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IN VITRO EVALUATION OF ANTIOXIDANT AND ANTIBACTERIAL ACTIVITIES OF *CAPPARIS SPINOSA L.* FRUIT EXTRACTS

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Abstract

The fruits of *Capparis spinosa L* have found in recent years a major application in traditional medicine. Since its known effects are limited as an antiseptic and anti-inflammatory, it is interesting to test other activities. The objective of this work is to evaluate in vitro the antioxidant and antibacterial activity of *C. spinosa L* fruit extracts obtained by two extraction methods (maceration and soxhlet) using three solvents, hexane, ethanol and distilled water. The antioxidant activity of these extracts was evaluated by the DPPH (1,1-diphenyl-2-picrylhydrzyl.) test with butylated hydroxytoluene as standard antioxidant. The antibacterial activity of the extracts was evaluated by the disk diffusion method against two bacterial strains (*Staphylococcus aureus* and *Escherichia coli*). The minimum inhibitory concentration (MIC) was determined by the broth microdilution method. The results showed that the ethanolic extracts have a very good antioxidant activity compared to the other extracts with IC50 values of 0.099 ± 0.005 mg/mL and 0.110 ± 0.001 mg/mL for the maceration technique and the soxhlet extraction technique, respectively. All extracts have activity on the bacteria studied. However, their effectiveness is best observed on *Staphylococcus aureus*.

Keywords: *Antibacterial activity, Antioxidant activity, Capparis spinosa L, MIC, DPPH, Extraction.*

Introduction

Crude plant extracts have recently attracted a great interest as a potential source of natural biologically active molecules. Therefore, these extracts are exploited as substitutes for the protection of food against oxidation and the treatment of infectious diseases. Since antiquity, medicinal plants have been utilized as therapeutic agents to manage health and treat diseases, because of their health benefits and bioactive compounds [1-2]. *C. spinosa* belong to the family *Capparidaceae* that includes around 350 species, it is a vivace species with deciduous leaves in winter of rounded and meaty forms and big white to white-pink flowers [3]. It grows wild all over the world [4-5]; This highlights the ability of this plant to adapt to various climatic factors such as dryness, elevated temperatures and high salinity [6]. Morocco is the first producer and exporter in the world. Indeed, the development of this plant has a strong socio-economic value, mainly in the peripheral zones of the provinces of Safi and Fez, because it provides thousands of working days to the rural population, it is one of the agricultural activities that generates incomes especially in arid and semi-arid environments [7]. The wide range of uses of *C. spinosa L*, is attributed to its impressive nutritional and medicinal properties, such as antihistamine, liver protection, antidiabetic, antispasmodic, antioxidant, antibacterial and antifungal [8]. It has been due to the different parts of the plant *C. spinosa* [9]. This plant is known for its edible fruits (caper berries). The fruits are spiny shrubs of oval shape, and contain high levels of antioxidant components, raw oil, fiber and sugars. As well as lipids proteins, sodium, potassium and phosphorus. However, few studies have focused on caper fruits. The objective of this study is to evaluate the antibacterial and antioxidant activities of fruit extracts of *C. spinosa L* harvested from the region of Fez-Meknes-Morocco. These extracts are obtained by different extraction methods (maceration and soxhlet) using 3 solvents of increasing polarity (Hexane, Ethanol, Distilled water).

Materials and methods

Plant material

Capparis spinosa L. harvested in July 2020 from plants which grew naturally in the village of Ouled jamaa, in the region of Fez. The collected fruits have been cleaned and dried in the open air and protected from light and humidity. They were then crushed and ground in an electromechanical mill; the resulting powder served as a support for the extractions.

