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Phytochemical screening, in vitro antioxidant and antibacterial activities of methanolic extracts of *Capparis Spionsa* L. different parts from Tunisia

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Abstract

The present work consisted in preparing methanolic extracts from the leaves, flowers, fruits and roots of Capparis spinosa L by maceration, in order to study certain biological activities. Antioxidant activities were assessed against radicals DPPH and total antioxidant capacity (TAC). Moreover, we have determined the contents of phenolic compounds (total phenols, flavonoids, tannins and anthocyanins). The total polyphenols contents are variable according to the organ of the plant studied the results obtained, reveal that the leaf extract of Capparis spinosa L is the richest in total phenols 77.7 ± 1.1 mg EAG / g DW. The contents of flavonoids, tannins and anthocyanins recorded equal to 39.6 ± 0.6 mg EQ / g DW,11.4 ± 0.9 mg EC / g DW and0.33±0.01 mg cyanidin-3-glucoside equivalent / kg dry mass) respectively. Then, we evaluated the antioxidant activity by the method of the total antioxidant capacity (TAC) which shows that all the extracts have different antioxidant activities. The analysis of GC/MS of the methanolic extract of C.spinosa L. fruits has identified 19 organic compounds, three of which are the majority, namely: β-sitosterol (28.70%), Hexadecanoic acid (26.25%) and 9,12,15-Octadecatrienoic acid, (Z, Z,Z) (7.61%) are the major compounds of the extract fruits. The FTIR analysis of the fruit methanol extract confirms these three products. The analysis of the results of the antiradical activity of the organic extracts of Capparis spinosa L. The DPPH radical shows that extract of the leaves has the most important activity (70.10 ± 0.94 mg / L). Furthermore, we evaluated the antimicrobial activity of the methanolic extracts of different parts of C. spinosa by the radial diffusion method (RDA), this activity was revealed on five reference bacterial strains (*Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Escherichia coli*, *Salmonella* and *Enterococcus*).

1/ Introduction

In recent years, several researchers are focused on the valorization of traditional medicine, in sight to verify the safety and efficacy of the plants used. The caper is the common name of the genus capparis, family Capparidaceae, it is among the rare shrub species[1].

Capparis spinosa L is a xerophyte plant have a wide distribution in the word,such as South Europe, North and East Africa, Madagascar, Southwest and Central Asia to Australia, Oceania and widely found in the Mediterranean region [2][3].The genus Capparis includes more than 250 species, grows naturally in mountainous regions, adapts to poor soils with a remarkable adaptability to harsh environments[4][5].This plant species is of great interest for its medicinal/ pharmacological properties and its culinary uses[6] .In fact, C.spinosa roots leaves and fruits are traditionally used for the treatment of various diseases gastrointestinal disorders, skin diseases, earache, kidney and liver diseases [7]. As well, biological studies show significant activity: anti-oxidant, anti-inflammatory, immunomodulatory, antiviral, antiplaque antimicrobials, anticarcinogenic, antidiabetic and antihepatotoxic [8-11]. Capers are also known in traditional medicine for their diuretic effect, astringent and antirheumatic

properties[12]. In Iran people use the roots, fruits and bark of the plant for its diuretic, tonic, anti-malarial and articular diseases[13]. In India, the buds and roots of Capparis spinosa L are used in the treatment of boils and to treat fever, rheumatism, paralysis, toothache, the bark is used in the treatment of cough, asthma and inflammation[14] . In Indonesia, the root of this species is used as a diuretic and against bronchitis, and the wood is used to treat upset stomach[15]. A study in Croatia has shown that the caper has molecules that play an important role in the prevention of colon cancer[8]. Capparis spinosa contain many benefits of natural biomoleculesin different part such as spermidine, rutin, quercetin, kaempferol, stigmasterol, campesterol, tocopherols, Glucosinolates and carotenoids[16][17] and [18]. Besides, presence of this metabolites that can have important antioxidant activity.

