a sufficient amount of the extract, as for the tests Microbial concentrations were prepared (200,400,600,800.1000). (mg/ml) and sterilized using the Millipore Filter with 0.22 µm holes.

3.6.2: Hot alcoholic extract

(Deshmuk and Borle ,1975) followed in the preparation of plant alcoholic extracts Study my agency:

200 g of vegetable powder was weighed into a thimble, its sides closed, and the sample was placed in a Soxhlet apparatus. Then ethanol alcohol was added to it at a concentration of 70% and a volume of 300-200 ml, The sample was left in ethanol for 24 h, after which extraction was carried out in a Sixolite apparatus for 16 h at 50–60 °C , The extract was taken and filtered with a type filter paper, then the remaining filtrate was dried in the century (Oven) to obtain the dry powder, and it was stored in the refrigerator until use. As for the microbial tests, the concentrations were prepared (600 , 1000) mg / ml and sterilized using The Millipore filter has 0.22µm holes.

3.7: determination of extract activity of *Portulaca oleracea* L

1. It was confirmed that the extract was not contaminated by planting a portion of the extract (0.1 ml) on the surface of the sterile medium (BHHIA), and incubated for 24 hours at a temperature of 37 °C.
2. Dishes were poured from Mueller-Hinton's medium, then 1:0 ml of the bacterial suspension was spread on the dishes by means of a sterile swab (Sterile swab) in several directions until the suspension was spread homogeneously on the surface of the dish and then left for a short period.
3. Then the graded concentrations were used from the original concentration of the aqueous and alcoholic extracts prepared previously with distilled and sterile water, which are (1000, 800, 600, 400, 200 0) mg/ml. 0.1 ml of the above concentrations was added to each previously selected and sterilized tablet. The plates were incubated at 37 °C for 24 hours.
4. Then, the effectiveness of all concentrations of each plant extract was determined by measuring the inhibition zone around each hole (Mahmood. *et al* ,.1989).

3.8: Statistical Analysis

The SPSS (Statistical Package for the Social Sciences) Version 25/2017 program was used in the statistical analysis to study the effect of salinity on *Portulaca oleracea* L content of important material and to explain the differences of plant activity as a microbial inhibitors (0.05) .

CHAPTER FOUR

RESULTS AND

DISCUSSION

4. Results and Discussion

It is well known that excess minerals in the plant is called salt stress and this phenomenon a major problem that negatively affects the physiological and metabolic processes in plant life, decreasing growth and ultimately yield. This editorial highlights the harmful effect of salt stress on the nutritional value of vegetables, including *Portulaca oleracea* L. (Prasad *et al.* , 2014) .

These inhibitory effects included stress on plant growth and biomass production which are well known (Hajer *et al.*, 2006, Alqurainy 2007, Long *et al.*, 2008, Ma *et al.*, 2013). In addition, salinity leads to dehydration and a decrease in the pressure potential (Munns 2002) .The present study showed that the NaCl stress effected all the studied growth traits as explained table (4-1) .

4.1: Soil characters

Soil is a highly dynamic and complex system that is a mixture of living and dead organisms, decaying and residual organic matter, minerals, air, and water. that support life together is difficult to understand the various physical, biological and chemical processes that occur in the soil; However, understanding soil properties helps in crop development and the influence of soil on plant content and growth (Schulte *et al.*, 2005) . *Portulaca oleracea* L. grows in full sun in almost any soil, from organic-rich clay to heavy clay, and tends to grow best in drier soils (Karkanis. 2017).

The soil texture used in the study is sandy mixture, and after conducting laboratory tests on it, the proportions of its components were (sand = 53.83%, salt = 29.84, clay = 16.33%) (Table 4-1 & Figure 4-1).

Table (4-1) properties of the soil used in the study

soil texture			Organic matter g/kg ⁻¹	EC dsm ⁻¹	PH
clay	silt	sand	7.6	4.22	8
16.33%	29.84%	53.83%			
sandy mixture					

Sandy soils have less charge of ions compared to clay soils, so they are less capable of absorbing ions, and thus the accumulation of salts in this soil decreases. Also, the higher the percentage of water in sandy soils, the higher the percentage of soil washing from ions, and the electrical conductivity values increase (Sonmez,2008) Soil texture influences soil slurry electrical conductivity (EC), anion and cation concentrations, and the retention of different soil-to-water ratios (Ozcan *et al.*, 2006) .

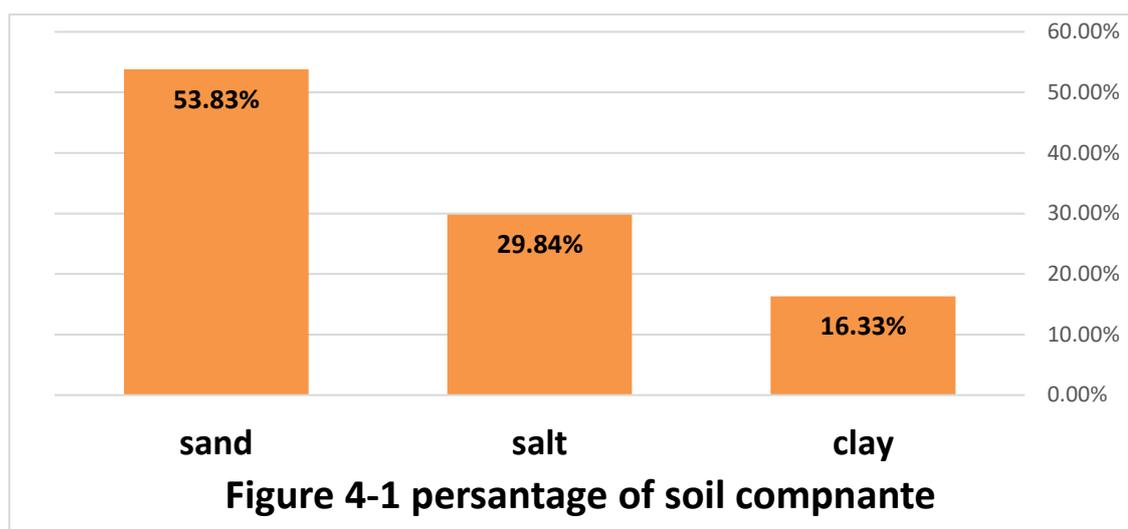
The results of laboratory analyzes of soil used in agriculture showed that it contains a high percentage of organic matter, and organic matter is a light substance with high porosity and has an important role in improving the composition of the soil and increasing its ability to conserve water in addition to its other effects on soil properties (Hagan *et al.*, 2013) ,The increase in the percentage of organic matter is accompanied by an increase in the value of the bulk density of the soil (Bonini *et al*, 2010) , As it is proven that with the increase in the bulk density, the percentage of total salts in the soil increases, as well as the percentage of exchanged sodium ions (Kadhim, 2015) , One of the causes of salinization is the poor physical and chemical properties resulting from the low content of soils of organic matter and thus turning the soil into saline, and that the more organic matter in the soil, the lower its salinity (Dweni, 2003) , This indicates that the soil used in agriculture is low in salt .

pH: In chemistry, denoting (potential of hydrogen) or (power of hydrogen) is a scale used to specify the acidity or basicity of an aqueous solution. Acidic solutions (solutions with higher concentrations of H⁺ ions) are measured to have lower pH values than basic or alkaline solutions (Jensen, 2004). The laboratory tests of the studied soil showed that the pH value is 8, and thus the soil is basic.

The addition of organic matter reduces the degree of soil reaction pH due to the role of organic matter in the production of CO₂ with water when it decomposes, forming carbonic acid and some organic acids resulting from decomposition such as humic and fulvic (Gascó and Lobo, 2007).

EC: Soil electrical conductivity, is a measure of the amount of dissolved salts in the soil (soil salinity). It affects crop yield, crop suitability, plant nutrient availability, and microorganism activity. Although EC does not provide a direct measurement of specific ions or salt compounds, it has been linked to nitrate, potassium, and sodium chloride concentrations, sulfates and ammonia. For some non-saline soils, determination of EC can be a convenient and economical way to estimate the amount of nitrogen (N) available for plant growth (Shakir, 2011).

the organic matter leads to the chemical properties of the soil, and the addition of the organic matter to the soil led to a decrease in the electrical conductivity (EC). The reason for the decrease is attributed to the fact that the organic matter contributes to increasing the water permeability in the soil, which leads to the washing of salts from the surface to depth (Amir, *et al.*, 2017). Organic matter increases the surface area of the soil by isolating soil particles from each other, which leads to an increase in water retention (Stock, 2008).



4.2 : Plant Biochemical responses

The table below shows us the extent of the effect of different salt concentrations compared to the control group on the plant's content of chemical response compounds and its effect on its general rates between high and low, as well as the value of LSD.

Table (4-2): Biochemical responses of *Portulaca oleracea* L. induced by treatment with NaCl

Treatments	Control (0 dsm ⁻¹)	Con1 (5 dsm ⁻¹)	Con2 (10 dsm ⁻¹)	Con3 (20 dsm ⁻¹)	LSD (0.05)
Parameters	Mean±S.D				
Total Chlorophyll (mg/g.F.W)	2.35±0.29	1.99±0.31	1.52±0.16	1.37±0.10	0.607
Chlorophyll a (mg/g.F.W)	1.39±0.16	1.13±0.18	0.97±0.33	0.83±0.10	0.326
Chlorophyll b (mg/g.F.W)	0.96±0.17	0.86±0.13	0.55±0.07	0.54±0.01	0.413
Carotenoids (mg/g.F.W)	1.24±0.22	0.39±0.12	0.55±0.14	0.40±0.03	0.230
Total Protein (mg/g.F.W)	31.29±3.88	9.07±2.35	3.69±0.73	4.43±2.03	3.820
Proline(μole/g.F.W)	0.47±0.19	0.93±0.10	0.86±0.08	2.12±0.06	0.588
Vitamin C (mg/g.F.W)	96.07±13.46	64.73±6.03	51.79±8.95	60.25±12.51	16.187
Total Antioxidant Activity (mg AEE/g.F.W)	10.50±0.76	5.13±0.62	13.49±1.13	16.27±1.31	2.078
Total Soluble Sugars (μg/g.F.W)	127.00±11.58	84.58±3.00	84.95±6.85	67.53±4.09	12.526

4.2.1 : Photosynthesis pigment

1- Total Chlorophyll

Reduction of chlorophyll contents can be as a result of either slow synthesis or fast breakdown, indicated that there was a photoprotective mechanism through reducing light absorbance by decreasing chlorophyll contents (Elsheery and Cao, 2008). The loss of chlorophyll under salt stress could be related to photoinhibition or ROS (reactive oxygen species) formation (Kato and Shimizu, 1985).

The study refers the value of total chlorophyll in the control group was 2.35 mg / g. The content of total chlorophyll decreased slightly with an increase in the concentration of NaCl salt, and noticed that there was a significant difference between other values.

The reduction in photosynthesis under salinity can also be attributed to a decrease in chlorophyll content. Salinity reduces the chlorophyll content in salt susceptible plants and increases it in salt tolerant plants salinity, level could be attributed to a reduction in leaf area expansion and hence to a lower light interception (Marcelis and Hooijdonk, 1999).

