

Antimicrobial activity of *Ceratonia siliqua* L. extract against diarrheagenic *E-coli*.

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Abstract

Ceratonia siliqua L. has been used in many Mediterranean and west Asian countries traditional medicine as antidiarrheal and diuretic. The present study was designed to determine the antimicrobial activity of both watery and methanol barks extract of the whole plant of *Ceratonia siliqua* L. against diarrheagenic *E. coli* isolated from cases of diarrhea in children less than 5 years old, in this study we use three different concentration of both watery and methanolic extract, and we find that the antibacterial activity of methanolic *Ceratonia siliqua* extract was best than antibacterial activity of watery extract.

Keywords: *Ceratonia siliqua* L., diarrheagenic *E. coli*

INTRODUCTION

Nowadays researchers are increasingly turning their attention to folk medicine, looking for new discovery to develop better drugs against micro-bial infections caused by various pathogens⁽¹⁾. The clinical efficacy of many existing antibiotics is being threatened by the emergence of multidrug-resistant pathogenic strains⁽²⁾. It is important to discover new antimicrobial compounds with diverse chemical structures and with novel mechanisms of action for new and re-emerging infectious disease⁽³⁾. The antibiotic resistance and failure of chemotherapeutic exhibited by pathogenic microbial infectious agent has led to the screening of several medicinal plants for their potential antimicrobial activity⁽⁴⁾.

The carob tree (*Ceratonia siliqua* L.), also called algarroba, is an evergreen tree which grows throughout the Mediterranean area, mainly in Morocco, Spain, Italy and Portugal⁽⁵⁾. The species belongs to the Cesalpinaceae sub-family of the family Leguminosae (syn Fabaceae)⁽⁶⁾. It's been reported that the genus of *Ceratonia*, consists of three species, Carob trees may be thus male, female, and hermaphrodite⁽⁷⁾. Carob has been cultivated for thousands of years as a forage crop or food for human consumption⁽⁸⁾. Recently, this species has attracted much attention and became economically important. Pods and seeds are used as raw material in food, pharmaceutical and cosmetic industries⁽⁹⁾. Everywhere in Morocco, pods are used to fight diarrhea in infants, children and adults⁽¹⁰⁾.

Infusion of carob leaves is used as an emetic for acute poisoning⁽¹¹⁾. In Turkish folk medicine, leaves and barks of carob tree are used as an antidiarrheal and diuretic⁽¹²⁾. The fruits of this plant are traditionally used as an antitussive and against warts⁽¹³⁾. Many recent activities are reported for carob pods such as antihyperglycemic, antioxidant, immunomodulating and antiproliferative on

mouse hepatocellular carcinoma cell line⁽¹⁴⁾.

Aim of the study: is to determine the effect of both of the watery and methanolic extract of *Ceratonia siliqua* on the Diarrheagenic *E. coli* isolated from diarrheal cases in children less than 5 years old.

MATERIALS AND METHODS

1. Bacterial Isolates and Culture Media

The diarrheagenic *E. coli* which are diagnosed by using of molecular methods was used for testing of plant extract effectivity. The isolates were subcultured from glycerol mixed broth media that was stored at -80°C, on fresh MacConkey agar plates for 24 hours prior to plant extract test, then testing of plant extract was done on Muller Hinton agar.

2. *Ceratonia siliqua* L. Extraction

Plant was air dried at room temperature, with no direct sunlight, and then pulverized using an electrical grinder, 260 g dried coarse powder of the bark was placed into the extractor. The extraction was carried out by using water and methanol as solvent. At the end of the extraction, the respective solvent were concentrated by evaporation to dryness by putting it in the oven, final methanolic and watery extract of *Ceratonia siliqua* was a dark brown semisolid in percentage dry weight of 15%. For assuring stability, the methanolic extract were stored at 4°C until use⁽¹⁵⁾.

3. Study of Antibacterial Activity by Using Agar Disc Diffusion Method for Aqueous Plant Extract and Alcoholic Extract

The Mueller Hinton Agar medium was prepared and sterilized at autoclaves for 15min, the sterilized agar medium was poured into sterile petri dishes under

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aseptic conditions and allowed to solidify. The 24 hours-old cultures of pathogenic *E. coli* strains were inoculated and evenly spread on the surface of the agar by sterile swab to get uniform lawn culture of the organism⁽¹⁶⁾.

The antimicrobial activity of the extracts and control was evaluated by the disc diffusion method described by Bauer *et al.*, (1966) with modifications⁽¹⁶⁾.

