Journal of Progressive Medical Sciences
ISSN: 3052-8518
J.P.M.S
DOI: https://doi.org/10.63939/swzcdf24
Copyright © The Author(s)
Vol. 2 , No. 7 , November 2026

RESEARCH Open Access

Preliminary Antibacterial Activity of *Carissa macrocarpa* Leaf Extract Against *Escherichia coli* and Molecular Screening of *intI3* and *fimH* Genes in *Enterobacter* and *Salmonella* Isolates

Alaa Mohammed Khaleel[1] , Zahraa K. Lawi[2], Sahar Mohammed Jawad[3]

[1]Faculty of pharmacy- Department of pharmacognosy/faculty of pharmacy/ kufa university – Iraq. Email: alaam.jabri@uokufa.edu.iq. https://orcid.org/0000-0002-2273-5628 , [2]University of kufa/ faculty of science/ department of biology. Email: zahraalawi234@gmail.com , [3]Middle Euphrates Cancer Research Unit, College of Medicine, University of Kufa, Iraq. Email: saharm.alkarawy@uokufa.edu.iq

Abstract

Background: The increasing prevalence of antimicrobial resistance among Gram-negative bacteria has stimulated interest in medicinal plants as potential sources of biologically active antibacterial compounds. *Carissa macrocarpa* (Eckl.) A.DC., a member of the *Apocynaceae* family, has attracted scientific attention because of its phytochemical diversity and previously reported antimicrobial properties. Molecular characterization of resistance-associated and virulence-associated genes in bacterial isolates represents a complementary approach to understanding their biological characteristics. **Objective:** This study aimed to describe the preliminary antibacterial activity of *C. macrocarpa* leaf extract against *Escherichia coli* and report polymerase chain reaction (PCR)-based screening observations for the *intI3* integron-associated gene and *fimH* adhesion-associated gene in *Enterobacter* and *Salmonella* isolates, respectively. **Methods:** The study was based on preliminary laboratory observations involving agar-well diffusion testing of *C. macrocarpa* leaf extract at three nominal concentrations. Inhibition-zone diameters were recorded against *E. coli*. Conventional PCR and agarose gel electrophoresis were described for screening the selected bacterial genetic targets. The available experimental findings were analyzed descriptively

Creative Commons Attribution-Non-Commercial 4.0 International License

because complete replicate-level measurements and molecular validation records were not available. **Results:** The reported inhibition-zone diameters against *E. coli* were 18 mm at 100 mg/mL, 16 mm at 50 mg/mL, and 14 mm at approximately 33.3 mg/mL. Preliminary molecular observations indicated amplification products corresponding to the expected 506-bp *fimH* target in selected *Salmonella* isolates and the expected 600-bp *intI3* target in selected *Enterobacter* isolates. However, interpretation of these findings was limited by the lack of independent experimental replicates, full control results, MIC/MBC measurements and corroborating molecular analyzes. **Conclusion:** The early results suggest that there is a need for additional research to find out the antibacterial effects of *C. macrocarpa* leaf extract on *E. coli*. The molecular observations reported are consistent with amplification of the selected genetic targets, but require independent confirmation. The evidence available so far does not establish that the plant extract had antibacterial activity against *Enterobacter* or *Salmonella*. It is not established that the molecular markers identified are directly related to susceptibility to the plant extract.

Keywords: Phytochemicals; antibacterial activity; antimicrobial resistance; *Carissa macrocarpa*; *Escherichia coli*; *Enterobacter*; *Salmonella*; *intI3*; *fimH*; PCR.

1. Introduction

Antimicrobial resistance (AMR) represents a major challenge to global public health because it compromises the effectiveness of antimicrobial therapy and increases the complexity of managing bacterial infections. The emergence and dissemination of resistant bacterial populations have encouraged the investigation of alternative antimicrobial strategies, including the identification of biologically active compounds derived from medicinal plants. Gram-negative bacteria are particularly important in this context because their structural characteristics and capacity to acquire resistance determinants may contribute to reduced susceptibility to multiple antimicrobial agents. Among these microorganisms, *Enterobacter* and *Salmonella* species are clinically relevant pathogens associated with a wide range of infectious diseases. *Enterobacter* species are opportunistic pathogens implicated in healthcare-associated infections, including urinary tract infections, respiratory tract infections, bloodstream infections,

Creative Commons Attribution-Non-Commercial 4.0 International License

Published by DAC Democratic Arabic Center
DAC Democratic Arabic Center GmbH, Berlin

and other invasive conditions. *Salmonella* species are important causes of foodborne gastrointestinal disease and may produce systemic infections in susceptible individuals. The emergence of antimicrobial resistance among these bacteria is influenced by several mechanisms, including alterations in antimicrobial targets, reduced membrane permeability, active efflux systems, enzymatic inactivation of antimicrobial agents, and acquisition of resistance-associated genetic elements. Horizontal gene transfer contributes to the dissemination of resistance determinants between bacterial populations. Mobile genetic elements, including plasmids, transposons, and integron-associated gene cassettes, are therefore important subjects of molecular microbiological investigation [11,14,15,24,17]. Understanding the genetic characteristics of bacterial isolates is relevant to antimicrobial resistance surveillance and may provide useful information for subsequent investigations of antibacterial agents.

Integrons are genetic platforms capable of capturing, integrating, and expressing gene cassettes through site-specific recombination mechanisms. Their biological significance is closely associated with the acquisition and dissemination of genetic determinants, including those involved in antimicrobial resistance.Integrons are classified according to the sequence and characteristics of their integrase genes. Class 1 integrons have been extensively investigated because of their widespread association with antimicrobial resistance among Gram-negative bacteria. Class 2 integrons have also been identified in different bacterial populations, whereas class 3 integrons have generally been reported less frequently [11,14,15,17]. The *intI3* gene encodes the integrase associated with class 3 integrons. This enzyme participates in site-specific recombination involving integron-associated recombination sites and gene cassettes. Collis et al. [15] characterized the class 3 integron recombination system and demonstrated the functional activity of the IntI3 integrase. Their findings contributed to understanding the molecular organization and recombination properties of this integron class. Additional investigations have reported different integron classes in Gram-negative bacterial isolates, highlighting the importance of integron screening in studies addressing bacterial resistance-associated genetic characteristics [14,24,17]. Nevertheless, the detection of an integrase gene does not independently establish phenotypic antimicrobial resistance. An integron may be present without demonstrating that the isolate carries a particular resistance-gene cassette or expresses a clinically relevant resistance phenotype. In addition to antimicrobial resistance, bacterial

Creative Commons Attribution-Non-Commercial 4.0 International License

Published by DAC Democratic Arabic Center
DAC Democratic Arabic Center GmbH, Berlin

pathogenicity is influenced by virulence-associated determinants that facilitate attachment, colonization, invasion, and persistence within host environments. Bacterial adhesion is an important early event in many infectious processes. Surface-associated structures, including fimbriae and adhesins, contribute to interactions between bacterial cells and host tissues. The *fimH* gene encodes a type 1 fimbrial adhesin involved in receptor recognition and bacterial attachment. This adhesin has been investigated in several members of the order *Enterobacterales*, including *Escherichia coli* and *Salmonella enterica*. Previous investigations have examined the role of type 1 fimbriae in *Salmonella* adhesion and pathogenicity. Yin et al. [13] investigated the influence of cinnamaldehyde on *Salmonella Typhimurium* adhesion through mechanisms involving type 1 fimbriae. Kisiela et al. [25] also investigated the relationship between mutations in a fimbrial adhesin and the evolution of *Salmonella enterica* virulence, demonstrating the importance of adhesin-associated molecular variation in host–pathogen interactions. Furthermore, molecular investigations of *Salmonella* isolates have included screening for virulence-associated genes as part of bacterial characterization [12,16,26]. However, the presence of the *fimH* gene does not necessarily demonstrate its transcriptional activity, production of a functional adhesin, or increased pathogenicity. Consequently, conventional PCR screening provides information about the potential presence of a genetic target but cannot independently establish the corresponding virulence phenotype.

Medicinal plants are a source of natural compounds with chemical diversity which have attracted attention in the antimicrobial research. Secondary metabolites of plants include phenolic acids, flavonoids, tannins, alkaloids, terpenoids, glycosides and other biologically active constituents. Various chemical classes have been investigated for antibacterial activity but the effects are dependent on molecular composition, concentration, extraction conditions and the properties of the target microbe. Previous studies investigated the antimicrobial activity of extracts of various medicinal plants and the effect of extraction methods and assay conditions on the results of the experiments [3,9,19,20]. Plant extracts can inhibit bacteria through several mechanisms, including interactions with bacterial membrane, proteins, enzymes and other cellular components. However, the presence of a particular phytochemical class does not mean that the compound is antibacterial, and the biological effects observed in crude extracts cannot be attributed to individual compounds without the necessary

Creative Commons Attribution-Non-Commercial 4.0 International License

Published by DAC Democratic Arabic Center
DAC Democratic Arabic Center GmbH, Berlin

analytical and experimental validation. In addition, differences in experimental procedures, plant material, geographical origin and solvents used for extraction may also affect the composition and apparent activity of the extracts produced. These considerations highlight the importance of distinguishing phytochemical findings reported in previous studies from constituents experimentally identified in the extract used in a particular investigation.