Salt stress affects chloroplast components of the main organs of plant photosynthesis, such as pigments, enzymes, proteins and membrane lipids, reduces plant photosynthesis and consequently reduces plant productivity. (Parida *et al.* 2003)

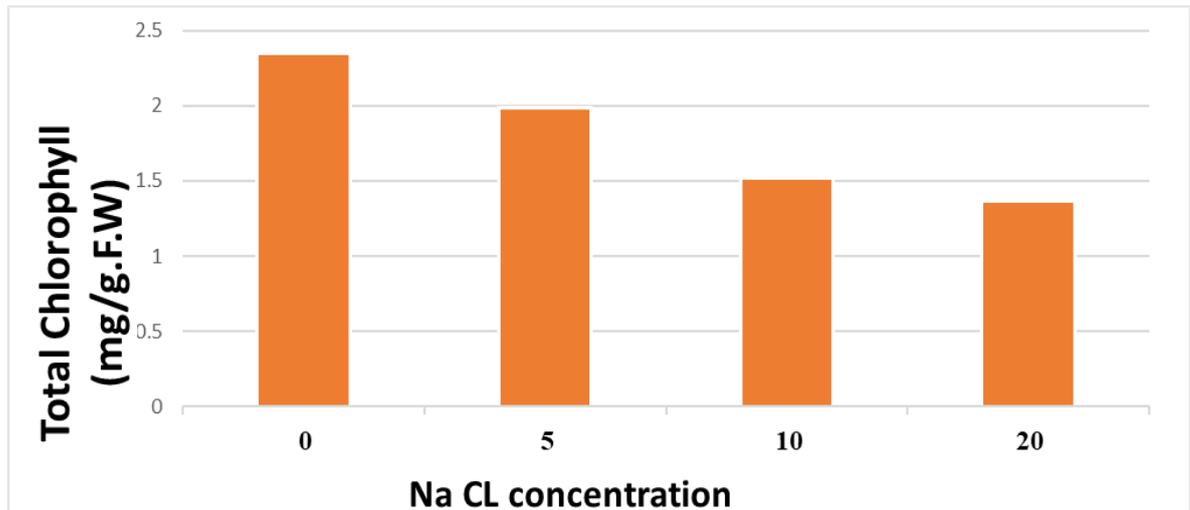


Figure (4-2) Effect of NaCl concentration on content of Total Chlorophyll (mg/g.f.w) in *P. oleracea* L

2- chlorophyll a and b

Higher plant is identified with their content of both chlorophyll a & b and chlorophyll. is used to as a bioindicator to explain the effect of environmental factor (Sitko,2017) plant growth and yield are directly related to photosynthetic performance. Chlorophyll is the main photosynthetic pigment and plays an important role in plant photosynthesis (Tang *et al.* 2020). Salt stress decreased chlorophyll synthesis and inhibited photosynthesis (Barhoumi *et al.* 2007) .

figure (4-2) explain the effect of NaCl on chlorophyll a and b of *Portulaca oleracea* L. respective. despite of purslane is a halophyte, but salinity decrease the chlorophyll a & b content, so the higher significant decrease wase in content ration.

The study refers the value of chlorophyll a and b in the control plant that was treated with distilled water was 1.39 mg / g for chlorophyll a, while the value of chlorophyll b was 0.96 mg / g. The content of chlorophyll decreased slightly with an increase in the concentration of NaCl salt, and we noticed that there was no significant difference.

Through our observation of each group that was irrigated with certain concentrations of NaCl, we noticed that with the increase of NaCl concentration in the irrigated water, this leads to a decrease in the proportions of both chlorophyll a and b generally. The maximum decrease was at 20 dsm^{-1} for NaCl, while the group irrigated with distilled water had vegetable chlorophyll a,b content with the highest value compared to the other three concentrations.

these results of chlorophyll a and chlorophyll b under salt stress which is in agreement with previous results of (Taïbi *et al.*, 2016) on *Phaseolus vulgaris L* and (Turan *et al.* 2007) and (Taffouo *et al.* 2010) on *Vigna subterranean L*.

Our results also agreed with the study (Heidari.2012), who concluded that the different levels of salinity and the interaction between salinity and different genotypes had a significant impact on the content of chlorophyll a, b and carotenoids. Also, by increase sing salinity levels, the three photosynthetic pigments are reduced.

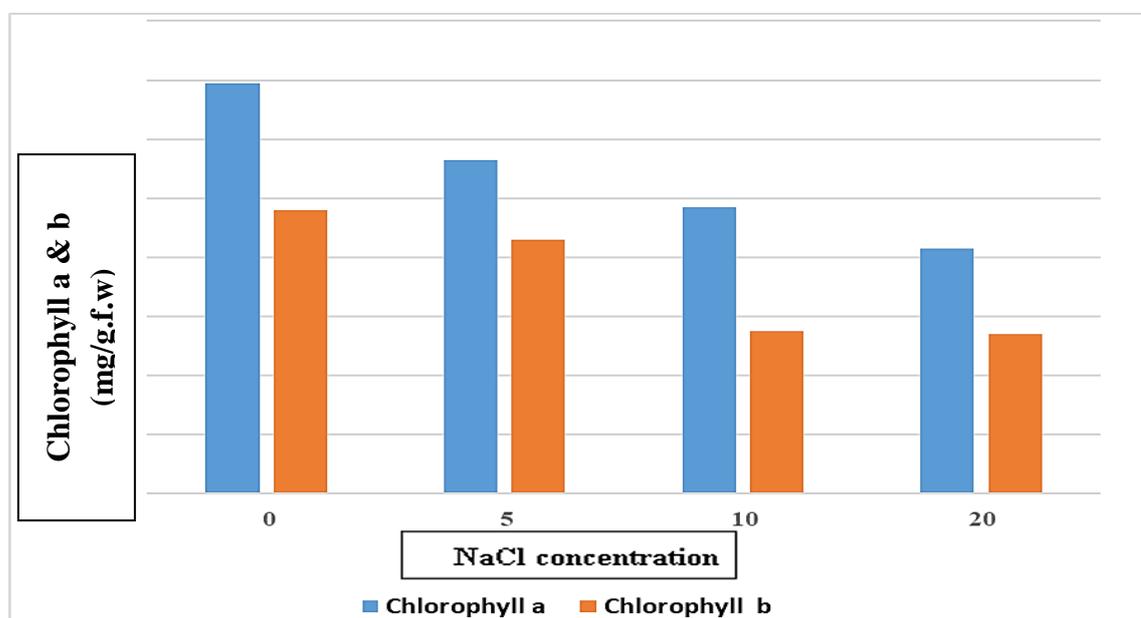


Figure (4-3) Effect of NaCl concentration on content of Chlorophyll a & b (mg/g.f.w) of *P. oleracea L*

4.2.2: Carotenoids

Carotenoids are pigments with several functions in plants, besides their direct role in photosynthesis, they are involved in the oxidative stress defence mechanisms (Gill and Tuteja, 2010) .

The data related to the carotene content are presented in (figure 4-4). The statistical analysis of the data showed that the salinity levels induced significant variator among group of the plant. Compared with the control value, the higher the salt stress on the plant, the lower the carotene value .

The most decreases wase (0.0230 mg/g) in the group concentration (20 dsm^{-1}) It found that the carotene content of the plant increased slightly in the second group compared to the first group and returned to decrease in the third group.

Similarly, to other studies, It is well known that carotenoids, being antioxidants, have the ability to detoxify plants from the effects of reactive oxygen species (Verma & Mishra, 2005). Salinity caused a decrease in carotenoid contents in all groups in which the concentration of NaCl was increased. These results are consistent with those found by (Jadallah ,1999) in *P. vulgaris* L. and (Singh *et al.* 2008) in the genetic structures of maize and wheat. Carotenoids They are known to act as light energy accumulators for photosynthesis and as dampers for trichotrophy and O_2 .

The decrease of carotenoid contents indicates that the protection by carotene was not one of the most important mechanisms under salt stress. Ali & Ismail, 2014) found that the use of 100 mm NaCl in the nutrient solution resulted in a significant decrease in tomato fruit carotenoids. In addition, salinity levels higher than 4.4 dsm^{-1} decreased carotenoid content (Pascale *et al* .,2001). This result could be due to the negative effects of saline stress on

carotenoid biosynthesis and the inhibition of lycopene accumulation (Riggi *et al* 2008)

The results of the mean values of the data showing the decrease in carotenoids under salinity stress thus it agree with the results obtained (Heidari ,2012) on *Ocimum basilicum* L .

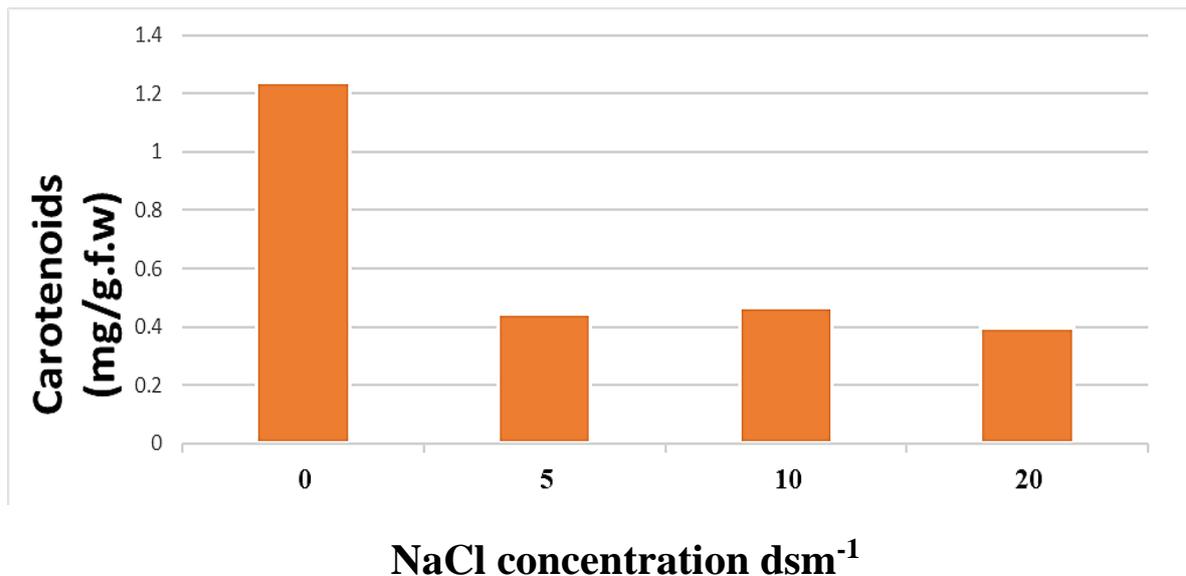


Figure (4-4) Effect of NaCl concentration on content of Carotenoids (mg/g.f.w) in *P. oleracea* L.

4.2.3: Total Proteins

Proteins are considered to be the building blocks of life and, in addition to their nutritional role, have other functions in living organisms.

All vital activities of plants are carried out through the interference and influence of proteins, and any interaction with external and internal factors depends on whether the plant has a mechanism for the production of supporting proteins or not (Musidlak , 2017) . This feature varies with different plants and their varieties in different regions. Therefore, these plant, climatic or

environmental differences that affect protein synthesis clearly indicate that the amount of some proteins, they can be an indicator of resistance or sensitivity to an external or internal factor and how resistant a plant is to environmental stresses (Hasanuzzaman, 2013).

The data related to the protein content was presented in (figure 4-5) protein content decreased significantly with the increase in the concentration of sodium chloride salt in the rest of the other three groups, and we noticed a significant difference, that it's decreased from (31.29 mg / g) in control group to (9.07 , 3.69 and 4.43 mg/g) in concentration (5 , 10 , 20 dsm⁻¹) respectively.

Also, when comparing the value of the third group, which was watered with the third concentration (10 dsm⁻¹) of NaCl salt and its value (3.69mg/g) with the fourth concentration (20 dsm⁻¹) of NaCl salt and its value (4.43 mg/g), the difference between them is (0.74 mg/g) which is lower than the LSD value of (3.820) and therefore no significant difference

Several salt-induced proteins have been identified in plant species and classified into two categories proteins, which accumulate only due to salt stress, and stress associated proteins, which also accumulate in response to heat, cold, drought, water, salinity and high and low mineral nutrients. Studies of Farghaly *et al.*, (2013), and Mansour *et al.*, (2000) revealed as salinity stress induced insignificant change in the soluble proteins of the tolerant and sensitive wheat cultivars. A higher content of soluble proteins has been observed in salt tolerant than in salt sensitive cultivars of sunflower Ashraf *et al.*, (1995), finger millet (Uma *et al.*, 1995) and rice (Pareek *et al.*, 1997)

In lentil Ashraf and Waheed , (1993) reported that leaf soluble proteins decreased due to salt stress in all lines, irrespective of their salt tolerance. (Ashraf and Harris ,2004) reported that the proteins produced under salt stress

are not always associated with salt tolerance, thus using proteins as salt tolerant indicators depending on the nature of the plant species or cultivar. The protein degradation under saline environment has been attributed to the decrease in protein synthesis, accelerated proteolysis, decrease in availability of amino acids and denaturation of enzymes involved in protein synthesis (Jaleel *et al.*, 2007 ; Lakhdar *et al.*, 2008).

It is note from the above values that the protein content in the third group returned slightly, and this indicates one of the plant's ways of resisting salinity, and this agrees with the study of (Gomathi. *et al.*, 2013) concluded that increasing protein content under salt stress involves activating transcription and translation processes for specific stress tolerance genes.

