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The Phytochemical Constituent Of Capparis Spinosa L. And Phenolic Activity On Pathogenic Bacteria And Blood Parameters

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ABSTRACT

Capparis Spinosa L. is one of the world's most edible plants that has provided researchers' attention in their study and has therapeutic value.. Therefore, That is why the most significant biologically productive compounds have been identified to be, namely (tannins, phlobatannins, Saponins, flavonoids, phenols, and glycosides). As the results of the detection showed the availability of excellent quantities of active chemical compounds in both parts and the compound Phlobatannins disappeared in the flowers, while the compound flavonoids did not appear in the fruits. Phenols were also studied in a spectrum to reveal the number of the secondary components that they consist of. The Spectral absorption was studied within the boundaries of the visible and ultraviolet region at the wavelength of 765 nanometers in to determine the basic groups in them. The number of components of phenols was six, while the fruits had three curves representing the types of secondary active compounds of phenols. The biological effectiveness of fruit and flower phenols was also tested against some bacterial pathogens by testing the lowest concentration of MIC inhibitor and the concentration of 100 fruits exceeded all the concentration against the isolates of bacteria that were used in the test, and the effectiveness of phenols in their effect on clotting time outside (vitro) and inside (vivo) , anti-coagulant effectiveness. And an important factor in blood flow were tested, the flowering exceeded the fruits by giving a ratio of clotting time of 4.33 minutes.

Keywords: Anti-coagulant , edible plants, flavonoids, The Spectral, MIC

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INTRODUCTION

Due to the global trend of expanding the use of plant raw materials in the manufacture of pharmaceutical preparations instead of chemicals with harmful side effects, the use of those raw materials with appropriate active substances and the appropriate dose has become more beneficial and safer in the treatment of many diseases⁽¹⁾ The Capparis Spinosa is a perennial shrub whose height ranges between (30-80) cm, and most of it lies on the ground, unless there is something attached to it, it can grow high, the evergreen plant has a bluish green color, the branches are creeping or its outgrowth, easy to break, the leaves are thick with ears Thorny, large flowers bloom in the morning in white color with a tendency to pink and wither before noon, giving a beautiful red color⁽²⁾ The fruit is fleshy in shape resembling a pear and is carried on a long neck, and when the fruit ripens it turns from yellowish green to crimson bright and its taste is sweet from the inside and bitter from Outside⁽³⁾ C. spinosa plants are one of the richest plants with active substances. The plant contains flavonoids, terpenoids and alkaloids.⁽⁴⁾ It also contains resins, glucosinolates, coumarins, saponins, tannins, sterols, in addition to organic acids and fatty acids⁽⁵⁾ Relieve stomach pain if properly prepared with vinegar.⁽⁶⁾ The roots are used as a diuretic, which is astringent, appetizing, menstrual, tonic, and repellent to intestinal worms. It is also used for treating infections, and foreskin is used to treat rheumatism in the joints.⁽⁷⁾ As for the flower buds, they are used as an organ regulator, diuretic and tonic, and for the treatment of scurvy and specialists in alternative medicine believe that C. spinosa is at the forefront of medicinal plants that should be taken care of by those who are keen to recover from rheumatic diseases, high blood sugar, and people with bloating and

liver disorders⁽⁸⁾ As the study aimed to detect the chemical content (Phytochemical Screening) of C. Spinosa for overland growth in Iraq and to diagnose some classes of chemical compounds present in it for the purpose of evaluating it from the chemical point of view, extracting phenols for quantitative evaluation of flowers and fruits and studying the inhibitory effect of them against some pathological bacteria, And its effect on some blood parameters such as blood clotting outside and inside the organism