Carissa macrocarpa (Eckl.) A.DC., commonly known as Natal plum, belongs to the family *Apocynaceae* and is native to southern Africa. The plant is an evergreen shrub cultivated for its ornamental characteristics and edible fruits. Different species belonging to the genus *Carissa* have also been investigated for their traditional medicinal applications and biological properties [22,23]. Phytochemical investigations have demonstrated that *C. macrocarpa* contains a variety of secondary metabolites with potential biological significance. Souilem et al. [1] investigated the phenolic composition and biological activities of *C. macrocarpa* leaves, stems, and flowers. Their study identified multiple phenolic compounds, including phenolic acids, flavan-3-ols, and flavonol glycoside derivatives, and evaluated the relationship between phytochemical composition and selected biological activities. Importantly, the investigators reported antibacterial activity associated with hydroethanolic extracts of different plant tissues, including bactericidal activity of the leaf extract against *E. coli* [1]. Allam et al. [5] also investigated phytochemical and biological characteristics of *C. macrocarpa*, contributing to the available scientific literature concerning the biological properties of this plant. Moodley et al. [10] examined antibacterial and anti-adhesion activities of pentacyclic triterpenoids isolated from *C. macrocarpa* leaves and edible fruits. Further investigations have addressed the influence of seasonal variation on phytochemical composition and biological activity, indicating that plant-derived bioactivity may depend on environmental and collection-related factors [2]. Studies involving other *Carissa* species have also investigated phytochemical composition and antimicrobial properties, providing broader information about the biological potential of this genus [4,6,8,22,23].

Nevertheless, findings obtained from different plant species, plant tissues, extraction methods, and bacterial strains cannot be considered directly interchangeable.

Creative Commons Attribution-Non-Commercial 4.0 International License

Published by DAC Democratic Arabic Center
DAC Democratic Arabic Center GmbH, Berlin

The chemical composition and antibacterial activity of an individual extract must therefore be evaluated according to its own experimental conditions.

Previous studies have reported antibacterial effects associated with extracts or isolated constituents of *Carissa* species. Souilem et al. [1] evaluated the antimicrobial activity of hydroethanolic extracts prepared from different parts of *C. macrocarpa* and reported activity against selected bacterial species. Moodley et al. [10] investigated antibacterial and anti-adhesion properties of isolated pentacyclic triterpenoids, supporting further investigation of chemically characterized constituents from this plant. Other investigations have examined antimicrobial activity in related *Carissa* species, including *Carissa edulis* and *Carissa opaca* [4,6]. Collectively, these studies provide a scientific rationale for investigating the antibacterial properties of *C. macrocarpa*. However, the reported biological activities vary according to extraction procedures, bacterial species, tested concentrations, and experimental endpoints. For example, inhibition-zone measurements obtained using agar diffusion techniques cannot be directly equated with minimum inhibitory concentrations or minimum bactericidal concentrations. Similarly, the observation of an inhibition zone does not independently establish bactericidal activity.

1.7. Research Gap and Scientific Rationale

Despite previous investigations into the phytochemical composition and biological activities of *C. macrocarpa*, important questions remain regarding the reproducibility and interpretation of antibacterial screening results under defined experimental conditions. In addition, the integration of plant-derived antibacterial investigations with molecular characterization of Gram-negative bacterial isolates requires careful consideration of the relationship between experimental endpoints. Previous investigations have provided information about the antimicrobial properties of *Carissa* extracts [1,5,10], while separate molecular studies have examined integron-associated resistance determinants and fimbrial adhesion-related genes in Gram-negative bacteria [12–16,24–17]. However, evidence of antibacterial activity against one bacterial species cannot be used to establish susceptibility among different molecularly characterized bacterial isolates. The present investigation addresses this issue by reporting preliminary antibacterial observations for *C. macrocarpa* leaf extract

Creative Commons Attribution-Non-Commercial 4.0 International License

Published by DAC Democratic Arabic Center
DAC Democratic Arabic Center GmbH, Berlin

against *E. coli* and separately documenting PCR-based screening observations for *intI3* and *fimH* in *Enterobacter* and *Salmonella* isolates.

1.8. Study Aim and Objectives

1.8.1. General Aim

To evaluate the available preliminary evidence on the antibacterial activity of Carissa macrocarpa leaf extract against *Escherichia coli* and to discuss the PCR-based screening results for the intI3 and fimH genes in the selected Enterobacter and Salmonella isolates.

1.8.2. Specific Objectives

1. 1. To report in detail inhibition-zone diameters reported to be associated with *C. macrocarpa* leaf extract against E. coli at three nominal concentrations.
2. To examine the numerical pattern of the reported inhibition-zone measurements without attributing statistical significance to unreplicated observations.
3. To document the preliminary PCR findings corresponding to the expected amplification-product sizes of the *intI3* and *fimH* targets.
4. To interpret the molecular observations in relation to the established biological functions of integron-associated and fimbrial adhesion-associated genes.
5. To compare the preliminary antibacterial observations with related published studies on C. macrocarpa and related plant species.
6. To identify the methodological and evidentiary limitations affecting the interpretation and reproducibility of the reported findings.

1.9. Study Hypothesis and Scope

The preliminary antibacterial component was guided by the expectation that *C. macrocarpa* leaf extract may produce measurable inhibition of *E. coli* growth under agar diffusion conditions. The molecular component was exploratory and focused on identifying amplification products corresponding to selected genetic targets in *Enterobacter* and *Salmonella* isolates. Because the antibacterial and molecular observations were not documented for the same bacterial isolates, the study was not designed to test a direct association between the presence of *intI3* or *fimH* and

Creative Commons Attribution-Non-Commercial 4.0 International License

Published by DAC Democratic Arabic Center
DAC Democratic Arabic Center GmbH, Berlin

susceptibility to the extract. Accordingly, no hypothesis concerning genotype-dependent antibacterial susceptibility was tested. The study is therefore interpreted as a preliminary descriptive laboratory investigation rather than a confirmatory assessment of antibacterial efficacy against molecularly characterized resistant or virulent pathogens.

2. Materials and Methods

2.1. Study Design

The present study was organized as a preliminary laboratory investigation comprising two complementary experimental components: antibacterial screening of *Carissa macrocarpa* leaf extract and PCR-based screening of selected genetic determinants in Gram-negative bacterial isolates. The antibacterial component focused on the assessment of growth inhibition in *Escherichia coli* using the agar-well diffusion method at three nominal extract concentrations. The molecular component involved screening for the class 3 integron-associated *intI3* gene in *Enterobacter* spp. and the type 1 fimbrial adhesin-associated *fimH* gene in *Salmonella* spp. The methodological process consisted of quantitative antibacterial evaluation, qualitative phytochemical screening, hydroethanolic extraction and plant preparation procedures.

2.2. Plant Material Collection, Identification, and Preparation

The study was done on the fresh leaves of Carissa macrocarpa (Eckl.) A.DC. belonging to *Apocynaceae* family. The leaves were collected from a specific collection site and identified by a botanical expert as per methodological report. The leaves were collected and washed thoroughly with distilled water to remove the dust, soil particles and other surface contaminants. The leaves were washed and dried in the shade at room temperature for about 10–14 days. The objective of this procedure was to reduce exposure to high temperatures and low moisture levels. The leaves were mechanically dried and powdered to a fine powder. The powdered material was kept in sealed containers at room temperature until extraction.