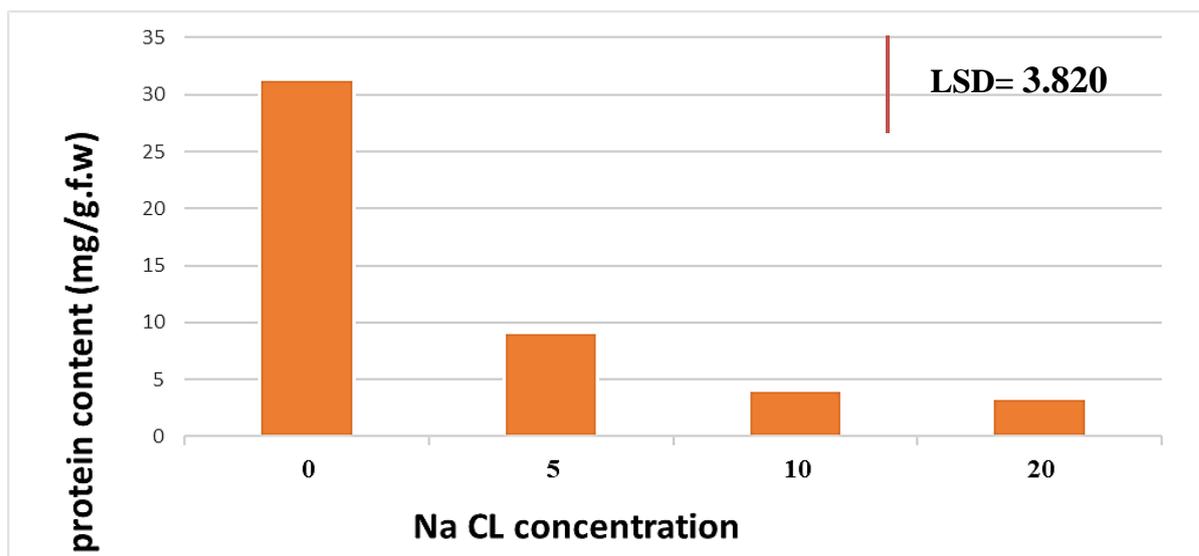


Figure (4-5) Effect of NaCl concentration on content of Protein (mg/g.f.w) in *P. oleracea* L.

4.2.4: Proline

A huge studies and data indicate a positive relationship between proline accumulation and plant stress. Proline, an amino acid, plays a very beneficial role in plants exposed to various stress conditions. In addition to being an excellent osmolality (Meena , 2019).

In response to various environmental stresses, many plant species synthesize proline in the cytosol and accumulates in the chloroplasts. Proline accumulation in plants is a well-recognized physiological reaction to osmotic stress prompted by salinity, drought and other abiotic stresses. Proline plays several protective functions such as osmoprotective, stabilizing cellular structures, enzymes, and scavenging ROS and keeps up redox balance in adverse situations. In addition, ample-studied osmoprotective capacity, proline has been also ensnared in the regulation of plant improvement, including flowering, pollen, embryo, and leaf enlargement (Meena *et al.*, 2009), Proline comprises less than 5% of the total pool of the free amino acids in plants under regular conditions (Shahbaz *et al.*, 2013).

After calculating the protein values of purslane in groups that were exposed to certain salt concentrations and comparing them with the control group, it noticed that they are clear significant differences and an increase in the value of proline with an increase in the concentration of sodium chloride, as it was noted that the value of proline in the control group is (0.47 $\mu\text{ole/g.F.W}$) while in the third group (2.12 $\mu\text{ole/gFW}$) in which the salt concentration is (20 dsm^{-1}) sodium chloride, and the difference between them is (1.65) which is greater than the LSD value which is (0.588), as there is a significant difference, as well as For the second and third group, as shown in in figure (4-6) .

Proline protects the plants from various stresses and also helps plants to recover from stress more rapidly. When applied exogenously to plants exposed to stress, Proline results in increased growth and other physiological characteristics of plants (Hayat. *et al*, 2012). The results of this study agreed with the study of Parvanehet *al.*, (2012) on *P. oleracea L*. An increase in the amount of leaf proline with an increase in sodium chloride, this increases half plant to reduce osmatic presser iv response to increased salinity (Ueda. *et al*, 2007).

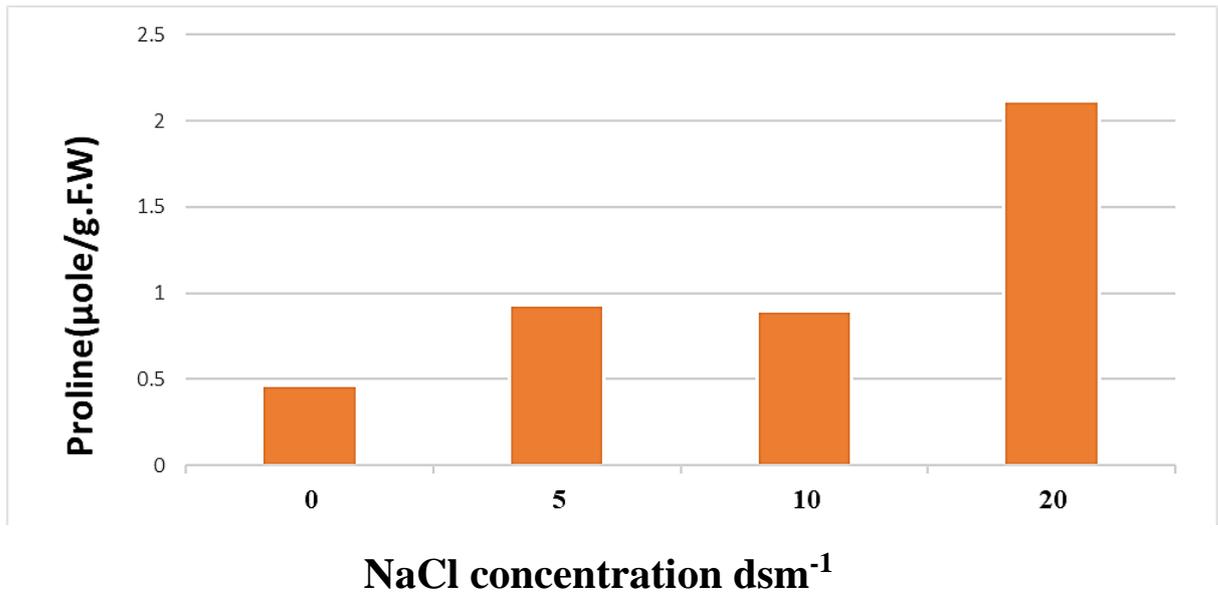


Figure (4-6) Effect of NaCl concentration on content of Proline (mg/g.f.w) in *P. oleracea* L

3.1.5 : Vitamin C

Salinity causes oxidative stress in plants by enhancing production of reactive oxygen species, so that an efficient antioxidant system, of which Vitamin C (ascorbic acid) is a key component, is an essential requirement of tolerance. Ascorbic acid (vitamin C) is an abundant component of plants. It reaches a concentration of over 20 mM in chloroplasts and occurs in all cell compartments, including the cell wall. It has proposed functions in photosynthesis as an enzyme cofactor (including synthesis of ethylene, gibberellins and anthocyanins) and in control of cell growth. Vitamin C (ascorbic acid) is very popular for its antioxidant properties (Arrigoni and De Tullio, 2002).

In the current study, the value of vitamin C decreases as the salt concentration increases, as its value recorded in the control group is (96.07 mg/gFW) while its value was (64.73, 51.79 and 60.25) mg/gFW respectively (figure 3-7) and, in the three treatment groups, we note a that high value of vitamin C in the third group, which was exposed to the highest value of sodium

chloride, and this indicates the adaptation of the plant to resist salinity stress. And through the statistical analysis, the value of LSD was (16.187) and from noting the difference between the control group and the first group, the difference between them is (64.73 mg/gFW) which means a significant.

In fact, several studies have shown that ascorbic acid plays an important role in improving plant tolerance to abiotic stress and intensifying its osmotic adaptability and growth activities (cell division and expansion) (Azzedine *et al* ,.2011). The results are also in agreement with the study conducted by Parida *et al*., (2004) who studied the effect of salt stress on the concentrations of ascorbic acid and Glutathione decreased in halophytic plants *Bruguiera parviflora*. The value of ascorbic acid during exposure to salt stress in the plant and this indicates a decrease in the levels of glutathione and ascorbic acid was due to consumption. One of these antioxidants in ascorbate is the oxidized glutathione.

Ascorbic acid (Vitamin C) plays a key role in salt tolerance of many halophytes (Jithesh *et al*. 2006), It also recycles the lipid-soluble antioxidant α -tocopherol (Lushchak and Semchuk 2012). It may also contribute to maintaining photosynthesis, cell-cycle progression, cell wall expansion, gene expression, synthesis of many hormones, anthocyanin and flavonoids (Smirnoff & Wheeler 2000).

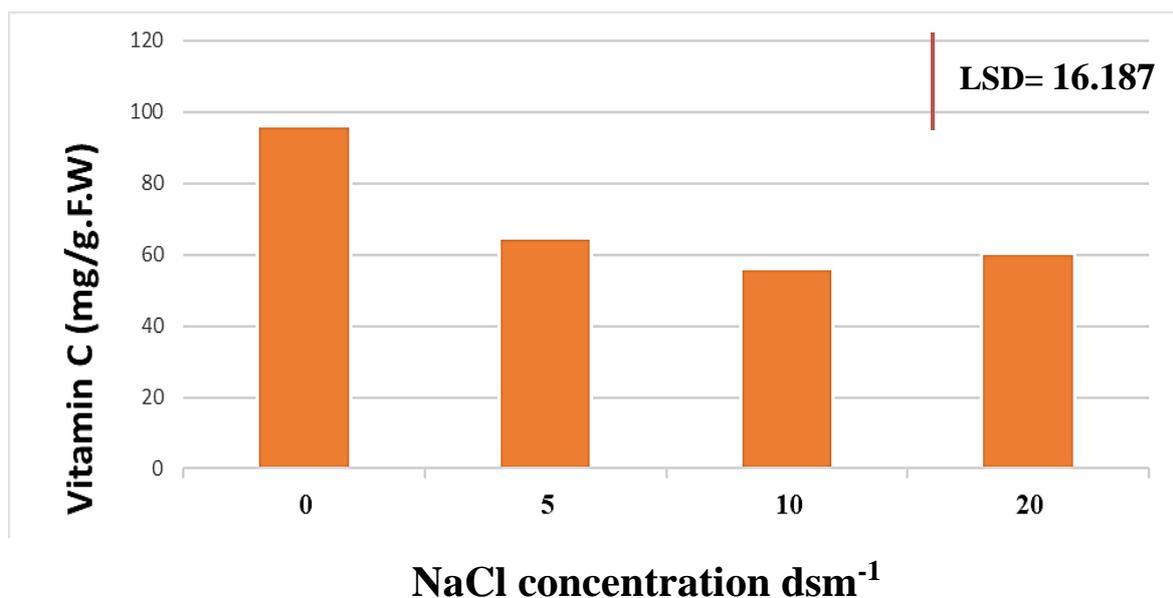


Figure (4-7) Effect of NaCl concentration on content of Vitamin C (mg/g.f.w) in *P.oleracea L*

4.2.6 : Total Antioxidant Activity (TAO)

Purslane is reported as an excellent source of the antioxidant vitamins α -tocopherol, ascorbic acid and β carotene, as well as glutathione (Dkhil *et al* ,. 2011) Salinity not only influences the nutrients of vegetable crops but also degrades the antioxidant property (Saira *et al* ,. 2012)

The results of the study showed that the amount of antioxidants in the purslane is in the control group is (10.50 mg AEE/gFW) and its quantity in the rest of the three groups that were irrigated with sodium chloride salt is (5.13, 13.49 and 16.27) mg AEE/gFW respectively,we note from During the statistical analysis, explain clear significant differences between the values of the four groups (Figure 4-8)

The plant contents of antioxidants increased in the third group, which was exposed to (20 dsm⁻¹) of NaCl salt and a clear increase from the values of the previous groups. This result indicates that the increase in antioxidants is one of the ways of plant adaptation and its resistance to Salt stresses. It agrees

with the result of the study Tang. *et al.*, (2020) in which they were concluded that Salt stress, which can provide a technical reference for the further improvement of saline and alkaline soils and the development of salinity-resistant plant germplasm resources. The role of antioxidant enzymes as components of key tolerance mechanisms developed in response to various environmental extremes has already been elucidated. Several studies have shown a relationship between resistance to environmental stresses and the efficiency of the antioxidant system (Demiral and Türkan, 2005) A study on green bean showed that the antioxidant enzyme activities, there were noticeable differences in the antioxidant enzyme activities between two green bean cultivars with an increase in NaCl concentration and the APX (Antioxidant enzymes activities) ability increased with an increase in NaCl concentration in both cultivars (Yasar *et al.*, 2008).