Filter paper disc which had previously been sterilized in an oven at 100°C for two hour were soaked with the three extract at 3 concentration (15%, 20%, 25%) of both water and alcoholic extract discs were placed on MH agar and inoculated with the test bacteria and incubated at 37°C for 18-24 hours.

After incubation, inhibition zone were measured to determine which concentration of the extract inhibited bacterial growth. The resulting haloes were compared with control discs, which composed of filter paper disk soaked with distal water and considered as control negative⁽¹⁶⁾.

4. Statistical Analyses

The program of spss version 18, was used to analysis data by T. test to determine the statistical significance of the

data. P value of <0.05 was considered significant.

RESULTS

The effect of three different concentration (15%, 20%, 25%) of each alcoholic and aqueous extract of *Ceratonia siliqua* were examined against *E. coli* species by using of disk diffusion method, and compared with control negative in this case the control was distilled water, we observed that antibacterial activity of plant extract increased with increasing the extract concentration, the inhibition zone of each alcoholic and aqueous at (15%) concentration was 5.8 mm, 5.6 mm respectively, when extract concentration increase to (25%) the inhibition zone of each alcoholic and aqueous extract also increase to 6.9 mm, 6.8 mm respectively as shown in table (1). Also it was noticed that there was difference between the aqueous and alcoholic extract in antibacterial activities that were in the same concentration, the alcoholic extract was better in action than aqueous extract as shown in table (2). Statistical analysis appears that there was significant differences between the concentration of 15% and 25% of both alcoholic and aqueous extract ($p < 0.05$).

Table 1. Inhibition zone of bacteria treated with extract of *Ceratonia siliqua*.

No.	No. of sample	15%		20%		25%	
		ALC/mm	W/mm	ALC/mm	W/mm	ALC/mm	W/mm
1.	2	6	7	7	7	7	8
2.	4	6	5	7	6	7	6
3.	5	7	6	7	6	8	7
4.	9	7	6	7	7	8	7
5.	11	6	7	7	7	7	8
6.	13	6	5	7	6	7	6
7.	25	5	5	6	6	6	6
8.	38	6	6	6	7	7	8
9.	40	5	6	6	6	7	7
10.	44	5	5	5	5	6	6
Total		58	56	63	62	69	68
Mean		5.8	5.6	6.3	6.2	6.9	6.8

Table 2. Statistical Analyses of the results of plant extract.

	No. of strains	Mean diameter (mm) ± SD	S. E. *	T- test (df=9)	95% C. I. **	P- value
15% <i>Ceratonia siliqua</i> in methanol	10	5.9 ± 0.74	0.1	11	-1.33 to -0.87	<0.0001***
25% <i>Ceratonia siliqua</i> in methanol	10	7 ± 0.67				

*S. E. = Standard Error / **C. I. = Confidence Interval / ***Highly Significant

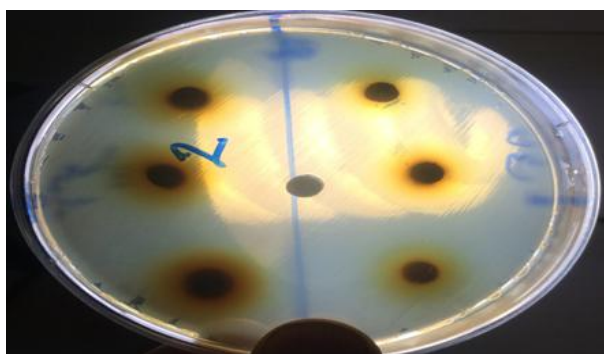


Figure 1. Disc Diffusion Method for *Ceratonia siliqua* Extract

DISCUSSION

The presented study was designed to obtain preliminary information on the antibacterial activity of *C. siliqua* fruits on pathogenic bacteria. The agar-well diffusion method was preferred to be used in this study. The results

showed a remarkable antibacterial activity of the methanol extracts of these plants. The strong antimicrobial activity of the *Ceratonia siliqua* against the tested microorganisms could be attributed to the presence of high percentage of hydrocarbo (51.06%), monoterpene (0.9%), and oxygenated monoterpene (1.19%) appreciated for their antibacterial potentials (16,17%). These results are in agreement with those reported in literature for methanol extract of *Ceratonia siliqua* showed strong action on *Enterococcus*, *Escherichia coli*, and *Staphylococcus aureus*⁽¹⁷⁾.

From the current result, there was clear effect of antibacterial activity of plant extract by increasing the concentration. And the alcoholic extract showed better action than the aqueous extract, and this was in agreement with the study of Basma *et al.* 2012⁽¹⁸⁾ who found that the methanolic extract of *Ceratonia Siliqua* show good antibacterial action against gram negative bacteria including *E. coli*.

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