Creative Commons Attribution-Non-Commercial 4.0 International License

Published by DAC Democratic Arabic Center
DAC Democratic Arabic Center GmbH, Berlin

2.3. Preparation of *Carissa macrocarpa* Leaf Extract

2.3.1. Proposed Soxhlet Extraction Procedure

The proposed a Soxhlet extraction procedure for the recovery of secondary metabolites from dried *C. macrocarpa* leaves. The choice of solvent extraction was due to previous studies which emphasized the phytochemical characterization and plant extracts [3,8,9]. The proposed procedure is to put about 50 g of powdered leaf material into a cellulose extraction thimble and then insert this into a Soxhlet apparatus. The main extractant solvent was 500 mL of 70% ethanol. The report also mentioned 80% methanol as an alternative solvent, but the two solvents were not reported as separate completed experimental treatments. The duration of extraction was suggested to be around 24 hours so that the solvent can be repeatedly evaporated, condensed and contacted with the powdered plant material. The liquid extract would then be filtered using Whatman No. 1 filter paper to remove any residual particulate matter. The filtrate was concentrated under vacuum on a rotary evaporator at temperature not exceeding 40 0 C. The concentrated crude extract would be weighed to determine the extraction yield and stock solution would be prepared thereafter. The first protocol proposed the extract be dissolved in 10% dimethyl sulfoxide (DMSO) with a stock concentration of 100 mg/mL as an example. The procedure proposed was filtration through 0.22-µm membrane and storage at 4°C.The study included antibacterial observations for a crude extract and its dilutions,

2.3.2. Extraction Yield

The extraction yield was intended to be calculated using the following equation: Extraction yield (%) = (Mass of dried crude extract / Mass of dried plant material) × 100. However, the report did not provide the measured mass of the recovered extract. Accordingly, no extraction yield was calculated or reported.

2.4. Qualitative Phytochemical Screening

Qualitative phytochemical screening was included investigate major classes of secondary metabolites potentially present in *C. macrocarpa* leaf extract. The selection of phytochemical classes was informed by previous investigations of *Carissa* species

Creative Commons Attribution-Non-Commercial 4.0 International License

Published by DAC Democratic Arabic Center
DAC Democratic Arabic Center GmbH, Berlin

and related medicinal plant extracts [1,2,4,5,8]. The proposed screening procedures are summarized below.

2.4.1. Detection of Alkaloids: A small portion of the extract was to be treated separately with Mayer's reagent and Dragendorff's reagent. The development of a cream-colored precipitate following the addition of Mayer's reagent or a reddish-brown precipitate following treatment with Dragendorff's reagent was specified as a presumptive positive reaction for alkaloids.

2.4.2. Detection of Flavonoids: The alkaline reagent test was proposed for the preliminary detection of flavonoids. A small quantity of the extract was to be treated with several drops of 10% sodium hydroxide solution. The development of an intense yellow coloration, followed by disappearance of the color after addition of dilute hydrochloric acid, was specified as a positive screening reaction.

2.4.3. Detection of Phenolic Compounds and Tannins: The ferric chloride test was proposed for detecting phenolic constituents and tannins. Approximately 2 mL of the extract was to be mixed with several drops of 5% ferric chloride solution. The appearance of blue-black or greenish coloration was considered a presumptive positive reaction for phenolic compounds or tannins.

2.4.4. Detection of Saponins: The froth test was proposed for detecting saponins. A small amount of the extract was to be mixed with approximately 5 mL of distilled water and vigorously shaken. The formation of stable foam persisting for approximately 15 minutes was specified as a positive screening response.

2.4.5. Detection of Terpenoids and Steroids: The Salkowski test was proposed for the preliminary detection of terpenoid- and steroid-associated constituents. The extract was to be dissolved in chloroform, followed by careful addition of concentrated sulfuric acid along the wall of the test tube. The development of a reddish-brown ring at the interface was considered a presumptive positive reaction.

2.4.6. Detection of Cardiac Glycosides: The Keller–Kiliani test was proposed for detecting cardiac glycoside-associated constituents. The extract was to be treated with glacial acetic acid containing a small quantity of ferric chloride solution, followed by

Creative Commons Attribution-Non-Commercial 4.0 International License

Published by DAC Democratic Arabic Center
DAC Democratic Arabic Center GmbH, Berlin

careful addition of concentrated sulfuric acid. The appearance of a brown ring at the interface or a blue-green coloration in the acetic acid layer was specified as a positive screening response.

2.4.7. Interpretation of Phytochemical Screening: According to qualitative tests, complete extract-specific results were unavailable. Therefore, the present study does not classify the tested extract as experimentally positive or negative for any of the proposed phytochemical groups. Information concerning the phytochemical composition of *C. macrocarpa* obtained from previously published investigations [1,2,5,10] was treated as background literature rather than as an experimental result of the present investigation.

2.5. Bacterial Isolates and Culture Conditions

The study identified *Enterobacter* spp. and *Salmonella* spp. as the bacterial groups selected for molecular screening. *Escherichia coli* was included in the preliminary antibacterial investigation. According to the methodological description, bacterial isolates were maintained on nutrient agar slants at 4°C and subcultured in Mueller–Hinton broth before experimental testing. The reported incubation conditions were 37°C for approximately 18–24 hours. Study described the *Enterobacter* and *Salmonella* isolates as carrying the selected genetic markers. However, because complete confirmatory molecular records were unavailable, this characterization was treated as preliminary rather than independently established. The total number of isolates, their clinical or environmental origins, collection period, species-level identification procedures, and complete antimicrobial susceptibility profiles were not adequately documented. Consequently, these characteristics could not be reported as verified study variables.

2.6. Assessment of Antibacterial Activity

2.6.1. Agar-Well Diffusion Assay: The antibacterial activity of *C. macrocarpa* leaf extract was investigated using the agar-well diffusion method described in the report. The general approach was consistent with commonly used plant-extract antibacterial screening procedures [3,9,20]. Bacterial inocula were prepared by suspending bacterial colonies in sterile physiological saline and adjusting the turbidity to approximately the

Creative Commons Attribution-Non-Commercial 4.0 International License

Published by DAC Democratic Arabic Center
DAC Democratic Arabic Center GmbH, Berlin

0.5 McFarland standard. The prepared bacterial suspensions were distributed over the surface of Mueller–Hinton agar plates using sterile cotton swabs. Wells approximately 6 mm in diameter were produced in the inoculated agar using a sterile cork borer. A volume of 100 µL of plant extract was specified for application to each well. The study reported three nominal extract concentrations:

- Crude extract: 100 mg/mL.
- First dilution (1:1): 50 mg/mL.
- Second dilution (reported as 2:1): approximately 33.3 mg/mL.

The third dilution was convention and volumes of preparation were not well enough documented to permit independent verification of the final concentration. The positive antimicrobial control was meropenem or ciprofloxacin and the negative solvent control was 10% DMSO as described in the methodological description. The test preparations were incubated for 18–24 h at 37°C reportedly after the preparation of the plates. The inhibition zone diameters were measured in mm using a digital caliper. The only experimental results were three inhibition-zone measurements against *E. coli*.

2.6.2. Antibacterial Controls: In the first methodological description, an antimicrobial positive control and a solvent negative control were included. The positive control proposed was ciprofloxacin or meropenem and the negative control was DMSO 10%.

2.6.3. Minimum Inhibitory Concentration and Minimum Bactericidal Concentration: The minimum inhibitory concentration (MIC) was proposed to be determined by broth microdilution assay. The proposed procedure consisted of serial dilutions of the extract in Mueller–Hinton broth, inoculation of the test wells with standardized suspensions of bacteria and evaluation of bacterial growth after incubation. The minimum inhibitory concentration (MIC) was defined as the lowest concentration of the extract, which inhibited detectable growth of the bacteria under the conditions described. The report also suggested that the minimum bactericidal concentration (MBC) should be determined by subculturing material from wells without visible growth onto fresh agar medium. However, the complete MIC or MBC values were not reported in the available literature. Thus, these procedures were not considered to be definitive experimental assays and the results did not include any quantitative MIC or MBC values.

Creative Commons Attribution-Non-Commercial 4.0 International License

Published by DAC Democratic Arabic Center
DAC Democratic Arabic Center GmbH, Berlin

2.7. Molecular Detection of Selected Genetic Targets

2.7.1. Genomic DNA Extraction: Genomic DNA was prepared by the boiling-lysis procedure described in the report (10). In brief, bacterial cells were pelleted by centrifugation from overnight cultures. The resulting bacterial pellet was resuspended in approximately 200 µL of nuclease-free water. The suspension was heated at 100°C for 10 min to facilitate the release of DNA and lysis of cells. The suspension was heated immediately and cooled on ice for ~ 5 min. The suspension was centrifuged at 10,000 rpm for 5 min. The supernatant obtained was used as the DNA template for PCR amplification. The DNA purity and concentration were also assessed spectrophotometrically using a NanoDrop device. However real DNA concentrations and absorbance ratios were not given.

2.7.2. Selection of Molecular Targets: Two genetic targets were selected for PCR-based screening.The *intI3* gene was selected as a molecular marker associated with class 3 integrons in *Enterobacter* isolates. Previous investigations have examined class 3 integrons and their recombination systems, as well as the distribution of integron-associated determinants in Gram-negative bacteria [11,14,15,24,27]. The *fimH* gene was selected as a molecular marker associated with type 1 fimbrial adhesion in *Salmonella* isolates. Previous studies have investigated the involvement of type 1 fimbriae and adhesin-associated genetic determinants in *Salmonella* adhesion and pathogenicity [12,13,16,25]. The selection of these targets was intended to provide preliminary molecular characterization rather than comprehensive assessment of antimicrobial resistance or bacterial virulence.

