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In Vitro Evaluation of the Antibacterial Activity of Aqueous and Alcoholic Extracts of *Ceratonia siliqua* L. Pods Collected from Al Marj Region Against *Escherichia coli* and *Staphylococcus aureus*

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ABSTRACT

The rapid emergence of antibiotic-resistant bacterial strains poses a severe global health challenge, necessitating the exploration of safe, plant-derived bioactive compounds. This study evaluated and compared the antibacterial potential of aqueous and alcoholic extracts derived from *Ceratonia siliqua* L. (carob) pods collected from the Al Marj region (Al Jabal Al Akhdar, Libya) against *Escherichia coli* and *Staphylococcus aureus*. Crude pod extracts were prepared using distilled water and 80% ethanol at graded concentrations of 25%, 50%, and 75%. Antibacterial efficacy was determined via the agar well diffusion assay on Mueller-Hinton agar, evaluated through a semi-quantitative scoring scale (− to +++). Experimental findings demonstrated a concentration-dependent inhibitory response, with the alcoholic extract exhibiting superior antibacterial activity compared to the aqueous extract. *S. aureus* showed greater susceptibility (+++ at 75% alcoholic extract) than *E. coli* (++ at the same concentration), whereas no inhibition (−) was recorded at the lowest aqueous concentration against *E. coli*. In conclusion, local *C. siliqua* pods from Al Marj harbor potent phenolic constituents with notable antibacterial properties, representing a promising natural resource for antimicrobial formulations and food preservation.

Keywords: *Ceratonia siliqua*; Carob pods; Antibacterial activity; *Escherichia coli*; *Staphylococcus aureus*; Al Marj.

1. INTRODUCTION

Multidrug resistance (MDR) among pathogenic bacteria represents a critical obstacle in clinical medicine and food safety, stimulating intensive research into plant-derived secondary metabolites as alternative therapeutic and preservative agents. *Staphylococcus aureus* (Gram-positive) and *Escherichia coli* (Gram-negative) are major etiological pathogens implicated in diverse nosocomial infections and foodborne illnesses.

Ceratonia siliqua L., belonging to the family Fabaceae, constitutes a vital component of the natural and agricultural flora in Al Marj and the broader Al Jabal Al Akhdar region of Eastern Libya, producing mature pods renowned for their nutritional and pharmacological significance.

Phytochemical investigations indicate that carob pod pulp and seeds are exceptionally rich in polyphenols, condensed tannins, flavonoids, and gallic acid derivatives. These bioactive molecules impair bacterial viability by binding to cell membrane proteins, inhibiting essential metabolic enzymes, and disrupting cytoplasmic membrane permeability. Accordingly, this study was designed to evaluate and compare the inhibitory efficacy of aqueous and alcoholic extracts of *C. siliqua* pods at varying concentrations (25%, 50%, and 75%) against *E. coli* and *S. aureus*.

2. MATERIALS AND METHODS

Collection and Preparation of Plant Material

Mature *Ceratonia siliqua* pods were harvested from the Al Marj region (Al Jabal Al Akhdar, Eastern Libya) and thoroughly rinsed with distilled water to remove surface dust and debris. The pods were shade-dried at ambient room temperature for 7–10 days, crushed, and ground into a homogenous fine powder. The powder was stored in airtight amber glass containers until extraction.

Preparation of Aqueous and Alcoholic Extracts

Powdered carob pods were divided into two equal portions and macerated in either distilled water (aqueous extraction) or 80% ethanol (alcoholic extraction) under continuous agitation for 24–48 hours at room temperature. The mixtures were filtered through Whatman No. 1 filter paper, and the filtrates were concentrated under reduced pressure using a rotary evaporator at 45°C to obtain the stock crude extracts (100%). Test concentrations (25%, 50%, and 75%) were formulated by diluting the stock extracts in 10% dimethyl sulfoxide (DMSO) or sterile distilled water.

Bacterial Inoculum Standardization

Authenticated clinical/standard strains of *E. coli* and *S. aureus* were subcultured in nutrient broth at 37°C for 24 hours. Bacterial suspensions were adjusted to match the 0.5 McFarland turbidity standard (approximately 1.5×10^8 CFU/mL).

