Society of Medical Education & Research

2024, Volume 4, Issue 2, Page No: 53-68
ISSN: 3108-4826

Journal of Medical Sciences and Interdisciplinary Research

Phytochemical Composition and Bioactivity of Leaf and Stem Extracts of Carissa bispinosa: Implications for Oral Health

Patrick Leo Sullivan[1]*, Brian Andrew Murphy[1], Eamon Declan Walsh[2], Ciaran Thomas Oreilly[2]

[1]Department of Management, College of Business, University College Dublin, Dublin, Ireland.
[2]Department of Management, Saïd Business School, University of Oxford, Oxford, United Kingdom.

***E-mail** ✉ p.sullivan.ucd@gmail.com

Abstract

Carissa bispinosa (L.) Desf. ex Brenan is traditionally employed in the management of oral infections. Nevertheless, scientific evidence supporting its therapeutic effects and phytochemical profile remains scarce. This research aimed to evaluate the protective potential of leaf and stem extracts from C. bispinosa against oral pathogens. Phenolic and tannin levels were quantified via the Folin-Ciocalteu assay following extraction with various solvents. Minimum inhibitory concentrations (MIC) of the extracts were determined through microdilution against fungal strains (Candida albicans and Candida glabrata) and bacterial strains (Streptococcus pyogenes, Staphylococcus aureus, and Enterococcus faecalis). Antioxidant capacity was examined using 2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging and ferric reducing power (FRP) assays. Cytotoxicity of the leaf acetone extract was assessed with the methylthiazol tetrazolium (MTT) method.

The highest phenolic content in leaf extracts was observed in the methanol fraction (113.20 mg TAE/g), while the hexane extract showed the greatest tannin level at 22.98 mg GAE/g. Among stem extracts, the acetone fraction exhibited the highest phenolic content (338 mg TAE/g), and the stem extract overall provided the maximum tannin content (49.87 mg GAE/g). The methanol leaf extract achieved the lowest MIC (0.31 mg/mL), matching the stem ethanol extract at 0.31 mg/mL. The stem methanol extract displayed the strongest DPPH scavenging activity (IC50, 72 µg/mL), whereas the stem ethanol extract demonstrated the highest FRP with an absorbance of 1.916. The leaf acetone extract showed low cytotoxicity, with an LC50 of 0.63 mg/mL. Findings from this investigation support the protective role of C. bispinosa in combating oral infections.

Keywords: Carissa bispinosa, Phytochemicals, Antimicrobial activity, Antioxidant activity, Cytotoxicity

Introduction

Oral infections represent a significant global health challenge, particularly affecting populations with low socioeconomic status due to inadequate oral hygiene and limited access to care [1]. The oral cavity provides an ideal environment for diverse microorganisms, including archaea, protozoa, bacteria, fungi, and viruses, with bacteria comprising approximately 96% and primarily belonging to phyla such as Bacteroidetes, Fusobacteria, Proteobacteria, Actinobacteria, Firmicutes, and Spirochaetes [2, 3]. More than 700 bacterial species are known to inhabit the oral cavity [4]. Many of these microbes are implicated in oral diseases, including dental caries and periodontitis [5]. Dental caries causes permanent enamel destruction through the acidic byproducts of sucrose fermentation by cariogenic bacteria, leading to demineralization [6]. Microbial plaque initiates both caries and periodontal disease, the latter being a serious inflammatory condition that damages gingival tissues and supporting dental structures, potentially resulting in tooth loss if unmanaged [7].

Oxidative stress is recognised as a major contributor to the pathogenesis of oral infections [8]. Microbial

Access this article online https://smerpub.com/

Received: 18 May 2025; **Accepted:** 27 July 2025

How to cite this article: Sullivan PL, Murphy BA, Walsh ED, Oreilly CT. Phytochemical Composition and Bioactivity of Leaf and Stem Extracts of Carissa bispinosa: Implications for Oral Health. J Med Sci Interdiscip Res. 2024;4(2):53-68. https://doi.org/10.51847/8qMqawahPE

invasion and toxins trigger excessive generation of oxygen-derived free radicals—such as superoxide anion, hydrogen peroxide, perhydroxyl, hydroxyl, alkoxy, and peroxyl radicals—as part of the host immune defence [9]. Excessive radical production establishes oxidative stress, which exacerbates oral disease progression by damaging key biomolecules like lipids and proteins [10]. Consequently, oxidative stress is closely associated with oral cancer and necrotising stomatitis [8]. Compounds capable of neutralising free radicals through chelation, enzyme inhibition, or direct scavenging have thus attracted considerable attention [11].

Conventional oral care products and therapeutic agents, including chlorhexidine, triclosan, fluoride, penicillin, erythromycin, and amoxicillin, are commonly used for preventing and treating oral infections owing to their antimicrobial and antioxidant effects [3, 12]. However, resistance has developed in many oral microbes against these agents [13]. Additionally, despite their effectiveness, these treatments raise concerns due to high cost, toxicity, and side effects such as hypersensitivity, tooth discolouration, tongue coating, taste alteration, and disruption of oral microbiota [14–16]. Such limitations have driven ongoing exploration of safer, plant-derived alternatives for antimicrobial development.

Plant secondary metabolites—including phenols, alkaloids, tannins, and flavonoids—offer promising complements to existing oral therapies because of their established antimicrobial, anti-inflammatory, and antioxidant actions [17, 18]. These natural compounds generally exhibit minimal side effects, lower cost, and greater accessibility, especially in developing regions like South Africa.

Carissa bispinosa, a member of the Apocynaceae family [19], is native to South Africa and traditionally valued for treating various conditions, including oral infections. Its stems and roots are used to enhance male hormones and manage oral ailments [20]. Previous studies identified terpenoids such as ursolic acid and tritriacontane in the plant, known for antioxidant, anti-inflammatory, and antimicrobial effects [21–23]. Recent work also detected various flavonoids with antioxidant properties in the fruit extract [24]. However, comprehensive data on the phytochemical composition and biological activities of leaf and stem extracts remain limited.

This study sought to assess the protective potential of constituents from C. bispinosa leaf and stem extracts by examining their antimicrobial effects against oral pathogens, along with antioxidant and cytotoxic profiles.

Materials and Methods

Chemicals and media

All chemicals and culture media employed were of analytical grade, sourced from Sigma-Aldrich, Whitehead Scientific, Adcock-Ingram, and Merck (Pty) Ltd. Distilled water was autoclaved at 121 °C for 15 min prior to use in the experiments.

Microbial strains

The fungal strains (Candida albicans and Candida glabrata) and bacterial strains (Streptococcus pyogenes, Staphylococcus aureus, and Enterococcus faecalis) utilised were clinical oral isolates obtained from Polokwane Hospital, National Health Laboratory Service (NHLS), South Africa.

Plant collection

Leaves and stems of the plant were harvested from the University of Limpopo Turfloop campus, Limpopo Province, South Africa (latitude −23.885425 S, longitude E, altitude 1335 m) on July 8, 2019. Ethical approval for plant usage was granted by the University of Limpopo research ethics committee. Voucher specimens were deposited at the Larry Leach Herbarium (UNIN), University of Limpopo (UNIN 1,220,078), with identification confirmed by Dr Bronwyn Egan. Plant extract utilisation complied with international guidelines [25]. Collected materials were rinsed with tap water to eliminate dirt and debris, then air-dried away from light and heat to preserve thermolabile constituents. Dried samples were pulverised into fine powder using an electric grinder (Sundy hammercrusher SDHC 150) and kept in opaque polyethylene bags until required.

Extraction

One gram of dried powdered material from each part (leaf and stem) was extracted with 10 mL of solvents ranging from non-polar to polar: hexane, chloroform, dichloromethane, ethyl acetate, acetone, ethanol, methanol, butanol, and water. Mixtures were agitated at 200 rpm for 20 min in a shaking incubator (New Brunswick Scientific Co., Inc). Extracts were then filtered through Whatman No.1 paper. Organic solvents were removed by evaporation under a fan, while aqueous and butanol fractions were concentrated via rotary evaporator (Buchi B-490) before fan drying. Dried extracts were weighed in pre-tared vials, and yield calculated as: yield = (Ao - A1), where Ao is the weight

of the vial plus extract (mg) and A1 is the empty vial weight (mg). All crude extracts were redissolved in acetone to achieve a stock concentration of 10 mg/mL.

Quantitative analysis of classes of phytochemicals

Total phenolic content

Phenolic levels in extracts (prepared in 70% aqueous acetone) were determined following the protocol outlined by Singleton *et al*. (1999). Results were reported as mg gallic acid equivalents per gram (mg GAE/g) of extract [26].

Total tannin content

Tannin concentrations were quantified employing the Folin-Ciocalteu assay. Values were expressed as mg GAE/g of extract [26].

