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Characterization and assessment of the antimicrobial function of total polyphenol extracts from pulps, leaves and seeds of two *Ceratonia siliqua L.* varieties

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ABSTRACT/RESUME

Abstract: *The overall phytochemical test results suggested that pulp, seeds, and leaves of both grafted and spontaneous varieties are especially rich in tannins , saponins, and leuco-anthocyanins. The result of the determination of total polyphenols Ceratonia siliqua L. showed that spontaneous leaves showed that the highest value of spontaneous leaves was 14 EAGmg / g, followed by grafted seeds (11.5 EAGmg / g), and spontaneous pulps (10.2 EAGmg / g). Infra -red spectroscopy revealed the presence of many functional groups in both varieties. The antimicrobial activity is particularly found with the spantaneous leaves against Escherichia coli (21.83 ± 0.93 mm) and Staphylococcus aureus (16.36 ± 0.63 mm) Micrococcus sp (15.83 ± 0.73 mm) and leaves grafted against Pseudomonas aeruginosa (14.30 ± 0.35 mm).*

I. Introduction

Algeria is a wide nation, its geographical position provides a climate biodiversity which has shaped the world of plants, the latter being very rich in plants used as medicinal herbs and natural foods. The Algerian flora is still very little scientifically utilized, particularly in combating infectious diseases [1].

Several natural substances were known from these plants, and many are used in traditional medicine [2].
These molecules may be produced during primary metabolism of plants (nucleic acids, amino acids, lipids, proteins and carbohydrates) also resulting from subsequent chemical reactions which they are called secondary metabolites. Among these, include phenolic compounds (including polyphenols) and nitrogen-containing compounds formed mainly of alkaloids and glycosides, which often play a defensive role [3].

The carob tree *(Ceratonia siliqua L.)* is an almost endangered plant in the Mediterranean, belonging to the large family of legumes, grown for a long time for its by-products but also because of its tolerance to water scarcity [4].

According to the literature search, carob contains up to 20% phenolic compounds, of which 24 different main structures have already been identified and their content determined by Owen *et al.* [5]. Recent studies have shown that other parts of the tree such as leaves [6] and bark [7] are also rich in phenolic compounds. It is currently proven that these compounds offer interesting biological and environmental properties such as antimicrobial and antioxidant properties [8].

For these purposes we chose to study the species *Ceratonia siliqua L.* We chose to research the species Ceratonia siliqua L for these purposes. Our study aims is to realize, a phytochemical screening of the powder of the leaves, pulp and seeds of the carob tree (grafted and spontaneous), an extraction and characterization of polyphenol *Ceratonia siliqua* L.; and finally an evaluation of the antimicrobial activity of polyphenolic substances extracted.

II. Material and methods

II.1. *Ceratonia siliqua* samples

Samples of both varieties of *Ceratonia siliqua L.* were harvested in the region of Lakhdaria (Bouira-Algeria).

Directly after harvest the plants are dried out in the open air and shielded from light and heat. They are ground up into powder after drying. The powder is contained in glass bottles, sealed free from light and moisture.

II.2. Microotganisms tested

Microbial strains used for testing the antimicrobial activity of polyphenols are represented by five bacteria: *Escherichia coli, Staphylococcus aureus, Bacillus thuringiensis Micrococcus sp, Pseudomonas aeruginosa,* fungus (*Aspergillus sp.*) and a yeast (*Candida albicans*) (Table 1). They were selected for their high frequencies to contaminate and for their pathogenicity.

Table 1. *Microorganisms used for evaluating the antimicrobial activity of polyphenol extracts of Ceratonia siliqua L.*

Microorganisms	Gram	References
Staphylococcus aureus	Gram +	**ATTC 43300**
Bacillus thuringiensis	Gram+	**ATTC 11178**
Micrococcus sp	Gram+	**Identified**
Pseudomonas aeruginosa	Gram -	**ATTC 27853**
Escherichia coli	Gram -	**ATTC 25922**
Candida albicans	/	**ATTC 2071**
Aspergillus sp	/	**Identified**

II.3. Chemical Screening

Phytochemical tests on the two varieties of *Ceratonia siliqua* L. aim to find substances existing secondary metabolites in these plants. They are either carried out on the powder of the plants, either on their infused at 20% [9].