Materials and methods

Collecting plant samples: The flowers and fruits of the plant were collected from the city of Habbaniyah - Iraq during the flowering season and the fruits of the plant from the month (3-6) of the year 2018, the species was identified on the basis of the keys mentioned in⁽⁹⁾ dried And milled and kept in dark bottles until use. Phytochemical analysis: The presence of many chemical substances in the pulverized plants samples of the Standard methods have been used such as tannins, flavonoids, saponin, phlobatannins, glycosides, alkaloids, and phenolic compounds⁽¹⁰⁾ Spectral study of phenols: in this test Weighed 50 grams of powdered flowers and fruits, each one of plant, and put it in a 1L glass beaker, add 500 ml of 75% ethanol alcohol to it, and filter soaked after a week of storage with filter paper, and were measured in UV apparatus between (200-600) nm lengths to appeared the peak⁽¹¹⁾ The effectiveness of phenols against bacteria : Pathological bacterial isolates were obtained from the laboratories of the College of Science - University of Baghdad namely (Pseudomonas aeruginosa, Staphylococcus aureus, Escherichia coli, Klebeslla sp., Bacillus subtilis) and after activating them with the nutrient broth, the previously extracted fruit and

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leaves phenols were placed at (2.3×10^9) cells of bacteria / ml in sterile test tubes according to the law (first concentration x first volume = second concentration x volume Second) and kept at a temperature of 37C for a period of (24) hours, as the MIC value of phenols is considered against the strains of bacteria, and the minimum dilution that inhibits the growth of bacterial pathogens after an incubation period of (24) hours, after which a bacterial sample of each extract was grown on the media of feeding⁽¹²⁾

Measuring the clotting time of blood

used in this test small glass tubes of similar size with a diameter of 1cm washed in distilled and sterile water, as it was coated from the inside with the fruit and flowers phenols at a concentration of 0.5 mg, then left in the laboratory atmosphere to dry completely, then blood was drawn from the radial vein by using a plastic syringe from a donor, as the venipuncture must be performed quickly and in a good manner, and the watch was set as soon as the blood entered the vein. Each tube (0.5) ml was filled with blood, after which the tube was closed with cotton, then placed in a water bath with a degree of (37) meters, then the tube was tilted at an angle of (45) degrees to see if the blood had clotted, and monitored every (15) seconds until the thrombus was seen, with Set the time and then record the clotting time⁽¹³⁾

Bleeding time in viva

In this study (12) laboratory mice of the Balb.c strain were used from males only, and their weights were (30 ± 5) gm at the age of about three months, and were divided into four groups that include each group (3) Mice, and the mice were placed in clean laboratory cages in the animal house of the Department of Life Sciences at the College of Science / University of Baghdad, at a temperature of (30 ± 2) C, and prepared with a special food pellet and quantities of water to ensure the need. The groups were numbered and dosed at a concentration of 0.5 mg of phenol. The fruits and flower phenols separately, at a volume of (0.4) ml, once during the day, at a rate of three replicates for each species for a week, and the fourth group was dosed with distilled water of the same size and represented the control group⁽¹⁴⁾

The bleeding time

was measured according to the method of⁽¹⁵⁾ since the mouse's tail was sterilized and then an incision was made with a sterile scalpel, as the blood should flow easily without the need to compress the mouse's tail, and adjust the time when the blood drops came out, and after (30) seconds the drop was taken The first blood was taken from the filter paper while avoiding touching the sin with the paper, then the other blood drops were taken every (30) seconds, arranged one after the other, until the size of the drops gradually became smaller, and when the blood flow stopped, the time was determined by the method of calculating the number of drops on the filter paper, then a procedure The process of multiplication by thirty seconds⁽¹⁶⁾

Platelet Count

Determine the number of platelets according to the visual method for the whole blood. As the count was done in one cubic millimeter, (400) microliters of ammonium oxalate solution were taken in a test tube, and then 0.02 milliliters of blood was placed by means of a capillary tube. A red mark was added to the content of the test tube, then the tube was shaken well and quietly and left (10) minutes after which it was placed on the counting slide Neubaer improved chamber and the slide was transferred to a glass dish with a wet cotton and the plate closed and after (10) minutes, the platelets were counted

Statistical analysis

The complete random design was adopted in the biological experiment with a probability level of 0.05 and with the least significant difference.

RESULTS AND DISCUSSION

Phytochemical analysis

The chemical disclosures in Table (1) showed the presence of a number of biologically important compounds. The compounds appeared (tannins, **Phlobatannins**, saponins and flavonoids, phenols and glycosides) and these results were identical to what was mentioned in previous studies⁽¹⁷⁾ which shows the vital and therapeutic importance. Which is characterized by this plant due to the effectiveness of these compounds in the physiological and defense processes against the bacterial and fungal pathogens that infect the living organism.