2.7.3. PCR Primers: The primer sequences and expected amplification-product sizes are presented in Table 1.

Table 1. Primer sequences and expected PCR amplification products

Target gene	Bacterial group	Primer sequence (5′–3′)	Expected product size
intI3	*Enterobacter* spp.	Forward: AGTGGGTGGCGAATGAGTG	600 bp

Creative Commons Attribution-Non-Commercial 4.0 International License

Published by DAC Democratic Arabic Center
DAC Democratic Arabic Center GmbH, Berlin

		Reverse: TGTTCTTGTATCGGCCAGGTG	
fimH	*Salmonella* spp.	Forward: GCAGTCACCTGCCCTCCGGTA	506 bp
		Reverse: TGCAGAACGGATAAGCCGTGG	

2.7.4. PCR Reaction Mixture: Conventional PCR amplification was described using a final reaction volume of 25 µL. The reported reaction mixture contained:

- 12.5 µL of 2× PCR Master Mix.
- 1 µL of forward primer (10 pmol/µL).
- 1 µL of reverse primer (10 pmol/µL).
- 2–3 µL of genomic DNA template.
- Nuclease-free water to achieve the final reaction volume of 25 µL.

The report did not specify the manufacturer or catalogue number of the PCR Master Mix or the final template DNA concentration. These details were therefore not added to the reconstructed methodology.

2.7.5. Thermal Cycling Conditions: The reported PCR amplification program consisted of initial denaturation, repeated amplification cycles, and final extension. The thermal cycling conditions are summarized in Table 2.

Table 2. Reported PCR thermal cycling conditions

Amplification stage	Temperature	Duration	Number of cycles
Initial denaturation	95°C	5 min	1
Denaturation	95°C	30 s	35
Annealing	55°C	30 s	35
Extension	72°C	45 s	35
Final extension	72°C	7 min	1

2.7.6. Agarose Gel Electrophoresis: The PCR amplification products were analyzed by agarose gel electrophoresis as described. A 1.5% (w/v) agarose gel was prepared in Tris–acetate–EDTA (TAE) buffer with adequate DNA-staining dye. Electrophoresis

Creative Commons Attribution-Non-Commercial 4.0 International License

Published by DAC Democratic Arabic Center
DAC Democratic Arabic Center GmbH, Berlin

was initially recommended to be run at 80 V for 45–60 min. A 100-bp DNA ladder was used as the molecular-size reference. Following electrophoresis, the amplified DNA products were visualized using a UV transilluminator and photographed. The expected amplification-product sizes were 600 bp for *intI3* and 506 bp for *fimH*.The preliminary interpretation was based on the approximate migration positions of the observed amplification products relative to the DNA ladder.

2.7.7. Interpretation of Molecular Screening Results: The molecular screening results were interpreted according to the reported presence of amplification products corresponding to the expected molecular sizes. The observation of a band at the expected position was considered consistent with amplification of the selected target. However, the identity of a PCR product cannot be conclusively established from electrophoretic size alone. Accordingly, the reported observations were not considered definitive molecular confirmation of *intI3* or *fimH*. Furthermore, the presence of a putative *intI3* target was not interpreted as proof of phenotypic antimicrobial resistance, and the presence of a putative *fimH* target was not interpreted as evidence of functional virulence. No prevalence estimates were calculated because the complete numbers of tested isolates and corresponding molecular outcomes were unavailable.

2.8. Data Management and Statistical Analysis

The antibacterial dataset available for analysis consisted of three preliminary inhibition-zone measurements against *E. coli*, corresponding to the three nominal extract concentrations. The molecular dataset consisted of reported PCR band interpretations for selected *Enterobacter* and *Salmonella* isolates. Complete replicate-level antibacterial measurements, quantitative molecular measurements, and statistical outputs were not available. The available inhibition-zone measurements were summarized descriptively in millimeters. The nominal extract concentrations were reported in mg/mL. Because only one inhibition-zone value was available for each concentration, no group means, standard deviations, standard errors, or confidence intervals were calculated. The numerical pattern across concentrations was described without assigning statistical significance. The statistic alanalysis includes the Shapiro–Wilk normality test, one-way analysis of variance, Tukey's post-hoc test, effect-size estimation, and 95% confidence intervals. . It also referred to technical triplicate measurements. However, the corresponding replicate-level data were not included in

Creative Commons Attribution-Non-Commercial 4.0 International License

Published by DAC Democratic Arabic Center
DAC Democratic Arabic Center GmbH, Berlin

the available source report. Therefore, the proposed statistical tests could not be performed or independently verified. No *p*-values or inferential statistical conclusions were reported. The nominal significance threshold of 0.05 described in the statistical plan was not applied to the preliminary observations. The molecular findings were summarized according to the reported presence of amplification products at the expected sizes. No statistical comparison was performed between the *intI3* and *fimH* findings because the targets were screened in different bacterial groups and the complete isolate-level dataset was unavailable. Similarly, no association analysis was performed between the molecular findings and antibacterial activity because the two experimental components did not provide matched isolate-level measurements.

3. Results

3.1. Experimental Findings

The present study comprised two preliminary experimental components: antibacterial screening of *Carissa macrocarpa* leaf extract against *Escherichia coli* and PCR-based screening of selected genetic targets in *Enterobacter* and *Salmonella* isolates. The available antibacterial findings consisted of three reported inhibition-zone diameters corresponding to three nominal extract concentrations. The molecular findings consisted of reported agarose gel electrophoresis observations interpreted as amplification products corresponding to the expected sizes of the *fimH* and *intI3* genes. The results are presented descriptively because complete experimental replicate records, numerical control measurements, and independently verifiable molecular confirmation data were unavailable.

3.2. Preliminary Antibacterial Activity of *Carissa macrocarpa* Leaf Extract Against *Escherichia coli*

The reported antibacterial screening demonstrated measurable inhibition zones around wells containing *C. macrocarpa* leaf extract at all three nominal concentrations tested against *E. coli*. The highest concentration, 100 mg/mL, was associated with an inhibition-zone diameter of 18 mm. The reported inhibition-zone diameter was 16 mm at a concentration of 50 mg/mL and the lowest nominal concentration estimated at 33.3

Creative Commons Attribution-Non-Commercial 4.0 International License

Published by DAC Democratic Arabic Center
DAC Democratic Arabic Center GmbH, Berlin

mg/mL, showed an inhibition-zone diameter of 14 mm. Table 3 summarizes the results of the findings.

Table 3. Preliminary inhibition-zone diameters of *Carissa macrocarpa* leaf extract against *Escherichia coli*

Extract concentration (mg/mL)	Reported inhibition-zone diameter (mm)
100	18
50	16
Approximately 33.3	14

The diameter of zone of inhibition was numerically higher at higher nominal extract concentrations. The inhibition-zone diameters between the highest and lowest reported values were 4 mm apart, and those between adjacent values were 2 mm apart. These differences are only indicative of the measured reported values and do not indicate a statistically significant concentration-dependent antibacterial effect.

3.3. Descriptive Pattern of Antibacterial Inhibition

The three concentrations tested showed a numerical pattern of decreasing inhibition-zone measurements. The diameter of the zone of inhibition was 18 mm at 100 mg/mL, 16 mm at 50 mg/mL and 14 mm at about 33.3 mg/mL. This pattern is in agreement with a greater inhibition observed at higher concentrations of the extracts. The absence of independent replicates of the experiments prevents the assessment of the variability of the measurements. Thus, the results were not analyzed statistically in an inferential way.

3.4. Antibacterial Control Results

The methodological description included the use of a reference antimicrobial agent as a positive control and 10% dimethyl sulfoxide as a negative solvent control. However, numerical inhibition-zone measurements for these controls were not available in the source dataset. Accordingly, no quantitative comparison could be made between the plant extract and the reference antimicrobial agent. Similarly, the available

Creative Commons Attribution-Non-Commercial 4.0 International License

Published by DAC Democratic Arabic Center
DAC Democratic Arabic Center GmbH, Berlin

findings did not permit an independent assessment of whether the solvent contributed to the observed inhibition zones.

3.5. Minimum Inhibitory and Minimum Bactericidal Concentrations

The study described the procedures proposed for determination of minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of the extract. However, no complete MIC or MBC measurements were obtained. Therefore, no quantitative inhibitory or bactericidal concentrations are reported. The MIC or MBC values cannot be derived from the available agar-well diffusion measurements.