II.4. Extraction and characterization of phenolic compounds

The process theory is based on the extraction of soli d-liquids [10]. The dosage of the phenolic compounds is conducted by a colorimetric spectrophotometer "Optizen 2120UV" by using the Folin-Ciocalteu reactive [11].

II.5. Infrared analyses

The infrared spectra were acquired using a JASCO FT spectrometer | IR-4100 Fourier transform computer, controlled to a range of frequencies between 400 and 4000 cm -1. The samples are deposited on KBr pellets. The wave numbers are given in cm-1.

II.6. Evaluation of the antimicrobial activity

To evaluate the antimicrobial activity of phenolic extracts, we used the method of susceptibility testing [12]. The purpose of this review is to assess the sensitivity or resistance of reference strains (Gram + bacteria, Gram, fungi and yeast) against the polyphenolic extract of *Ceratonia siliqua* L.

III. Results and discussion

III.1. Phytochemical screening

The results of the phytochemical screening realized on the pulp, seeds and leaves of the grafted variety (GP, GS and GL) as well as pulp, seeds and leaves of the spontaneous variety (SP, SS, SL) are summarized in Table n°2.

The overall results of phytochemical tests indicated that pulp, seeds and leaves of both grafted and spontaneous varieties of *Ceratonia siliqua* L. are rich in total tannins, gallic tannins and catechin tannins. However, spontaneous leaves contain more tannin than those grafted leaves. The same result was noted by Shawakfeh and Ereifej [13]. Also, studies have reported that the condensed tannin content of fruits carob represented 16 and 20% of the dry mass [14]. It should also note the presence of saponins and alkaloids in both varieties. According to the result of our study, the rate of total sugars (glucoside) at the wild and cultivated pulp is slightly higher than that of the seed but these glycosides are absent in the leaves of both varieties (grafted and spontaneous). It should be noted that spontaneous seeds are rich in glucoside compared to the grafted seeds. The results of the chemical screening of our two varieties show that the spontaneous varieties are rich in mucilage and coumarin compared to grafted varieties. It is noted the importance of mucilaginous gum seeds of the carob used in many commercial products as a stabilizer thickener [15].

Table 2. *Results of phytochemicl screening of pulp, seeds and leaves of the grafted and spontaneous variety of Ceratonia siliqua L.*

	GP	*GS*	*GL*	*SP*	*SS*	*SL*
Total tannins	+	+	+++	+++	+++	+++
Catechin tannins	+	-	++	+	-	+
Gallic tannins	++	+	+++	+++	+++	+++
Flavonoids	+	+	+++	+	++	+++
Anthocyanins	-	-	-	-	-	-
Leuconthocyanin	+++	++	++	+++	+++	+
Free quinones	++	-	+	+	-	-
Saponosides	+++	++	++	+++	+++	++
Alkaloids	+++	++	+	+++	++	+
Irridoittes	-	-	-	-	-	-
Coumarins	+	+	+	+++	+++	+++
Mucilage	+	+	-	++	+++	+

III.2 . *Dosage of total polyphenols*

Spectrophotometric method enables us to determine the total polyphenol concentration that presents each sample, as mentioned in the following table:

Table 3. *Concentration of total polyphenols in different extracts*

	GP	**GS**	**GL**	**SP**	**SS**	**SL**
Concetration mgEAG/g	10 ± 1,2	11.5 ± 0,85	10.2 ± 0,65	10.5 ± 0,43	8 ± 0,61	14 ± 0,53

The result of the determination of total polyphenols *Ceratonia siliqua* L. showed that spantaneous leaves is more superior than the other extracts. The results we have obtained is greater than 1,9mg EAG / g .. Other studies have shown that the carob could hold a lot more phenolic compounds, up 13,51mg EAG / g [16] and 19,2mg EAG / g [17]. The difference observed in the different studies may be explained by geographical origin, cultivar, variety and especially the degree of maturity, which has been proven by some studies [18].