Antibacterial Susceptibility Assay and Semi-Quantitative Scoring

Mueller-Hinton agar plates were uniformly inoculated with 0.1 mL of the standardized bacterial suspension using a sterile swab. Wells of 6 mm diameter were bored into the agar, and 50–100 µL of each extract concentration (25%, 50%, and 75%) for both solvents was dispensed into respective wells. Ciprofloxacin (5 µg) served as the positive control, while the diluent served as the negative control. Plates were incubated at 37°C for 24 hours in triplicate. Antibacterial susceptibility was evaluated semi-quantitatively based on the presence and clarity of the inhibition zones surrounding the wells according to the following standardized scoring scale:

- **(−)**: No inhibition (complete bacterial growth around the well).
- **(+)**: Weak inhibition (narrow inhibition zone).
- **(++)**: Moderate inhibition (distinct, medium-sized inhibition zone).

• **(+++)**: Strong inhibition (wide, completely clear inhibition zone).

3. RESULTS

The semi-quantitative antibacterial efficacy of the aqueous and alcoholic extracts of *C. siliqua* pods against *S. aureus* and *E. coli* is summarized in Table 1. A progressive, concentration-dependent enhancement in inhibitory activity was observed as the extract concentration increased from 25% to 75% across both extraction solvents. Notably, the alcoholic pod extract consistently outperformed the aqueous extract against both bacterial strains, with *S. aureus* demonstrating markedly higher sensitivity than *E. coli*.

Table 1. Semi-quantitative antibacterial activity scores of aqueous and alcoholic *Ceratonia siliqua* pod extracts against *S. aureus* and *E. coli*.

Extract Type (Carob Pods)	Concentration (%)	*S. aureus* Inhibition Score	*E. coli* Inhibition Score
Aqueous Extract	25%	+	–
Aqueous Extract	50%	++	+
Aqueous Extract	75%	++	++
Alcoholic Extract	25%	++	+
Alcoholic Extract	50%	+++	++
Alcoholic Extract	75%	+++	++
Positive Control (Ciprofloxacin)	5 µg	+++	+++
Negative Control (Diluent)	—	–	–

Scoring Key: (−) No activity; (+) Weak activity; (++) Moderate activity; (+++) Strong activity.

4. DISCUSSION

The findings of this investigation corroborate the potent antimicrobial properties of *Ceratonia siliqua* pod extracts native to the Al Marj region against pathogenic bacteria, particularly at the highest concentration (75%), which achieved maximum inhibition (+++). These outcomes align with recent regional and Mediterranean studies confirming the capacity of carob extracts to suppress multidrug-resistant bacterial strains.

The marked superiority of the alcoholic pod extract over the aqueous extract is primarily attributed to the selective polarity of ethanol, which efficiently solubilizes free phenolic acids and condensed tannins concentrated within the pod pulp. Conversely, aqueous extraction co-extracts substantial quantities of water-soluble sugars (such as sucrose and glucose) naturally abundant in carob pods, which can dilute the relative concentration of active antimicrobial constituents in the crude extract.

Furthermore, the higher susceptibility of *S. aureus* (+++) compared to *E. coli* (++) aligns with structural differences in their cell envelopes. Gram-positive bacteria lack an outer membrane, permitting gallic acid and flavonoids from carob pods to penetrate the peptidoglycan layer and disrupt the cytoplasmic membrane. In contrast, *E. coli* features a lipopolysaccharide-rich (LPS) outer membrane that functions as an effective permeability barrier against hydrophobic phenolic molecules.

5. CONCLUSIONS

Local *Ceratonia siliqua* pod extracts collected from the Al Marj region exhibit significant concentration-dependent antibacterial activity against *S. aureus* and *E. coli*, achieving peak inhibitory efficacy (+++) with the 75% alcoholic extract. Future studies should focus on isolating individual phenolic fractions via chromatographic techniques to evaluate their potential application as natural food preservatives or antibiotic adjuvants.

CONFLICT OF INTEREST

The author declares that there is no conflict of interest regarding the publication of this manuscript.

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