The results of the study also agree with the study conducted by Carrasco-Ríos & Pinto. (2014). on the corn plant under saline stress, it contains an enzymatic antioxidant system that is more active, and therefore this property can be considered as a defense mechanism that contributes to bearing the oxidative effect of salinity, and therefore the oxidative damage is less.

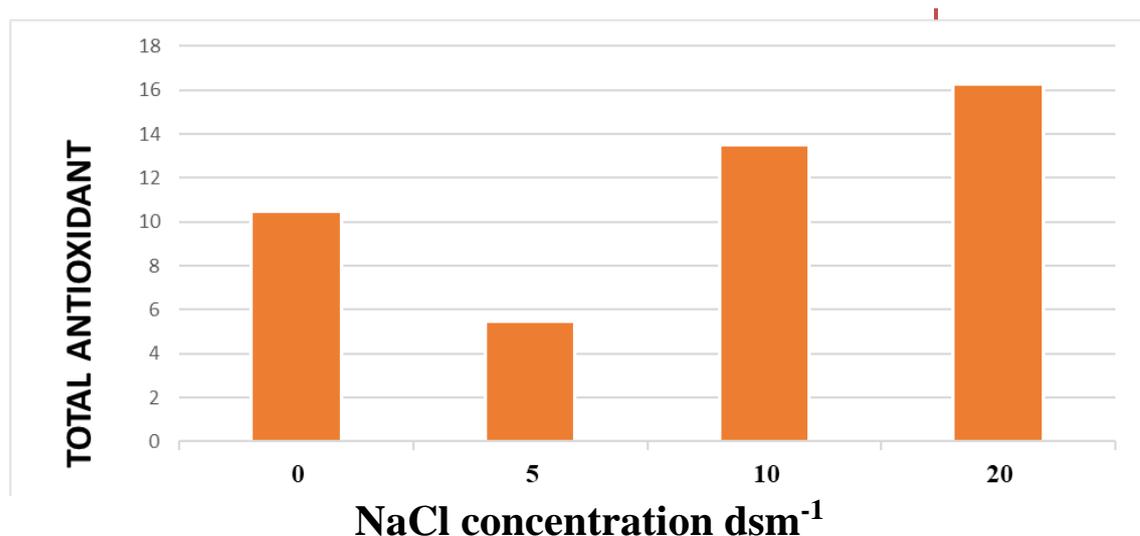


Figure (4-8) Effect of NaCl concentration on content of Total antioxidant activity ((mg AEE/g.F.W)) in *P. oleracea* L

4.2.7 : Total Soluble Sugars (TSS)

It has been known for a long time that soluble sugars are among the main metabolites that sustain plant growth and development (Irigoyen *et al.*,1992)Soluble sugars do not only function as metabolic resources and structural constituents of cells, they also act as signals regulating various processes associated with plant growth and development (Loreti *et al* ,.2001) Differences in environmental factors, such as salinity and others, lead to a significant decrease in the efficiency of photosynthesis in plant tissues, thus reducing the supply of soluble sugars to flood the tissues. Under conditions of sugar deprivation, significant physiological and biochemical changes occur to maintain respiration and other metabolic processes in the life cycle of plants (Yu, 1999).

the results of laboratory tests to measure the plant's total sugar content show that the value of sugars is (127.00 $\mu\text{g/gFW}$) in the control group, which is the highest value for the rest of the three groups, which were treated with different concentrations of NaCl salt, and its value was (84.58, 84.95 and 67.53) $\mu\text{g/gFW}$, respectively, and the LSD value was (12.526), these there was clear significant differences between the values and that the rate of sugars decreased with the increase in the plant's exposure to salt stress .

Different studies on this species explained value of sugars decreased with salinity stress increasing (Kazeroni *et al.*, 2005) . Some results indicated a decrease in the salinity stored sugars, Increasing of sugar content under condition of salinity stress in such plants as tomato Amini and Ehsanpour, (2005) and barley Bagheri and Sadeghipour, (2009) was reported increasing of sugar under environmental stress was recognized as a result of starch degradation, sugar synthesis by non-photosynthesis pathways, non-converting of these components to other productions and decreasing of transporting from

leaves . Sugar is the main molecule symptoms which influence on different physiological response in regulated genes in photosynthesis, metabolism and defensive Reponses (Crowe. *et al.*, 2002). The low level of sugars in the purslane plant is one of the ways of adapting the plant to the stresses, as the accumulation of sugar particles in the staplast causes osmotic pressure (Bartels and Ramanjulu, 2005 ; Khavarinejad. *et al.*,2002). The concentrations of sugar solution of two types of plants have led to an increase in the severity of drought due to preventing plasma dissolution and modifying osmosis (Hosseini, 2019).

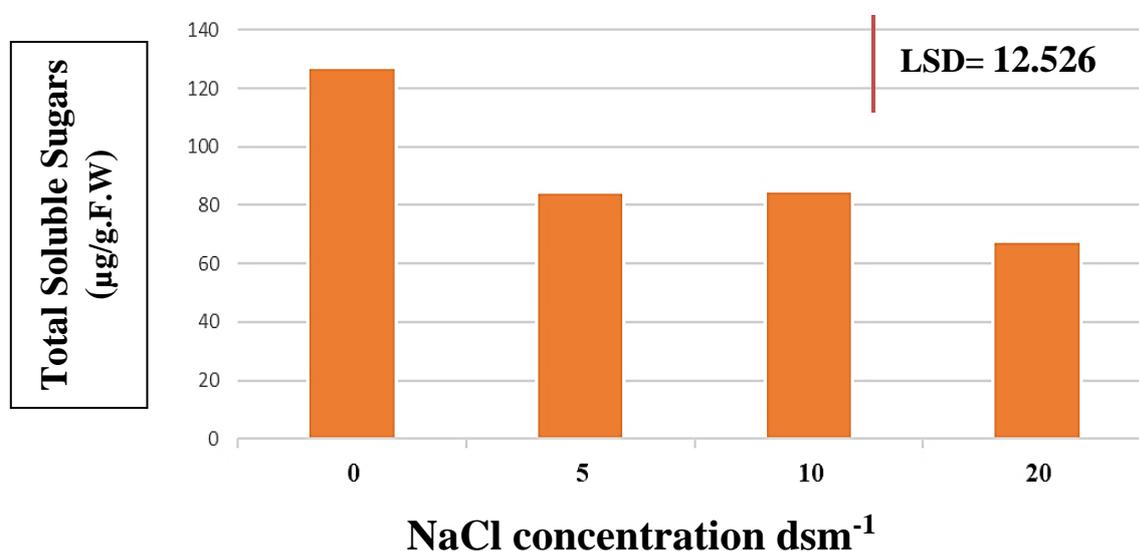


Figure (4-9) Effect of NaCl concentration on content of Total Soluble Sugars (µg/g.F.W) in *P. oleracea* L

4.3 : Secondary Metabolite Product of *Portulaca oleracea* L.

The results shown below show the effect of different salt concentrations, combined with the control group, on the amount of the plant's content of secondary metabolites, as we note the high and low values of alkaloids, flavonoids and vitamin A.

Table (4-3): Secondary Metabolite Product *P. oleracea* induced by Salinity

Treatments Parameters	Total Alkaloids content (%)	Total flavonoid content (mg Rutin / gm)	Vitamin A (IU)
	Mean±S.D		
Control	2.92±0.08	0.58±0.03	651.07±3.60
5 dsm ⁻¹	2.47±0.07	0.37±0.02	430.07±4.14
10 dsm ⁻¹	2.05±0.04	0.27±0.02	328.4±8.16
20 dsm ⁻¹	3.48±0.13	0.84±0.04	883.73±24.03
LSD _(0.05)	0.135	0.048	19.719

4.3.1 : Total Alkaloid

Alkaloids are alkaline nitrogenous organic compounds, which extracted from this plant. These compounds have antibacterial, anti-inflammatory, antitumor, anti-hypertensive and antidepressant effects (Di Chen *et al.*,2019) ,Alkaloids are final or intermediate results of primary metabolic processes and are part of a group of natural products in plants and are called secondary compounds (Graf *et al* ,. 2007) Amino acids are the basic units of many alkaloid compounds (Nahar& Sarker, 2019).

The results of the study showed that exposure of purslane to saline concentrations led to a decrease in the plant's total alkaloid content, and as shown in Table No. (3) that the value of alkaloids in the control group was (3.48%), while the alkaloids were evaluated in the other three groups that were irrigated with NaCl salt It is (2.92%, 2.47%, 2.05%), respectively, and the results of the statistical analysis showed that there is a significant difference between the four groups and that the LSD value is (0.135).

The environmental factors affecting the metabolism of photosynthesis affect the production and quantity of alkaloids in plants (Saad Al-Din *et al.*, 2005). Because of the saline pressures, therefore, we notice a decrease in the plant's content of total alkaloids as the salt concentrations increase in a proportional relationship, and the decrease in the medicinal content of the plant under the influence of salinity is due to chemical and physiological fluctuations and the manufacture of these compounds.

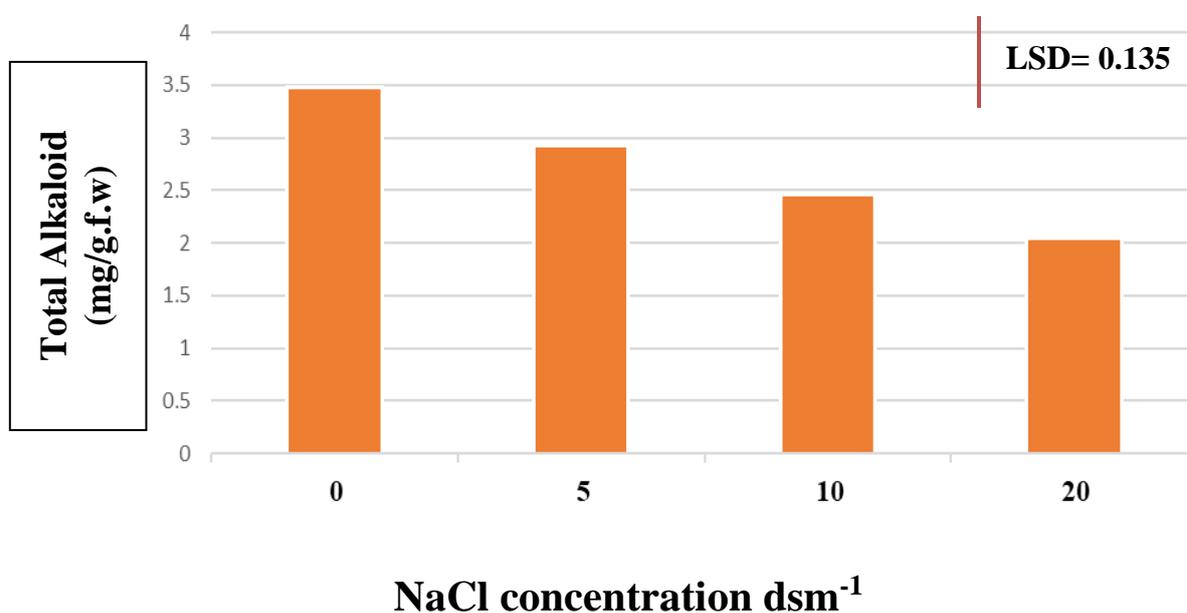


Figure (4-10) Effect of NaCl concentration on content of Total Alkaloid (mg/g.f.w) in *P. oleracea* L

4.3.2 : Total flavonoid

Flavonoids are structurally diverse secondary metabolites in plants, with a multitude of functions. These span from functions in regulating plant development, pigmentation, and UV protection, to an array of roles in defence and signaling between plants and microorganisms. Because of their prevalence in the human diet, many flavonoids constitute important components of medicinal plants and are used in the control of inflammation and cancer prevention (Mathesius. 2018). Flavonoids (flavonols, flavones, flavan-3-ols,

anthocyanidins, flavanones, isoflavones and others) they are commonly found conjugated to sugars and organic acids , Several works reported the presence of flavonoids as main bioactive purslane constituents (Erkan , 2012).

The results of the study showed (Figure 4-11)) that the total flavonoids in the purslane plant in the control group was (0.84mg Rutin / gm), and that its value in the rest of the groups that were irrigated with NaCl salt was (0.58, 0.37 and 0.27) mg Rutin / gm, and as shown to us The decrease in the plant's content of flavonoids with the increase in the amount of salinity to which the plant was exposed, the results of the statistical analysis showed that the LSD value is (0.048), and from calculating the differences between the value of the control and the values of the other groups together, it is clear that there are significant differences between them.