Table (1): The chemical detection of flowers and fruits of *Capparis Spinosa*

No.	Bioactive materials	Reagent used	Color after detection	Fruits	Flowers
1	Tannins	1% ferric chloride	bluish green color	+	+
2	Glycosides	Benedict's reagent	precipitate red	+	+
3	Phlobatannins	1% hydrochloric acid	red precipitate	+	-
4	Saponins	distilled water mercuric %1 chloride	White precipitate Foam	+	+

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5	Flavonoids	M3 ammonia solution	yellow color	-	+
6	Phenols	potassium freecyanide	dark bluish green	+	+

The presence of active groups in natural flowers and fruits such as flavonoids, alkaloids, glycosides, Phlobatannins and Saponins indicates great importance and reveals their pharmacological importance that was used in the past and still is. As an excellent antioxidant, it makes the plant effective against many bacterial pathogens⁽¹⁸⁾ and effective against mycotic poisoning⁽¹⁹⁾ in addition to its therapeutic importance as a pain reliever and a nerve agent⁽²⁰⁾

The quantitative and spectral concentration of phenols

The total amount of phenols present in both *C. pinosa* in fruits and flowers, whose color was dark yellow, was estimated using a spectrophotometer at the wavelength

Table (2): The concentration of phenols in the fruits and flowers of the *C. spinosa* mg/g of the plant

adjectives	fruits	flowers
Phenol concentration mg / g plant	0.51	0.89

Secondary metabolites in plants give an important role in the vital activities of the organism, as they act as antioxidant compounds that repel free radicals and have nutritional and therapeutic value at the same time⁽²¹⁾ as a result of what this plant contains of chemical components and nutrients, making it one of the important medicinal plants that support the body's immunity. They prevent

of 765 nm, depending on the concentrations of the standard lioncalc solution, as the results of the examination of phenolic compounds showed the superiority of flowers In the amount of phenols, which amounted to 0.89 mg/g, while the quantity in the fruits was 0.51 mg/g, the number of active ingredients was calculated in the urea device based on the presence of peaks curves, and this is a good evidence for the presence of many types of phenols in *C. spinosa* plant, and the spectroscopic examination showed the superiority of flowers in the injustice of the secondary active compounds and they were 7 compounds. As for the fruits, they were only three and as shown in Figures (1 and 2).

the abnormal division of the cells of the organism, which causes many diseases, and inhibit many mutations by removing free radicals that interfere with the genetic material⁽³⁾ and prevent the growth of some microscopic pathogens such as bacteria, due to its chemical content, which also supports the work of some Important organs in the body such as the liver and bile⁽²⁾