Phytochemical analysis by chromatography

Compounds in leaf and stem extracts of C. bispinosa were characterised using Sciex Exion LC-MS coupled to a Sciex X500R QTOF system with electrospray ionisation detection, as detailed by Mpai *et al*. [27]. Chromatographic separation was performed on a Phenomenex Luna C18 column (2.5 µm, 100 × 2 mm). Mobile phase A consisted of methanol and phase B of water, both containing 20 mM ammonium acetate. Gradient elution was programmed as: 0 min, 5% A and 95% B for 1 min; to 95% A and 5% B over 22 min; held at 95% A and 5% B for 27 min; return to 5% A and 95% B at 27.10 min; equilibrated at 5% A and 95% B until 30 min. Flow rate was 0.4 mL/min at 40 °C, with 10 µL injection volume. Mass spectrometry operated in negative electrospray ionisation mode using nitrogen for desolvation. Ion source gas 1 and 2 pressures were 50 and 70 psi, curtain gas 30 psi, CAD gas 7, source temperature 500 °C, spray voltage 4500 V, and declustering potential −80 V. Data were acquired and analysed with Analyst software.

Antimicrobial testing

Culturing of test organisms

Microbial stock solutions were created by transferring the isolates into 150 mL volumes of Sabouraud dextrose broth (for fungi) or nutrient broth (for bacteria), with incubation carried out at 30 °C for fungi and 37 °C for bacteria. Both fungal and bacterial cultures were standardized to 1×10^8 CFU/mL by dilution in sterile distilled water.

Bioautographic screening

Qualitative assessment of antimicrobial properties in the extracts was performed against chosen fungal and bacterial strains via bioautography, following the procedure detailed by Begue and Kline [28]. Each extract (10 mg/mL) was spotted in 20 µL aliquots onto TLC plates, which were then eluted in three solvent systems: benzene/ethanol/ammonium hydroxide [BEA] (9:1:0.1), chloroform/ethyl acetate/formic acid [CEF] (5:4:2), and ethyl acetate/methanol/water [EMW] (10:1.35:1). Zones of clearance on the developed bioautograms signified inhibitory effects from the extracts.

MIC determination and total activity calculation

Minimum inhibitory concentrations (MICs) for the extracts were established through serial microdilution assays [29]. The MIC was taken as the lowest concentration preventing detectable microbial growth. Total activity for each extract was derived by dividing the yield (in mg) from 1 g of C. bispinosa material by its MIC value (in mg/mL) [30].

Evaluation of antioxidant properties

DPPH scavenging activity

Free radical scavenging potential of the extracts against DPPH was measured according to the protocol of Brand-Williams *et al*. [31], incorporating minor changes. Inhibition percentage was computed as: %Inhibition = [(Ao – A1)/Ao] × 100, with Ao being the control absorbance and A1 being the sample absorbance.

Ferric ion reducing capacity assay

Reductive ability of the extracts toward ferric ions was assayed via the ferric reducing power method adapted from Oyaizu [32]. Elevated absorbance readings at 700 nm reflected greater reducing strength. A blank control was set up identically, but substituting acetone for the extract.

Assessment of cytotoxicity

Cytotoxic potential of the acetone leaf extract was tested on THP-1 human monocytic leukemia cells using the MTT assay, based on Mosmann's method [33] with adjustments. Cells were grown in RPMI 1640 medium fortified with 10% fetal bovine serum (FBS). Viability was quantified as: %Cell viability = [(Ao – A1)/Ao] × 100, where Ao is absorbance from untreated cells and A1 from treated cells.

Setup of ligands and target proteins

Three-dimensional models of gentamicin and oleamide were retrieved from the NCBI PubChem database, whereas protein structures were acquired from the Protein Data Bank (www.rcsb.org). Targets selected included ABC transporter (PDB ID 6aal) and DNA gyrase subunit A (PDB ID 3g7b). Proteins underwent preparation by removing waters, non-protein atoms, and bound ligands, then adding polar hydrogens, all via Discovery Studio v4.1 [34].

Computational docking analysis

In silico docking was executed with AutoDock Vina. Grid boxes were centered at coordinates (−0.302947, 21.052211, −23.891211) for 6aal and (−27.830552, −5.584207, 8.598638) for 3g7b, defined from active pockets using Discovery Studio 4.1. Affinity scores were generated by AutoDock Vina [35], and poses with the most negative binding energies were retained as optimal. Resulting complexes were then loaded into Discovery Studio v4.1 for 2D and 3D interaction visualization [34].

Statistical treatment

All assays were conducted in triplicate, with results reported as mean ± standard deviation. One-way ANOVA was applied for analysis, and differences were deemed significant at $p < 0.05$.

Results and Discussion

Yield from extraction

Figure 1 displays the quantities (in mg) of extracts derived from leaves and stems post-extraction. Yields from leaves generally exceeded those from stems. Additionally, solvents of higher polarity produced the greatest amounts of plant compounds.

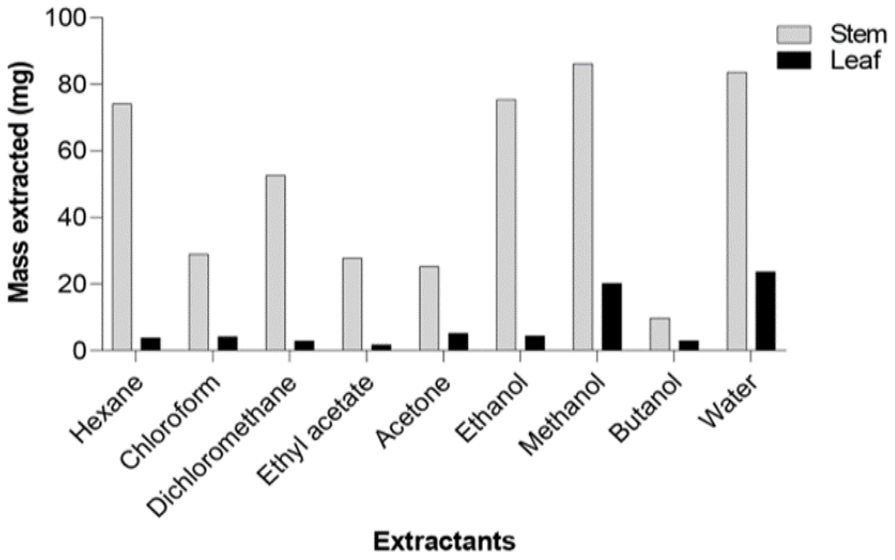
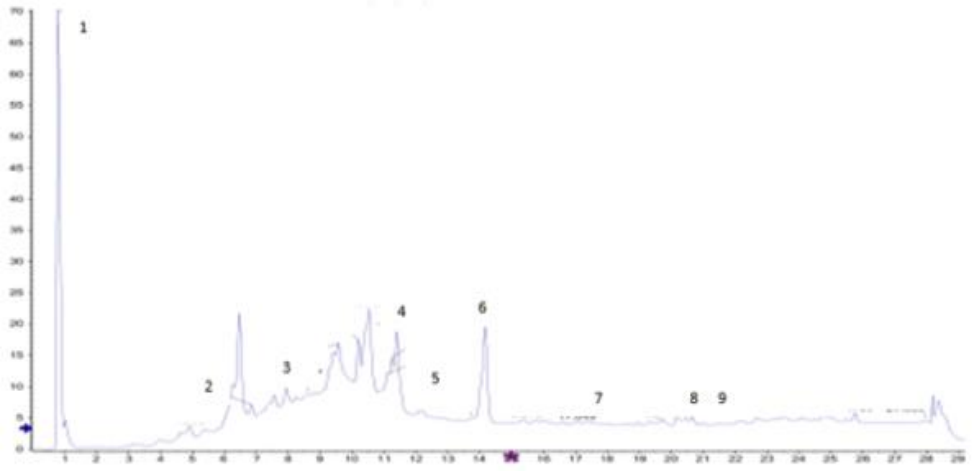


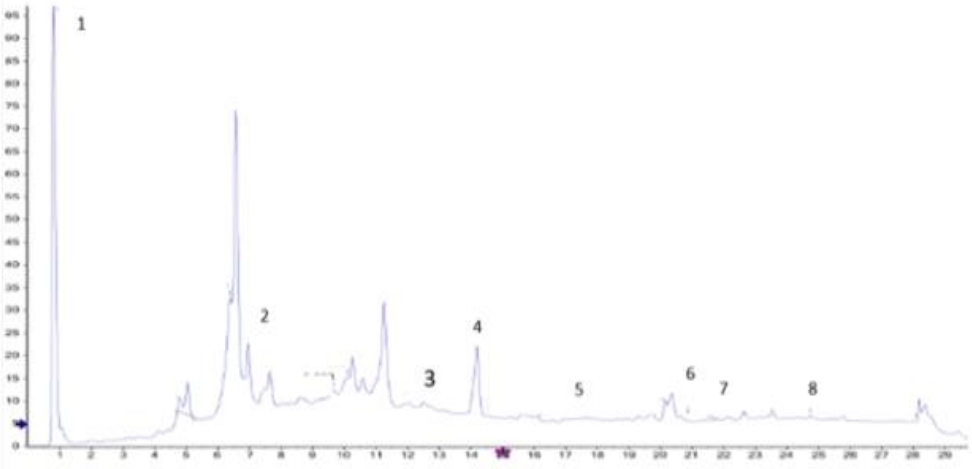
Figure 1. Extraction yields from leaves and stems of Carissa bispinosa using various solvents