Compared with previous studies, it was concluded (Yuan *et al.*, 2010) that the total phenol content of 5–7-day old radish sprouts was significantly decreased due to moderate salinity compared to the control group, Rezazadeh *et al.*, (2012) showed in his study on *Cynara scolymus L* that the amount of flavonoids in plant leaves decreased as the concentration of NaCl salt increased, and an increase in the response of phenolic compounds to salinity in extracts of different tissues of some other plants was also reported (Agastian *et al.*, 2000) .

Salt stress limits plant growth more than photosynthesis; As a result, the plant converts carbohydrate synthesis to produce secondary metabolites (Coley.1986). In contrast, at a high salt concentration, the uptake of phosphorous and potassium, which are the main material for secondary metabolites such as total flavonoids, decreases (Waring and Pitman.1985). Also, due to the disturbance of enzymatic activities at high salinity,

photosynthesis decreases. Therefore, the production of flavonoids will decrease (Wong.*et al.*, 2006).

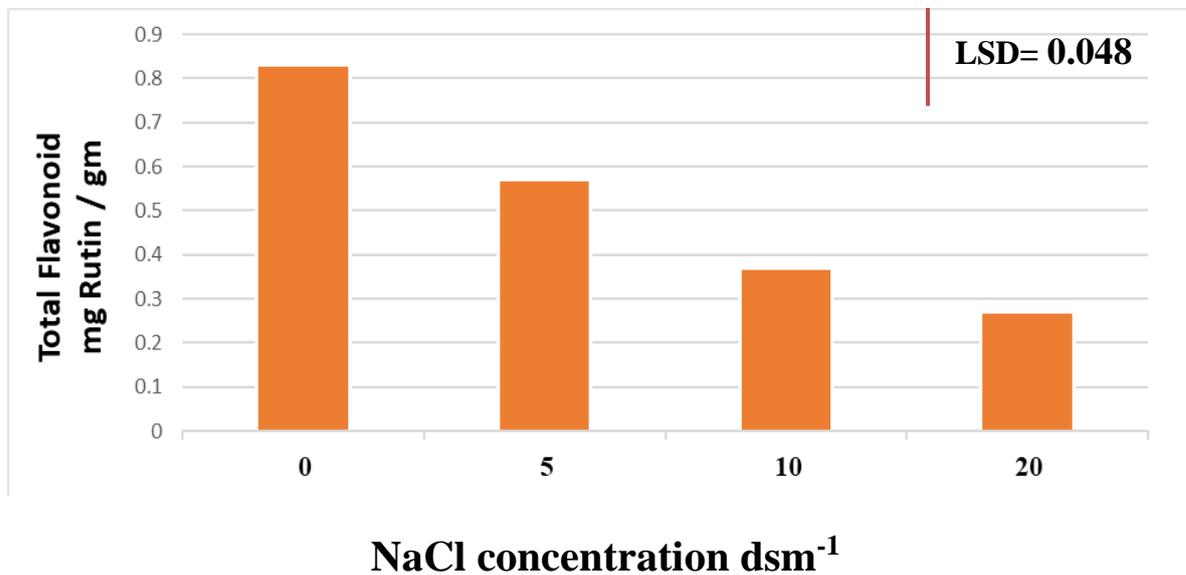


Figure (4-11) Effect of NaCl concentration on content of Total flavonoid (mg/g.f.w) in *P.oleracea* L

4.3.3 : Vitamin A

P.oleracea L Rich in vitamin A, a natural antioxidant, it can play a role in the health of the mucous membranes of vision and to protect against lung and oral cavity cancer, purslane contains the highest percentage of vitamin A among leafy vegetables (Uddin *et al.*, 2014) The content of vitamins in purslane varies with genotype, origin and environmental conditions (Petropoulos *et al.*, 2015).

From (Figure 4-12), the increase in the concentration of NaCl led to a decrease in the content of *P. oleracea* L of vitamin A, where the percentage of vitamin A in the control group was (883.73 IU), while its percentage in the rest of the groups was (651.07, 430.07 and 328.4) IU, we conclude that increase in salinity led to a significant decrease in the vitamin content. From the results of the statistical analysis, there is a clear significant difference.

Studies of salt stress effect on vitamin A content of vegetables are scarce. Carotene concentrations are commonly determined as these vegetable yellow pigments are converted to vitamin A in the human body and are accordingly refer to as provitamin A (Ratnakar and Rai, 2013), In the current study, the content of vitamin A in *Portulaca oleracea* significantly decreased with the increase in the concentration of NaCl in the growing medium, WouYou *et al.*, (2017) study on *Amaranthus cruentus* showed a decrease in the amount of vitamin A at 30 mm of NaCl , It was also observed in *Trigonella foenum-graecum* that the content of vitamin A decreased under salt stress (Rapala-Kozik *et al.*, 2008) .

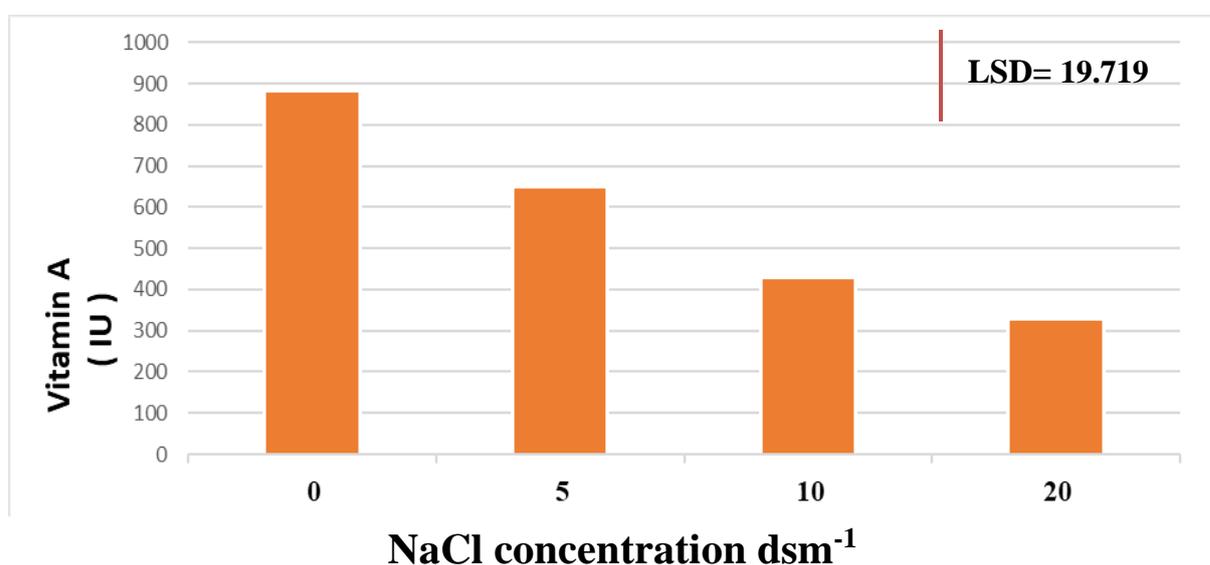


Figure (4-12) Effect of NaCl concentration on content of Vitamin A (mg/g.f.w) in *P. oleracea* L

4.4 : Fatty acids

The table below shows the values of the seven important fatty acids that were isolated and their values measured in the plant, and the values of the extent of the decreasing amount of these acids in the plant appear with the increase in the salt concentrations to which the plant was exposed, according to what is shown in front of each of them.

Table (4-4): Salinity Effect on Fatty acids content in *P. oleracea* L

Treatments	Control	5 dsm ⁻¹	10 dsm ⁻¹	20 dsm ⁻¹	LSD (0.05)
Parameters (%)	Mean±S.D				
a- linolenic acid	8.33±0.80	6.93±1.05	5.57±0.60	4.77±0.65	1.573
Ecosopentaenoic	7.80±0.80	6.63±0.90	5.33±0.65	4.40±0.55	1.467
Docosahexaenoic	9.17±0.85	7.90±0.95	6.50±0.55	5.77±0.65	1.501
Linolic acid	10.77±0.70	9.47±1.01	7.87±0.70	7.43±0.55	1.502
Palmatic acid	4.47±0.65	3.50±0.72	2.53±0.51	1.97±0.51	1.134
Oleic acid	10.47±1.06	8.87±0.85	7.77±0.65	7.07±0.70	1.530
Stearic acid	4.90±0.85	3.83±0.70	2.80±0.50	2.27±0.45	1.215

P. oleracea L is one of the richest plant sources of polyunsaturated fatty acids, the most important of which is the omega-3 linolenic acid (Yan *et al.*, 2009) , Environmental conditions affect the content and compositions of fatty acids in plants (Yeilaghi, *et al.*, 2012) , The findings on the effect of salt stress on fatty acid content are contradictory in purslane. (Carvalho *et al.*, 2009)

Table (4-4) Shows the fatty acid values of *P. oleracea* L by exposure to saline concentrations, From the above data, The results of data analysis of fatty acids in the seven fatty acids that were detected in *Portulaca oleracea* L under control conditions and saline pressure, as it appears that the greater the plant's exposure to salt, the lower the value of fatty acids, and the results of the statistical analysis showed a significant difference and the value of LSD is as shown in front all kind of acids .

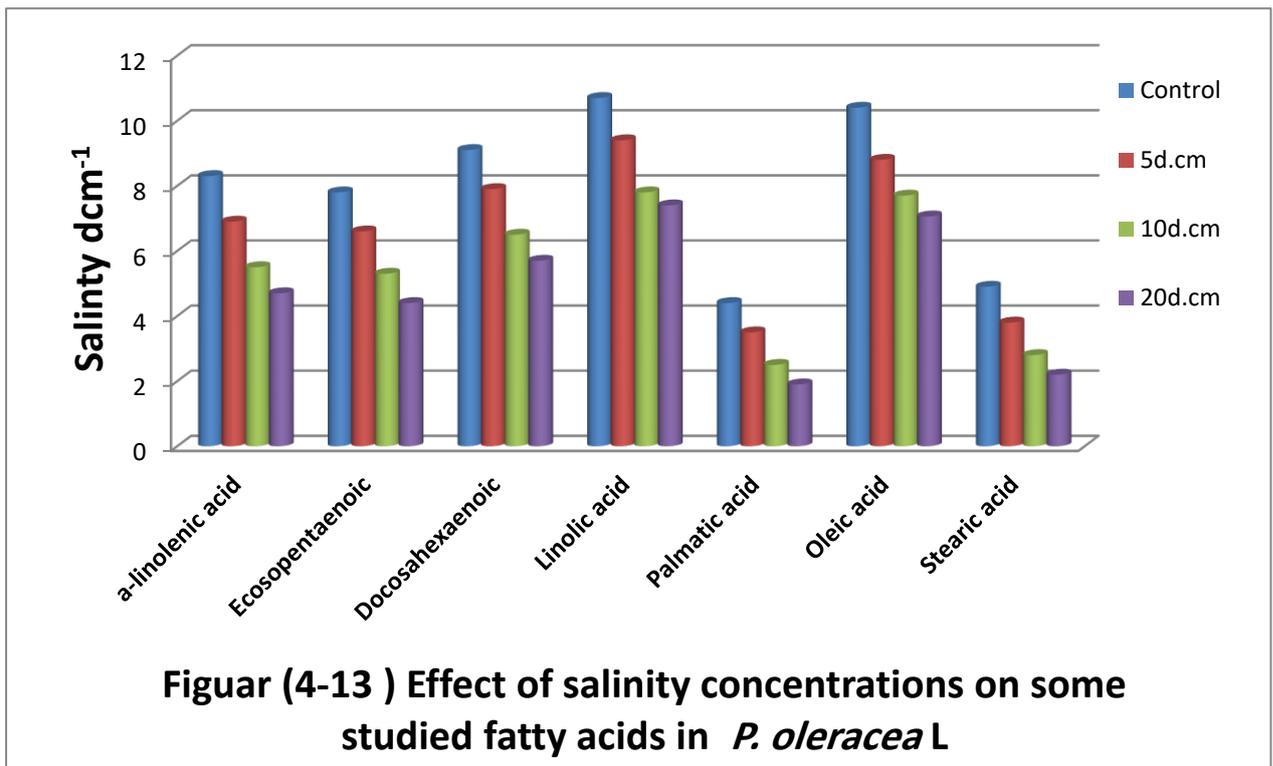
From previous studies, salinity changes the composition of fatty acids and is very important in stress tolerance for plants (Malkit *et al.*, 2002), Salt

tolerant species have the ability to adjust membrane lipid fluidity by increasing the proportion of unsaturated fatty acid (Anastácio and Carvalho, 2013) Free alpha-linolenic acid is itself a salt stress signal (Ivanova *et al.*, 2016) At higher levels of plant salt exposure decreased lipid synthesis in purslane leaves (Teixeira and Carvalho 2019), (Szalai *et al.*, 2010) indicated the effect of fatty acid values on purslane leaves with the variety and their exposure to sunlight, also (Zaman *et al.*, 2019) found that at 200 mM of NaCl stress, the percentage of ω -6 and ω -3 acids decreased significantly in the leaves of the purslane plant.

