

Phytochemical and biological studies of *Spergularia diandra* and *Spergularia marina* (Caryophyllaceae) growing wild in western Iraq

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Abstract

The present research dealt with an anatomical and chemical study comparing the *Spergularia Diandra* and *Spergularia marina* Caryophyllaceae family. The leaf, stem, and root also showed clear anatomical properties in terms of the cross-section shape and thickness of both the epidermis and cortex in both species. Some effective chemical compounds in the aerial part, Phenols, Saponin, glycoside, Flavonoid, and tannin, were detected, positive detection was found in them except for the alkaloids that were appeared in *Spergularia Diandra* only. Phenols were also quantitatively estimated, and *Spergularia marina* exceeded its quantity by 515.4 mg\ 50g plant, while in *Spergularia Diandra* it reached 461.75 mg\ 50g plant. It is considered suitable materials suitable to differentiate between species. The results of lymphocytes showed that the phenolic compounds extracted from plants at a concentration of 0.31 mg/ml had a significant positive effect on the viability of the human lymphocytes implanted, with significant differences at the probability level ($P < 0.01$), as *Spergularia marina*, was superior, as it gave a vital percentage of 700% while *Spergularia Diandra* was less than 600%.

Key words: Active , Iraq, Materials, plant, lymphocytes ,wild

Introduction

The Caryophyllaceae pink family or the pink clove family is one of the large families, as it contains 2100 species belonging to almost eighty genera as reported by Lawrence in 1951(Chakravarty,1976). This came identical to what (Al-Rawi,1964).mentioned, and the two studied species are one of the species of the family that spreads in several regions from Iraq especially western ones (Sheikh and Ahmed,2006).*Spergularia diandra* is characterized by the stem- leaves (the diameter of

the blade is 0.8 - 1.0 mm), the stamens are 2 and rarely the 3 stamens while *Spergulariamarina* the leaves are cylindrical in shape the diameter of the blade is 1.7 - 2.6 mm, the stamens are only 5 (Rechinger,1964).*Spergulariamarina* plant takes several synonyms, the first of which was the equivalent name *Spergulariasalina*, then it was transferred to the genus *Arenaria*, and Linnaeus considered it a variant multiplication under this name *Arnariarubra* L. var. *marina* L. Then under the name *Arenariamarina* (L.)All. And finally, according to (Flora of Egypt) mentioned, all the names mentioned earlier are names that are equivalent to the *Spergularia marina*(The kateb,2000).Rechinger was Described *S. diandra* and *Spergularia marinain* 1964 , which would become in different regions within the Western Desert, Diyala, Baghdad, Rumaila, and areas in Basra and Safwan, as indicated by the spread of *Spergulariadiandra* in the cities of Ramadi, Rutba, Baghdad, DhiQar Governorate, and Basra in Nukhaib Umm Qasr, he also mentioned the presence of the species *S.marina* in the cities of Ramadi, Qaim, Basra, and the Sanam Mountains. She studied many species of the family, the two were anatomically and morphylocally, which grows in western Iraq and in the Western Desert there (Musa and Al-Nasery,2016). The researchers stated that most species of Caryophyllaceae contain saponin, which gives foam when mixed with water (STEVANOVICet al.,2019). Toxic substance would be achieved if injected into the blood; Because it removes and breaks the red blood cell membrane, causing hemoglobin to emerge (Iriadam et al.,2006), confirmed that this article contains family species. It also contains species of the pink family together the two species *Spergulariadiandra* and *Spergularia marina* on the pigments of the flowers and their colors. The yellow color represents Flavonoids (PARK et al.,2020). which is about pigments insoluble in water found in the juice of cells and chloroplasts That most species in the pink family contain steroids and steroids, which are among the natural products that are widely spread in animal and plant tissues and Glycosides, which are water-soluble solids and most of them taste.The study aimed to shed light on the anatomical and chemical characteristics that differentiate between the two species.