In this context fits this present work, as part of a contribution to a better knowledge of this medicinal plant in the region of Tunisia. Thus, we have determined the content of phenolic compounds, flavonoids, condensed tannins of methanolic extracts from different parts of caper (leaves, fruits, flowers and roots).Then, we have studied the antioxidant activity of C.spinosa L by means of two tests: The free radical scavenging of DPPH (2, 2 - diphenyl-1-picrylhydrazyl) and the total antioxidant capacity ((TAC).On the other hand, Caper fruits have been used for centuries in traditional herbal medicine because they have important medicinal qualities and have been widely used as food and added in many dishes[19].Moreover, Tunisian rural population has forged solid links with this plant (fruits of C.spinosa L.) because it has many therapeutic properties that are carefully described in local surveys that why we focused our study in the composition by GC/MS of methanol extract. In order to confirm the major product of the fruit methanol extract, we study the FTIR of this extract. Finally, we evaluated in vitro the antimicrobial activity of extracts against some bacteria gram positive and gram negative by the RDA methods.

2/ Materials and Methods

2.1/ Plant material

Roots leaves and fruits of Capparis spinosa; the caper (Kebbar) were collected in June 2017 from Siliana in the north of Tunisia. The harvested plant material was washed with water, dried at room temperature until the weight was stable, after was milled into a fine powder and stored in a closed container before the analysis.

2.2/ Preparation of Extracts

The methanolic extracts were prepared successively by maceration. For this, 10 g of the powdered plant material (roots leaves and fruits) are macerated in 100 Ml methanol at room temperature for 48hwith stirring. The macerate was then filtered through Whatman No. 4 paper and finallythe filtrate is concentrated in a rotavapor at 40 ° C.

2.3/Determining phytochemical compounds of C. spinosa L.

2.3.1/Total phenols content (TP)

The total phenolic contents of methanolic extracts were determined by a colorimetric method, using the Follin Ciocalteu's reagent [20] and [21]. Each extract (0. 1mL) was mixed with Folin Ciocalteu reagent (0.2N). The mixture was shaken and incubated at room temperature for 5 min. Then was added with sodium carbonate solution (75 $g.L^{-1}$ in water; 0.4 mL). After 30 min in the dark, the absorbances were read at 765 nm against a water blank. Astandard calibration curve was carried at same operating conditions using gallicacid(0 - 300 $mg.L^{-1}$) as a positive control. The results were expressed as mg of gallic acid equivalent (GAE) /g dry weight (mg GAE/g DW). All samples were analyzed in six replications

2.3.2/ Total flavonoid content (TF)

The total flavonoid content was measured by using the $AlCl_3$ colorimetric assay [22]. 0.25 mL of each extract was dissolved in 1 mL of distilled water and 0.075 mL of 5% $NaNO_2$ was added. After 5 min, 0.075mLof a 10% (w / v) $AlCl_3$ was added. Left for 6 minutes, the solution was neutralised with 1 mL 4% NaOH and the total volume was adjusted to 2.5 mL with distilled water. The absorbance of the mixture was measured at 510 nm, after 15 min against prepared water blank and the quantification was done using the calibration curve.TF content of the extract was obtained by using quercetin as standard. The results were expressed as mg of quercetin equivalent/g of dry weight (mg QE/g DW).

Tests were carried out in triplications.

2.3.3/Condensed tannins content

Condensed tannins were determined by the vanillin method[20]. This method was based on the ability of vanillin to react with condensed tannin units in the presence of acid to produce colored complex measured at 500nm. 0.350 mL of each methanolic extract was added to 0.750 mL of the vanillin (1% in 7 M H2SO4) the mixture was allowed

to stand for 15 minutes. The absorbance was measured at 500 nm against a blank using a spectrophotometer. A calibration curve was performed in parallel under the same operating conditions using catechin a positive control. The results obtained were expressed as milligram(mg) catechin equivalent gram of dry weight (mg GAE/g DW).

2.3.4/ Total anthocyanin content

The anthocyanin content was estimated by pH differential method[23][24].

This method uses the property of anthocyanins to be in a colored form and in a colorless form depending on the pH of the medium. The absorbance of each sample was measured at 510–700 nm using two buffer systems: 0.025 M potassium chloride buffer (pH1 = 1, 0) 0.025 M and sodium acetate buffer (pH2 =4, 5). 0.4 M. 0.2 mL of each methanolic extract was mixed with 1.8 mL of corresponding buffers.