3.6. Phytochemical Screening Findings

Results gave the qualitative phytochemical screening procedures for a number of classes of secondary metabolites including alkaloids, flavonoids, phenolic compounds, tannins, saponins, terpenoids, steroids and cardiac glycosides. However, the screening tests did not give complete experimental results. Therefore, the available data did not allow determination of the presence or absence of individual phytochemical classes in the tested extract. Chromatographic profiles and extraction yield and concentrations of phytochemicals were not shown. The phytochemical composition of *C. macrocarpa* obtained in previous studies was not considered as an experimental result in the present study.

3.7. PCR-Based Screening of the *fimH* Gene in *Salmonella* Isolates

The study gave the qualitative phytochemical screening procedures for a number of classes of secondary metabolites including alkaloids, flavonoids, phenolic compounds, tannins, saponins, terpenoids, steroids and cardiac glycosides. However, the screening tests did not give complete experimental results. Therefore, the available data did not allow determination of the presence or absence of individual phytochemical classes in the tested extract. Chromatographic profiles and extraction yield and concentrations of phytochemicals were not shown. The phytochemical composition of *C. macrocarpa* obtained in previous studies was not considered as an experimental result in the present study.

Creative Commons Attribution-Non-Commercial 4.0 International License

Published by DAC Democratic Arabic Center
DAC Democratic Arabic Center GmbH, Berlin

Table 4. Preliminary PCR screening observations for the *fimH* target in *Salmonella* isolates

Target gene	Expected amplicon size	Reported lanes with expected-size bands	Interpretation
fimH	506 bp	1, 2, 3, 4	Presumptive amplification of the target

3.8. PCR-Based Screening of the *intI3* Gene in *Enterobacter* Isolates

The report stated that PCR amplification products in selected Enterobacter isolates were approximately the expected 600-bp intI3 target. Lanes 7, 8, 10 and 11 showed amplified products of the expected size. These results were taken as evidence for intI3 target amplification. The observations reported are summarized in Table 5.

Table 5. Preliminary PCR screening observations for the *intI3* target in *Enterobacter* isolates

Target gene	Expected amplicon size	Reported lanes with expected-size bands	Interpretation
intI3	600 bp	7, 8, 10, 11	Presumptive amplification of the target

3.9. Molecular Screening Observations

The preliminary PCR findings for the two selected targets are summarized in Table 6.

Table 6. Summary of reported molecular observations

Bacterial group	Molecular target	Expected product size	Reported lanes
Salmonella spp.	*fimH*	506 bp	1, 2, 3, 4
Enterobacter spp.	*intI3*	600 bp	7, 8, 10, 11

The findings indicate that amplification products corresponding to the expected molecular sizes were reported for both selected targets.

Creative Commons Attribution-Non-Commercial 4.0 International License

Published by DAC Democratic Arabic Center
DAC Democratic Arabic Center GmbH, Berlin

3.10. Relationship Between Antibacterial and Molecular Findings

The antibacterial screening results were reported for *E. coli*, whereas the molecular screening observations involved *Enterobacter* and *Salmonella* isolates. In particular, the available observations do not demonstrate that the extract inhibited *Enterobacter* isolates carrying the putative *intI3* target or *Salmonella* isolates carrying the putative *fimH* target. No genotype–susceptibility comparison, correlation analysis, or resistance-stratified antibacterial assessment was performed.

3.11. Summary of the Principal Findings

The principal findings of the study were as follows:

1. *C. macrocarpa* leaf extract was associated with reported inhibition-zone diameters of 18, 16, and 14 mm against *E. coli* at nominal concentrations of 100, 50, and approximately 33.3 mg/mL, respectively.
2. The reported inhibition-zone diameters exhibited a numerical decrease with decreasing extract concentration, but statistical significance could not be assessed.
3. PCR amplification products corresponding to the expected 506-bp *fimH* target were reported in lanes 1, 2, 3, and 4 of the *Salmonella* screening analysis.
4. PCR amplification products corresponding to the expected 600-bp *intI3* target were reported in lanes 7, 8, 10, and 11 of the *Enterobacter* screening analysis.
5. Complete phytochemical screening results, MIC/MBC measurements, quantitative antibacterial controls, and independently verified molecular confirmation were unavailable.

Creative Commons Attribution-Non-Commercial 4.0 International License

Published by DAC Democratic Arabic Center
DAC Democratic Arabic Center GmbH, Berlin

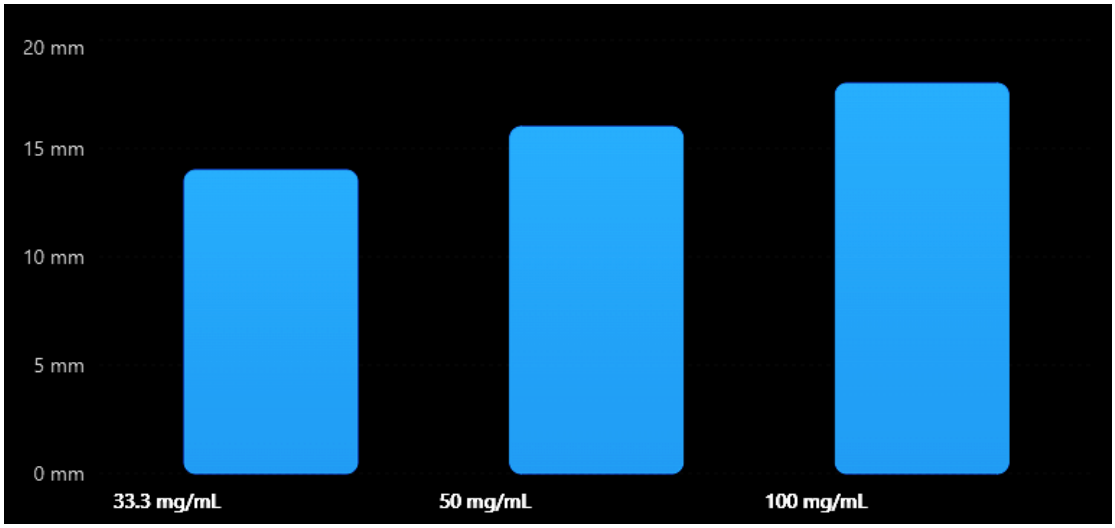


Figure 1. Descriptive representation of the reported inhibition-zone measurements. Each bar represents one source-reported value, not a replicated mean; therefore, error bars and statistical significance indicators are not shown.

4. Discussion

4.1. Overview and Interpretation of the Principal Findings

The present study reports preliminary observations on the antibacterial activity of Carissa macrocarpa leaf extract against *Escherichia coli* and some discrete PCR based screening observations of specific genetic targets in Enterobacter and Salmonella isolates. The antibacterial component yielded inhibition-zone diameters of 18, 16, and 14 mm at nominal extract concentrations of 100, 50, and approximately 33.3 mg/mL, respectively. The molecular component documented amplification products corresponding to the expected sizes of the *intI3* and *fimH* targets in selected electrophoretic lanes. These observations provide two distinct forms of preliminary laboratory evidence. The agar-well diffusion findings describe apparent growth inhibition of *E. coli* under the reported assay conditions, whereas the PCR findings indicate presumptive amplification of selected resistance-associated and adhesion-associated genetic targets in different bacterial groups. The distinction between these components is fundamental to the interpretation of the study. The antibacterial findings were not obtained from the *Enterobacter* or *Salmonella* isolates subjected to molecular screening. Consequently, the available results cannot establish a direct relationship between the selected genetic determinants and susceptibility to *C. macrocarpa* extract.

Creative Commons Attribution-Non-Commercial 4.0 International License

Published by DAC Democratic Arabic Center
DAC Democratic Arabic Center GmbH, Berlin

The following discussion evaluates the findings in relation to the phytochemical and antibacterial literature on *Carissa* species and previous molecular investigations of integrons and fimbrial adhesion in Gram-negative bacteria.

