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Phytochemical, Free Radical Scavenging and Antimicrobial Activities of Ceratonia Siliqua L. Fruits Collected of Jijel (Algeria)

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ABSTRACT

The purpose of this study is to realize a phytochemical screening and determine the antioxidant and antibacterial activities of the phenolic compound of Ceratonia siliqua L. fruits. The phenolic compounds were extracted by four solvents of increasing polarity (dichloromethane, ethyl acetate, butanol and methanol) respectively and analyzed using different tests in vitro in order to evaluate their total phenolic and flavonoid contents, antioxidant and antibacterial activities. The phytochemical study of the c. siliqua fruits showed the presence of certain chemical compounds such as: alkaloids, flavonoids, saponins, reducing compounds, tannins and volatile oils, comarins, triterpenes, resins and the total absence of imodols. The content of the total phenols by the Folin-Ciocalteu method and flavonoids by the AlCl3 method with the methanoic extract gave higher values which estimated: (42.65 ± 4.90 mg AGE / g Ext) and (8.83 ± 1.24 mg QE / g Ext) respectively. The quantitative evaluation of the anti-radical activity has proved that methanolic extract is the most active (IC50 = 0.029 mg / ml); however, ascorbic acid (used as control) showed approximately equivalent activity (IC 50 = 0.017mg / ml), indicating the presence of compounds effective in the chemical composition of the plant that have a high capacity in reducing DPPH. The phenolic extracts from the fruits C. siliqua showed considerable activity against all bacterial tested.

Keywords: *Ceratonia Siliqua, Phytochemical Analysis, Phenolic Compounds, Antioxidant Activity, Antimicrobial Activities.*

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1. INTRODUCTION

Interest in dietary supplements of various plants has increased recently and for this we have chosen the plant of Carob (Ceratonia *siliqua* L.), which belongs to the family of leguminoseae, distributed mainly in the Mediterranean region (Seghir et al., 2016), it is cultivated in Algeria for ornamental and industrial purposes. *C.silliqua* is an evergreen, drought resistant tree. It has a good nutritional value, a long shelf-life (2-3 years) and it is relatively cheap (Bulica, 2016), is well known for its high content of phenolic compounds, carbohydrates and contain low amounts of insoluble dietary fibers, minerals, lipids and proteins (Avallone et al., 1997). Several studies have shown various biological activities and pharmacological characteristics of *C. siliqua* such as Antioxidant (Custodio et al., 2011; Benchikh et al.,2014) antibacterial activities (Ouis and Hariri, 2018) antiproliferative activity (Corsi et al., 2002; Roseiro et al., 2013) and their probable role in the prevention of various diseases and of inflammation (Vauzour et al., 2010). This study was aimed to determine the phytochemical analysis, antioxidant and antimicrobial activities of phenolic extracts from the fruits of *Ceratonia siliqua.*

2. MATERIALS AND METHODS

Plant material collection and identification

Fruits of *C.siliqua* were collected from Ziama region (Center of Jijel, Algeria) in September 2016. The plant was identified by Dr. Y. Halis Researcher in Scientific and Technical Research Center for Arid Areas (Touggourt) Algeria

Phytochimical screening

The fruits were used as plant material, the fruits is dried in an oven at 30 ° C to eliminate total moisture, then finely ground and weighed with an electric balance at the Laboratory of Biomolecules and Plant Amelioration, Larbi Ben m'hidi University of Oum El Bouaghi, Algeria. Phytochemical screening was performed according to the protocols described in Sofowara (1993), Trease and Evans (1989), Harbome (1979). The phytochemical tests used are presented in Table 1.

Preparation of phenolic extracts from fruits of *C. Siliqua*

According to the Markham (1982) protocol, the different types of extracts were prepared from the pulverized fruits (100 g) using 1L of increasing polarity solvents (dichloromethane, ethyl acetate, butanol, methanol). At the end of the extraction, the four organic extracts were concentrated under vacuum at Rotavapor at temperatures of 35 ° C, 40 ° C. and 50 ° C respectively. The dried sample of each extract was weighed and the yield of soluble constituents was calculated from the following equation: Yield (%) = [Final weight of dried extract / initial weight of carob powder] x100

The extracts were dissolved in methanol (1 mg / ml) to measure the total phenol and flavonoid contents as well as the antioxidant activity. The experiment was repeated in triplicate.