Absorbance (A) was calculated as: A = (A510 - A700) pH1 - (A510 - A700) pH2

Total anthocyanin content of extracts (cyanidin-3-glucoside (mg/L)) was calculated by following equation:

[Anthocyanin]= A · MW · DF · 1000/ (MA)

MW: molecular weight (449.2)

DF: dilution factor

MA: molar absorptivity (26.900)

The total anthocyanin content was expressed as cyanidin-3-glucoside (mg/100 g).

2.4. Determining antioxidant activity of C. spinosal.

2.4.1 Total antioxidant activity

The total antioxidant capacity (TAC) of the extracts was evaluated by using the assay of a green phosphate/Mo5+complex[25]. This technique was founded on the reduction of Mo(VI) to Mo(V) by the sample analyte and the ensuing formation of a green phosphate/Mo(V) complex at acidic pH [26]. 0.1 mL of each methanolic extract was mixed with 1 ml of reagent solution (0.6 M sulfuric acid, 28 mM sodium phosphate, and 4 mM ammonium molybdate). The tubes were screwed and incubated at 95 ° C for 90 min. After cooling, the absorbance of the samples was measured at 695 nm.

Total antioxidant capacity was expressed as milligrams of ascorbic acid equivalents per gram of dry extract (mg EAA / g dry extract). The experiments are repeated in 3 times.

2.4.2/Free radical scavenging capacity by DPPH

Radical scavenging activity was determined by a spectrophotometric method based on the reduction of the methanolic samples of 1,1-diphenyl-2-picrylhydrazyl (DPPH)[27] and [28]. To 0.9 mL of methanolic solution of DPPH (0.2 mM) was added 0.1mL of each extract, the whole was mixed with a vortex for 30 s, and then kept in the dark at laboratory temperature for 30 min. The absorbance was measured against a blank at 520 nm. The positive control is represented by a solution of a standard antioxidant, ascorbic acid whose absorbance was measured under the same conditions as the samples studied. The antiradical activity was expressed as a percent inhibition of DPPH (I %)according to the following equation:

$$I\% = [A\ (blank) - A(sample)/A(blank)] \times 100$$

IC50 (inhibitory concentration 50%) is the concentration values to inhibit or reduce 50% of the initial concentration of DPPH radicals were determined graphically by linear regression. Since there is no absolute measure of the antioxidant capacity of a compound, the results are often based on a reference. Ascorbic acid was used as the standard[29][30].

2.5/ GC-MS analysis in methanolic extract of fruit Capparis spinosa L.

The GC - SM coupling allowed us to highlight the main constituents of fruit extract of C. spinosa. Then, 10 µL of the methanolic extract of caper fruit injected into the gas chromatograph (7890A) coupled to an Agilent mass spectrometry detector (5975C). Indeed, an injection of 1.0 µL was carried out in split mode with a ratio of (10: 1), and a temperature of the injector was detained at 280° C. The temperature of the mass spectrum source was 250 ° C. The carrier gas is N_2 at a constant flow rate of 0.7 mL / min.

The components are identified using the internal mass spectra library NISL version 2.0 MS Search Program (Agilent Techn) using the mass finder 3 software.

2.6/ Methanolic extract fruit Capparis Spinosa l .analysis by Fourier Transform Infrared Spectroscopy (FT-IR)

The FT-IR analysis has been used to identify the nature and functional groups of bioactive molecules present in fruit extract. The FT-IR analysis was carried out on a PerkinElmer Spectrum Two FT-IR spectrometer operating

in transmission mode equipped with a platinum ATR with internal reflection diamond crystal lens. The acquisitions were made by performing 8 scans between 4000 and 600 cm^{-1}, with a resolution of $4cm^{-1}$.

2.7/ Determination of antibacterial activity by RDA

2.7.1/Bacterial strains tested

The bacterial strains used are: Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa, Salmonella sp and Enterococcus faecalis.

2.7.2/Test of antibacterial activity

The methanolic extracts were dissolved in dimethyl sulfoxide (DMSO). The test was carried out at different concentrations of the extracts: 1 g / mL, 0.5 g / mL (1/2), 0.25 g / mL (1/4) and 0.1 g / mL (1/10).