Materials and Methods

Plant Collection: The study relied on soft vegetable samples (stem, leaves and flower), collected during field trips to western Iraq, Al-Qaim City during the spring of the year 2018 and 2019. Some of which were preserved with alcoholic solution 70%. The anatomical study: relied on the soft vegetable samples collected from the field and some

dry plant samples. The anatomical study covered the stems, leaves and roots, the waxy method was adopted (Al-lami, 2002), and was photographed using a compound microscope with the camera for imaging and on the power of magnification (40 X). General Phytochemical disclosures: "in order to test the General Phytochemical takes 50g of powder of two studied species were weighed and placed in a 1 liter glass beaker and 500 mL of ethanol alcohol was added 75% concentration and left for a week in the dark" after which the marinated filtrate was filtered and filtered drying was done for each plant separately using Rotary evaporator at 45°C, dry product weight, and kept in dark containers until the rest of the analyzes and disclosures (Xing et al., 2019):

- **Tannins:** adding 1% drops (3-5) of lead acetate solution to 2ml of the extract. When a gelatinous precipitate is formed, this indicates a positive detection.

- **Glycosides:** To complete the test, mix (1 ml) of the plant's alcoholic extract with (2ml) of Benedict reagent, forming an orange precipitate, with a reference to the presence of glycosides.

- **Flavonoids:** To complete the test, mix (1ml) of sodium hydroxide solution with the extract of the plant and leave it for a short period, when a clear yellow color appears, indicating the positive.

- **Alkaloids:** For detection, the alkaloids of the stock alcohol extract are added to the plant and Dandruff's reagent, so if the precipitate appears to be a nutty orange, it indicates the presence of alkaloids.

- **Saponins:** Shake the extract (alcoholic) well when it was notice foaming evidence of the presence of Saponins

- **Phenolic:** Phenols were measured by adding 1 ml of ferric chloride with a concentration of 1% to 3 ml of alcoholic extract. When the color turned to bluish green, it was a positive sign of "the test both the presence of phenols and the total value was estimated according to the method (Freshney, 2012) in which the solution turns blue with absorption at a wavelength (765nm) it can be estimated by a UV-VIS spectrometer compared to a phenolic acid that was prepared by dissolving" 2mg of Gallic acid in 10 ml of pure methanol where the concentration becomes 200 mg/ml. Preparing of sodium carbonate [7.5% $\text{CO}_3 \text{Na}_2$] by dissolving 7.5% g of Na_2CO_3 in 100 ml of pure methanol. I prepared 6 dilutions of the standard solution of calcium acid in a group of tubes and in different concentrations, left after completing the two-hour isolation steps in dry and dark

conditions, after that the absorbance of each concentration was measured at length 760 Nm vs. blank solution by spectrophotometer.

The effect of total phenols extracted from the plants on normal human blood lymphocytes cultures.

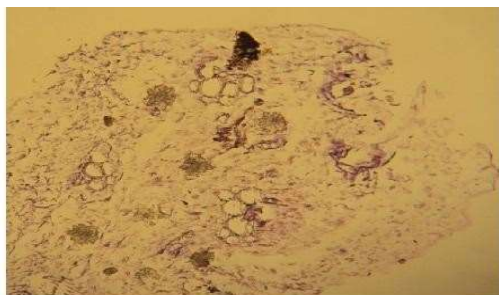
The effect of total phenols extracted from plants on the cultures of normal human lymphocytes by the MTT method was studied by treating the cells implanted in the Micro Titer Plate 96-Well, U-Bottom which contains 96 holes and 7 different concentrations of phenols were added. Repeats for each concentration, then compared with the control model, which included 12 holes, represented by lymphocytes without treatment (Freshney,2012), Stock solution was prepared from total phenols at 250 mg in 25 mL distilled water to a final concentration of 10 mg / mL and 0.31 mg / mL of stock was prepared.