4.2. Antibacterial Activity of *Carissa macrocarpa* Leaf Extract

The reported inhibition-zone diameters indicate that *C. macrocarpa* leaf extract was associated with measurable growth inhibition of *E. coli* at all three nominal concentrations. The largest reported inhibition zone was observed at 100 mg/mL, followed by progressively smaller zones at 50 and approximately 33.3 mg/mL. These observations are broadly compatible with previous investigations reporting antibacterial properties of *C. macrocarpa* extracts and isolated constituents [1,5,10]. However, the magnitude and reproducibility of the present findings cannot be established because replicate-level measurements and complete control results were unavailable. Souilem et al. [1] investigated the phenolic profile and biological properties of different *C. macrocarpa* tissues, including leaves, stems, and flowers. Their findings provide a relevant basis for considering the antibacterial potential of extracts obtained from this plant. Allam et al. [5] also examined phytochemical and biological characteristics of *C. macrocarpa*, contributing to the available evidence concerning its pharmacologically relevant constituents. Moodley et al. [10] also investigated the antibacterial and anti-adhesion activities of pentacyclic triterpenoids isolated from the edible fruits and leaves of *C. macrocarpa*. What is particularly relevant about their research is that they studied the biological characteristics of isolated constituents rather than just crude-extract screening. Nevertheless, for a direct numerical comparison of the present inhibition-zone measurements with those reported in previous studies, similar methods for extraction, test organisms, preparation of inoculum, diffusion conditions, concentrations of extracts and experimental controls are necessary.

4.3. Concentration-Related Variation in Inhibition-Zone Diameters

The present study reported inhibition-zone diameters of 18 mm at 100 mg/mL, 16 mm at 50 mg/mL, and 14 mm at approximately 33.3 mg/mL. The numerical pattern suggests that the highest nominal extract concentration was associated with the greatest observed inhibition under the reported assay conditions. Such a pattern is compatible

Creative Commons Attribution-Non-Commercial 4.0 International License

Published by DAC Democratic Arabic Center
DAC Democratic Arabic Center GmbH, Berlin

with a concentration-related effect, in which increasing the amount of extract applied to an agar well may increase the quantity of antibacterial constituents available to interact with the surrounding bacterial population. However, inhibition-zone diameter is influenced by more than antibacterial potency. Diffusion through agar, extract solubility, molecular size, chemical composition, and interactions with the culture medium may all affect the measured zone. Consequently, a larger inhibition zone does not necessarily indicate a proportionally greater antibacterial effect. The present study did not provide sufficient replicate-level observations to evaluate measurement variability or establish a statistically significant concentration–response relationship. Moreover, the reported concentration of approximately 33.3 mg/mL was derived from a dilution designation requiring clarification. This introduces an additional uncertainty when interpreting the numerical pattern. Accordingly, the results support only the descriptive observation that larger inhibition zones were reported at higher nominal extract concentrations. They do not establish a validated dose–response model or permit quantitative estimation of antibacterial potency.

4.4. Phytochemical Basis of the Reported Antibacterial Observations

The potential antibacterial properties of *C. macrocarpa* have been investigated in relation to its diverse phytochemical constituents. Previous studies have described phenolic compounds, flavonoids, and triterpenoid-associated constituents in *Carissa* species [1,2,5,10,22,23]. Souilem et al. [1] examined the phenolic composition of *C. macrocarpa* tissues and evaluated associated biological activities. Their findings support the scientific rationale for investigating the antibacterial properties of this plant. Moodley et al. [10] investigated isolated pentacyclic triterpenoids and reported antibacterial and anti-adhesion properties, suggesting that selected nonpolar plant constituents may contribute to the biological activity observed in some preparations. Additional phytochemical investigations of *Carissa* species have provided information concerning the diversity of their secondary metabolites [4,6,8,22,23]. Phenolic compounds and flavonoids have been widely investigated for their potential interactions with bacterial cellular structures and metabolic processes. Terpenoid constituents have also attracted interest because of their chemical diversity and reported biological properties. Nevertheless, the present study did not provide completed phytochemical screening results, chromatographic profiles, or quantitative

Creative Commons Attribution-Non-Commercial 4.0 International License

Published by DAC Democratic Arabic Center
DAC Democratic Arabic Center GmbH, Berlin

measurements of individual constituents in the tested extract. It is therefore not possible to attribute the observed inhibition zones to phenolic compounds, flavonoids, triterpenoids, or any other specific phytochemical class. The previously published phytochemical findings provide a plausible scientific context but do not establish the chemical composition of the extract evaluated in the present study. Further chemical characterization would be required before proposing specific compound-mediated antibacterial mechanisms.

4.5. Influence of Extraction Procedures on Antibacterial Activity

Extraction methodology represents an important consideration when interpreting the antibacterial activity of medicinal plant preparations. The chemical composition of a crude extract may vary according to the solvent system, extraction duration, temperature, plant-to-solvent ratio, and characteristics of the starting material. Previous investigations have used different extraction approaches to evaluate the biological properties of medicinal plants [3,8,9,20]. The present study described a proposed Soxhlet extraction procedure using 70% ethanol.

4.6. Relationship to Previous Studies of *Carissa* Species

The antibacterial observations reported in the present investigation are relevant to a broader body of literature examining the biological activities of *Carissa* species. Souilem et al. [1] investigated *C. macrocarpa* extracts from different plant tissues, providing evidence that biological activity may vary according to the plant part examined. Allam et al. [5] contributed further information concerning the phytochemical and biological characteristics of *C. macrocarpa*. Moodley et al. [10] investigated antibacterial and anti-adhesion activities of isolated pentacyclic triterpenoids, emphasizing the potential importance of chemically defined constituents. Other studies have examined related species, including *Carissa edulis*, *Carissa opaca*, and *Carissa carandas* [4,6,8]. The review literature on the genus *Carissa* has also described its ethnopharmacological, phytochemical, and pharmacological characteristics [22,23]. Collectively, these investigations support continued scientific interest in the genus as a source of biologically active natural products.

Creative Commons Attribution-Non-Commercial 4.0 International License

Published by DAC Democratic Arabic Center
DAC Democratic Arabic Center GmbH, Berlin

However, findings obtained from different *Carissa* species cannot be directly generalized to *C. macrocarpa*. Similarly, results obtained from fruits, stems, or other plant tissues cannot automatically be applied to leaf extracts. Differences in bacterial species and assay conditions further limit direct comparison. Therefore, the present study should be considered a preliminary contribution to the existing literature rather than an independent confirmation of previously proposed antibacterial mechanisms.

4.7. Interpretation of the *intI3* Molecular Findings

The molecular component of the present study reported amplification products corresponding approximately to the expected 600-bp *intI3* target in lanes 7, 8, 10, and 11 of the *Enterobacter* screening analysis. The *intI3* gene encodes the integrase associated with class 3 integrons, which are genetic platforms capable of participating in site-specific recombination involving gene cassettes. Collis et al. [15] characterized the class 3 integron and its associated recombination system, providing important molecular evidence concerning the activity of the IntI3 integrase. Other studies have examined the distribution of integrons in Gram-negative bacteria and their relationships with antimicrobial resistance-associated genetic characteristics [11,14,24,17]. The reported amplification products in the present study are therefore relevant to molecular investigations of integron-associated determinants. However, the interpretation of these findings requires several qualifications. First, the observation of an amplification product at the expected molecular size does not independently confirm the identity of the target sequence. Second, the presence of an integrase gene does not establish that a particular resistance-gene cassette is present. Third, even when resistance-associated genetic elements are identified, phenotypic antimicrobial susceptibility testing is required to characterize the corresponding resistance profile.

4.8. Interpretation of the *fimH* Molecular Findings

The present study also reported amplification products corresponding approximately to the expected 506-bp *fimH* target in lanes 1, 2, 3, and 4 of the *Salmonella* screening analysis. The *fimH* gene is associated with type 1 fimbrial adhesion and has been investigated in relation to bacterial attachment and host–pathogen interactions. Previous studies have addressed the biological significance of fimbrial adhesion in *Salmonella*. Yin et al. [13] investigated the effect of

Creative Commons Attribution-Non-Commercial 4.0 International License

Published by DAC Democratic Arabic Center
DAC Democratic Arabic Center GmbH, Berlin

cinnamaldehyde on *Salmonella Typhimurium* adhesion through mechanisms involving type 1 fimbriae. Kisiela et al. [25] examined the evolution of *Salmonella enterica* virulence in relation to mutations affecting a fimbrial adhesin. Additional molecular investigations have examined virulence-associated genetic determinants in *Salmonella* isolates [12,16,26].

4.9. Integrons, Fimbrial Adhesion, and Their Distinct Biological Roles

The selection of intI3 and fimH reflects two distinct aspects of bacterial molecular biology. The intI3 gene is involved in integron-mediated genetic recombination, and fimH is related to fimbrial adhesion. These genetic targets therefore have distinct biological functions and should not be interpreted as interchangeable indicators of bacterial pathogenicity or antimicrobial resistance. Previous studies have investigated integron-associated determinants in relation to antimicrobial resistance [11,14,15,24,17]. Other investigations have examined fimbrial adhesins and their involvement in bacterial attachment and virulence-associated processes [12,13,16,25]. The presence of these molecular targets may provide useful information for bacterial characterization, but the biological consequences depend on additional genetic, regulatory, and phenotypic factors. The two targets were explored in different bacterial groups during the present investigation. Thus, the observations available cannot be directly compared in terms of prevalence, expression or functional significance. Also, the results do not allow us to estimate the frequency of any of the genetic determinants in the bacterial populations investigated, because the total number of isolates screened was not properly documented.