In order, to evaluate the antibacterial activity of the prepared extracts by the radial diffusion method. This method consists in culturing bacteria at a concentration of 4.106 were included with 10 mL Underlay gel containing 10 mM sodium phosphate buffer, 0,3 mg/mL Tryptone Soya Broth (TSB) powder and 1% (w/v) agarose, was maintained at 42 °C. The gel was stirred and poured over 80 mm petri dish on a level space, and sited at 4 °C for 10 min for perfect gelation. These petri dishes were placed on the model 16 see 24 wells they are made using a 4 mm punch. After, the plates were oriented and 10 µL of every extract to be tested was deposited in each well, the plates were covered, inverted and first incubation during 3h at 37°C. After, thencovered with 10 mL of nutrient-rich overlay gel. The petri dishes were turned and followed by a second incubation at 37 °C for overnight[31].

2.6/Statistical Analysis

Experimental data from the assay and evaluation of the antioxidant activity obtained were expressed as the mean ± standard deviation. The confidence limits were at $p \leq 0.05$considered statistically significant, the correlation coefficient of the antioxidant properties was determined using the IBM SPSS Statistics 21 software.

3/ Results and Discussion

3.1/Phenolic compounds of C. Spinosa L.

3.1.1/ Total phenolic contents

The determination of phenolic was carried out according to the Folin-Ciocalteu method. We used Gallic acid as standard[32] . Based on the absorbance value of the extract solution, reacted with the reagent of Folin-Ciocalteu and compared with the standard solution in equivalence of Gallic acid, the results of the colorimetric analysis total phenolic compounds **(Table 1)** revealed that phenolic contents vary according to the organ of the studied plant leaves of capparis spinosa was the richest in phenolic (77,7 ± 1,1mg gallic acid equivalent/g DW), however the flowers occupies the 2nd position (50.5±1.6
mggallic acid equivalent/g DW), followed by the fruits (43±1.8mg gallic acid equivalent/g DW) while the roots come to the last position with 24,2 ± 0,3 mg gallic acid equivalent/g DW. Also found that the C. spinosa L leaf contained important level of polyphenols (23.37 ± 0.40 mg GAE /g) [33].

The results of our work are in perfect agreement with those of Lekhmici Arrar et al[34].Indeed, where well confirmed in the methanolic extract of C. Spinosa leaves contain the highest amount of polyphenols (133.6 ± 58.8 mg GAE/g DW), followed by fruits (58.8 ± 4.7mg GAE/g DW), flowers (33.5 ± 3.4 mg GAE/g) and roots (9.2 ± 2.2 mg GAE/g DW).

This variation in values can be explained by the fact that the phenolic content is influenced by different parameters such as time and place of harvest, climate, geographical conditions, method and time of extraction, solubility and degree maturation of the plant[6].

Table 1. Composition of CapparisspinosaL.extracts.

Methanolic extract	*Phenolics (mg gallic acid equivalent/g of DW)*	*Flavonoids (quercetin mg equivalent/g DW)*	*Tannins (mg catechin equivalent/g DW)*	*Anthocyanins (mg cyanidin-3-glucoside equivalent/kg drymass)*
Leaves	77.7±1.1	39.6±0.6	11.4±0.9	0.33±0.01
Flowers	50.5±1.6	29.7±0.17	9.2±0.5	0.11±0.08
Fruits	43±1.8	14.5±0.9	9.2±0.3	0.47±0.04
Roots	24.2±0.3	13.9±0.6	6.7±0.07	0.30±0.03

3.1.2/Determination of Flavonoids

The concentrations of flavonoids for various extracts of Capparis spinosa L were determined using the colorimetric method of aluminum and soda. Aluminum trichloride forms a yellow complex with flavonoids, and soda forms a complex of pink color that absorbs in the visible at 510 nm. According to the results obtained, we have noticed that the leaves (39,6 ± 0.6 mg EQ / gDW) and the flowers (29.7 ± 0.2 mg EQ / g DW) were higher in flavonoids, subsequently comes the fruits (14.5 ± 0.9 mg EQ / g DW) followed by the roots (13.9± 0.6 mg EQ / g DW).

3.1.3/Determination of tannins content

The condensed tannins were determined by the method of vanillin in acidic medium. Our results revealed that the leaves had higher content of tannin(11,4 ±0.9 mg catechin equivalent/g of DW), followed by the fruits and the flowers with equal concentrations (9.2 ± 0.03 mg catechin equivalent/g DW) and (9.2 ± 0.5 mg catechin equivalent/g DW).

