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Effects of extraction methods on total polyphenols, free radical scavenging and antibacterial activity of crude extracts of *Cleome arabica* L. growing in Oued Souf region

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ABSTRACT

This study aims to phytochemical study, antiradical scavenging and antibacterial Activity of the extracts of *Cleome arabica* L. from the region of Oued Souf (East South Algerian).
The crud extracts two methods obtained methanolic were maceration (EM) and ultra-sound (EU). The yields were: 8.95% and 9.60%, respectively. The quantitative estimation of total polyphenols is 11.57 mg EAG/g Ex (EM) and 13.46 mg EAG/g Ex (EU). The flavonoids contents in (EM) and (EU) are respectively 5.48 and 6.19 mg EQu/g Ex. the anti-free radical activity in the extracts at (concentration 0.1mg/ml) showed a great capacity to scavenge of the DPPH• radical and the percentage of inhibition was 41% in (EM) and 60.67 % in (EU). The results of the antibacterial activity of two bacterial strains: *Escherichia coli* and *Staphylococcus aureus*, revealed that Cleome arabica L. has a significant effect on the two strains with inhibition zones variable from 0 to 12 mm for *Escherichia coli* and 0 to 13 mm for *Staphylococcus aureus*.

1. Introduction

Cleome arabica L. is herb common in desert and rises in the Mediterranean sea on stony slopes and sandy ravines up to 2300m altitude [1]. In the Sahara, is found on rock, sand and gravel, is the only species of the Capparidaceae family in the region of Oued Souf -East South Sahara Algerian [2]. Perennial plant 30 cm tall, with upright and branched stems; small leaves hairy and trifoliate, the flowers have petals whose color turns yellow to dark purple, the fruit is a hairy pod 2 to 5 cm long [3], with a foul odor, toxic and has hallucinogenic effects [4].
According to [1], the camels, goats and sheep refuse this plant; the natives use it as a diuretic and against rheumatism [2], also is used in traditional medicine by the Nomads as analgesic of neuralgic pains [5].
This study aims to phytochemical study, antiradical scavenging and antibacterial activity of the extracts of *Cleome arabica* L., collected in the region of Oued Souf (East South Sahara Algerian).

2. Materials and Methods

2.1. Plant material

The *Cleome arabica* L. was collected in November 2014 from the Oued Souf region (East South Algerian). The plant is then dried, crushed, stored and protect from light

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and moisture.

2.2. Preparation of extracts

We prepared the extracts of plant material with two extraction methods:

2.2.1. Maceration

50g of plant material with 500 ml of methanol for 24 h, after filtration, the macerated is evaporated in Rota-vapor at 55°C [6].

2.2.2. Extraction with ultra-sound

According to [7], 200 ml of methanol is added to 20 g of plant material then take mixtures to ultra-sound Type JP Selecta (3.1A; 720 W) under the conditions: 30°C for 30 min, the extract is evaporated in Rota-vapor.

2.3. Estimation of total polyphenols

The total phenols content are determined according to the method described by [8], 0.2 ml of the extract was mixed with 1 ml of Folin-Ciocalteu reagent (10%). Then 0.8 ml of a solution of Na_2CO_3 (7.5%) was added to the mixture. The mixture is incubated at room temperature, protected from light, for about 30 minutes. The absorbance is measured at 760 nm. The results are expressed in mg equivalent Gallic acid/g Ex).

2.4. Dosage of Flavonoids Content

The flavonoids were estimated using method citing in [9], 0.5 ml of extract with 0.5 ml of $AlCl_3$ (2%). After 1h at room temperature, the absorbance was measured at 420 nm. The flavonoid content was estimated as (mg Quercetin equivalent /g Ex)

2.5. Anti-free radical scavenging

In this study, we used the DPPH• free radical to evaluate the scavenging activity of the plant's crude extracts.

The Anti-free radical activity of different extracts was measured by the method described by [10], 1ml of extract with 1ml DPPH (0.1 mM). The tubes are incubated at 37°C for 15 min. The absorbance is estimated at 515 nm. The following formula determines the inhibition:

% DPPH radical scavenging = [(Ac – As)/Ac] x100.

Where Ac is the absorbance of the control and As is the absorbance of the sample.

2.6. Antibacterial activity:

2.6.1. Source of pathogens and cultures medium

One Gram negative bacteria (*Escherichia coli* ATCC 25922) and one Gram positive bacteria (S*taphylococcus aureus* ATCC 25923) obtained from the Pasteur Institute, Algiers. Nutrient agar Mueller Hinton was used as a growth medium for investigated microorganisms.

2.6.2. Antibacterial activity

The agar diffusion method determined the antibacterial activity of extracts and antibiotic (AMC_{30}: Amoxyclav 30µg/disk) [11].

and 1mg/ml diluted in DMSO) are then deposited on the agar surface previously seeded with bacterial suspension (10^6 CFU/ml) in exponential growth phase. The Petri dishes were incubated at 37°C for 18-24 h. The inhibition of microbial growth is determined by measuring each disk's zones of inhibition diameter (mm) [12].

3. Results and Discussion

3.1 The Yield, Content of total polyphenols and flavonoids

The yield of extraction (Fig. 1) was registered as the maximum value in the extract of the Ultra-sound method (EU) and the minimum in the extract of the Maceration method (EM).

The total phenols and flavonoids of methanolic extracts from *Cleome arabica* L. are presented in (Fig. 1). We observed the relationship in the quantitative content of the polyphenols and the flavonoids.