Identification of phytochemicals via LC-MS

Phytochemicals identified along with their retention times from LC-MS are presented in **Figure 2a** (leaves) **and 2b** (stems), as well as **Table 1** (leaves) and **Table 2** (stems). Several compounds, including oleamide, sorbitan monopalmitate, and tributylamine, were present in both leaf and stem samples.



a)



b)

Figure 2. LC–MS/MS total ion chromatograms of methanol extracts from C. bispinosa: (a) leaves and (b) stems

Phytocompounds identified in the methanol leaf extract of Carissa bispinosa by liquid chromatography-mass spectrometry (LC-MS).

Table 1. Phytocompounds identified in the methanol leaf extract of Carissa bispinosa by liquid chromatography-mass spectrometry (LC-MS)

Peak #	Rt (min)	Area %	MS-MS (m/z)	MS (m/z)	Molecular Formula	Chemical class	Tentative identification	Bioactivity
1	0.60	3.963e + 04	185.1, 203.0	365.106 [M + H]+	C19H24OS3			
2	7.20	6.772e + 04	74.1, 130.2	186.222 [M + H]+	C12H27N	Tertiary amines	Tributylamine	
3	7.69	2.895e + 02	67.1, 119.1, 189.0	207.174 [M + H]+	C14H22O	Phenols	2,4-Di-tert-butylphenol	Antibacterial, antifungal [36]
4	11.24	9.879e + 03	437.2, 527.1	527.246 [M + H]+	C23H38N6O4S2			

5	12.77	7.628e + 03	149.1, 236.9, 28.1	434.289 [M + H]+	C25H39NO5	Acetamide	N-[1,1-Bis[(acetyloxy) methyl]-3-(4-octylphenyl)propyl]acetamide	
6	14.15	2.017e + 05	121.0, 347.2	347.231 [M + H]+	C21H30O4	21-hydroxysteroids	Corticosterone	
7	17.99	4.267e + 04	403.3	403.294 [M + H]+	C22H42O6	Fatty acid esters	Sorbitan monopalmitate	
8	21.10	2.236e + 04	95.3	194.115 [M + H]+	C11H15NO2	Carbamate ester	Isoprocarb	
9	22.37	5.995e + 06	69.0, 83.1, 95.1, 107.1, 282.1	282.279 [M + H]+	C18H35NO	Fatty amide	Oleamide	Antibacterial [37, 38]

Table 2. Phytocompounds identified in the methanol stem extract of Carissa bispinosa by liquid chromatography-mass spectrometry

Peak #	Rt (min)	Area %	MS-MS (m/z)	MS (m/z)	Molecular Formula	Chemical class	Tentative identification	Bioactivity
1	0.60	3.963e + 04	185.1, 203.0	365.106 [M + H]+	C19H24OS3			
2	7.20	6.772e + 04	74.1, 130.2	186.222 [M + H]+	C12H27N	Tertiary amines	Tributylamine	
3	12.77	8.033e + 03	81.1, 149.1, 258.1	434.289 [M + H]+	C25H39NO5	Acetamide	N-[1,1-Bis[(acetyloxy)methyl]-3-(4-octylphenyl)propyl]acetamide	
4	14.15	2.017e + 05	121.0, 347.2	347.231 [M + H]+	C21H30O4	21-hydroxysteroids	Corticosterone	
5	17.99	4.267e + 04	403.3	403.294 [M + H]+	C22H42O6	Fatty acid esters	Sorbitan monopalmitate	
6	20.93	5.867e + 04	67.1, 123.1, 408.4, 474.4	547.521 [M + H]+	C28H66N8S			
7	22.37	5.995e + 06	69.0, 83.1, 95.1, 107.1, 282.1	282.279 [M + H]+	C18H35NO	Fatty amide	Oleamide	Antibacterial [37, 38]
8	25.54	1.933e + 06	282.2, 299.3, 369.2, 537.5	537.395 [M + H]+	C40H56	Carotenoids	β-Carotene	Antioxidant [39]

Quantification of phenolics and tannins in leaf and stem samples

Levels of total phenolics and tannins were measured, with results summarized in **Table 3**. Stem samples contained greater amounts of both total phenolics and tannins compared to leaf samples. The methanol extract from leaves showed the peak phenolic level at 113.20 ± 10.4 mg GAE/g, while the acetone extract from stems recorded the highest at 338 mg GAE/g. The lowest phenolic value, 29.21 ± 13.4 mg GAE/g, came from the acetone leaf extract. Tannin concentrations followed a similar pattern, being higher in stems; the hexane leaf extract had the maximum tannin content among leaves (22.98 mg GAE/g), and the ethanol stem extract topped the list at 49.87 mg GAE/g.

Table 3. Contents of total phenolics and tannins (expressed as mg GAE/g) in extracts from leaves and stems of Carissa bispinosa

Solvent	Tannin content (Stem extract)	Tannin content (Leaf extract)	Phenolic content (Stem extract)	Phenolic content (Leaf extract)
Hexane	47.29 ± 2.8 e	22.98 ± 2.2 a,c	216 ± 30.6 b	29.98 ± 18.0 a
Dichloromethane	8.89 ± 0.7 a, b	8.36 ± 1.0 b	50 ± 18.2 a	70.41 ± 34.8 a,b
Ethyl acetate	6.79 ± 1.7 a	16.64 ± 2.6 c,d,e	36 ± 7.8 a	69.64 ± 25.5 a,b
Acetone	47.65 ± 2.5 e	15.04 ± 2.3 b,d,e	338 ± 14.9 c	29.21 ± 13.4 a
Ethanol	49.87 ± 5.0 e	13.46 ± 0.7 b,d	216 ± 59.4 b	33.79 ± 11.3 a
Methanol	28.07 ± 7.5 c,d	9.35 ± 5.0 b,f	163 ± 26.8 b	113.20 ± 10.4 a
Butanol	12.23 ± 3.3 a,b	10.65 ± 1.9 b,d	31 ± 10.7 a	40.01 ± 7.6 b
Chloroform	18.21 ± 4.4 b,c	15.37 ± 1.2 d,e,f	46 ± 17.6 a	30.23 ± 19.3 a

Within columns, means sharing identical letters indicate no significant statistical difference (Tukey's multiple comparison test)

Evaluation of antimicrobial effects from leaf and stem extracts

Bioautographic screening of leaf and stem extracts

Bioautography was employed to assess the inhibitory potential of leaf and stem extracts against microbes associated with oral infections. Clear zones on the chromatograms marked antibacterial effects **(Figure 3)**. Activity against bacteria appeared exclusively in BEA-developed plates across all tested strains. Overall, extracts from leaves demonstrated stronger inhibition than those from stems. Every leaf extract, apart from the aqueous one, inhibited the growth of the pathogens tested (S. aureus, E. faecalis, and S. pyogenes).