The lowest concentration of tannins was measured in the roots (6.7 ± 0.07 mg catechin equivalent/g of DW).

3.1.4/ Determination of Anthocyanins content

The methanolic extracts of Capparis spinosa L contain relatively low concentrations of anthocyanins (**Table 1**). The concentrations were between 0.47 ±0.04 mg cyanidin-3-glucoside equivalent/kg dry mass (Fruits of Capparis spinosa L) to 0.11 ±0.08 mg cyanidin-3-glucoside equivalent/kg dry mass (Flowers of Capparis spinosa L). However, the difference among the anthocyanins contents according to the organs of the plant is not significant.

3.2 Antioxidant activities of Capparis spinosa L

3.2.1/Total antioxidant capacity TAC

Establishing the relationship between the total phenol, flavonoid and tannin contents and the total antioxidant capacity of the different plant extracts studied the results clearly showed that all the extracts were have different antioxidant activity (**Table 2**). The methanolic extract of C. spinosa L. roots has the best total antioxidant capacity (175.7 ±2.3mg EAA / g DW), followed by leaf and fruits extract where the values were (112.4 ±4.7 mg EAA/g DW) and (97 ±6.1 mg EAA / g DW). However, the flowers extract showed the lowest total antioxidant activity and (67.3±0.9 mg EAA / g DW).

Table 2: Evaluation of antioxidant activity of C. spinosa by DPPH and total antioxidant capacity

Methanolic extract	***1, 1-diphenyl-2 picrylhydrazyl (IC50 mg/L)***	***Total antioxidant capacity (mg EAA / g of dry extract)***
Leaves	70.1±2.32	112.4±4.7
Flowers	137.1 ±2.08	67.3±0.9
Fruits	172.5±1.9	97±6.1
Roots	329±2.4	175.7±2.3

The correlation coefficients for the different parts of C. spinosa show a considerable correlation between total polyphenols and total antioxidant capacity with indices of correlations are between - 0.439to- 0.600.

3.2.2/ Free radical-scavenging capacity

The DPPH radical is generally one of the most used substrates for rapid and direct evaluation of antioxidant activity because of its stability (in radical form) and simplicity of analysis[35]. The radical scavenging ability of methanolic extracts and the standard antioxidant (acid ascorbic) were evaluated spectrophotometric ally using the 1, 1-diphenyl-2-picrylhydrazyl. The results obtained show that the methanolic extracts of leaves of Capparis spinosa L presented the lowest (IC_{50}= 70.10 ± 2.32 mg / L) therefore the most important antioxidant activities. Followed by extracts of flowers and fruits (IC_{50} = 137.14 ± 2.08 mg / L) (IC_{50} = 172.50 ± 1.96mg / L) respectively. However, a lower antiradical activity and that of roots (IC_{50} = 329.20 ± 2.44mg / L), which is three times less active than leaves. Ascorbic acid was used as positive control(IC50 = 4 ± 0.2 mg/L).

Indeed, divers researchers have suggested the antiradical activity of extracts was related to their concentrations of polyphenols and flavonoids [36][37][38]. In our present study, we were established the relationship between secondary metabolites and antioxidant activity (IC50 for DPPH).The results presented in (**Table 3**) prove a significant (negative) correlation. In addition, the correlation coefficient between the polyphenol content and the

antioxidant activity is high (R =-0.940), which means that 94% of the antioxidant capacity of the extracts is due to the contribution of phenolic compounds and they are the dominant antioxidants in these extracts. Although also tannins have a high (negative) correlation coefficient R = -0.973.
After all, conducted combinations, we recorded linear regression between the IC_{50} of DPPH and total phenolics. However, our results are in agreement with those of the others works in the literature and have proved a significant linear correlation between antioxidant activity and level of total polyphenol, tannin and flavonoid [39][40].

Table 3: Correlation between secondary metabolites, anti-radical activity and total antioxidant capacity.

	Polyphenol	**Flavonoids**	**Tannins**	**DPPH (IC50) mg/L**
DPPH (IC50) mg/L	-0,940	- 0,820	- 0,973	1
TAC (mg/g)	- 0.507	- 0.439	-0.600	0.763

Moreover,a positive and significant connection existed between the two techniques of antioxidant power TAC and DPPHradical scavenging capacity with a coefficient of correlation R = 0.763.
The level of correlation between the phenolic content and the antioxidant activity is an interesting aspect, but it must be taken into consideration that phenolic compounds respond differently in the analysis, according to the number of phenolic groups and that the total phenolic compounds do not necessarily incorporate all the antioxidants that may be present in an extract[41].