Extraction methods

Two extraction methods were adopted: the maceration technique and the Soxhlet extraction technique. The maceration technique consists in shaking 35 g of *C. spinosa* L fruit powder with successively, 250 mL of hexane, ethanol and distilled water in a conical flask for 48h at ambient temperature (20±1°C). The soxhlet extraction technique consists of placing 35g of the sample in the filter cartridge in a Soxhlet apparatus and subjecting it to extraction by the same solvents for 24h. The conditions used to compare Soxhlet and maceration extraction are given in Table 1. All obtained extracts were filtered and concentrated via rotary evaporator at 45°C. After filtration and evaporation of the solvent under vacuum, the total obtained extracts are weighed in order to evaluate their yield. The latter is calculated according to the following formula:

$$Yield\,(\%) = \frac{W_1 \times 100}{W_2}$$

Where W_1 represents the mass of the extract after evaporation of the solvent and W_2 is the dry mass of the plant sample. The organic solvents used in the extraction were recycled by rotary evaporator to eliminate any pollution, and used to operate other extraction.

Antioxidant properties

The antioxidant potential of extracts obtained from the fruit of *C. spinosa L* was evaluated in vitro using the DPPH (2,2 diphenyl-1-picryl hydrazyl) test **[10]**. 100 µL of the extracts with several dilutions are added to 750 µL of a solution of DPPH (0.004%), previously prepared in methanol. The reaction mixture was stirred and then held in the dark for 30 minutes at ambient temperature to complete the reaction. The absorbance of the reaction medium was measured at 517 nm withe the

spectrophotometer. Butylated hydroxytoluene (BHT) was used as a reference antioxidant for the inhibition of DPPH˙ (I%) having the expression:

$$I(\%) = \frac{A_1 - A_2}{A_1} \times 100$$

Where

A_1: Absorbance of the negative control (consisting of the solution DPPH without extract)

A_2: Absorbance of the sample.

The result is evaluated by calculating the inhibitory concentration at 50% of DPPH. The plot of the inhibition rate I (%) as a function of the extract concentrations indicates the concentration allowing to reduce 50% of DPPH (IC50); deduced by extrapolation on the abscissa axis.

Antibacterial activity

The antibacterial activity of several fruits extracts of *C. spinosa L* was assessed against two pathogenic strains namely: *Staphylococcus aureus* and *Escherichia coli.* These strains were isolated from contaminated salads, identified on the basis of Gram stain, classical morphological and biochemical characteristics and stored in glycerol at -80°C [11].

The extracts were tested for their antibacterial activity by the disk diffusion test. The Bacterial inoculum was prepared and standardized to correspond to the turbidity norm of 0.5 McFarland, which is equivalent to 10^8 CFU mL^{-1}. The bacterial suspensions were inoculated by flooding onto Mueller Hinton (MH) agar medium surface. Sterile discs of 6 mm diameter were soaked with 10 µl of each extract and deposited on the surface of the culture medium already inoculated with the tested strains. After 24 hours of incubation at 37°C, the inhibition zones formed around the discs were observed and measured with a graduated ruler. Each test was repeated three times for reliability of the results, the result is the average of the measured values [12].

The broth microdilution method is employed to assess the minimum inhibitory concentration (MIC) using dimethylsulfoxide (DMSO) as an emulsifier, and triphenyltetrazolium chloride (TTC) as a bacterial growth indicator. From the 2nd to the 12th well of the 96-well microplate (Greiner, VWR) 20 µL of DMSO were distributed. Further, 40 µL of extract was added to the first test well of each microplate line, from which 20 µL geometric base-2 dilution was performed from the second to the 11th well. The 12th well was considered a growth control. Then, 160 µL of Brain Heart Infusion Broth (BHI) is added to all wells. and 20 µL of a bacterial suspension at 10^6 CFU.ml^{-1} was added to each well.

After 18 hours of incubation at 37°C, the reading was performed by adding 10 µL of triphenyltetrazolium chloride diluted in sterile distilled water to the order of 0.2 g.mL-1, followed by incubation for 10 min at 37°C. The TTC acts as a redox indicator because it allows to differentiate living bacteria by the appearance of a red coloration [13-14].