Determination of total polyphenols

The concentration of total phenols in dry extracts of *C. siliqua* fruits was determined by the Folin-Ciocalteau method (Singleton and Rossi, 1965)

200 µl of each extract were mixed with 1 ml of Folin-Ciocalteu reagent (10%) and incubated at room temperature for 4 minutes. After adding 800 µl of sodium bicarbonate (7.5 %) to the mixture, the total polyphenols were determined after 2 hours of incubation at room temperature. The absorbance of the blue color was measured at λ max = 765 nm with a spectrophotometer of CECIL2041 UV-VS. Quantification was done using a standard curve of gallic acid. The results were expressed in milligrams of equivalents of gallic acid per 100 g of dry matter (mg GAE / g Ext)

Determination of total flavonoids

The concentration of total flavonoids was measured by the colorimetric method of aluminum chlorite ($AlCl_3$) (Bahorun et al., 1996). A quantity of 1 ml of each sample and standard (prepared in methanol) was added to 1 ml of the AlC_3 solution (2% dissolved in methanol). The mixture was left to react for 10 min at room temperature, and the absorbance of each mixture was read at λ max = 430 nm. The flavonoid concentrations were deduced from the calibration curve established with quercetin. The results were expressed in milligrams equivalents of quercetin per 100 g of the dried material (mg EQ / 100 g Ext).

DPPH radical scavenging assay

Radical scavenging activity of the different extracts was measured according to the procedure described by Brand-Williams et al. (1995). One milliliter of DPPH solution (60 µM) was mixed with 100 µL of sample at different concentrations. The decrease in absorbance was determined at 517 nm, after 30 min of incubation. The DPPH radical scavenging activity was calculated according to the following equation:

% inhibition = [(absorbance of control - absorbance of the sample)/ absorbance of control]x100

IC_{50} was calculated as the concentration of extract causing a 50% inhibition of DPPH radical; a low IC_{50} value corresponds to a high antioxidant activity of sample.

Evaluation of the antibacterial activity

Sources and maintenance of microorganisms

Bacterial strains of Gram+ : *Staphylococcus aureus* ATCC25923 ; Bacterial strains of Gram : *Escherichia coli* ATCC25922 and *Pseudomonas aeruginosa* ATCC 27853 (American Type Culture Collection), obtained from the Pasteur Institute of Algiers, maintained by subculture on nutrient agar medium favorable to their growth and incubated at 37 ° C for 24 h . So we obtain a bacterial suspension density of 10^6 colony forming units per milliliter (cfu/ml).

Preparing Disks

The phenolic extracts of fruits were recovered by dissolved in DMSO 2% (dimethyl-sulfoxide) to obtain an initial solution concentration of C_0 = 100mg/ml, from C_0 is conducting a series of dilutions. The sterile filter paper discs (6 mm diameter) were impregnated with 100 µl of different concentrations of each extract.

Diffusion method on agar medium

The antibacterial activity of different plant extracts was evaluated using the method of agar diffusion (Benbott et al, 2012).From colonies of 18 to 24 h, a bacterial suspension was made in sterile distilled water for each strain. The turbidity of this suspension is adjusted to 0.5 McFarland and then diluted 1/100. This gives an estimated inoculum to 10^6 cfu/ml.

This inoculum is inoculated by flooding on Petri dishes containing Mueller-Hinton agar. Disks impregnated with various concentrations (12.5 %, 25 %, 50 % and 100 %) were then delicately deposited on the surface of the agar. The Petri dishes left for 1 h at room temperature for pre-release substances, before being incubated at 37 ° C in an oven for 24 h. The antibacterial activity is determined by measuring the diameter of the inhibition zone around each disc

3. RESULTS AND DISCUSSION

Phytochemical screening

The results of phytochemical analysis on *C. Siliqua* fruits showed the presence of numerous chemical compounds, such as: phenols, tannins, flavonoids, reactive compounds, alkaloids, saponins, coumarins, resins, triterpenes and sterols. On the other hand, we note the absence of the imodols. The presence of these actives compound in the fruits of *C. Siliqua* indicates the importance of this plant in traditional and modern medicine, some of these results are close to those obtained by Ouis and Hariri (2017) and Nahla (2014)

Table 1. Phytochemical screening of extracts *C. siliqua* fruits

Extract / Metabolites	Result
Alkaloids	+
Tannins	+
Flavonoids	+
Saponins	+
Triterpenes and Sterols	+
Reducing compounds	+
Volatile oils	+
Comarins	+
Resins	+
Imodols	-

+: Presence, **-:** Absence

Yield of phenolic compound in various extracts

Table 2 shows that the phenolic yield was varied depending on the organic solvents used in the extraction. There is a correlative relationship between the polarity of the solvent and its solubility due to the solubility nature of these compounds in the solvents. The best yield of phenolic compounds in *C. Siliqua* fruits was with the methanolic extract (10.05± 0.21 %). The lowest yield was recorded with the dichloromethane extract (0.26± 0.13%).