RESULTS AND DISCUSSION

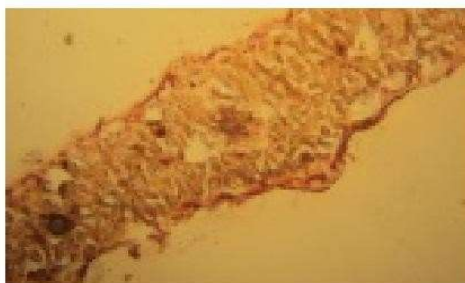
Anatomical study

The leaf :

The epidermis represents the first layer in the plant, which is covered by a protective waxy layer of Cuticle, which is added in the form of a continuous and soft outer layer. The thickness differed between the two species studied, with an average thickness of 2.6mm in *S. marina* and 3.6mm in *S. diandra*, and the next layer, which represents The intermediate tissue did not distinguish into a clear, palisade and spongy region within the section. Rather, it was a homogeneous and small cells that leave between them interstitial spaces rich in green chloroplasts, and with a large number, as they appear within the section, or they are large or giant cells lengthy or elliptical



Spergularia marina



Spergularia diandra

Fig. (1) : Transverse section of leaf 40x

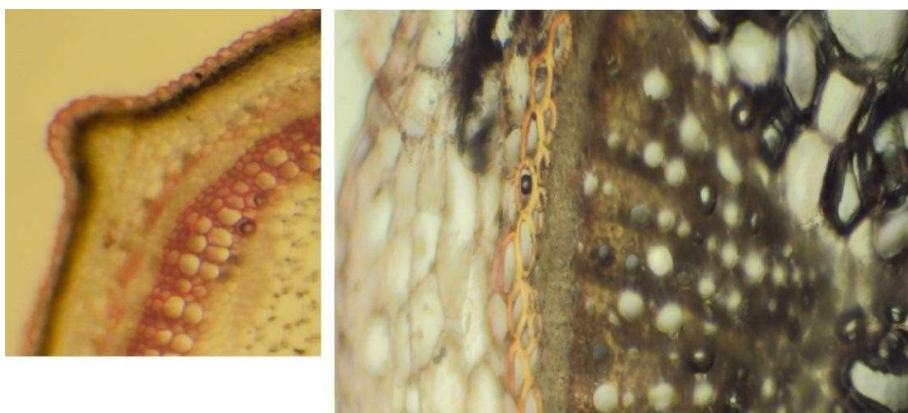
The nature of leaves tends a lot to the nature of succulent leaves with delicate tissues and a high water content more than other species in the same family(Tohma et al.,2018).have indicated the scarcity of the existence of this pattern in plants with two groups, this pattern is often observed or The style Dorsiventral leaf(AL-Rajabet *al.*,2018).As for the vascular bundles, they are the central one-bundle located in the center of the section. The numbers of xylem and phloem arms varied in their numbers by two species , as shown in Table (1), and in their thickness and sizes.

Table(1): The dimensions of the leaf blade of measured in Micrometers.

Species	Cuticle thickness	Epidermis thickness	Vascular Bundle diameter	Mesophyll thickness	NO. arms of xylem	NO. arms of phloem
<i>S. diandra</i>	3.6	54.8	976	890	4	4
<i>S. marina</i>	2.6	57.5	1326	1085	6	5

The stem

Stem epidermis was a single layer, followed by inward several rows of parenchyma cells representing the Cortex. The number of layers ranged between 4 layers in *S. marina*, and 3 layers in *S. diandra*, and there is no specific endodermis. Which was represented by a ring of outer fibers, including lignified narrow cavities, more than the cells inside, which were distinguished by the capacity of their cavities and their thickest walls. The sclerenchyma ring is also a variation, depending on the size of the cells. The number of rows is not important in the nature of the thickness, the Vascular Cylindrical has the advantage of being ring-shaped and there are no separate vascular bundles as it is common in most dicot plants. phloem formed a continuous ring inside the section, and in this capacity the wood is also accompanied by the direction towards the center, where the first xylem. Protoxylem, is inward. Phloem recorded a thickness of 70 Mm in *S. marina* and 55 in *S. diandra*. This is what was also observed in the same species spread in the Western Desert of Iraq(Issaet *al.*,2020).