III.3. Qualitative analysis of phenolic compounds

IR spectroscopy is a method of rapid and sensitive characterization of most existing organic molecules. IR spectra representing transmittance (% T) as a function of wave number (cm-1) of the residue of grafted extracts are represented by the following figures (1):

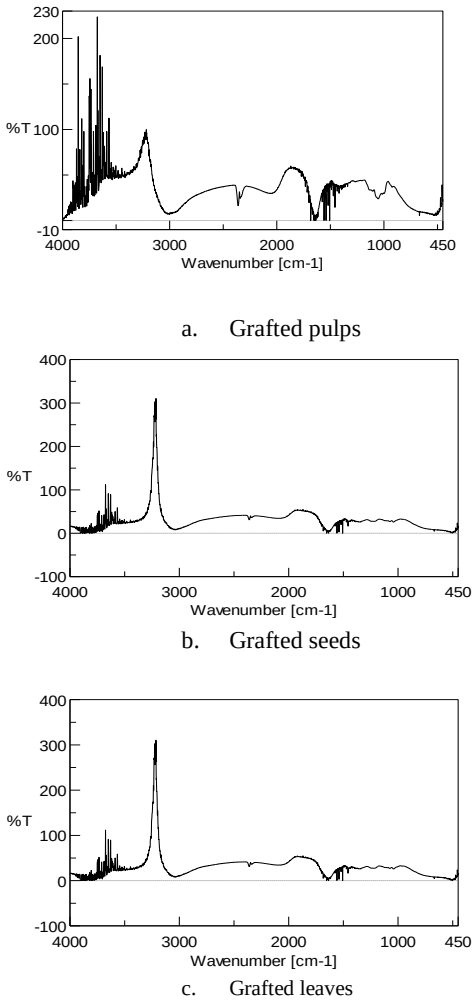
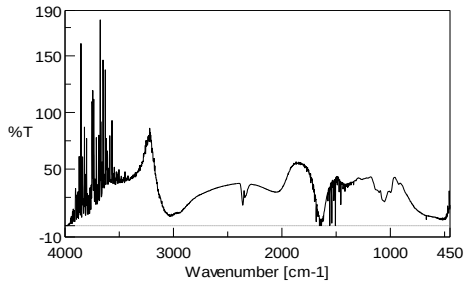


Figure 1. *IR Spectroscopy of phenolic compounds (grafted variety)*

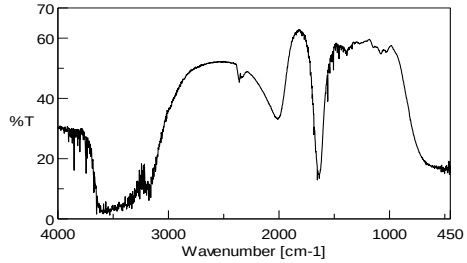
In the phenolic extract of grafted pulps *Ceratonia siliqua* L. Broadband around 3002.14cm-1 corresponds to the vibration of elongation of the CH bond of the alkene, it of 2360.93cm-1 is attributed to the stretching vibration of the C≡C bond nitriles both 1684.03cm 1634.38cm-1 and 1-bands are allocated to the stretching vibration of C-O bond. Bands 1,1506.13cm 1558.20 cm-1 are generally associated with the vibration of OH bond of carboxylic acid. Concerning the phenolic extract cultured seeds, broadband around 2047.55 cm-1 is associated with the C≡C bond stretching vibration of Nitriles and elongation of the bond C- O .the aldehyde two tape 1558.68 cm-1 and 1384.16cm-1 are attributed to the OH bond of vibration of carboxylic acid, the four band 513 936 cm-1470.064 cm-1, 458.01cm-1, 452 708 cm-1 are generally associated with the vibration of elongation of the CH bond of alkenes. For the phenolic extracts grown leaves, broadband around 3221.99 cm-1 corresponds to the vibration of the OH

bond Alcohol and elongation of the bond O. The two C- band 1456.88 cm-1, 504.290cm-1 are generally associated with the vibration of elongation of the CH bond of alkenes.

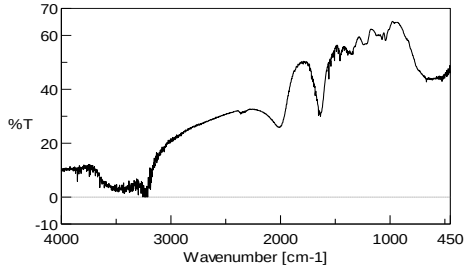
IR spectra representing transmittance (% T) as a function of wave number (cm-1) of the residue of spontaneous extracts are represented by the following figures (2):