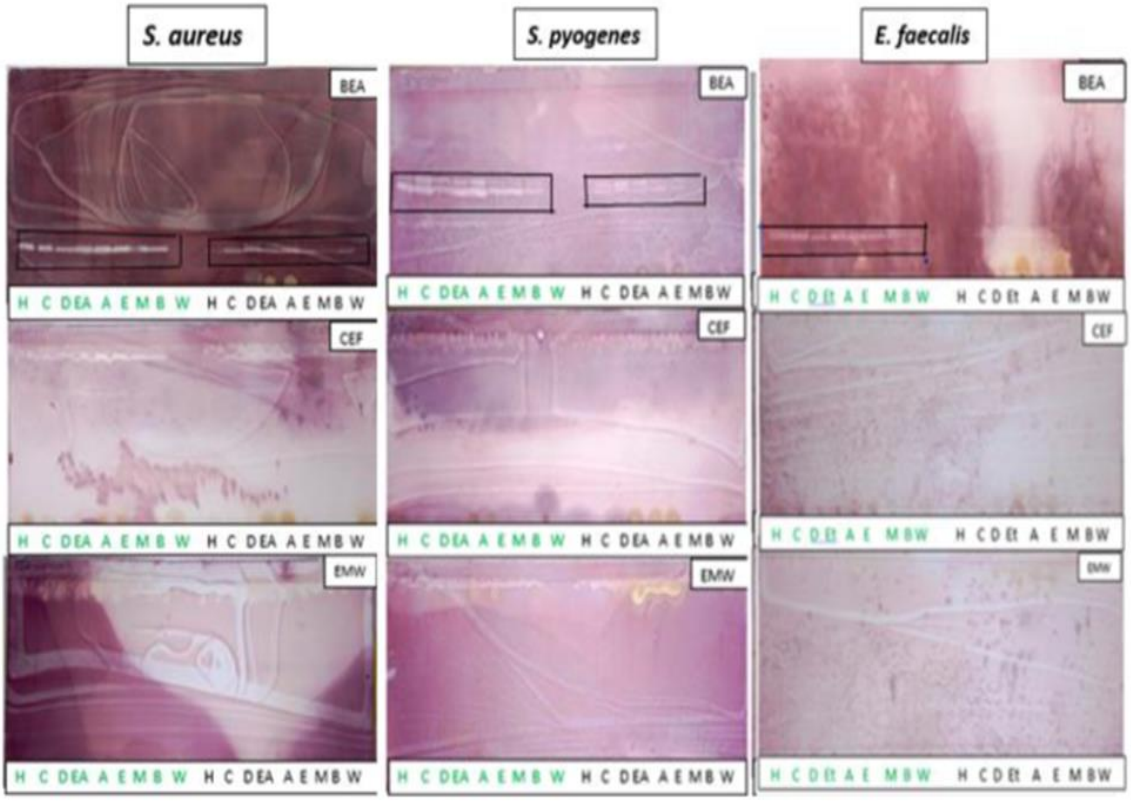


Figure 3. Bioautograms showing activity of various Carissa bispinosa leaf and stem extracts against Staphylococcus aureus, Streptococcus pyogenes, and Enterococcus faecalis, developed using mobile phases: (benzene/ethanol/ammonium hydroxide 9:1:0.1), (chloroform/ethyl acetate/formic acid 5:4:2), and (ethyl

acetate/methanol/water 10:1.35:1). Legend: (H) hexane, (C) chloroform, (D) dichloromethane, (EA) ethyl acetate, (A) acetone, (E) ethanol, (M) methanol, (B) butanol, (W) water. Green outlines denote leaf extracts; black outlines denote stem extracts

Antifungal effects of leaf and stem extracts from C. bispinosa against C. albicans and C. glabrata are depicted in **Figure 4**. Weak clearance zones appeared solely on BEA plates inoculated with C. albicans, suggesting mild antifungal action from the leaf extract against this species. No inhibition was detected against C. glabrata.

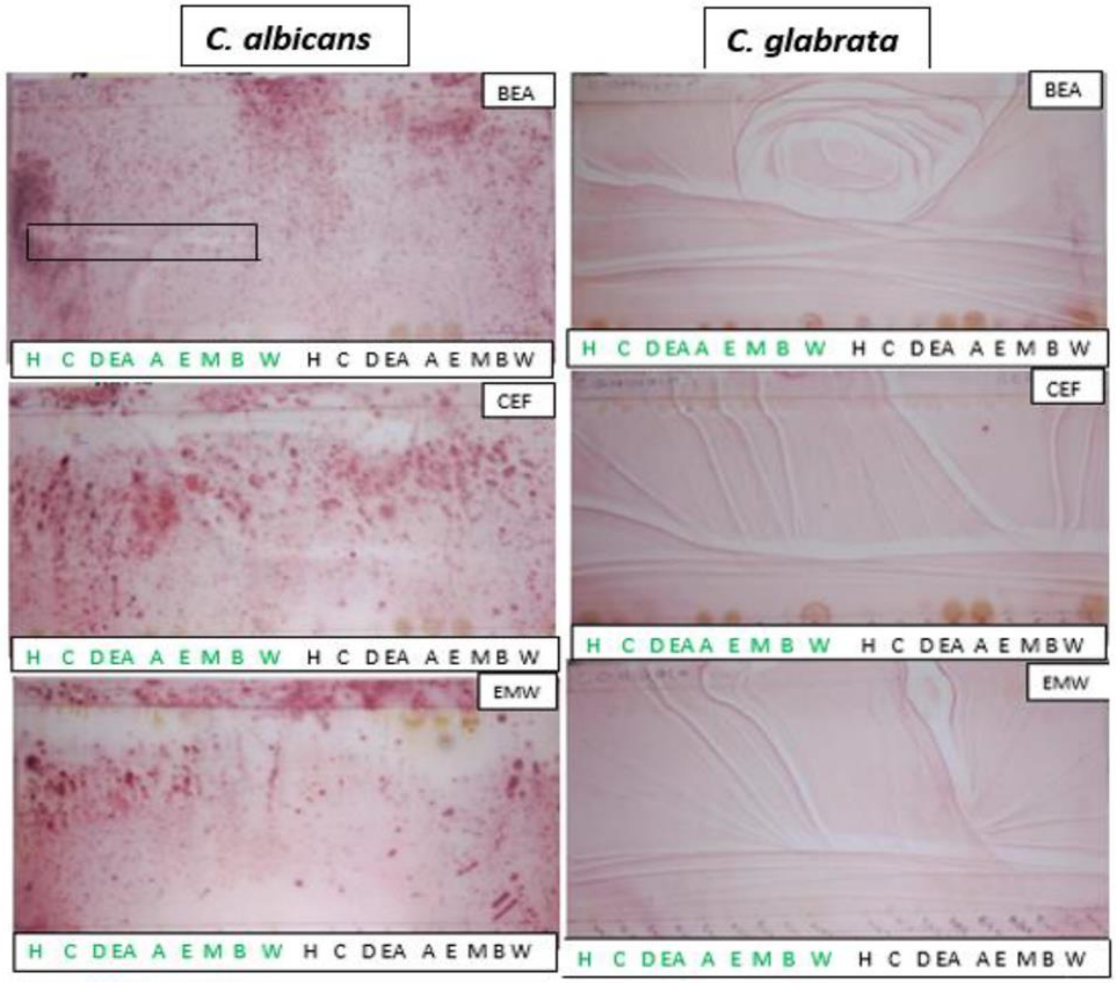


Figure 4. Bioautograms illustrating activity of assorted Carissa bispinosa extracts from leaves and stems versus Candida albicans and Candida glabrata, using elution systems: (benzene/ethanol/ammonium hydroxide 9:1:0.1), (chloroform/ethyl acetate/formic acid 5:4:2), and (ethyl acetate/methanol/water 10:1.35:1). Symbols: (H) hexane, (C) chloroform, (D) dichloromethane, (EA) ethyl acetate, (A) acetone, (E) ethanol, (M) methanol, (B) butanol, (W) water. Green borders highlight leaf samples; black borders highlight stem samples

Lowest concentrations inhibit growth for leaf and stem samples

Inhibitory concentrations from leaf samples

Broth dilution in microplates was used to measure precise inhibitory levels of leaf samples against bacteria and fungi. The strongest performance came from the methanol version, achieving 0.31 mg/mL as its lowest effective level across several organisms. Weakest results appeared with ethyl acetate and butanol versions, both requiring ≥ 1.25 mg/mL. Sensitivity varied by pathogen: C. albicans responded best to low doses (0.31 mg/mL),

while C. glabrata required the highest (≥ 1.25 mg/mL) **(Table 4)**.

Table 4. Lowest inhibitory concentrations (mg/mL) recorded for Carissa bispinosa leaf samples, plus gentamicin and amphotericin-B, versus chosen pathogens

Microorganism	H	C	D	EA	A	E	M	B	W	Gen/Amp
S. aureus	1.25	0.63	0.63	2.5	2.5	0.63	0.31	2.5	0.31	0.15
S. pyogenes	1.25	1.25	0.63	1.25	1.25	0.63	0.31	1.25	1.25	0.08
E. faecalis	2.5	2.5	2.5	2.5	0.63	1.25	0.63	1.25	1.25	0.15
C. albicans	0.63	0.63	1.25	1.25	0.63	0.31	0.31	1.25	0.31	0.08
C. glabrata	2.5	2.5	2.5	1.25	1.25	2.5	2.5	2.5	1.25	0.08

Symbols: (H) hexane, (C) chloroform, (D) dichloromethane, (EA) ethyl acetate, (A) acetone, (E) ethanol, (M) methanol, (B) butanol, (W) water, (Gen/Amp) gentamicin/amphotericin-B

Pathogens tested: Fungal strains (Candida albicans, Candida glabrata); bacterial strains (Streptococcus pyogenes, Staphylococcus aureus, Enterococcus faecalis)

Inhibitory concentrations from stem samples

Corresponding measurements were taken for stem samples **(Table 5)**. Ethanol version proved most potent at 0.31 mg/mL, whereas chloroform required ≥ 1.25 mg/mL. Among organisms, S. pyogenes showed the greatest vulnerability to minimal doses, with C. glabrata again proving hardest to inhibit (≥ 1.25 mg/mL).