Sparganium angustifolium . *Sparganium angustifolium*

Fig. (2) Transverse section of stem 40x.

As for the region of Pith which occupies the center and consists of thin-walled and large parenchyma cells as we go towards the center of the section where its size has increased until its walls have been crushed as the plant ages, so the stems of it have become hollow later, and they differed in thickness shown in table (2).

Table (2) The quantitative characteristics of stems measured in Micrometers..

Species	Epidermis thickness	Cortex thickness	Cortex layers	Xylem arms	Xylem thickness	Phloem thickness	Pith thickness
<i>S. diandra</i>	25	75	3	6	122(Ring)	55	27
<i>S. marina</i>	28	78	4	8	131(Ring)	70	42

Root

The roots were distinguished in both species by being cylindrical roots and tap roots, the average thickness of the skin is 13 micrometers in *S. diandra* and 10 Mm in *S. marina*, and the cortex area that followed the skin was wide and was characterized by flat oval cells to irregular shape characterized by the presence of starch granules in Its cells, *S. diandra* recorded a thickness of that reached 134 Mm, either the bark was in the form of regular rectangular or oval cells and interfering with the epidermis cells with ripples, and then the cambium with a narrow area unlike the xylem that occupied a large area of the vascular cylinder and its transport units were characterized by the formation of dicots or groups of The units may reach up to six transporting units per group. As for the *S. marina*, the cells of the cortex appeared large, irregular in shape, followed by inward oval cells with a thickness of the cortex of 186 Mm.

Table (3) The quantitative characteristics of Roots, measured in Micrometers

Species	Epidermis thickness	Cortex thickness	xylem thickness	Phloem thickness
<i>S. diandra</i>	31	134	425	55
<i>S. marina</i>	33	186	407	38

The phloem appeared in the form of rises and depressions linked by the rectangular parenchyma cells of the cortex, whereas the cambium appeared in the form of rectangular and undulating cells that occupied a narrow space of the cylinder, while the transporting elements in the xylem texture seemed wide to many, and the beams of xylem of a single row were Uniseriate and the xylem was occupied in it The center of the root is in the *S. diandra*, while vessels were less thick and number in *S. marina*, and the marrow region in the two species, where the wood occupied the largest space, in other areas near the Jordanian border, the two species appeared with the lowest number and capacity in the xylem vessels (Qasimet *al.*,2017).All these differences in anatomical characteristics are an important issue in separating the species and studying the relationship of the environment to the anatomical traits and variations of that family.

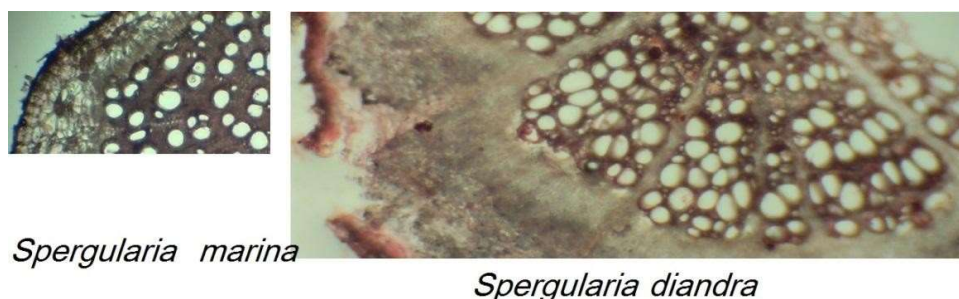


Fig. (3) Transverse section of root 40x.