The lipid peroxidation of leaves was suppressed at 70 and 140 mM NaCl by increasing the ability to scavenge reactive oxygen species. Therefore, the ability of purslane to withstand the effects of salinity stress in leaves depends on a balance between the accumulation of total fatty acids and chemical degradation. Salt concentrations up to 120 mM NaCl also affected the total fat content (Anastácio & Carvalho. 2013)

a study Yeilaghi *et al.*, (2012) on *Carthamus tinctorius* L showed that all fatty acids in the plant were affected Significantly, under pressure and increase in salinity, and also under stress conditions, the oil content in olives decreased and the fatty acid composition also changed. Generally, salinity stress induces inactivation of PSI and PSII, Unsaturated fatty acids in membrane lipids shelter PSI (first photovoltaic system) and PSII (second photovoltaic system) inactivation as one of effective protective strategy. Where it affects dually; alleviating the salinity induced damage to PSI and PSII and improving the healing of injury (Allakhverdiev *et al.*, 2011) These high amounts of Linolic acid present in the leaves of *P. oleracea* of plants of the control group are unusual. Previous studies have reported 17% (Liu *et al.*, 2000), 18% (Pa'ez *et al.* 2007) and 24% (Palaniswamy *et al.* 2001) of this percentage in the total fatty acids detected.

This study specifically identified salt stress as an important factor affecting both the concentrations of fatty acids in the purslane plant and thus its effect on the nutritional value. Severe salt stress reduces the concentration of monounsaturated fatty acids, present results expanded our knowledge of the composition and proportions of fatty acids in purslane under salt stress, and the extent of the effect of salt on the nutritional value of purslane rich in fatty acids.



4.5: Effect of aqueous and alcoholic extracts of *Portulaca oleracea* L on some types of bacteria and yeast

A study of the effect of cold aqueous extracts and warm alcoholic extracts of *Portulaca oleracea* plant on some types of bacteria *E.coli*, *Staph.aurues* and yeast, which included *C.albicans*,

The effect of different concentrations of cold and hot aqueous and alcoholic extracts, as well as extracts from the salt groups that were previously

studied, was studied on the tested microorganisms. Table (4-5) and when the values of the inhibition areas for the cold aqueous extract for the control group and the rest of the three groups reached (12.44 , 4.5 and 0 , 0) mm, respectively, at the concentration (600) mg / ml to reach (13.44, 6.5 and 1.5,0) mm Respectively at a concentration of (1000) mg / ml, The hot alcoholic extract was significantly more effective compared to the cold aqueous extract when the inhibition zone reached (17.33) ml at a concentration of 1000 mg/ml compared to (13.44) for the cold aqueous extract at the same concentration. It does not dissolve well in water, it dissolves in the presence of organic solvents such as ethanol and methanol (Abo-Shanab *et al* ,. 2004), While the decrease in the inhibitory activity of the types of extracts with the increase in salt concentrations can be explained by the decrease of the active substances in the plant such as alkaloids, flavonoids and terpenes, and as we indicated for the results of this study , Previously, all of these compounds were reduced with an increase in salt concentrations, thus reducing the content of the extract and reducing its inhibitory ability. The hot alcoholic extract was significantly more effective compared to the cold aqueous extract when the inhibition area reached (17.33) ml at the concentration of 1000 mg / ml in the control group compared to (13.44) for the cold German extract in the control group and respectively at the same concentration, the reason may be due In addition, many effective plant compounds that do not dissolve well in water dissolve in the presence of organic solvents such as ethanol and methanol (Abo-Shanab *et al*,.2004)

This is consistent with what Al-Dhahab , (1998) found that ethyl alcohol has a high ability to withdraw active compounds from the plant sample due to its high polarity. It also agrees with Tepe *et al*,.(2004) that the alcoholic ethanolic extract of sage had a high inhibition effect against *Staphylococcus aureus* and *C. albicans* yeast were less affected than bacteria of the alcoholic

extract and it could explain the inhibitory activity of the *Portulaca oleracea* plant because it contains some active compounds such as alkaloids, terpenes, tannins, flavonoids, resins and soaps, as shown in Table (4-6). (Hassan *et al* ,.2000) indicated that soaps reduce sugar inside the bacteria which leads to cell death.

The results showed that the toxic effect of the plant depends on the method of extraction, the concentration of the extract and the type of bacteria, as shown by the statistical analysis. (Augustit ,1996) indicated that the alcoholic extract of the plant *Portulaca oleracea* has high activity against *Staphylococcus* positive bacteria and activity against one negative bacteria *E.coli*.

Also (Parkh and Chanda. 2016) indicated that the reason for the discrepancy in the inhibitory activity of the extracts is due to the variation in the method of extraction, the type of solvent and the nature of the membranes of the microorganisms. The plant was exposed to it.

Table (4-5) Effect of type of the extract (1000 mg / ml) of *P.oleracea L* on some microorganism

Extract 1000 mg / ml		Isolation type											
		<i>Staph aureus</i>				<i>E.coli</i>				<i>C. albicans</i>			
		Salinity dsm^{-1}											
		0	5	10	20	0	5	10	20	0	5	10	20
Inhibition	cold aqueous	12.5	4.5	0	0	9.33	1.5	0.00	0.00	0.00	0.00	0.00	0.00
	warm alcoholic	16.33	10.5	2.5	0	8.5	3.02	0.00	0.00	11.26	5.5	0.00	0.00

LSD(0.05) for extract*bacteria.*treatment=0.719

Table (4-6) Effect of type of the extract (600mg / ml) of *P.oleracea L* on some microorganism

Extract 1000 mg / ml		Isolation type											
		<i>Staph aureus</i>				<i>E.coli</i>				<i>C. albicans</i>			
		Salinity dsm^{-1}											
		0	5	10	20	0	5	10	20	0	5	10	20
Inhibition	cold aqueous	13.20	4.5	0	0	6.5	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	warm alcoholic	17.06	9.5	2.5	0	8.91	2.5	0.00	0.00	9.83	6.5	0.00	0.00

LSD(0.05) for extract*bacteria*treatment=0.358

The above results are in agreement with it in the response of bacteria *Staphylococcus aureus* (Gram-positive) to the effect of the extract more than *E.coli*. This may be due to the structural structure of the bacterial wall, as Gram-positive bacteria lack an outer membrane layer that makes the permeability of materials to the interior of the cell greater compared to Gram-negative bacteria Hassouni & Liqaa. (2013)

In order to reach the most efficient plant extract, the results were compared. The results of the statistical analysis showed that there were significant differences at the probability level (0.5) in the effect of the cold water extract and the hot alcoholic extract of paraben on the tested organisms, and as shown in Table (4-6), it was The hot alcoholic extract had the most effect on *Staph aureus* and *E.coli* and in the control group when the diameter of inhibition was (17.06) mm on *Staph aureus*, at a concentration of (600) mg/ml, and also, the diameter of inhibition was (9.33) on *E.coli* bacteria, while the was hot alcohol extract at a concentration (1000) mg/ml on the control group has

the highest activity on *C. albicans* yeast when the inhibition diameter is (11.26) mm.

Conclusions

&

Recommendations

Conclusion

Conclusion

1. Despite of *P.olearacea* L. is a halophyte , but it can't tolerant the elevated concentrations of salinity.
2. Elevated salinity reduce medical important of *P. olearacea* L. as a source to antioxidant substances
3. The value of *P.olearacea* L. as a source to omega-3 fatty acids decrease according to the level of salinity.
4. *P.oleracea* L. to action as a microbial inhibitor depended on each of type of extraction , salinity concentration in the environment whre plant lived in it.

Recommendations

- 1) Study the effect of salinity on *P.oleracea* L genome.
- 2) Study how can reduce salinity stress on plants by interacting it with another environmental factor.
- 3) Study the effects of pollution on some important medical vegetables.

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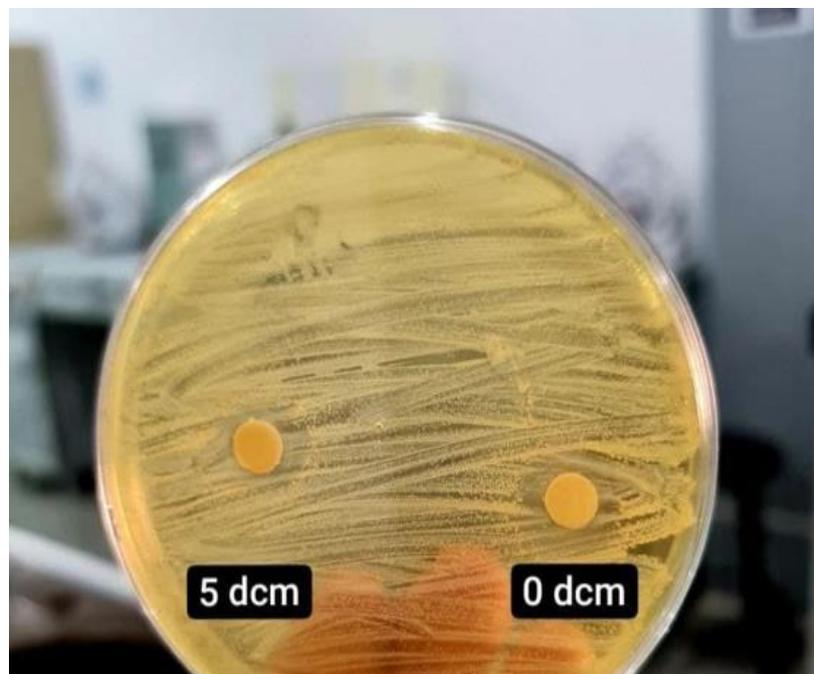
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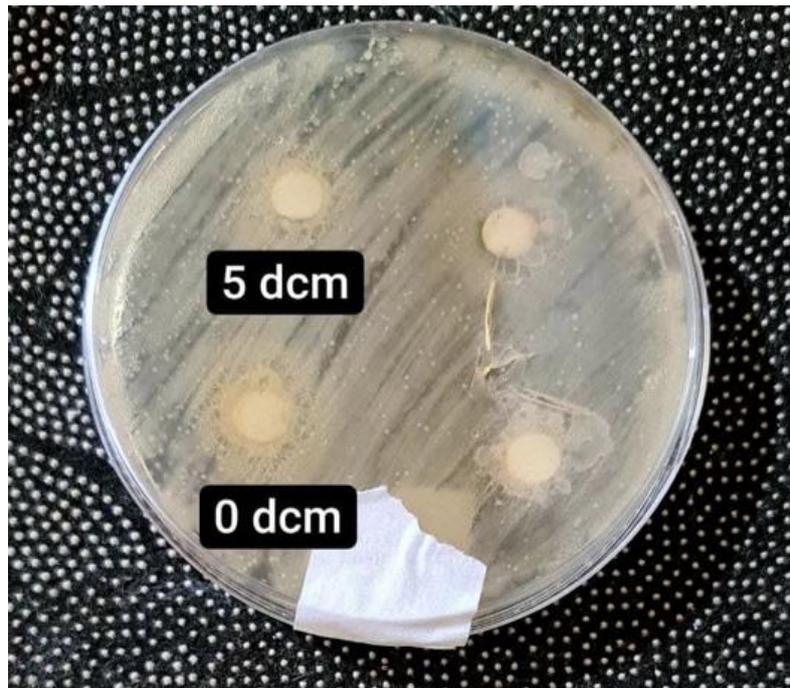
Appendices