Table 5. Lowest inhibitory concentrations (mg/mL) recorded for Carissa bispinosa stem samples, plus gentamicin and amphotericin-B, versus chosen pathogens

Microorganism	H	C	D	EA	A	E	M	B	W	Gen/Amp
S. aureus	1.25	5	1.25	2.5	1.25	0.31	2.5	2.5	2.5	0.31
S. pyogenes	5	1.25	0.63	0.63	0.31	0.63	1.25	0.63	1.25	0.15
E. faecalis	1.25	2.5	2.5	1.25	1.25	0.63	1.25	2.5	1.25	0.15
C. albicans	2.5	2.5	2.5	5	0.63	0.31	0.63	2.5	2.5	0.31
C. glabrata	5	5	2.5	2.5	1.25	1.25	2.5	2.5	2.5	0.15

Symbols: (H) hexane, (C) chloroform, (D) dichloromethane, (EA) ethyl acetate, (A) acetone, (E) ethanol, (M) methanol, (B) butanol, (W) water

Pathogens tested: Fungal strains (Candida albicans, Candida glabrata); bacterial strains (Streptococcus pyogenes, Staphylococcus aureus, Enterococcus faecalis)

Combined potency measures for leaf and stem samples

Combined potency from leaf samples

Calculated combined inhibitory capacities for leaf samples appear in **Table 6**. The maximum value reached 278 mL/g with the methanol version, dropping to a minimum of 3.9 mL/g for butanol.

Table 6. Combined inhibitory capacities (mL/g) of Carissa bispinosa leaf samples versus chosen pathogens

Microorganism	H	C	D	EA	A	E	M	B	W	Microorganism
S. aureus	59.3	46.0	83.5	11.1	10.1	119.7	278.1	3.9	270	*S. aureus*
S. pyogenes	59.3	23.2	83.5	22.2	20.2	119.7	278.1	7.8	67.0	*S. pyogenes*
E. faecalis	29.6	11.6	21.0	11.1	26.4	60.3	136.8	7.8	67.0	*E. faecalis*
C. albicans	117.6	46.0	42.1	22.2	40.2	243.2	278.1	7.8	270	*C. albicans*
C. glabrata	29.6	11.6	21.0	22.2	20.2	30.2	34.5	3.9	67.0	*C. glabrata*

Symbols: (H) hexane, (C) chloroform, (D) dichloromethane, (EA) ethyl acetate, (A) acetone, (E) ethanol, (M) methanol, (B) butanol, (W) water

Pathogens tested: Fungal strains (Candida albicans, Candida glabrata); bacterial strains (Streptococcus pyogenes, Staphylococcus aureus, Enterococcus faecalis)

Overall potency measures for stem samples

Table 7 presents the calculated overall inhibitory capacities of stem samples. The methanol version achieved the peak value at 32.06 mL/g, whereas ethyl acetate recorded the minimum at 0.36 mL/g.

Table 7. Overall inhibitory capacities (mL/g) of Carissa bispinosa stem samples versus selected pathogens

Microorganism	H	C	D	EA	A	E	M	B	W	Microorganism
S. aureus	3.04	0.84	2.32	0.72	4.16	14.19	0.08	1.2	9.48	*S. aureus*
S. pyogenes	0.76	3.36	4.60	2.86	16.77	6.98	16.16	4.76	18.96	*S. pyogenes*
E. faecalis	3.04	1.68	1.16	1.44	4.16	6.98	16.16	1.2	18.96	*E. faecalis*
C. albicans	1.52	1.68	1.16	0.36	8.25	14.19	32.06	1.2	9.48	*C. albicans*
C. glabrata	0.76	0.84	1.16	0.72	4.16	3.52	8.08	1.2	9.48	*C. glabrata*

Legend: (H) hexane, (C) chloroform, (D) dichloromethane, (EA) ethyl acetate, (A) acetone, (E) ethanol, (M) methanol, (B) butanol, (W) water
Pathogens tested: Fungal strains (Candida albicans, Candida glabrata); bacterial strains (Streptococcus pyogenes, Staphylococcus aureus, Enterococcus faecalis)

Radical scavenging and reducing capacities of leaf and stem samples

DPPH neutralization by leaf and stem samples

Radical neutralization potential of both leaf and stem samples was measured via DPPH assay, with outcomes listed in **Table 8**. Methanol and ethanol versions from leaves displayed peak effectiveness, both reaching IC_{50} of 95 µg/mL.

Table 8. IC_{50} values (µg/mL) for Carissa bispinosa leaf and stem samples in DPPH assay

Extractant	Stem Yield	Leaf Yield
Hexane	198	–
Dichloromethane	–	–
Ethyl acetate	–	–
Acetone	–	206
Ethanol	105	95
Methanol	72	95
Butanol	221	–
Water	172	75
Ascorbic acid	11.6	11.6

Legend: (–) Not determined

Ferric ion reduction capacity of leaf and stem samples

Figure 5 depicts reduction capabilities across concentrations for leaf and stem samples. Leaf versions showed dose-responsive reduction, increasing with higher doses. Highly polar versions—methanol from leaves (absorbance 1.308) and ethanol from stems (absorbance 1.916)—delivered the strongest performance. L-ascorbic acid outperformed all leaf versions but fell slightly below the top stem version (ethanol, absorbance 1.916).

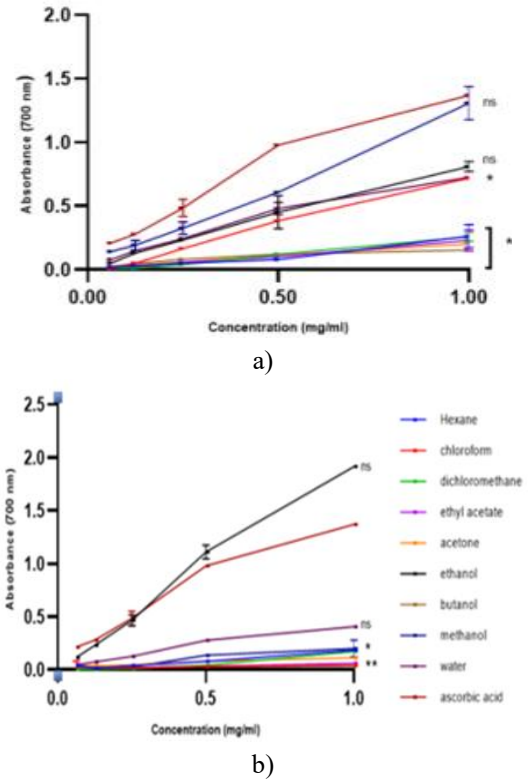


Figure 5. Reduction capacity profiles for Carissa bispinosa leaf (left panel) and stem (right panel) samples. Values shown as mean ± SD from triplicate runs. Statistical evaluation via one-way ANOVA ($p < 0.05$). Legend: () indicates a significant difference, (ns) indicates a non-significant difference

Cell viability impact of the acetone leaf sample

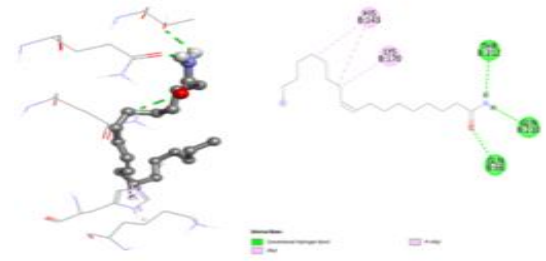
Cell viability reduction by the acetone leaf sample was assessed through the MTT protocol. Dose-dependent response was observed, yielding LC_{50} of 0.63 mg/mL.

Docking affinity scores for ligand-protein complexes

Compounds oleamide and gentamicin were computationally bound to proteins 6aal and 3g7b to determine interaction strengths. Oleamide produced scores of -5.6 kcal/mol (versus 6aal) and -4.2 kcal/mol (versus 3g7b). Gentamicin achieved stronger scores: -7.7 kcal/mol (versus 6aal) and -5.8 kcal/mol (versus 3g7b).