Results and discussion

Extraction

Successive extractions with solvents of increasing polarity allow the separation of compounds from the plant material according to their degree of solubility in the extraction solvent (Table 2).

The best extraction efficiency was obtained by the maceration method with water (14.6%), the most polar solvent, against 12.47% acquired by the Soxlet extraction with the same solvent. The extraction efficiency in decreasing order by method and solvent is:

M-water > M-ethanol > M-hexane > S-water > S-ethanol > S-hexane

The difference is essentially due to the polarity of both the solvent and the compounds present in the caper fruits [15].

Antioxidant activity

The DPPH radical scavenging assay reveals that the antioxidants in *C. Spinosa* fruits extracts react with DPPH while accepting an electron or hydrogen radical and convert it to yellow colored α,α-diphenyl-β-picryl hydrazine [16]. The degree of discoloration indicates the radical scavenging potential of the antioxidant. As shown in Fig.1, all extracts exhibited radical scavenging activity which is dependent on concentration. The calculation of the concentrations of extracts causing 50% inhibition or reduction of DPPH (IC_{50}) is given in Table 3.

The results show that for both extraction techniques, the IC_{50} values of the different extracts are classified as follows: ethanolic extract > aqueous extract > hexanolic extract. In comparison with the standard antioxidant (BHT), The DPPH radical scavenging activity of the ethanolic extract showed almost identical activity to the control (BHT) with IC_{50} values of 0.099 ± 0.005 mg/mL and 0.110 ± 0.001 mg/mL, for the maceration method and the soxhlet extraction method, respectively. For the aqueous and hexanolic extracts acquired by maceration and soxhlet methods, the antioxidant activity is less significant than BHT respectively. M. S. Bhoyar et al. (2018) [17] showed that extracts of *C. spinosa L* have high antioxidant capacity, especially those from the edible parts (leaves, flowers, roots and fruits). Furthermore, F. Elshibani et al. (2020) [18] showed that the extract of *C. spinosa* by maceration in methanol displays a significant scavenging effect on the DPPH radical, reflecting a high antioxidant capacity compared to gallic acid as a pure reference antioxidant.

Antibacterial activity

The results related to the antibacterial activity of *Capparis spinosa L* fruit extracts, obtained by different extraction techniques (Table 4 and 5), denote that all extracts possess inhibitory activity on the tested strains with inhibition zone diameters ranging from 7 to 13 mm. Moreover, comparing the extraction methods, it appears that the extracts have a similar behavior, since the diameters of the inhibition zones remain very close for the two methods but different from one bacterium to another and from one extract to another. Based on minimum inhibitory concentrations (MICs), the aqueous extracts were the most active against the two tested strains with MIC values in the range of 3.25 to 30 ug/mL followed by ethanolic and hexanolic extracts. In the presence of the hexanolic extract, *Escherichia coli* was more resistant with an inhibition concentration of 60 mg/mL. In the present investigation, it was found that *Staphylococcus aureus* (Gram+) seemed to be more sensitive to the different obtained extracts than *Escherichia coli* (Gram-). This could be explained by the lipopolysaccharide structure of the external membrane of (Gram-) bacteria, which makes them intrinsically impermeable to external agents [19-20]. On the other hand, the spatial configuration of the molecules prevents them from crossing the transport proteins (porin) of the outer membrane, and therefore from reaching the peptidoglycan of the bacterial wall [21]. As for Gram-positive bacteria, including *S. aureus*, they can easily be altered by external agents. The obtained findings are similar to other research studies which revealed that this plant has a strong antibacterial activity against the same strains, and that the aqueous and ethanolic extracts of *C. spinosa. L* exert significant antimicrobial activity on *S. aureus* and *Escherichia coli* strains [22].