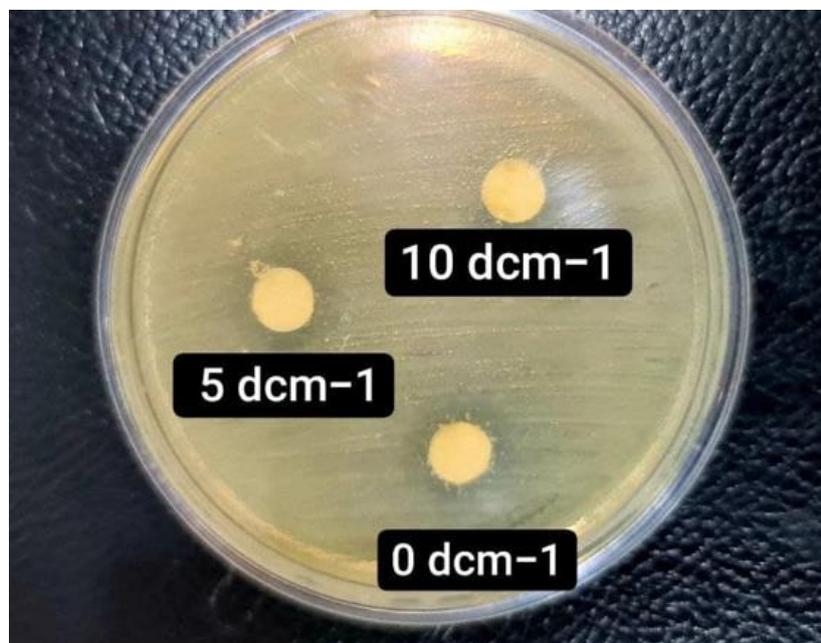
Appendix No. (1) The inhibition of *staph aureus* by warm alcoholic of *P. oleracea L* in concretion 1000mg/ml



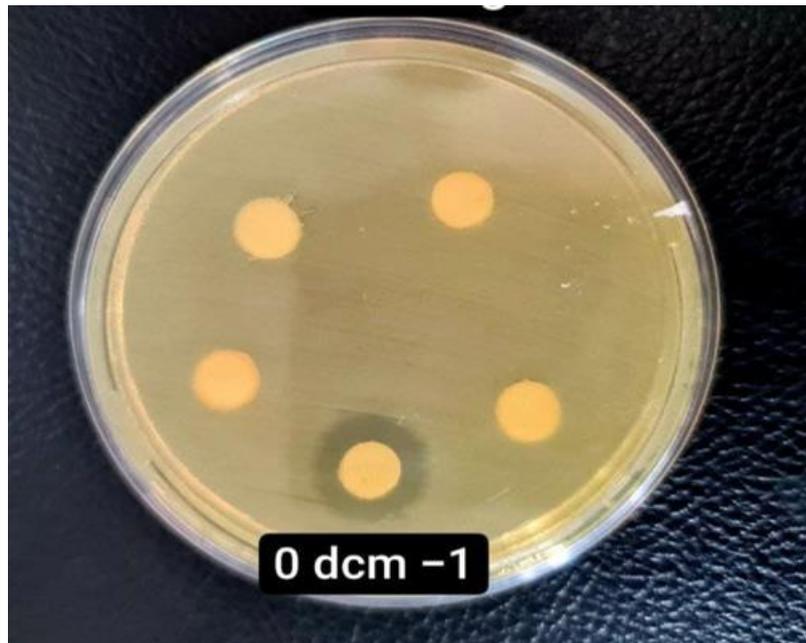
Appendix No. (2) The inhibition of *staph aureus* by cold aqueous of *P. oleracea L* in concretion 1000mg/ml



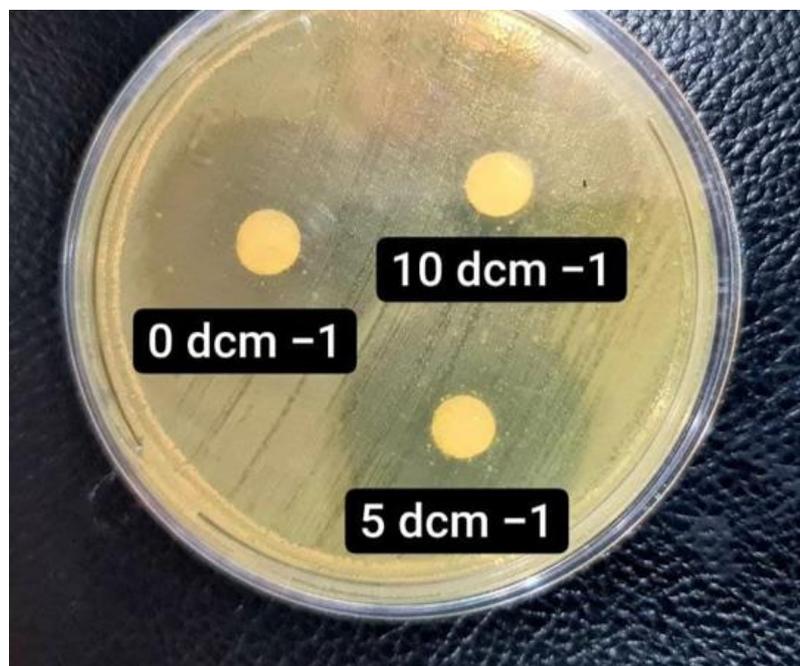
Appendix No. (3) The inhibition of *E.coli* by cold aqueous of *P. oleracea L* in concretion 1000mg/ml



Appendix No. (4) The inhibition of *staph aureus* by warm alcoholic of *P. oleracea L* in concretion 600mg/ml



Appendix No. (5) The inhibition of *E.coli* by cold aqueous of *P. oleracea L* in concretion 600mg/ml



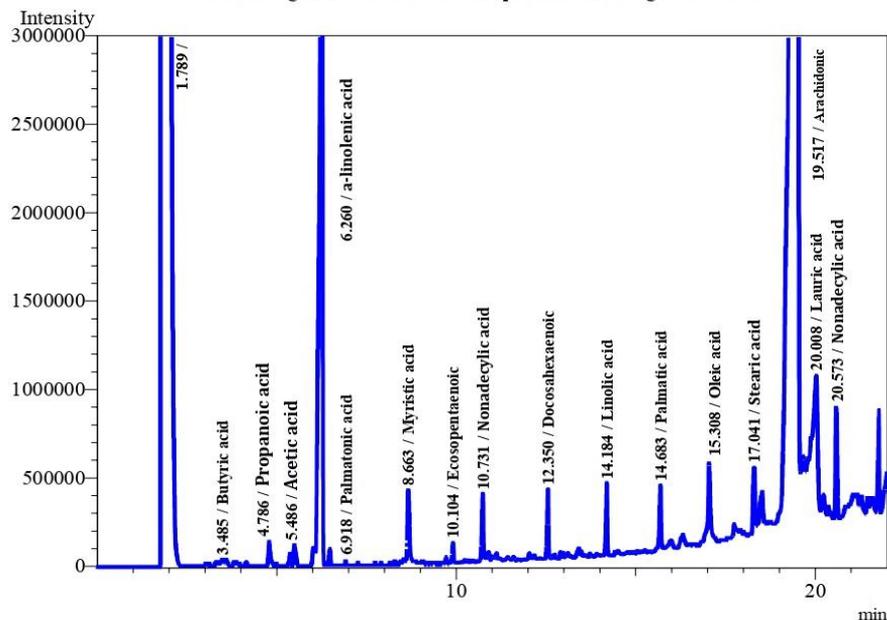
Appendix No. (6) The inhibition of *staph aureus* by warm alcoholic of *P. oleracea L* in concretion 600mg/ml

Appendices

Sample Information

Sample Name = FAME 0.5 %
 Injection Volume = 1 uL
 Tem Injector = 280 C
 Tem Detector (FID) = 330 C
 Column Oven (ZB-5) = 100 (hold 1 min) - 300 c (hold 2 min) (10 c / min)
 pressure= 100kpa

Chromatogram C:\GCsolution\Sample\Data1L3056.gcd - Channel 1



Peak Table - Channel 1

Peak#	Ret.Time	Area	Area%	Height	Name
1	1.789	3757736973	96.5843	79923849	
2	3.485	78248	0.0020	17354	Butyric acid
3	4.786	446524	0.0115	123856	Propanoic acid
4	5.486	498134	0.0128	111467	Acetic acid
5	6.260	24476180	0.6291	4119974	a-linolenic acid
6	6.918	53932	0.0014	27438	Palmitonic acid
7	8.663	1139321	0.0293	366464	Myristic acid
8	10.104	283710	0.0073	113578	Ecosopentaenoic
9	10.731	1006479	0.0259	366429	Nonadecylic acid
10	12.350	841200	0.0216	351995	Docosahexaenoic
11	14.184	1099883	0.0283	404266	Linolic acid
12	14.683	1081835	0.0278	355923	Palmitic acid
13	15.308	469989	0.0121	64080	Oleic acid
14	17.041	1863019	0.0479	425211	Stearic acid
15	19.517	91285767	2.3463	7436458	Arachidonic
16	20.008	5090487	0.1308	630853	Lauric acid
17	20.573	1915554	0.0492	625830	Nonadecylic acid
Total		3890628660	100.0000	95811193	

Appendix No. (7) fatty acids isolated from *P. oleracea* L by HPLC device

Appendices

05/04/2021 01:13

Chromatogram F:\A 1.PRM

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Chromatography Laboratory

HPLC, GC

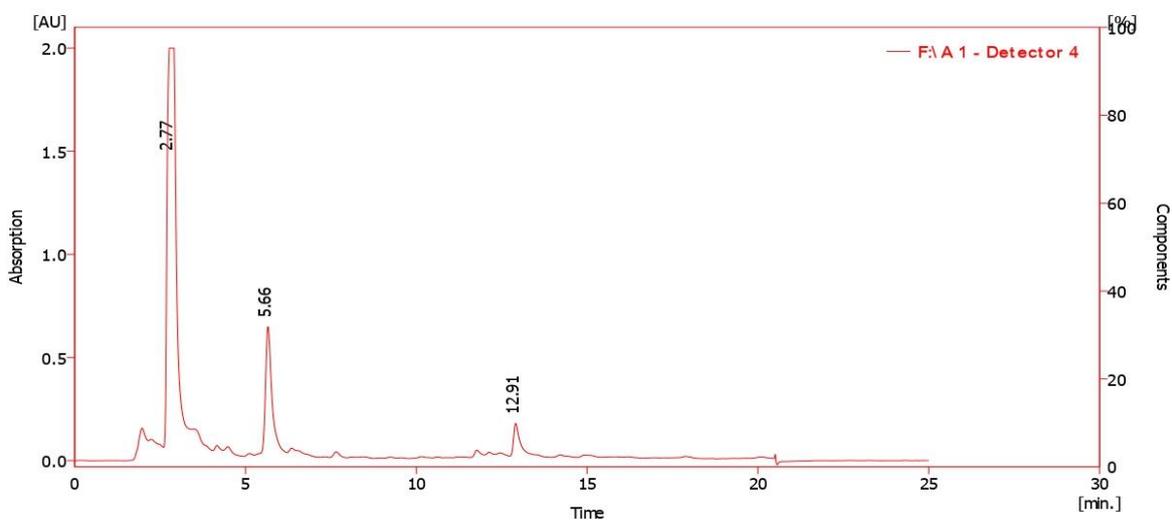
Sample Info:

Sample ID : A 1
 Sample : A 1
 Inj. Volume [mL] : 0.01

Amount : 0
 ISTD Amount : 0
 Dilution : 1

Autostop : 25.00 min
 Detector 1 : Detector 4
 Subtraction Chromatogram : (None)

External Start : Start - Restart, Down
 Range 1 : Bipolar, 2000 mAU, 10 Samp. per Sec.
 Matching : No Change



Result Table (Uncal - F:\A 1 - Detector 4)

	Reten. Time [min]	Area [mAU.s]	Height [mAU]	Area [%]	Height [%]	W05 [min]	Compound Name
1	2.770	22358.356	1591.975	84.3	75.5	0.26	
2	5.657	3575.475	437.584	13.5	20.8	0.14	
3	12.907	586.305	78.119	2.2	3.7	0.11	
	Total	26520.136	2107.678	100.0	100.0		

Appendix No. (8) Vitamin A isolated from *P. oleracea* L by HPLC device in C₁ (5 dcm⁻¹)

Appendices

05/04/2021 01:16

Chromatogram F:\B 1.PRM

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Chromatography Laboratory HPLC, GC