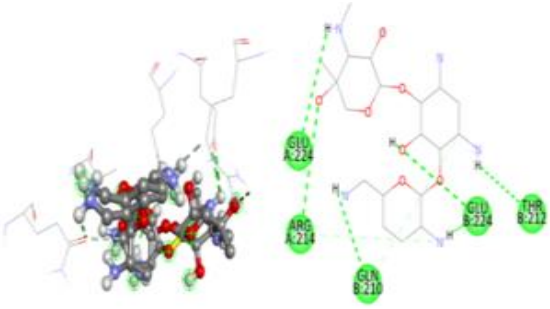
Binding interactions within docked complexes

Specific contacts between oleamide and target proteins (6aal, 3g7b) were examined, as visualized in **Figure 6**. With 6aal, oleamide established two hydrogen contacts (SER22, PHE23) reinforced by multiple alkyl contacts (VAL12, TYR18, ILE37, PHE53, LEU61). Against 3g7b, it formed three hydrogen contacts (GLN66, GLN210, THR212) plus two alkyl contacts (HIS143, LYS170). The gentamicin-6aal pairing featured five hydrogen contacts (GLU81, HIS95, ASP188, ASN189, SER190), one covalent contact (GLU133), and one alkyl contact (MET186). Gentamicin with 3g7b generated five hydrogen contacts (GLN210, THR212, ARG214, GLUA224, GLUB224) alongside van der Waals contributions.

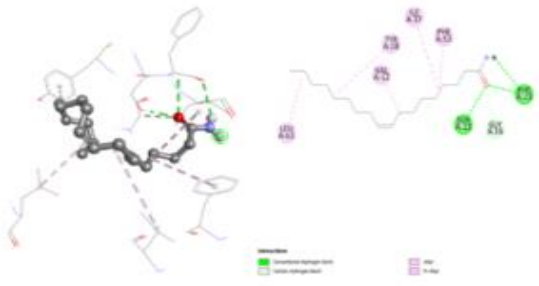


Oleamide-DNA Gyrase A complex

a)

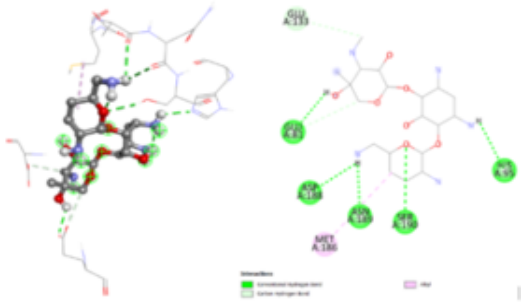


b)



Oleamide-abc transporters complex

c)



d)

Figure 6. Two-dimensional and three-dimensional representations of the bound complexes

Plants are well-known for producing a wide array of pharmacologically valuable phytochemicals in abundance. Achieving higher yields and enhanced biological activity from plant extracts requires careful solvent selection. In this research, multiple solvents were tested for phytochemical extraction. Methanol and water showed the greatest capacity to recover numerous phytochemicals from the leaf (methanol) and stem (water) sections of C. bispinosa, suggesting that these polar solvents are ideal for isolating compounds from this species. Methanol's superior metabolite yield matches observations by Masoko and Eloff [40], while water's effectiveness aligns with findings from Bouhafsoun *et al*. [41].

The therapeutic effects of botanical materials depend on the types and amounts of their phytochemical constituents. This investigation quantified phenolics and tannins, finding higher total levels in stem extracts compared to leaves. Studies indicate that greater phenolic concentrations are typically linked to stronger antioxidant effects [42–46]. Accordingly, C. bispinosa

offers promising potential as an antioxidant resource. In addition, LC-MS analysis revealed compounds like oleamide, known for antimicrobial properties, and the antioxidant β-carotene, which are likely responsible for much of the plant's biological activity [37, 38, 47].

In managing oral conditions, potent antimicrobial agents are essential to control infections from oral microbes. Botanical agents have demonstrated significant effectiveness against such pathogens [48]. Qualitative testing here showed that all leaf extracts, aside from the aqueous one, inhibited the tested bacteria and Candida albicans, but C. glabrata resisted every extract. Inhibition zones mostly appeared in BEA chromatogram sections, indicating that the active antimicrobial components are likely non-polar in nature.

Quantitative analysis measured minimum inhibitory concentrations (MICs) of the extracts. The methanol leaf extract had the lowest average MIC, showing the broadest efficacy among the tested organisms. The ethanol stem extract similarly recorded the minimal average MIC. Extracts with MICs under 1 mg/mL are classified as having substantial antimicrobial potency [49]. Thus, the methanol leaf and ethanol stem extracts stand out as valuable sources of agents against oral pathogens. Additionally, methanol extracts from leaves and stems displayed the greatest overall antimicrobial potency, meaning 1 gram of leaf or stem material could be diluted to 201.10 and 16.11 mL/g, respectively, while maintaining inhibition against vulnerable strains [28].

Oxidative stress results from free radicals produced by physiological and external factors, disrupting redox equilibrium and contributing to oral diseases. Identifying effective antioxidants to halt oxidative damage-linked infections is therefore a priority. Methanol and ethanol extracts in this work exhibited robust antioxidant capabilities via DPPH scavenging and ferricyanide reduction, reinforcing methanol's role in isolating antioxidants as noted by Ebrahimzadeh *et al*. [50]. IC50 values for both ethanol and methanol leaf extracts reached 95 µg/mL, rated as strong per the scale: very strong (IC50 < 50 µg/mL), strong (50 ≤ IC50 < 100 µg/mL), moderate (100 ≤ IC50 < 150 µg/mL), and low (IC50 > 150 µg/mL) [51]. The observed effects stem from interactions among the detected phytochemicals. A clear positive link existed between antioxidant performance and phenolic levels, indicating phenolics as primary contributors to this activity (unpublished).

High levels of toxicity in botanical extracts and isolated compounds are responsible for more than 54% of preclinical failures during drug development [52]. According to the International Organization for Standardization, cell viability exceeding 80% indicates non-cytotoxicity; 60–80% suggests weak cytotoxicity; 40–60% moderate; and below 40% strong cytotoxicity [53]. The cell viability percentages for the leaf acetone extract varied from 51 to 93%. Consequently, this extract demonstrates considerable biosafety, displaying effects ranging from moderate to non-toxic. Nonetheless, employing lower concentrations is recommended to prevent potential adverse impacts that could arise at higher doses. Muleya *et al*. [20] documented the traditional application and efficacy of C. bispinosa roots in managing toothache. Thus, the demonstrated effectiveness of these extracts against oral microbes and reactive oxygen species, combined with their elevated safety profile, positions the leaf and stem as viable substitutes for roots, aiding in the conservation of the species [54].

Molecular docking techniques were employed to forecast and clarify the inhibition mechanisms underlying the in vitro antimicrobial effects seen in this investigation. The selected target proteins were the ABC transporter (ID 6aal) and DNA gyrase A (ID 3g7b). DNA gyrase A is essential for bacterial DNA replication and supercoiling [55], while ABC transporters are transmembrane proteins that mediate the transport of substances like nutrients across cell membranes and catalyze ATP hydrolysis [56]. These two proteins, therefore, represent key targets in the design and development of antimicrobial agents.

The reduced binding energy values recorded here reflect strong accommodation of oleamide within the active sites of both proteins, indicating favorable binding affinity and stable interactions with the targets. This supports the role of oleamide in driving the antimicrobial effects detected in the experiments [57]. In contrast, gentamicin showed superior binding affinities to both proteins compared to oleamide, suggesting greater inhibitory potential. However, the robust antimicrobial performance of the extracts may primarily result from synergistic interactions among multiple constituents.

The inhibitory potential of ligands depends not solely on binding energy but critically on the specific interaction types formed with receptor sites [58]. The complexes involved hydrogen bonds, alkyl interactions, covalent linkages, and van der Waals forces with the target proteins, implicating these bonds in the antimicrobial outcomes observed. Hydrogen bonds play a crucial role in stabilizing ligand-receptor assemblies, thereby

promoting suppression of microbial proliferation [59]. Alkyl interactions, being covalent in nature, typically enhance complex stability. Covalent bonds are particularly robust, often resulting in irreversible associations [60]. Accordingly, the docking results suggest that extracts from C. bispinosa possess therapeutic promise for oral infections through the disruption or elimination of associated pathogens.

Conclusion

This research assessed the phytochemical composition, antioxidant capacity, antimicrobial efficacy, and cytotoxic profile of leaf and stem materials from C. bispinosa. Leaf extracts displayed low MIC values toward the evaluated oral microorganisms. These extracts also showed substantial antioxidant properties, highlighting their possible role in safeguarding against oxidative stress-related oral disorders. Additionally, the leaf acetone extract exhibited limited cytotoxicity, particularly at reduced doses (93% cell viability at 0.25 mg/mL), confirming a strong safety margin. Moreover, docking simulations indicated that oleamide contributes to antimicrobial action via varied bonding with selected protein targets. The recorded biological effects are likely due to the combined actions of the detected phytochemicals. Future investigations could prioritize purifying and identifying the key antimicrobial agents, along with conducting in vivo evaluations.