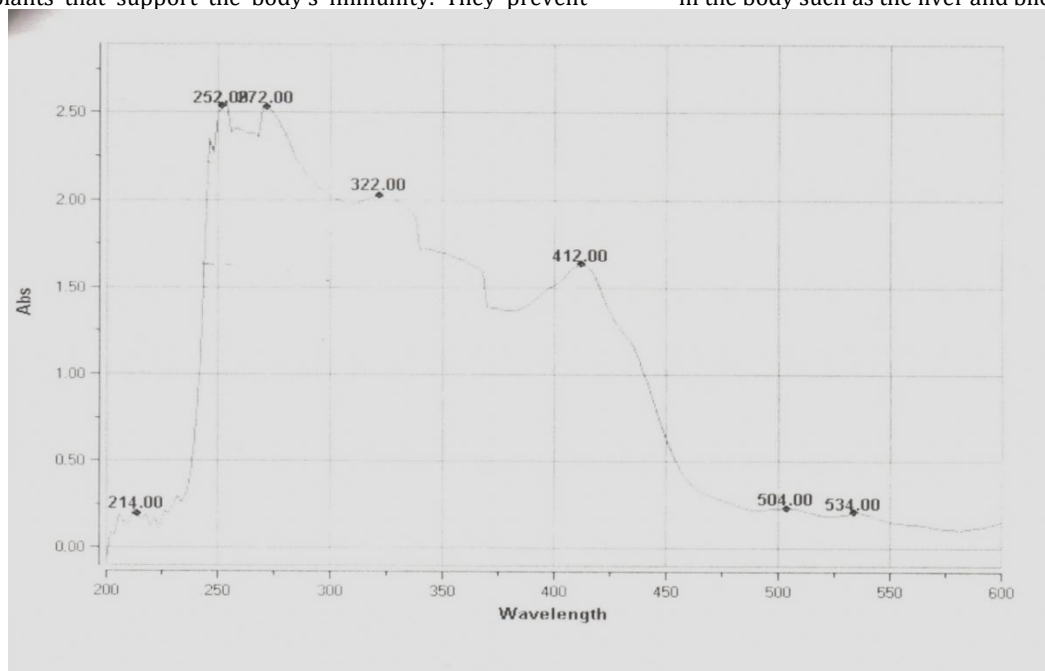


Fig 1: Ultraviolet spectra of total phenolic from n flower 7 compound

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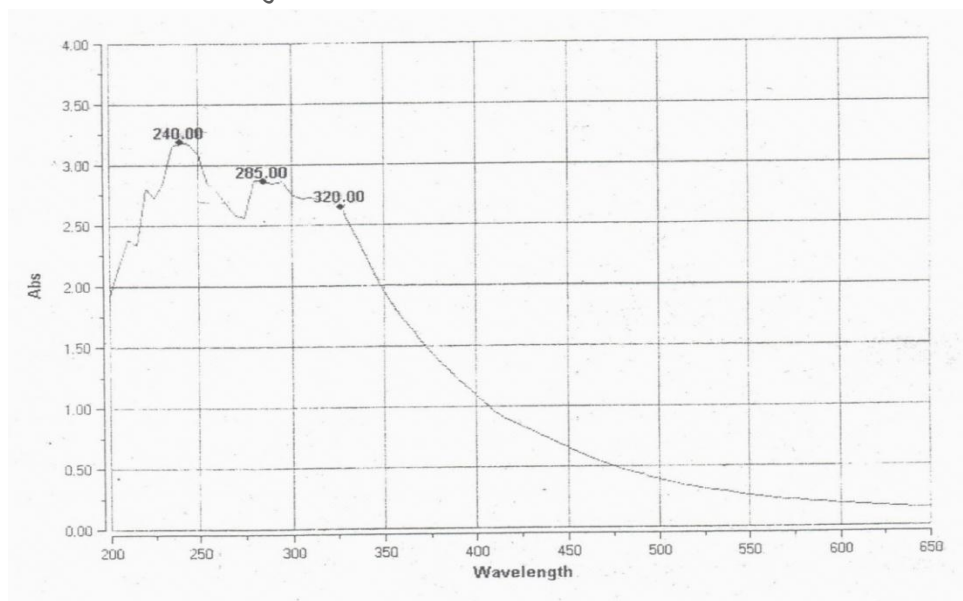


Fig 2: Ultraviolet spectra of total phenolic from n fruits 3 compound

Testing of the minimum concentration of bacterial inhibition (MIC)

The MIC values were investigated for the phenols of the flowers and fruits of the *C. spinosa* plant against the pathogenic species, and there was a discrepancy in the values recorded against the pathogenic species, as shown in Table (2). Phenols have the highest inhibitory value, reaching 23mm.

Table 3 : Minimum inhibitory concentration MIC of phenols of *C. sponosa* flowers and fruits. LSD= 0.325

Tested bacteria	MIC(mg/ ml ⁻¹)				Control (70 %)	
	Fruit phenol		flower phenol		Hexane	Ethanol
	50	100	50	100		
<i>Pseudomonas aeruginosa</i>	22	23	20	23	7.5	6.2
<i>Staphylococcus auris</i>	11	12	11	14	7.3	5.9
<i>Escherichia coli</i>	13	15	8	19	8	11.5
<i>Klebsella sp.</i>	14	15	16	20	8.8	9.3
<i>Bacillus subtilis</i>	10	13	9	12	6.6	5.4