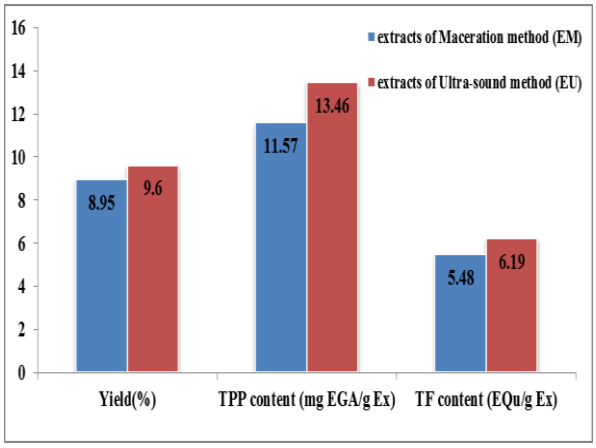

Fig 1. The yield (%), the content of total polyphenols and flavonoids of different extracts methanolic of *C. arabica* L.

3.2 Antibacterial activity

The antibacterial activity of extracts *Cleome arabica* L. are summarized in (Table 1 and Fig. 2).

Table 1. Diameter of inhibition zones (mm) of different concentrations of extracts of *Cleome arabica* L.

	Concentrations (mg/ml)	*Escherichia coli* (ATTC 25922)	*Staphylococcus aureus* (ATTC25923)
extract (EM)	1	12	11.5
	0,75	9	9.5
	0,5	8.5	8
	0,25	8	0
extract (EU)	1	8	13
	0,75	0	9.5
	0,5	0	9
	0,25	0	8
Antibiotic Amoxiclav	30µg/disk	44	45.5

The comparison of the activities of different extracts of *C. arabica* L. reveals a high sensitivity of the tested germs, especially with: EU extract (13 mm in C: 1 mg/ml), also showed very expressive inhibition diameters (44-45.5 mm) with antibiotic tested (Amoxiclav).

Escherichia coli is very sensitive to different concentrations of the EU extract (8 mm with a concentration of 1 mg/ml. Also, we did not notice the effect of the EU extract with this strain.

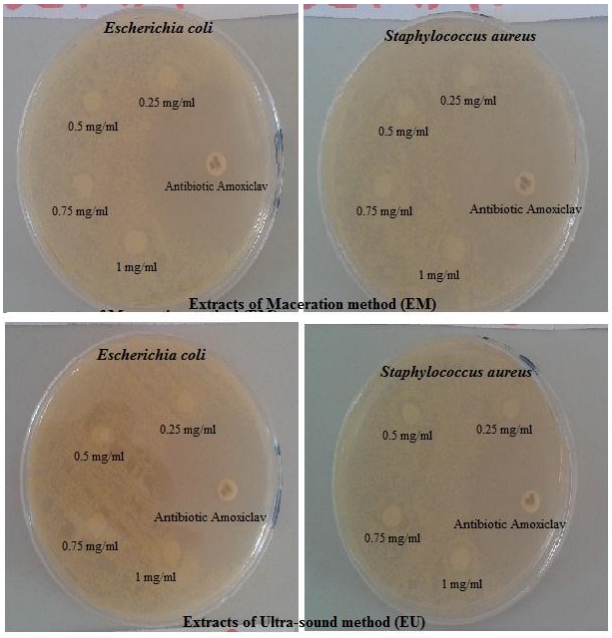

Fig 2. Antibacterial activity of different concentrations of extracts of *C. arabica* L. against strains bacteria.

3.3 DPPH˙ radical scavenging

The results of radical scavenging activity of extracts at concentration 0.1 mg/ml showed a great capacity to scavenge of the DPPH• radical and the percentage of inhibition was 41% in extracts of the Maceration method (EM) and 60.67 % in extracts of the Ultra-sound method (EU). The results indicate that extracts of *C. arabica* L. have a reduced potential with radical scavenging activity.

The variability observed in the values of yield, polyphenols, and flavonoid contents could be attributed to the difference in the samples studied [13].

In the result of anti-free radical activity, it was clearly noticed superiority extracts of the Ultra-sound method to extracts of the Maceration method. This was due to antioxidation's activity, which was closely linked to phenolic compounds' structure and quality than the concentration and quantity of these compounds within the plant tissue [14]. The strong effect of antioxidants in some ethanol extracts samples could be explained by the difference in antioxidant activity between samples for different behavior to give a proton and an electron between samples [15].

The results of present investigation indicated that the antibacterial activity varies with methods extraction used.

The observed differences in medium sensitivity the different concentrations of extracts between Gram-positive and Gram-negative bacteria can probably be attributed to the structural and compositional variations in the nature of the cell wall between the two groups [16].

This inhibitory effect of *C. arabica* L. extracts might be due to the action of special organic compounds such as flavonoids [17, 18].

4. Conclusion

This work is comparison to phytochemical study, antiradical and Antibacterial Activities of the extracts of *Cleome arabica* L. The crude methanolic extracts were obtained by two methods Maceration (EM) and Ultra-sound (EU). The contents of total polyphenols and flavonoids are proximities in two methods of extraction. The anti-free radical activity showed a great capacity to scavenge the DPPH• radical, and in the antibacterial activity revealed that *Cleome arabica* L. extracts have a significant effect on the two strains used with inhibition zones variable.

Conflict of Interest

The authors declare that they have no conflict of interest.

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