Acknowledgments: None

Conflict of Interest: None

Financial Support: None

Ethics Statement: None

References

1. Melo BADC, Vilar LG, Oliveira NRD, Lima POD, Pinheiro MDB, Domingueti CP, Pereira MC. Human papillomavirus Infection and oral squamous cell carcinoma-a systematic review. Braz J Otorhinolaryngol. 2021;87:346–52. doi: 10.1016/j.bjorl.2020.10.017
2. Džunková M, Martinez-Martinez D, Gardlík R, Behuliak M, Janšáková K, Jiménez N, Vázquez-Castellanos JF, Martí JM, D'Auria G, Bandara HMHN, Latorre A. Oxidative stress in the oral cavity is driven by individual-specific bacterial communities. NPJ Biofilms Microbio. 2018;4(1):1–10. doi: 10.1038/s41522-018-0072-3
3. Milho C, Silva J, Guimarães R, Ferreira IC, Barros L, Alves MJ. Antimicrobials from medicinal plants: an emergent strategy to control oral biofilms. Appl Sci. 2021;11(9):4020. doi: 10.3390/app11094020
4. Deo PN, Deshmukh R. Oral microbiome: unveiling the fundamentals. J Oral Maxillofac Pathol. 2019;23(1):122. doi: 10.4103/jomfp.JOMFP_304_18
5. Besra M, Kumar V. In vitro investigation of antimicrobial activities of ethnomedicinal plants against dental caries pathogens. 3 Biotech. 2018;8(5):1–8. doi: 10.1007/s13205-018-1283-2
6. dos Santos Letieri A, Siqueira WL, Solon-de-Mello M, Masterson D, Freitas-Fernandes LB, Valente AP, de Souza IPR, da Silva Fidalgo TK, Maia LC. A critical review on the association of hyposalivation and dental caries in children and adolescents. Arch Oral Biol. 2022;144:105545. doi: 10.1016/j.archoralbio.2022.105545
7. Sczepanik FSC, Grossi ML, Casati M, Goldberg M, Glogauer M, Fine N, Tenenbaum HC. Periodontitis is an inflammatory Disease of oxidative stress: we should treat it that way. Periodontology. 2000;84(1):45–68. doi: 10.1111/prd.12342
8. Picciolo G, Mannino F, Irrera N, Minutoli L, Altavilla D, Vaccaro M, Oteri G, Squadrito F, Pallio G. Reduction of oxidative stress blunts the NLRP3 inflammatory cascade in LPS stimulated human gingival fibroblasts and oral mucosal epithelial cells. Biomed Pharmacother. 2022;146:112525. doi: 10.1016/j.biopha.2021.112525
9. Sardaro N, Della Vella F, Incalza MA, Di Stasio D, Lucchese A, Contaldo M, Laudadio C, Petruzzi M. Oxidative stress and oral mucosal Diseases: an overview. In vivo. 2019;33(2):289–96. doi: 10.21873/invivo.11474
10. Ishii K, Hamamoto H, Imamura K, Adachi T, Shoji M, Nakayama K, Sekimizu K. 2010. Porphyromonas gingivalis peptidoglycans induce excessive activation of the innate immune system in silkworm larvae. J Biol Chem. 2010; 285(43): 33338–47.
11. González-Palma I, Escalona-Buendía HB, Ponce-Alquicira E, Téllez-Téllez M, Gupta VK, Díaz-Godínez G, Soriano-Santos J. Evaluation of the antioxidant activity of aqueous and methanol

extracts of Pleurotus ostreatus in different growth stages. Front Microbiol. 2016;7:1099. doi: 10.3389/fmicb.2016.01099
12. Galvão LC, Furletti VF, Bersan SM, da Cunha MG, Ruiz AL, Carvalho JE, Sartoratto A, Rehder VL, Figueira GM, Teixeira Duarte MC, et al. Antimicrobial activity of essential oils against Streptococcus mutans and their antiproliferative effects. Evid-Based Complement Altern Med. 2012;2012:751435. doi: 10.1155/2012/751435
13. Hassan M, Shafique F, Bhutta H, Haq K, Almansouri T, Asim N, Khan D, Butt S, Ali N, Akbar N. A comparative study to evaluate the effects of antibiotics, plant extracts and fluoride-based toothpaste on the oral pathogens isolated from patients with gum Diseases in Pakistan. Braz J Biol. 2021;83:e242703. doi: 10.1590/1519-6984.242703
14. Jain I, Jain P, Bisht D, Sharma A, Srivastava B, Gupta N. Use of traditional Indian plants in the inhibition of caries-causing bacteria-Streptococcus mutans. Braz Dent J. 2015;26:110–15.
15. Kanth MR, Prakash AR, Sreenath G, Reddy VS, Huldah S. Efficacy of specific plant products on microorganisms causing dental caries. J Clin Diagnostic Res. 2016;10(12):ZM01.
16. Kathiravan MK, Salake AB, Chothe AS, Dude PB, Watode RP, Mukta MS, Gadwe S. The biology and chemistry of antifungal agents: a review. Bioorg Med Chem. 2012;20:5678–98. doi: 10.1016/j.bmc.2012.04.045
17. Salehi B, Jornet PL, López EPF, Calina D, Sharifi-Rad M, Ramírez-Alarcón K, Forman K, Fernández M, Martorell M, Setzer WN, Martins N. Plant-derived bioactives in oral mucosal lesions: a key emphasis to curcumin, lycopene, chamomile, aloe vera, green tea and coffee properties. Biomolecules. 2019;9(3):106. doi: 10.3390/biom9030106
18. Tsilo PH, Maliehe ST, Shandu JS, Khan R. Chemical composition and some biological activities of the methanolic Encephalartos ferox fruit extract. Pharmacogn J. 2020;12(5):1190–97. doi: 10.5530/pj.2020.12.167
19. Dhatwalia J, Kumari A, Verma R, Upadhyay N, Guleria I, Lal S, Thakur S, Gudeta K, Kumar V, Chao JCJ, Sharma S. Phytochemistry, pharmacology, and nutraceutical profile of Carissa species: an updated review. Molecules. 2021;26(22):7010. doi: 10.3390/molecules26227010
20. Muleya E, Ahmed AS, Sipamla AM, Mtunzi FM. Free radical scavenging and antibacterial activity of crude extracts from selected plants of medicinal value used in Zululand. Pak J Nutr. 2014;13(1):38. doi: 10.3923/pjn.2014.38.42
21. Kaunda JS, Zhang YJ. The genus Carissa: an ethnopharmacological, phytochemical and pharmacological review. Nat Prod Bioprospect. 2017;7(2):18–99. doi: 10.1007/s13659-017-0123-0
22. Takaba K, Hirose M, Yoshida Y, Kimura J, Ito N, Shirai T. Effects of n-tritriacontane-16, 18-dione, curcumin, chlorophyllin, dihydroguaiaretic acid, tannic acid and phytic acid on the initiation stage in a rat multi-organ carcinogenesis model. Cancer Lett. 1997;113(1–2):39–46. doi: 10.1016/S0304-3835(96)04579-X
23. Mlala S, Oyedeji AO, Gondwe M, Oyedeji OO. Ursolic acid and its derivatives as bioactive agents. Molecules. 2019;24(15):2751. doi: 10.3390/molecules24152751
24. Gwatidzo L, Dzomba P, Mangena M. TLC separation and antioxidant activity of flavonoids from Carissa Bispinosa, Ficus sycomorus, and Grewia bicolar fruits. Nutrire. 2018;43(1):1–7. doi: 10.1186/s41110-018-0062-5
25. World Health Organization. WHO guidelines on good agricultural and collection practices [GACP] for medicinal plants. World Health Organization, 2003.
26. Tambe VD, Bhambar RS. Estimation of total phenol, tannin, alkaloid, and flavonoid in Hibiscus tiliaceus Linn. Wood extracts: Research and Reviews. J Pharmacogn Phytochem. 2014;2:41–7.
27. Mpai S, Mokganya LM, Raphoko L, Masoko P, Ndhlala AR. Untargeted metabolites and chemometric approach to elucidate the response of growth and yield attributes on different concentrations of an amino acid based biostimulant in two lettuce cultivars. Sci Hortic. 2022;306:111478. doi: 10.1016/j.scienta.2022.111478
28. Begue WJ, Kline RM. The use of tetrazolium salts in bioautographic procedures. J Chromatogr A. 1972;64:182–84. doi: 10.1016/S0021-9673(00)92965-0
29. Pfaller MA, Andes D, Diekema DJ, Espinel-Ingroff A, Sheehan D, CLSI Subcommittee for Antifungal Susceptibility Testing Wild-type MIC distributions, epidemiological cutoff values and species-specific