The reason may be attributed to the ability of phenolic compounds with high concentrations to affect the viability of pathological bacteria⁽⁴⁾ because the biologically active compounds work to reduce the activity of negative and positive bacteria such as *S. aureus*. The effect of the active ingredients of *C. spinosa* is due to an increase in the readiness of active substances such as phenols, glycosides, tannins and other substances that have a role in alternative treatment and the elimination of pathogens such as bacteria and fungi⁽⁶⁾. The results of the study showed and through the results we noticed that the majority of phenols, regardless of their source from the plant, have shown efficacy against bacteria, and this indicates an increase in the concentration of the active components in the reproductive parts represented by the flower and the fruits, to which the medicinal or physiological effect of the plant and its medicinal value are attributed. *C. spinosa* are one of the richest herbs with active ingredients, because of the diversity in the tremendous chemicals found in plants their mechanisms of action are not fully known. On the basis of the different studies conducted on it appears that different phytochemicals target different levels of an organization ranging from the molecular level to the level of the organism, as well as in specific situations such as biofilms. The diversity of mechanisms of action demonstrated by

phytochemicals appear to be very promising in treating the problem of antibiotic resistance is often observed in pathogens causing infectious diseases. At the molecular level, various antimicrobial phytochemicals interact with different biomolecules present on the job site and thus modify them chemically and physically to the point where they lose their vital functions either partially or completely. During these phytochemical reactions or their own, the biologically active products are linked to many biomolecules and compounds such as protein and nucleic acid by active or non-covalent bonds⁽³⁾.

Measuring blood clotting time in vitro

The effect of phenols extracted from the fruits and flowers of the *C. spinosa* plant on the blood clotting time outside the body was tested, and the normal blood clotting time was recorded for (3.16) min. The clotting time of blood was recorded when using laboratory tubes coated from the inside with phenols extracted from fruits and flowers, a clotting time capacity of (4.0, 4.33) min. respectively, as we notice an increase in the clotting time when using the flowering phenol compared to the control as shown in Table 3. With the findings of previous studies, that *C. spinosa*, as one of the effective and medicinal ones, affect the aggregation of blood platelets, thus inhibiting their aggregation. Thus, thrombus formation is inhibited as the effect of Aspirin, Warfarin⁽⁴⁾.

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Table (4): Effect of fruit and flower phenols on coagulation time in vivo at a concentration of 0.05 mg.

adjectives	Phenols 0.5 mg		
	Control	Fruits	Flowers
Coagulation time outside the vivo minutes	3.16	4.0	4.33

The effect of phenols on clotting time in vitro

The results in Table (4) showed that administration of phenols to fruits and flowers through dosing to treated mice for seven consecutive days led to an increase in clotting time, bleeding time, and platelet count compared to the fourth group (control group). Significant differences were observed at <0.05 level. as the clotting time for the control group was recorded (0.30) minutes and (0.30) min. for the fruiting phenols, while the flowering results were (0.14) min. As for the bleeding time, the highest value was recorded for the fruits and reached (3.75) minutes, while in the control it was (0.28) min. , And the normal blood platelet count was (420,000) cells / mm³, while the fruiting phenols administration readings recorded an increase of (778000) cells / mm³. We note a direct relationship between the studied traits

and the increased concentration, and it was noticed that the number of blood platelets corresponds to the time required for the clotting and hemorrhage process. Stem cells which are the origin of platelet formation as well as the formation of other blood components of red blood cells and eggs. It also has the ability to increase blood fluidity by interfering with the function of platelets and thus increases the bleeding time and clotting time, which is in agreement with this. With what he reached ⁽²²⁾ This effectiveness of *C. spinosa* in phenols in preventing blood clot formation and in inhibiting platelet aggregation may be attributed to the chemical composition and amount of variation in the subtypes of phenols, which are a large group of biologically active chemical compounds that reduce platelet aggregation ⁽²⁾.

Table (4): The effect of fruit and flower phenols of *C. spinosa* on clotting time in the studied blood characteristics of laboratory animals at a concentration of 0.05 mg.

adjectives	Phenols 0.5 mg		
	Control	Fruits	Flowers
Bleeding time in vivo minutes	0.28	3.75	2.9
The clotting time outside the body	0.30	0.30	0.14
Platelet count (C / mm ³)	42000	778000	600000

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