a. Spontaneous pulp



b. Spontaneous seeds



C . Spontaneous leaves

Figure 2. *IR Spectroscopy of phenolic compunds (spontaneous variety)*

In the phenolic extract of spontaneous pulp *Ceratonia siliqua* L. , there are a broad band around 3029 .14cm-1 corresponds to the vibration of elongation of the CH bond of the alkene followed by that of 2361.41cm-1 is attributed to the stretching vibration of the bond C≡ N nitriles. Both 1684.03cm-1 band, 1634.86 cm-1 are respectively associated with the stretching vibration of C = O double bond. And for 1558.20cm-1 bands, 1506.61cm-1 are associated with the vibration of the OH bond elongation of carboxylic acid. Furthermore, the phenolic extract of spontaneous seeds, broadband author 2012.36cm-1 corresponding to the stretching vibration of C = O double bond; both 1559.17cm-1 bands, 1506.61cm-1 are attributed to the vibration of the OH bond elongation of carboxylic acid. The three bands (1456.96cm-1 1386.57cm-1 501CM-1, 884cm-1) are respectively associated with the vibration of elongation of the CH bond of the alkene. The phenolic wild leaf extract has two strong band (3128.94cm-1, 1456cm-1) are respectively associated with the CH bond of alkenes, both 1558.68cm band 1506cm-1 corresponding to the elongation of single bond OH, and a strong band at 1635.34 cm-1 corresponding double bond.

According to the infrared spectra obtained, it can be said that the various phenolic extracts from two varieties of *Ceratonia siliqua* L. have several functional groups with high and medium band.

III.4. Evaluation of the antimicrobial activity of phenolic compounds

The evaluation of the antimicrobial activity of phenolic extracts of *Ceratonia siliqua* L, both grafted and spontaneous varieties performed on five bacteria and two fungal strains (yeast, fungi), is done by measuring the diameters of inhibition zone (clear zone).

Table 4. *antimicrobial activity of different phenolic extracts*

	GP	**GL**	**GS**	**SP**	**SL**	**SS**
Staphylococcus aureus	6.35 ± 0.043	14.66 ± 2.18	10 ± 0.58	8.73 ± 0.56	16.36 ± 0.63	7.06 ± 0.58
Bacillus thuringiensis	7.63 ± 0.55	8.13 ± 0.18	6.3 ± 0.06	6.43 ± 0.18	9.73 ± 0.16	7.5 ± 0.30
Micrococcus sp	0	13 ± 0.58	11.56 ± 0.30	0	15.83 ± 0.73	9.7 ± 0.18
Pseudomonas aeruginosa	0	14.30 ± 0.35	0	6.26 ± 0.12	13.8 ± 0.15	0
Escherichia coli	15.76 ± 0.23	20.63 ± 0.88	13.66 ± 2.66	17.8 ± 0.25	21.83 ± 0.93	11.33 ± 1.20
Candida albicans	0	0	0	7.56 ± 0.23	0	6.7 ± 0.41
Aspergillus sp	0	0	0	0	0	0

According to the infrared spectra obtained, the specific phenolic extracts from two varieties of *Ceratonia siliqua L.* can be state have several functional groups with high and medium band.

Concerning the antimicrobial activity, the best antimicrobial activity against different strains was recorded by spontaneous leaves against *Escherichia coli* and *Staphylococcus aureus*, *Microccus sp* and by leaves grafted against *Pseudomonas aeruginosa*. It should also be noted that *Aspergillus* sp is resistance to all extract of *Ceratonia siliqua* L.,

Compared to the literature, our results are different. This difference can be attributed to several factors such as the inherent factors (variety, environmental conditions, ecological, seasonal variations), extraction methods [19, 20], preparation of the extract, the solvent used the sensibility of bacteria [21] and finally the part of the plant used [22, 23].

VI. Conclusion

Our work consists of a phytochemical study, extraction, characterization and evaluation of the antimicrobial activity of total phenolic compounds of *ceratonia siliqua* L. (grafted and sponatneous). The overall results of phytochemical tests indicated that pulp, seeds and leaves of both varieties are rich in total tannins, gallic tannins and catechin tannins. However, spotaneous leaves contain more tannin than those grown leaves. It should also note the presence of saponins and alkaloids in both varieties.

Relate to the quantitative analysis was carried out by spectrophotometry; it appears that the spontaneous leaves have the highest content (14mg EAG / g). However, the qualitative analysis by infrared spectroscopy revealed the presence of several functional groups in both varieties

Experimental results showed that for all phenolic compunds extracts of *Ceratonia siliqua* L. act only on bacteria but in different degree and have no effect on the tested fungi.

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