In the same context, it has been suggested that the aqueous extract of the immature fruits of *C. spinosa L* has very good antibacterial activity against *S. aureus* and that it is inactive against *Escherichia. coli* but presents a suspicious activity against *S. aureus* [23-24]. On the other hand, ethyl acetate and n-butanol extracts of the leaves showed high to moderate activity of 10-30 mm against *Escherichia coli*, and *S. aureus* [25].

Conclusion

The study conducted on the evaluation of the antioxidant and antibacterial activities of extracts (hexanolic, ethanolic and aqueous) of fruits of *capparis spinosa L*, obtained by two extraction techniques; maceration and soxhlet. Led to the following conclusions:

- The best extraction yield of *C. spinosa L* fruits is that obtained by the maceration method in an aqueous medium with a yield of 14.6%.
- All the extracts of *C. spinosa L*, have an antioxidant potential. However, ethanolic extracts showed better antioxidant activity with IC50 values lower than those of BHT.
- All the extracts of *C. spinosa L* exerted an antibacterial effect on the two strains tested and more particularly on the *Staphylococcus aureus* strain.
- The aqueous and ethanolic extracts showed the highest antibacterial activity against the tested strains.
- The minimum inhibitory concentrations (MIC) of the most active aqueous extracts are respectively 3.25 ug/mL and 15 ug/mL for *Staphylococcus aureus* and *Escherichia coli.*

On the basis of these results it is clear that the fruits of *C. spinosa L* possess antioxidant and antibacterial properties and that they can therefore be used in the pharmaceutical and/or food industries. It is therefore interesting to continue this study by testing the antibacterial effect of *C. spinosa L* extracts on other bacteria of animal or plant origin and to determine their mode of action.

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Table 1. Extraction operating conditions.

Parameter	Extraction method	
	Maceration	Soxhlet
Sample size (g)	35	35
Solvent	Hexane, ethanol and water	Hexane, ethanol and water
Solvent volume (ml)	250	250
Temperature (°C)	20°C	n- Hexane (65 °C), ethanol (79 °C) and water (100°C).
Time (h)	48	24

Table 2. Extraction yield (%) of different extracts from the *Capparis spinosa L.* fruits.

Extraction Method	Hexanolic	Ethanolic	Aqueous
Maceration	12.19	13.27	14.6
Soxhlet	9.28	12.6	12.47

Table 3. Inhibitory concentrations (IC_{50}) in mg/mL) of the tested extracts compared to BHT (IC_{50} = 0.113 mg/mL).

Extract	Maceration	Soxhlet
Ethanolic	0.099 ± 0.005	0.110 ± 0.001
Aqueous	0.253 ± 0.001	0.134 ± 0.001
Hexanolic	0.322 ± 0.004	0.580 ± 0.002

Table 4. Diameter of the inhibition zone (mm) of *Capparis spinosa L* fruits extracts obtained by maceration and soxhlet.

Strain	Meceration			Soxhlet		
	Hexanolic	Ethanolic	Aqueous	Hexanolic	Ethanolic	Aqueous
S. aureus	7.00 ± 0.29	12.00 ± 0.21	13.00 ± 0.50	7.00 ± 0.76	11.00 ± 0.28	12.00 ± 0.50
E. coli	8.00 ± 0.25	9.00 ± 0.00	12.00 ± 0.40	8.00 ± 0.15	10.00 ± 0.05	11.00 ± 0.64

***** The inhibition zone includes the diameter of the disc (6 mm).
Results are expressed as the mean ± standard deviation of three measurements

Table 5. Concentration Minimum inhibition concentration (ug/mL) of *Capparis spinosa* fruit extracts.

Strain	Meceration			Soxhlet		
	Hexanolic	Ethanolic	Aqueous	Hexanolic	Ethanolic	Aqueous
S. aureus	-	60	3.25	60	30	7.5
E. coli	60	60	15	60	60	30

Figure 1. Radical scavenging activity of the three extracts obtained by soxhlet (A) and maceration (B) compared to BHT.