3.3/ Methanolic extract analysis by GC-MS
Qualitative and quantitative analysis (GC/MS) of the extract identified 56 compounds representing a total of 98.77% (**Table 4**).

Table 4: Chemical composition of methanolic extract fruit capparisspinosa L. by GC–MS

Retention time	***Compounds***	***Molecular formula***	***Area (%),***
3.158	***1,3,5-Cycloheptatriene***	C_7H_8	***1.59***
22.500	***Hexadecanoic acid, methyl ester***	$C_{17}H_{34}O_2$	***3.38***
22.855	***n-Hexadecanoic acid***	$C_{16}H_{32}O_2$	***26.25***
24.194	***9,12-Octadecadienoic acid, methyl ester, (E,E)***	$C_{19}H_{34}O_2$	***1.02***
24.256	***11,14,17-Eicosatrienoic acid, methyl ester***	$C_{21}H_{36}O_2$	***1.68***
24.460	***Tetradecanoic acid, 12-methyl-, methyl ester***	$C_{16}H_{32}O_2$	***0.93***
24.531	***9,12-Octadecadienoic acid (Z,Z)***	$C_{18}H_{32}O_2$	***3.49***
24.602	***9,12,15-Octadecatrienoic acid, (Z,Z,Z)-***	$C_{18}H_{30}O_2$	***7.61***
24.761	***Octadecanoic acid***	$CH_3(CH_2)_{16}COOH$	***4.63***
26.570	***9-Octadecenamide, (Z)***	$C_{18}H_{35}NO$	***2.86***
27.307	***Pentadecane***	$C_{15}H_{32}$	***2.38***
27.599	***NI***	-	***0.96***
28.894	***Heptacosane***	$C_{27}H_{56}$	***1.31***
29.222	***Hentriacontane***	$C_{31}H_{64}$	***1.46***
30.907	***Tetrapentacontane***	$C_{54}H_{110}$	***1.14***
31.351	***Eicosane***	$C_{20}H_{42}$	***2.39***
37.319	***NI***	-	***0.90***
37.824	***NI***	-	***0.97***
38.702	***β-Sitosterol***	$C_{29}H_{50}O$	***28.70***
Total			***93.65***

*NI:not identified

The methanolic extract consists mainly of compounds of which (**Figure 1**): β-Sitosterol (28.70%) ; n-Hexadecanoic acid(26.25%); 9,12,15-Octadecatrienoic acid, (Z, Z, Z) (7.61%) are major components, followed by other low-content molecules such as: Octadecanoic acid(4.63%), 9,12-Octadecadienoic acid (Z,Z)(3.49%), 9-Octadecenamide, (Z)(2.86%) Eicosane(2.39 %).The results obtained indicate that the fruit of caper represents a rich source of bioactive compounds. Majority compounds that prove to constitute a branch of Phytotherapy and is mainly used in diseases. The results obtained indicate that the fruit of caper represents a rich source of bioactive compounds. However, the majority compounds which prove to constitute a branch of Phytotherapy. Indeed, β-sitosterol used for therapeutic purposes, especially in the diseases of colon, prostate and breast cancer [42][43], cardiovascular[44], diabetic[45]and hypercholesterolemic[46]. Thus, n-Hexadecanoic acid plays an important role in the treatment of inflammation[47].

3.4/ Analysis by Fourier transform infrared spectroscopy (FTIR)

Fourier transform IR spectrometry used with the aim of the methanolic extract. The spectrum obtained by FTIR confirms the presence of different bands each of which corresponds to a given functional group, the results are shown in **Figure 1**, which shows a weak band located at 3450 cm^{-1} is associated with the presence of stretching vibrations OH. As it can be observed an intense band between 2850 cm^{-1} and 2920 cm^{-1} corresponds (aliphatic C-Hstretching) and a band at 1680 cm^{-1} characteristic of the carbonyl group indicating the presence of the compound hexadecanoic acid. Moreover, we can see a band at around 1450 cm^{-1} oscillations of the CH bond of the alkyl groups CH_3, CH_2 and CH. Infrared spectroscopy has collected more information to identify the bioactive compounds contained in the fruit extract.