Table 2. Yields of extracts

Extracts	Yield (%)
dichloromethane	0.26± 0.13
ethyl acetate	0.68± 0.28
butanol	2.24± 0.51
methanol	10.05±0.31

It should be noted that the yield of the phenolic extracts obtained differs from the yield of phenols which are studied by Ouis and Hariri (2017) and Ydjedd et al. (2017), this difference

being able to be due to: the quality of the fruits, the maturity, the geographical zone, weather conditions, harvest times, and storage and extraction methods

Content of total phenols and flavonoids in dry extracts:

Table 3 shows that *C. Siliqua* fruits contain varying amounts of total flavonoids and total phenols, due to the type of solvent used for extraction. We conclude that the methanol and butanol extracts have the greatest ability to extract these compounds from other extracts, with high amounts in the methanolic extract, compared to the results of Sebai et al. (2013).

Table 3. Total phenols and flavonoids content of *C. siliqua* fruits

Extracts	Total Phenols (mg EAG / g Ext)	Total flavonoids (mg EQ / g Ext)
dichloromethane	4.19 ±0.22	1.92± 0.28
ethyl acetate	12.27±0.18	3.99±0.14
butanol	22.66±0.37	5.66±0.32
methanol	42.65±0.55	8.93±0.45

DPPH free radical-scavenging activity

DPPH is one of the most important tests used to estimate antioxidant activity. In this test, we observed the colouring of all the extracts tested in yellow, proof that these extracts gave a hydrogen atom to the DPPH compound. In this study, we used ascorbic acid as a control.

Figure. 1 shows a correlative relationship between the DPPH radical scavenging activity and the increase in phenol concentration in all the extracts used, the highest inhibitory level of DPPH was recorded at a concentration of 0.1 mg / ml in all extracts, ranging from 52.41% to 63.09%. Whereas Dallali et al (2018) showed that DPPH radical scavenging activity was highest with methanol extract in leaves of *C. siliqua* (%70 ,83)

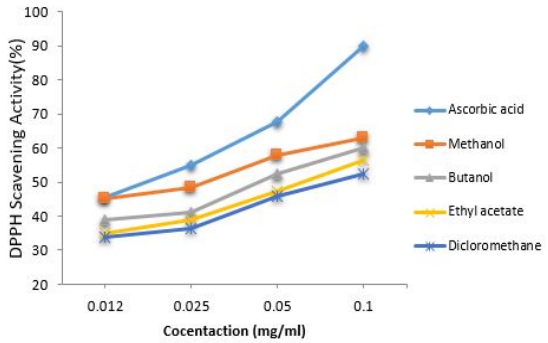


Figure 1: Free radical scavenging activity of the phenol extracts of *C. siliqua* fruits

Figure. 2 shows that the high values of IC50 correspond to a low antioxidant activity, which explains that the methanolic extract showed a high DPPH radical scavenging activity (IC50 = 0.029 mg / ml) but was less effective than the ascorbic acid (IC50 = 0.017mg / ml) followed by butanol extract (IC50 = 0.054mg / ml), whereas we recorded the lowest efficacy with dichloromethane extract (IC50 = 0.083mg / ml). These results show a high efficiency compared to the results of Ouis and Hariri (2017) and Ydjedd et al. (2017).

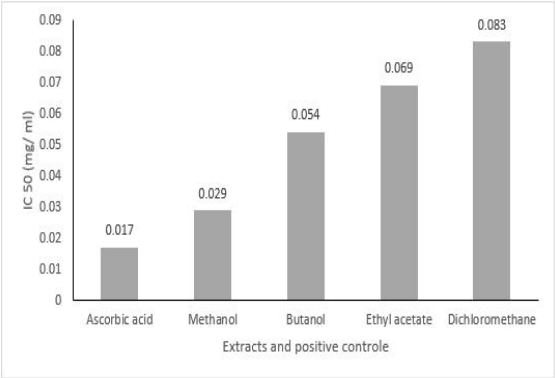


Figure 2. Antioxidant capacities of the ascorbic acid and the phenols of *C. siliqua* fruits.

Determination of the antibacterial activity

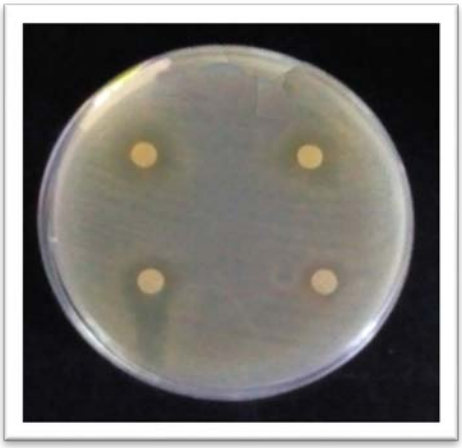
Table 4 and Figures 3, 4 and 5 show that there is inhibitory activity of C. Siliqua methanol and butanol extracts against three reference bacterial strains (*E. coli, P. aeruginosa and S. aureus*). The highest antibacterial activity against *S. aureus* was recorded with the methanolic extract at the highest concentration with an inhibition diameter of 15 mm, compared to butanol extract with a 12 mm inhibition diameter. With regard to the *E. coli* and *P. aeruginosa* bacterial strains, the diameter of the inhibition zones is between 6.5 and 9 mm.