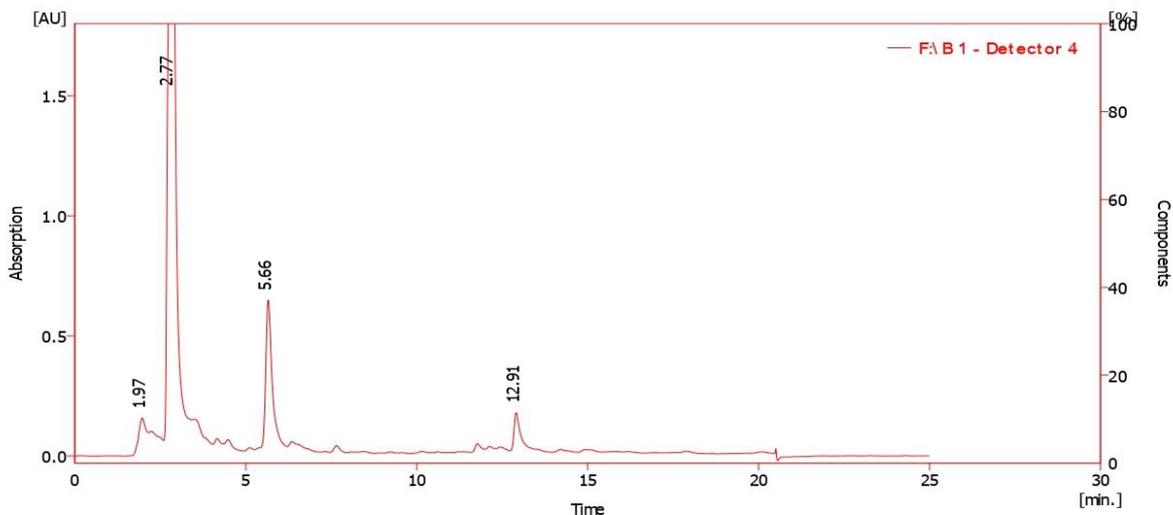
Sample Info:

Sample ID : B 1
Sample : B 1
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ISTD Amount : 0
Dilution : 1

Autostop : 25.00 min
Detector 1 : Detector 4
Subtraction Chromatogram : (None)

External Start : Start - Restart, Down
Range 1 : Bipolar, 2000 mAU, 10 Samp. per Sec.
Matching : No Change



Result Table (Uncal - F:\B 1 - Detector 4)

	Reten. Time [min]	Area [mAU.s]	Height [mAU]	Area [%]	Height [%]	W05 [min]	Compound Name
1	1.970	898.628	87.989	7.2	5.9	0.18	
2	2.770	7810.540	905.185	62.4	60.9	0.18	
3	5.657	2870.542	381.195	22.9	25.7	0.13	
4	12.907	943.584	111.424	7.5	7.5	0.14	
	Total	12523.294	1485.793	100.0	100.0		

Appendix No. (9) Vitamin A isolated from *P. oleracea* L by HPLC device in C_2 (10 dcm^{-1})



Chromatography Laboratory HPLC, GC

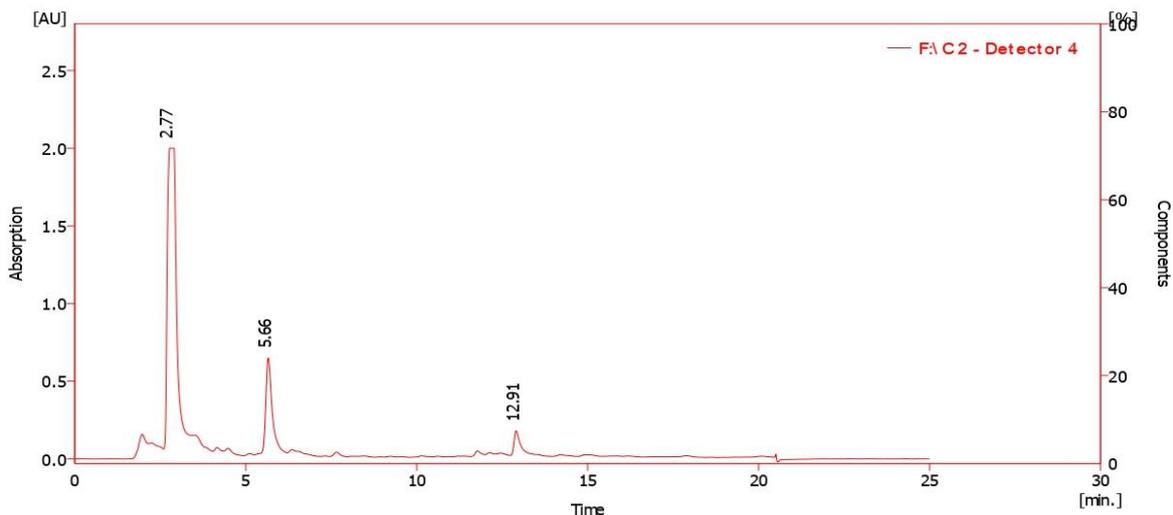
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Sample ID : C 2
 Sample : C 2
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Amount : 0
 ISTD Amount : 0
 Dilution : 1

Autostop : 25.00 min
 Detector 1 : Detector 4
 Subtraction Chromatogram : (None)

External Start : Start - Restart, Down
 Range 1 : Bipolar, 2000 mAU, 10 Samp. per Sec.
 Matching : No Change



Result Table (Uncal - F:\C 2 - Detector 4)

	Reten. Time [min]	Area [mAU.s]	Height [mAU]	Area [%]	Height [%]	W05 [min]	Compound Name
1	2.770	4382.439	553.337	69.9	63.7	0.19	
2	5.657	1307.034	238.022	20.9	27.4	0.10	
3	12.907	578.854	77.951	9.2	9.0	0.11	
	Total	6268.327	869.310	100.0	100.0		

Appendix No. (10) Vitamin A isolated from *P. oleracea* L by HPLC device in C₃ (20 dcm⁻¹)

Appendices

05/04/2021 01:25

Chromatogram F:\D 2.PRM

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Chromatography Laboratory
 HPLC, GC

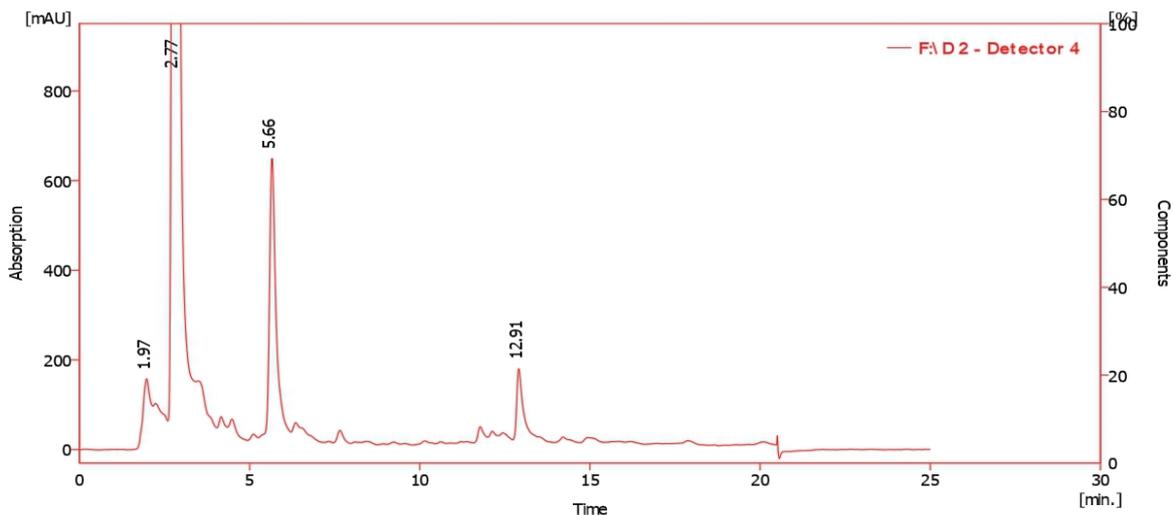
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Sample ID : D 2
 Sample : D 2
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Amount : 0
 ISTD Amount : 0
 Dilution : 1

Autostop : 25.00 min
 Detector 1 : Detector 4
 Subtraction Chromatogram : (None)

External Start : Start - Restart, Down
 Range 1 : Bipolar, 2000 mAU, 10 Samp. per Sec.
 Matching : No Change



Result Table (Uncal - F:\D 2 - Detector 4)

	Reten. Time [min]	Area [mAU.s]	Height [mAU]	Area [%]	Height [%]	W05 [min]	Compound Name
1	1.970	1897.001	119.978	4.3	4.4	0.27	
2	2.770	34101.646	1914.523	77.2	69.4	0.28	
3	5.657	6050.072	567.442	13.7	20.6	0.17	
4	12.907	2149.787	154.778	4.9	5.6	0.18	
	Total	44198.505	2756.722	100.0	100.0		

Appendix No. (11) Vitamin A isolated from *Portulaca oleracea* by HPLC device in Control (0 dcm^{-1})

الخلاصة

هدفت الدراسة الحالية لمعرفة تأثير مستوى الملوحة على محتوى نبات البربين *Portulaca olearacea L.* من المركبات ذات الأهمية الغذائية و الطبية اذ زرعت بذور النبات في أصص نباتية ثم سُقيت بماء الحنفية و بعد مرور 10 أيام تم تقسيم تلك الاصصن الى أربعة مجاميع ليتم سقيها حسب حاجة النبات بمحاليل dsm^{-1} (0,5,10,20) و أستمر سقي تلك المحاليل لمدة ٣٠ يوم حتى بلغ النبات مرحلة بداية التزهير و في نهاية تلك المدة تم قياس كل من الكلوروفيل a,b والكلوروفيل الكلي والكاروتينات و البروتين الكلي والسكريات الكلية ومضادات الاكسدة والبرولين والاحماض الدهنية وفيتامين C وفيتامين A والالكلويدات والفلافونويدات .

أظهرت النتائج أن قيم الكلوروفيل أ ، ب تراوحت بين (1.39 - 0.83) و (0.96 - 0.54) (ملغم / وزن الجسم) على التوالي ، بينما انخفضت في كل من (dsm^{-1} 10) و (20 dsm^{-1}) مع زيادة تركيز الملوحة. وكمية السكريات الذائبة الكلية أعلى قيمة (127) بتركيز (dsm^{-1} 0) ، وتقل القيمة (67.53) ميكروغرام / غم ، عند التركيز (dsm^{-1} 20) ، كما أظهرت الفحوصات أن المجموع البروتين هو أعلى قيمة (31.29) في التركيز (dsm^{-1} 0) ، وأقل قيمة (3.69) (dsm^{-1} 10) g / gFW in ، وقيمة Proline هي أعلى قيمة (2.12) في (dsm^{-1} 20) ، القيمة الأقل هي (0.47) ole / gFW عندما يكون التركيز (0 dsm^{-1}) ، الكاروتينات هي أعلى قيمة (1.24) في (dsm^{-1} 0) ، أقل قيمة هي (0.39) (dsm^{-1} 5) (mg / mg gFW) في (dsm^{-1} 5) ، وأعلى قيمة لمضاد الأكسدة الكلي هي (16.27) في (dsm^{-1} 20) ، وأقل قيمة هي (5.13) (ملغ AEE / g.F.W) عند التركيز (dsm^{-1} 5).

أظهرت نتائج فحوصات المركبات الأيضية الثانوية أن أعلى قيمة لفيتامين أ (IU 883.73) عند (dsm^{-1} 0) بينما أدنى قيمة هي (IU 328.4) عند (dsm^{-1} 20) ، بينما أعلى قيمة لإجمالي الفلافونويد هي (0.84) (mg Rutin / gm) كانت عند (dsm^{-1} 5) و أقل قيمة كانت (0.27) عند (dsm^{-1} 20) ، وأعلى قيمة من إجمالي القلويات (3.48%) عند (dsm^{-1} 5) والحد الأدنى للقيمة (2.05%) عند (dsm^{-1} 20). ان فعالية نبات الرجلة كمثبط للبكتريا قد

الخلاصة

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اختلفت حسب تركيز النبات وكذلك نوع المستخلص , ولقد أظهرت نتائج الدراسة ان اعلى
تثبيط لبكتيريا (*Staph aureus*) قد كانت (10.5) مم عند المستخلص الكحولي الحار , وقد
سجل نفس المستخلص مدى تثبيط اقل على خميرة (*C. albicans*) واقل منه على بكتيريا
(*E.Coli*) وقد بلغ التثبيط (5.5) و (3.2) على التوالي في النباتات المروية في (5 dsm^{-1})



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جامعة بابل / كلية العلوم
قسم علوم الحياة

**تأثير الملوحة على بعض مركبات نبات *Portulaca oleracea* L.
وتأثيره التثبيطي على بعض الكائنات الحية الدقيقة**

رسالة

مقدمة إلى مجلس كلية العلوم / جامعة بابل
جزءاً من متطلبات نيل درجة الماجستير في العلوم / علوم حياة

قدمها

ضرغام خالد ناصر تاج الدين
بكالوريوس علوم حياة / جامعة المثنى

اشراف

أ.م.د بتول محمد حسن