clinical breakpoints for fluconazole and Candida: time for harmonization of CLSI and EUCAST broth microdilution methods. Drug Resist Updat. 2010;13(6):180–95. doi: 10.1016/j.drup.2010.09.002
30. Eloff JN. A sensitive and quick microplate method to determine the minimal inhibitory concentration of plant extracts for bacteria. Planta Med. 1998;64:711–13. doi: 10.1055/s-2006-957563
31. Brand-Williams W, Cuvelier ME, Berset CLWT. Use of a free radical method to evaluate antioxidant activity. LWT - Food Sci Technol. 1995;28:25–30. doi: 10.1016/S0023-6438(95)80008-5
32. Oyaizu M. Studies on products of browning reaction: antioxidative activities of products of browning reaction prepared from glucosamine. Jpn J Nutr Diet. 1986;44:307–15. doi: 10.5264/eiyogakuzashi.44.307
33. Mosmann T. Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxicity assays. J Immunol Methods. 1983;65:55–63. doi: 10.1016/0022-1759(83)90303-4
34. Afriza D, Suriyah WH, Ichwan SJA. August. In silico analysis of molecular interactions between the anti-apoptotic proteinsurvivin and dentatin, nordentatin, and quercetin. J Phys Conf Ser. 2018; 1073(3): 032001.
35. Trott O, Olson AJ. Autodock Vina: improving the speed and accuracy of docking with a new scoring function, efficient optimization, and multithreading. J Comp Chem. 2010;31:455–61. doi: 10.1002/jcc.21334
36. Varsha KK, Devendra L, Shilpa G, Priya S, Pandey A, Nampoothiri KM. 2, 4-Di-tert-butyl phenol as the antifungal, antioxidant bioactive purified from a newly isolated Lactococcus Sp. Int J Food Microbiol. 2015;211:44–50. doi: 10.1016/j.ijfoodmicro.2015.06.025
37. Elmi A, Spina R, Risler A, Philippot S, Mérito A, Duval RE. Abdoul-Latif FM, aurain-Mattar D. evaluation of antioxidant and antibacterial activities, cytotoxicity of Acacia seyal Del Bark extracts and isolated compounds. Molecules. 2020;25:2392. doi: 10.3390/molecules25102392
38. Farha AK, Hatha AM. Bioprospecting potential and secondary Metabolite Profile of a Novel sediment-derived Fungus Penicillium sp. Arcspf from Continental Slope of Eastern Arabian Sea. Mycology. 2019;10(2):109–17. doi: 10.1080/21501203.2019.1572034
39. Mueller L, Boehm V. Antioxidant sctivity of β-carotene compounds in different In vitro sssays. Molecules. 2011;16:1055–69. doi: 10.3390/molecules16021055
40. Masoko P, Eloff JN. Screening of twenty-four South African Combretum and six Terminalia species (Combretaceae) for antioxidant activities. Afr J Tradit Complement Altern Med. 2007;4:231–39. doi: 10.4314/ajtcam.v4i2.31213
41. Bouhafsoun A, Boga M, Boukeloua A, Temel H, Kaid-Harche M. Determination of anticholinesterase and antioxidant activities of methanol and water extracts of leaves and fruits of Chamaerops humilis L. J Appl Nat Sci. 2019;11:144–48. doi: 10.31018/jans.v11i1.2001
42. Moure A, Cruz JM, Franco D, Domínguez JM, Sineiro J, Domínguez H, Núñez MJ, Parajó JC. Natural antioxidants from residual sources. Food Chem. 2001;72:145–71. doi: 10.1016/S0308-8146(00)00223-5
43. Tlili N, Elfalleh W, Hannachi H, Yahia Y, Khaldi A, Ferchichi A, Nasri N. Screening of natural antioxidants from selected medicinal plants. Int J Food Prop. 2013;16:1117–26. doi: 10.1080/10942912.2011.576360
44. Tohma H, Gülçin İ, Bursal E, Gören AC, Alwasel SH, Köksal E. Antioxidant activity and phenolic compounds of ginger (Zingiber officinale Rosc.) Determined by HPLC-MS/MS. J Food Meas Charact. 2017;11:556–66. doi: 10.1007/s11694-016-9423-z
45. Granato D, Shahidi F, Wrolstad R, Kilmartin P, Melton LD, Hidalgo FJ, Miyashitag K, Camph JV, Alasalvari C, Ismailj AB, Elmorek S, Birchk GG, Charalampopoulosk D, Astleyl SB, Peggm R, Zhoun P, Finglas P. Antioxidant activity, total phenolics and flavonoids contents: should we ban In vitro screening methods? Food Chem. 2018;264:471–75. doi: 10.1016/j.foodchem.2018.04.012
46. Rahman MJ, Ambigaipalan P, Shahidi F. Biological activities of Camelina and Sophia seeds phenolics: inhibition of LDL oxidation, DNA damage, and pancreatic lipase and α-glucosidase activities. J Food Sci. 2018;83:237–45. doi: 10.1111/1750-3841.14007
47. Gkotsis G, Nika MC, Athanasopoulou AI, Vasilatos K, Alygizakis N, Boschert M, Osterauer R, Höpker

KA, Thomaidis NS. Advanced throughput analytical strategies for the comprehensive HRMS screening of organic micropollutants in eggs of different bird species. Chemosphere. 2023;312:137092. doi: 10.1016/j.chemosphere.2022.137092

48. Yadav R, Rai R, Yadav A, Pahuja M, Solanki S, Yadav H. Evaluation of antibacterial activity of Achyranthes aspera extract against Streptococcus mutans: an In vitro study. J Adv Pharm Technol Res. 2016;7:149–52. doi: 10.4103/2231-4040.191426
49. Uche-Okereafor N, Sebola T, Tapfuma K, Mekuto L, Green E, Mavumengwana V. Antibacterial activities of crude secondary metabolite extracts from Pantoea species obtained from the stem of Solanum mauritianum and their effects on two Cancer cell lines. Int J Environ Res Public Health. 2019;16(4):602. doi: 10.3390/ijerph16040602
50. Ebrahimzadeh MA, Pourmorad F, Bekhradnia AR. Iron chelating activity, phenol and flavonoid content of some medicinal plants from Iran. Afr J Biotechnol. 2008;7:3188–92.
51. Ngidi LS, Nxumalo CI, Shandu JS, Maliehe TS, Rene K. Antioxidant, anti-quorum sensing and cytotoxic properties of the endophytic Pseudomonas aeruginosa CP043328. 1's extract. Pharmacog J. 2021;13(2):332–40. doi: 10.5530/pj.2021.13.43
52. López-García J, Lehocký M, Humpolíček P, Sáha P. HaCaT keratinocytes response on antimicrobial atelocollagen substrates: extent of cytotoxicity, cell viability and proliferation. J Funct Biomater. 2014;5:43–57. doi: 10.3390/jfb5020043
53. Jena AK, Karan M, Vasisht K. Plant parts substitution-based approach as a viable conservation strategy for medicinal plants: a case study of Premnalatifolia Roxb. J Ayurveda Integr Med. 2017;8:68–72.
54. Kitchen DB, Decornez H, Furr JR, Bajorath J. Docking and scoring in virtual screening for drug discovery: methods and applications. Nat Rev Drug Discov. 2004;3(11):935–49. doi: 10.1038/nrd1549
55. Weber SG, Gold HS, Hooper DC, Karchmer AW, Carmeli Y. Fluoroquinolones and the risk for methicillin-resistant Staphylococcus aureus in hospitalized patients. Emerg Infect Dis. 2003;9(11):1415–22. doi: 10.3201/eid0911.030284
56. Ye Z, Lu Y, Wu T. The impact of ATP-binding cassette transporters on metabolic Diseases. Nutr Metab. 2020;17(1):1–14. doi: 10.1186/s12986-020-00478-4
57. Maliehe TS, Selepe TN, Mthembu NN, Shandu JS. Antibacterial and anti-quorum sensing activities of Erianthemum dregeis Leaf Extract and Molecular Docking. Pharmacogn J. 2023;15(2):279–85. doi: 10.5530/pj.2023.15.39
58. Chakraborty C, Mallick B, Sharma AR, Sharma G, Jagga S, Doss CGP, Nam JS, Lee SS. Micro-environmental signature of the interactions between druggable target protein, dipeptidyl peptidase-IV, and anti-diabetic Drugs. Cell J. 2017;19(1):65. doi: 10.22074/cellj.2016.4865
59. Amer HH, Eldrehmy EH, Abdel-Hafez SM, Alghamdi YS, Hassan MY, Alotaibi SH. Antibacterial and molecular docking studies of newly synthesized nucleosides and Schiff bases derived from sulfadimidines. Sci Rep. 2021;11(1):17953. doi: 10.1038/s41598-021-97297-1
60. Oyedele AQK, Ogunlana AT, Boyenle ID, Adeyemi AO, Rita TO, Adelusi TI, Abdul-Hammed M, Elegbeleye OE, Odunitan TT. Docking covalent targets for drug discovery: stimulating the computer-aided drug design community of possible pitfalls and erroneous practices. Mol Divers. 2022:1–25.