Table 4. Results of antibacterial activity of *C. siliqua* fruits

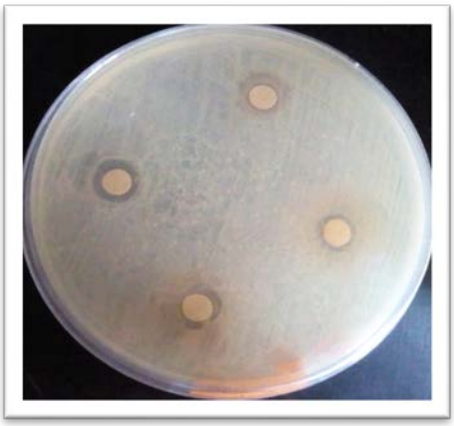
Tested bacteria	Inhibition zone diameter in (mm) at different concentrations of methanol and butanol extracts in (mg/ml)							
	Methanol extract of the fruits				Butanol extract of the fruits			
	100%	50%	25%	12%	100%	50%	25%	12%
S. aureus	15± 0.5	11± 1.01	8± 1.09	8± 0.6	12± 0.3	10± 16	8± 0.65	7± 0.77
E. coli	9± 0.8	9± 0.4	9± 0.19	7± 0.4	8± 0.55	8± 0.41	7± 0.11	-
P. aerugenosa	8± 0.33	8± 0.2	7± 0.4	6± 0.3	6.5± 0.06	-	-	-

The results of the study were in agreement with Abd Razik et al. (2012), who recorded the highest antibacterial activity with *S. aureus* and *Proteus sp* strains with inhibition diameters of 17.09 mm and 17.05 mm. respectively, at a concentration of 1000 mg / mL and the lowest bacterial activity against *E. coli* is 12 mm. On the other hand, Ouis and Hariri (2018) showed that the essential oils extracted from the pulp and the seeds of *C. siliqua* have a good antibacterial activity.

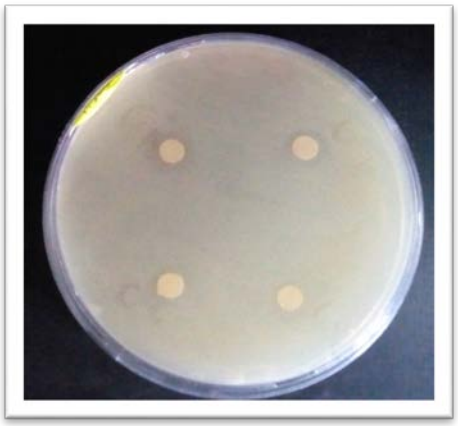
S. aureus (ATCC 25923)



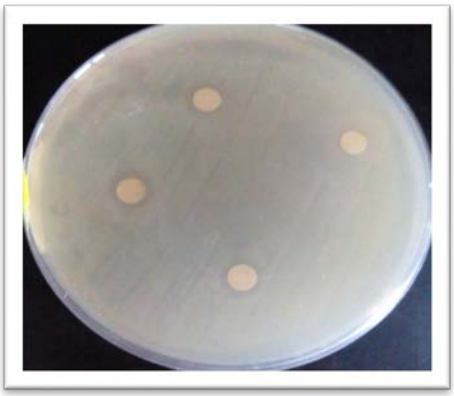
S.aureus (ATCC 25923)



E. coli (ATCC 25922)



E.coli (ATCC 25922)



P. aeruginosa (ATCC 25853)

Figure 3. The effect of the different concentrations of methanolic extracts (100%, 50%, 25% and 12.5%) on the growth of three bacteria tested

P.aeruginosa (ATCC 25853)

Figure 4. The effect of the different concentrations of butanolic extracts (100 %, 50 %, 25 %, and 12.5 %) on the growth of three bacteria tested

4. CONCLUSIONS

In conclusion, we can confirm that the fruits of the *Ceratonia Siliqua* plant in the Jijel region of Algeria are rich in active

substances and have a relatively high percentage of total phenols and flavonoids. It is considered as a potential natural source of antioxidant and antimicrobial drugs. It is particularly important in combating the recent trend in the emergence of multiple drug resistant organisms.

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