4.10. Relationship Between Plant-Derived Antibacterial Activity and Molecular Characteristics

In the present study, the methodological consideration of separating the antibacterial and molecular experimental parts is critical. The molecular screening was done for selected *Enterobacter* and *Salmonella* isolates and antibacterial assay was reported for *E. coli*. Hence, the present data does not indicate the susceptibility of the molecularly screened isolates to *C. macrocarpa* leaf extract. This distinction limits the extent to which the findings can be integrated into a single biological interpretation. For example, the presence of a putative *intI3* target in *Enterobacter* isolates does not

Creative Commons Attribution-Non-Commercial 4.0 International License

Published by DAC Democratic Arabic Center
DAC Democratic Arabic Center GmbH, Berlin

establish whether the plant extract would inhibit those isolates. Similarly, the presence of a putative *fimH* target in *Salmonella* isolates does not establish whether the extract would interfere with their growth, adhesion, or virulence-associated functions. Previous investigations have explored antibacterial and anti-adhesion properties of *Carissa*-derived constituents [1,10]. However, those findings cannot be used to infer that the extract tested in the present study exerted comparable effects on the molecularly characterized isolates. A direct investigation of these relationships would require antibacterial testing and molecular characterization of the same isolates, together with appropriate controls and quantitative endpoints. Accordingly, the present study provides preliminary observations from two related but experimentally distinct areas of investigation.

4.11. Scientific Contribution and Interpretation of the Available Evidence

The principal scientific contribution of the present study is the descriptive reporting of preliminary antibacterial observations and selected molecular screening findings. The antibacterial component provides initial information concerning the reported response of *E. coli* to three nominal concentrations of *C. macrocarpa* leaf extract. The molecule detects amplification products of the size expected for genetic targets of interest in *Enterobacter* and *Salmonella* isolates. The study also stresses the importance of differentiating between molecular detection and functional characterization, and between agar diffusion observations and quantitative antibacterial potency. This distinction is of particular importance when considering the potential of natural products as antibacterial agents. The reported inhibition-zone measurements are consistent with antibacterial activity under the stated conditions, but do not provide sufficient data to establish reproducible efficacy. The molecular findings are, in turn, relevant for preliminary genetic screening, but do not independently establish antimicrobial resistance or virulence phenotypes. Taken together, the results provide a basis for exploration in future studies and not conclusive evidence of a correlation between bacterial genetic traits and plant-derived antibacterial activity.

Creative Commons Attribution-Non-Commercial 4.0 International License

Published by DAC Democratic Arabic Center
DAC Democratic Arabic Center GmbH, Berlin

4.12. Study Limitations

Several limitations should be considered when interpreting the present findings. First, the antibacterial dataset available contained only one inhibition zone measurement for each nominal extract concentration. The lack of independent experimental replicates prevented the statistical evaluation of significance and the quantification of measurement variability. Second, there was a lack of full positive-control and negative-control measurements. This limited evaluation of the assay's performance and possible solvent-related effects. Complete phytochemical characterization or extraction yield was also unavailable. Fourth, the study did not offer full MIC or MBC measurements. Consequently, the antibacterial findings could not be extrapolated to quantitative inhibitory or bactericidal concentrations. Fifth, the molecular observations were based on reported sizes of amplification products in the absence of complete records of assay controls or of sufficient confirmatory sequence data. Sixth, the total number and the full characteristics of the screened bacterial isolates were poorly recorded, which made it difficult to accurately estimate the prevalence. Seventh, molecular and antibacterial components affected different bacterial groups. This precluded direct assessment of genotype-susceptibility relationships. Finally, the available data did not allow mechanistic inferences on the antibacterial activities of the individual phytochemical compounds or the functional consequences of the identified molecular targets. These limitations constrain the generalizability and interpretive strength of the findings and should be considered when evaluating the scientific significance of the findings.

4.13. Implications for Future Research

Preliminary observations from the current study suggest several areas for further investigation. Further research should be directed toward establishing a complete documented and reproducible extraction process including botanical authentication, extraction yield, solvent composition and chemical characterization of the final extract. Antibacterial investigations should include standardized quantitative endpoints, suitable positive and negative controls, and independent biological replicates. The MIC and MBC of the extract will further demonstrate the inhibitory and bactericidal effect of the extract. Molecular investigations should include validated primer systems, complete assay controls and confirmatory identification of amplification products. The

Creative Commons Attribution-Non-Commercial 4.0 International License

Published by DAC Democratic Arabic Center
DAC Democratic Arabic Center GmbH, Berlin

biological significance of intI3 should be studied in the light of molecular findings, antimicrobial susceptibility testing and characterization of relevant resistance-associated gene cassettes. Similarly, investigations of *fimH* should distinguish gene detection from gene expression and functional adhesion. Importantly, subsequent studies aiming to examine relationships between plant-derived antibacterial activity and bacterial genetic characteristics should perform molecular characterization and antibacterial testing on the same bacterial isolates. Such an integrated approach would permit more direct evaluation of potential genotype-associated differences in susceptibility.

5. Conclusions and Recommendations

5.1. Conclusions

1. In this preliminary laboratory study, PCR-based screening observations were recorded for intI3 and fimH genetic targets in Enterobacter and Salmonella isolates respectively, in relation to the reported antibacterial activity of Carissa macrocarpa leaf extract against Escherichia coli. The available experimental results allowed to make the following conclusions. Initially, inhibition zones were observed against E. coli for the *C. macrocarpa* leaf extract at all three nominal concentrations. The diameters of inhibition zones were reported to be 18 mm, 16 mm and 14 mm at 100 mg/mL, 50 mg/mL and 33.3 mg/mL, respectively. These observations are preliminary evidence compatible with an antibacterial activity under the experimental conditions reported.
2. Diameters of inhibition zones increased numerically with increase in nominal extract concentration. However, the lack of independent experimental repeats and full control measurements limited the possibility to verify a statistically significant concentration-dependent effect.
3. Preliminary PCR screening showed amplification products of the predicted size for the 600-bp intI3 target in selected Enterobacter isolates and the predicted 506-bp fimH target in selected Salmonella isolates. These observations are consistent with presumed amplification of the selected genetic targets, but do not provide definitive molecular confirmation.
4. The molecular data do not independently prove functional virulence or phenotypic antimicrobial resistance. In particular, the presumptive detection of

Creative Commons Attribution-Non-Commercial 4.0 International License

Published by DAC Democratic Arabic Center
DAC Democratic Arabic Center GmbH, Berlin

intI3 cannot be taken as evidence of multidrug resistance and the presumptive detection of fimH does not imply increased bacterial adhesion or pathogenicity.

5. The molecular and antibacterial components influenced diverse bacterial groups. Thus, the present evidence does not support the existence of a direct link between the selected genetic determinants and susceptibility to *C. macrocarpa* leaf extract.
6. The absence of complete phytochemical analysis, extraction-yield values, MIC and MBC values, antibacterial data at replicate level, and confirmatory molecular analyzes reduces the strength and generalizability of the findings. In general, this study presents preliminary descriptive observations in favor of further study of *C. macrocarpa* leaf extract as a source of antibacterial constituents. However, additional experimental validation is needed to draw conclusions regarding the reproducibility of antibacterial efficacy, the specific modes of action, or the activity against molecularly characterized Enterobacter and Salmonella isolates.

5.2. Recommendations

The investigations suggested below are based on the findings and methodological limitations found in this study.

1. The extraction protocol needs to be fully documented in future work, including botanical authentication, collection details, extraction solvent, duration, temperature and extraction yield. Standardization would enhance reproducibility of experimental results and allow for comparison of investigations.
2. Qualitative and quantitative analytical methods validated should be used to study the chemical composition of *C. macrocarpa* leaf extract. Constituents that may be associated with antibacterial activity can be identified and characterized by chromatographic and spectroscopic methods.
3. Antibacterial screening should be carried out under standardized experimental conditions with adequate positive and negative controls and independent biological replicates. This would allow for the estimation of experimental variability and would help in establishing valid statistical comparisons.