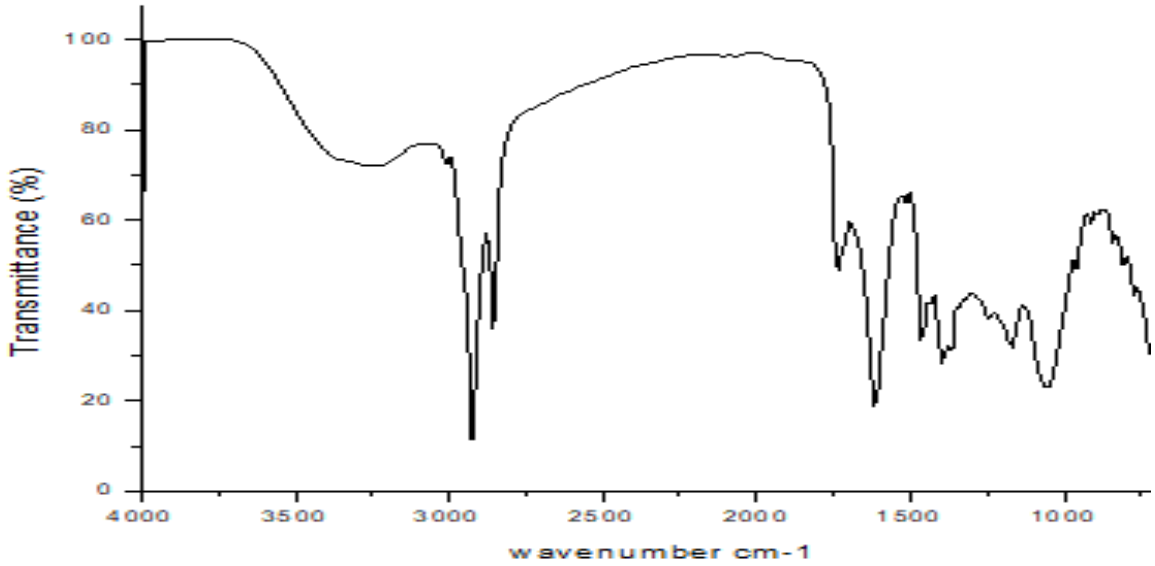

Fig. 1. FT-IR spectra of methanolic extract ofCapparisspinosa L. at the region (4000–600 cm^{-1}).

3.5/ Determination of antibacterial activity by RDA

The results of the antimicrobial activity of the methanolic extracts of the different parts of C. spinosa tested at four different concentrations (1 g / mL, 0.5 mg / mL, 0.025 g / mL and 0.1 g / mL) are recorded in (**Table 5)**.

The results obtained show that the bacterial strains behave differently against all extracts. In fact, the flowers have the most important antibacterial activity, versus to Staphylococcus aureus, Enterococcus faecalis, Escherichia coli, Salmonella, and Pseudomonas aeruginosa with diameters of inhibition 13, 12, 12, 10, and 10 mm, respectively. However, the fruits had a major inhibitory activity at the concentration of (1 g / mL), towards Staphylococcus aureus with a diameter of inhibition of 14 mm may be due to its richness in flavonoids which are good inhibitors of the sortase.

On the other side, low sensitivity was observed with E. coli strains. feacalis and E. coli for the leaves with respective inhibition diameters of 4 ± 0.7 mm and 8 ± 0.7 mm at a concentration (0.1 g/mL).

Whereas, for the salmonella strain, no zone of growth inhibition was found in extracts of the leaves, fruits and roots, which shows the resistance of this strain. The latter is due to the difference in the wall structure of gram-negative bacteria [48]. On other hand, it is noted that the increasing concentrations of root extract and leaf (0.1; 0.25; 0.5;1 mg / mL) causes the growth of the strains can be induced by a gradual decrease and dose-dependent turbidity.