Chemical study

The chemical study showed great significance, as it was found through the study that the two species that are considered plants in the fields of folk medicine, ancient and modern, as it showed between the initial detection of the two species that they contain phenolic compounds, flavonoids, glycosides, tannins, Saponins and other active substances while alkaloid of the *S.marina* disappeared.

Table (4) Phytochemical study of Aerial part of species extract.

Species	Phytochemical compounds					
	Alkaloid	Saponin	Flavonoid	Glycoside	Tannins	Phenols
<i>S.marina</i>	-	+	+	+	+	+
<i>S. diandra</i>	+	+	+	+	+	+

The results of the investigation showed phenolic compounds in table (5) and fig. (4,6) , which are among the important chemicals that are used in the field of plant classification and have the importance in establishing developmental relationships(Mohammed *et al.*,2020). The results showed the difference between the two species in the amount of phenols, as the *S.marina* outperformed with a total phenolic rate of 515.4 mg/50 g plants, compared to *S. diandra* in which the percentage of phenols was 461.7 mg /50 g.

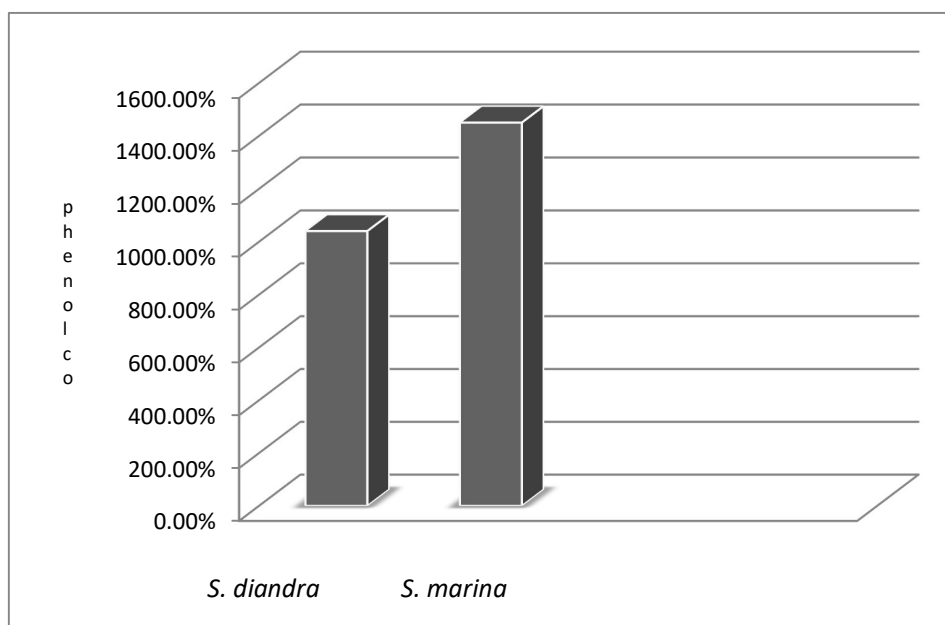


Fig. (4): The percentage ratio % of phenol in two species.

Table (5) Absorption value of total phenolic compounds in each species .

Species (2.5mg/ml)	Absorption(760nm)
<i>S. marina</i>	0.223
<i>S. diandra</i>	1.532

Table (6) The amount of total phenols (mg \ 50 mg) of each plant

Species	<i>Spergularia marina</i>	<i>Spergularia diandra</i>
Mg of phenols	515.4	461.75

Phenolic compounds are found in many wild plants, especially the vegetative part of them ^(18,19) . The wild plants of western Iraq are distinguished by the abundance of phenolic compounds in them, are used taxonomically to differentiate between them and these compounds are important for human health; This is because they protect cells from damage by the action of their antioxidant properties, which are closely related to their chemical composition⁽²⁰⁾, and play a very important role in preventing inflammation and anti-cancer ⁽²¹⁾.