Creative Commons Attribution-Non-Commercial 4.0 International License

Published by DAC Democratic Arabic Center
DAC Democratic Arabic Center GmbH, Berlin

4. Future studies should use validated methods applicable to plant extracts for determination of MICs and MBCs. Such measurements would provide a more informative assessment of the antibacterial activity than inhibition zone diameters alone.
5. Further studies should be done to investigate the antibacterial activity of *C. macrocarpa* leaf extract against the same Enterobacter and Salmonella isolates that had been subjected to molecular screening. This would allow for direct assessment of potential correlations between antibacterial susceptibility and genetic features. If necessary, confirmatory sequencing or other validated molecular methods must be used to confirm the identity of the amplified targets.
6. PCR-based screening results must be supported by appropriate positive and negative controls.
7. Studies on intI3 should be complemented with antimicrobial susceptibility testing and, if possible, integron gene cassette characterization. Gene detection is different from gene expression and functional adhesion, and these should be distinguished in fimH studies.
8. Future studies could focus on the possible effects of chemically defined components of *C. macrocarpa* on bacterial growth, membrane stability, or other relevant cellular processes. Such investigations should be designed to establish mechanisms experimentally rather than infer them from results of crude-extract screening.
9. Future research should evaluate the cytotoxicity and biological safety of the extract or of isolated constituents using appropriate experimental models before considering any possible therapeutic applications.
10. Future studies should incorporate standardized extraction, phytochemical analysis, quantitative antibacterial testing, molecular characterization and appropriate statistical analysis in a unified experimental design. The adoption of this approach would provide stronger evidence for the antibacterial potential of *C. macrocarpa* and its possible importance against Gram-negative bacterial pathogens.

5.3. Final Scientific Perspective

The results of this preliminary study can be considered as a first contribution to the experimental evaluation of *Carissa macrocarpa* leaf extract and to the molecular

 Creative Commons Attribution-Non-Commercial 4.0 International License Published by DAC Democratic Arabic Center
DAC Democratic Arabic Center GmbH, Berlin

screening of selected bacterial genetic determinants. The inhibition-zone measurements reported are in agreement with antibacterial activity against *E.coli*. The scientific impact of the study depends on preliminary observations and the design of specific questions to be further studied. Future studies will be needed to determine whether C. *macrocarpa* has reproducible potency or therapeutic applicability, as the available evidence is not sufficient to establish these properties. Similarly, the reported PCR amplification products are preliminary molecular observations and do not, in themselves, demonstrate antimicrobial resistance, bacterial virulence, or susceptibility to the plant extract. The antibacterial activities of macrocarpa-derived constituents against clinically relevant Gram-negative bacteria are consistent.

References

1. Souilem F, Dias MI, Barros L, Calhelha RC, Alves MJ, et al. Phenolic profile and bioactive properties of *Carissa macrocarpa* (Eckl.) A.DC.: An in vitro comparative study between leaves, stems, and flowers. Molecules. 2019;24(9):1696. doi: 10.3390/molecules24091696.
2. Ramasar R, Naidoo Y, Dewir YH, El-Banna AN. Seasonal change in phytochemical composition and biological activities of *Carissa macrocarpa* (Eckl.) A. DC. leaf extract. Horticulturae. 2022;8(9):780. doi: 10.3390/horticulturae8090780.
3. Soni A, Suryakant S. Evaluation of antibacterial activity of various sample extracts against *Bacillus subtilis*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, and *Salmonella typhi*. Journal of Advances in Science and Technology. 2024. doi: 10.29070/ny0x7910.
4. Opande GT. Phytochemical screening and antimicrobial properties of *Carissa edulis* extracts obtained from Kaimosi Forest, Vihiga County, Kenya. European Journal of Medicinal Plants. 2022;33(10). doi: 10.9734/ejmp/2022/v33i1030492.
5. Allam KM, Abd El-kader AM, Fouad MA, Mostafa MAH. Phytochemical and biological studies of *Carissa macrocarpa*, F. Apocyanaceae. Journal of Advanced Biomedical and Pharmaceutical Sciences. 2020. doi: 10.21608/jabps.2020.40128.1098.

Creative Commons Attribution-Non-Commercial 4.0 International License

Published by DAC Democratic Arabic Center
DAC Democratic Arabic Center GmbH, Berlin

6. Bhat B, Dar R, Ashgar RI, Mangral ZA, Abdullah N, et al. Metabolic profiling and antibacterial activity of *Carissa opaca* leaf extracts: Bridging experimental and computational approaches. Chemistry & Biodiversity. 2025. doi: 10.1002/cbdv.202501424.
7. Khalil HE, Mohamed ME, Morsy MA, Kandeel M. Flavonoid and phenolic compounds from *Carissa macrocarpa*: Molecular docking and cytotoxicity studies. Pharmacognosy Magazine. 2018.
8. Monika MS, Thirumal M, Kumar PR. Identification of bioactive phytochemicals by GC-MS profiling, phytochemical screening, and TLC using methanolic extract of *Carissa carandas* Linn leaf. Journal of Pharmaceutical Negative Results. 2022. doi: 10.47750/pnr.2022.13.s08.568.
9. Abah SE, Egwari LO. Methods of extraction and antimicrobial susceptibility testing of plant extracts. 2011.
10. Moodley R, Chenia HY, Jonnalagadda SB, Koorbanally NA. Antibacterial and anti-adhesion activity of the pentacyclic triterpenoids isolated from the leaves and edible fruits of *Carissa macrocarpa*. 2011.
11. Moghadam A, Nazarian S. Evaluation of class 1, 2 and 3 integrons in clinical *Salmonella enteritidis* strains by PCR method. 2017.
12. Molecular detection of some virulence genes in *Salmonella* species isolated from clinical samples in Iraq. Archives of Razi Institute. 2022. doi: 10.22092/ari.2022.357983.2129.
13. Yin L, Dai Y, Chen H, He XD, Ouyang P, et al. Cinnamaldehyde resist *Salmonella Typhimurium* adhesion by inhibiting type I fimbriae. Molecules. 2022;27(22):7753. doi: 10.3390/molecules27227753.
14. Mohammadalipour Z, Kargar M, Doosti A. High frequency of class 2 and 3 integrons related to drug-resistance in clinical isolates of diarrheagenic *E. coli* in Iran. 2017.
15. Collis, C. M., Kim, M.-J., Partridge, S. R., Stokes, H. W., & Hall, R. M. (2002). Characterization of the class 3 integron and the site-specific recombination system it determines. *Journal of Bacteriology, 184*(11), 3017–3026. https://doi.org/10.1128/JB.184.11.3017-3026.2002
16. **Ugorski, M. (2025).** Role of type 1 fimbriae in the pathogenesis of *Salmonella* infections: Twenty five years of personal research. *Medycyna Weterynaryjna, 81*(10), 506–510.

Creative Commons Attribution-Non-Commercial 4.0 International License

Published by DAC Democratic Arabic Center
DAC Democratic Arabic Center GmbH, Berlin

17. Kargar M, Mohammadalipour Z, Doosti A, Lorzadeh S, Japoni-Nejad A. High prevalence of class 1 to 3 integrons among multidrug-resistant diarrheagenic *Escherichia coli* in southwest of Iran. 2014.
18. Irfan T, Adeel J, Arshad L, Qaiser D, Mehmood T. Bioactive phytochemicals of *Carissa macrocarpa*: In vitro and in silico investigations. 2025.
19. Syed IZ. Antimicrobial potential and phytochemical screening of leaf extracts in *Cassia tora* L. 2018.
20. Saif MMS, Al-Fakih AA, Hassan MAM. Antibacterial activity of selected plant (aqueous and methanolic) extracts against some pathogenic bacteria. 2017.
21. Glassman M. Potential of African medicinal plants against Enterobacteria: Classification of plants antibacterial agents. 2023.
22. Dhatwalia J, Kumari A, Verma R, Upadhyay NK, Guleria I, et al. Phytochemistry, pharmacology, and nutraceutical profile of *Carissa* species: An updated review. 2021.
23. Kaunda JS, Zhang YJ. The genus *Carissa*: An ethnopharmacological, phytochemical and pharmacological review. 2017.
24. Xu YH, Zhang Q, Li T, Ling HZ, Xiong ZZ, et al. Distribution and typing of integrons in gram-negative bacilli from sputum. 2007.
25. Kisiela DI, Chattopadhyay S, Libby SJ, Karlinsey JE, Fang FC, et al. Evolution of *Salmonella enterica* virulence via point mutations in the fimbrial adhesin. 2012.
26. Hossain, S., De Silva, B. C. J., Dahanayake, P. S., Shin, G.-W., & Heo, G.-J. (2019). Molecular characterization of virulence, antimicrobial resistance genes, and class one integron gene cassettes in *Salmonella enterica* subsp. *enterica* isolated from pet turtles in Seoul, Korea. *Journal of Exotic Pet Medicine, 28*, 209–217.

Creative Commons Attribution-Non-Commercial 4.0 International License

Published by DAC Democratic Arabic Center
DAC Democratic Arabic Center GmbH, Berlin