Tale 5: Antibacterial activity of methanolic extracts of C. spinosa

	Leaves				***Fruits***				***Roots***				***Flowers***			
	1 g/mL	**0,5 g/mL**	**0,25 g/mL**	**0,1 g/mL**	**1 g/mL**	**0,5 g/mL**	**0,25 g/mL**	**0,1 g/mL**	**1 g/mL**	**0,5 g/mL**	**0,25 g/mL**	**0,1 g/mL**	**1 g/mL**	**0,5 g/mL**	**0,25 g/mL**	
coli	0	0	0	8±0,70	0	0	0	8	0	0	7	12,67±0,58	12	0	!	6,3
aph aureus	0	0	0	0	14	0	0	0	0	0	0	0	13±1,70	0	0	
aeruginosa	0	0	0	0	9,3±0,58	0	0	0	0	0	0	0	10	8,3±0,70	0	
feacalis	0	0	0	4±0,70	11	10,3±0,58	0	0	0	0	0	0	12	8,6±1,50	7±1	
lmonella	0	0	0	0	0	0	0	0	0	0	0	0	10,3±0,58	7,5±0,70	0	

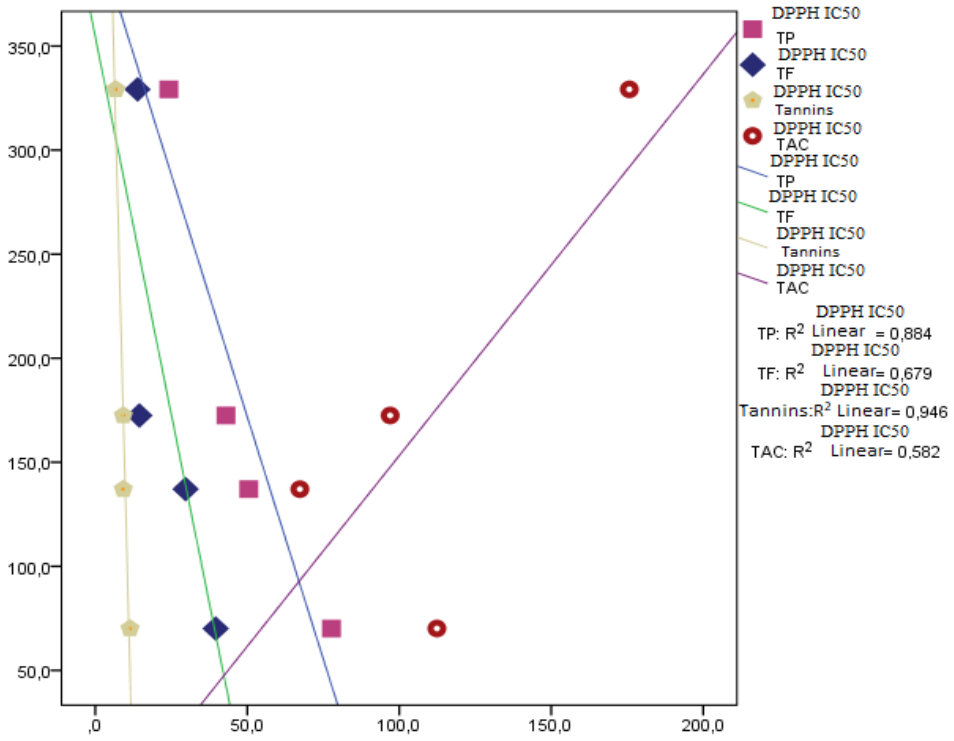

Fig.2. Correlation between DPPH (IC_{50}), TP, TF, Tannins and TAC

Conclusion

The results of this work suggest the importance of C. spinosa L species for use in pharmacy and phytotherapy. Indeed, phytochemical screening reveals the richness ofour plant to secondary metabolites that have culinary proportion, alimentary and medicinal. Moreover, C. spinosa L can constitute an interesting source of natural antioxidants towards the free radicals in comparison with other provenances. In fact, leaves are endowed with a great antioxidant power so is a very good preservative in the field of pharmacology, agri-food, and cosmetics. Furthermore, the results obtained for the antimicrobial activity show that the flower extracts are the most active against the five strains. Therefore, antibacterial properties of these extract may have important industrial applications. For greater efficiency, many perspectives can be envisaged, such as, extend the panel of antioxidant activities in vitro, in vivo and in-depth studies on biological tests: antidiabetic, anti-inflammatory, antitumor, and anticancer. Thus, better understand the mechanism of action of bioactive molecules of this plant, their therapeutic dose and their site of action at the cell level.

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