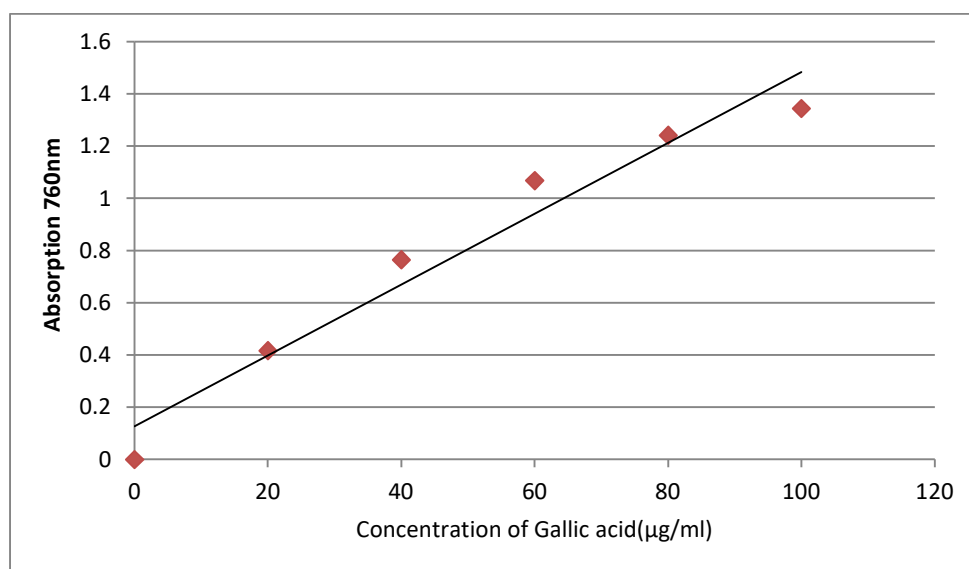


Fig. (5) Gallic acid standard curve

Effect of phenols on lymphocyte cultures

The study showed that the phenolic extracts of the aerial parts of plants contain phenolic acids as well as flavonoids, which have proven their biological and physiological efficacy to improve immunity, especially after noticing the increase in the number of lymphocytes when treated with these extracts, especially when we used a medium concentration between high and low, The results of the statistical

analysis of *S. marina* extract showed an increase in the vitality of human lymphocytes and significant differences at ($P < 0.01$), while the extract of *S. diandra* plant showed that it had the least effect. As noted in a table(7).

Table (7): Effect of plant-derived phenols(0.31 mg\ml) on normal human lymphocyte viability LSD = 0.0425.

Species	Viability%
<i>S. marina</i>	700
<i>S. diandra</i>	600
Control	300

The discovery and identification of new drugs that can enhance immune function has become an important goal of research in pharmacology and immunology, especially if they are from a natural source (vegetable) and have fewer side effects. The test for the inhibitory effect on normal cells is one of the basic things that must be investigated when studying the effect of A substance for the purpose of using it as a treatment, and among the most important of these cells are immune cells, especially lymphocytes, which have the ability to divide outside the body under the influence of cleavers ⁽²²⁾. This study agrees with the study by ⁽²³⁾ which demonstrated that phenols and flavonoids help increase human lymphocyte activity and protect them from damage and from oxidative stress.

Conclusion

Some effective chemical compounds in the aerial part, Phenols, Saponin, glycoside, Flavonoid, and tannin, were detected, positive detection was found in them except for the alkaloids that were appeared in *SpergulariaDiandra* only. The leaf, stem, and root also showed clear anatomical properties in terms of the cross-section shape and thickness of both the epidermis and cortex in both species. Some effective chemical compounds in the aerial part, Phenols, Saponin, glycoside, Flavonoid, and tannin, were detected, positive detection was found in them except for the alkaloids that were appeared in *SpergulariaDiandra* only.

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