




Submitted: 12/09/2025

Revised: 15/11/2025

Accepted: 29/11/2025

Published: 31/12/2025

Phytochemical profiles, chemical composition, molecular docking, and antibacterial activity of the *Opuntia* cladode from Al Zawia, Libya

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ABSTRACT

Background: *Opuntia* spp. are prickly pear cactus species that have long been used for medical purposes. Mature cladodes are also used to feed animals during droughts.

Aim: The phytochemical and chemical composition of cladode extracts from *Opuntia streptacantha* and thornless *Opuntia ficus indica* cultivated in Al-Zawia-Libya and to evaluate their antibacterial activity through both *in vitro* assay and molecular docking analysis.

Methods: Cladode extracts from two *Opuntia* species cultivated in Libya were analyzed in this study to compare their chemical composition and evaluate their antibacterial properties explained by structure-function relationships studies.

Results: The ethanolic extract of *O. streptacantha* registered the highest total polyphenolic content, total flavonoid content, and tannin contents (112.00 ± 0.58 mg GAE/g, 64.67 ± 0.88 mg CAT/g, and 0.408 ± 0.046 mg CAT/g, respectively). The ethanolic extract of thornless *O. ficus-indica* exhibited strong antibacterial activity against several bacterial strains, particularly colistin-resistant *Escherichia coli*. Rutin and luteolin-7-O-glucoside interact with key active-site residues, including the catalytic residues Thr285 and His395.

Conclusion: *Opuntia* spp. cultivated in Libya are a valuable source of biomolecules with significant antibacterial properties and may be exploited as a functional food.

Keywords: Phytochemical, *Opuntia* spp., Molecular docking, Antibacterial.

Introduction

Prickly pear cactus has long been used for medicinal purposes. The cladode of *Opuntia* spp. exhibits antibacterial activity due to its bioactive compound content (Maniaci *et al.*, 2024). Bacterial pathogens, such as *S. aureus* and *Escherichia coli*, are major causes of mastitis in dairy cows, leading to milk spoilage, reduced shelf life, and costly veterinary treatments. Cladodes of *Opuntia* contain bioactive substances (such as polyphenols and flavonoids) that possess antibacterial properties and can exhibit the growth of pathogenic bacteria. Cladodes can help prevent milk spoilage by reducing bacterial infections, thereby improving milk quality and yield (Keba *et al.*, 2020; Liu and Kong,

2021). Healthier cows produce more milk with superior compositional quality, which can result in higher market value, particularly for small-scale farmers without advanced refrigeration facilities (Kousta *et al.*, 2010; Barkema *et al.*, 2015). Cladodes can reduce infection rates and the need for medical interventions and antibiotics as a natural alternative. Exploration of new safe and natural bioactive compounds from plant extracts has become essential due to growing concerns about antibiotic resistance and drug safety (Oumato *et al.*, 2016). Cladodes are often considered agricultural waste, making them an inexpensive and sustainable resource (Auteri *et al.*, 2023). Mature cladodes are also used as animal feed during droughts, emphasizing

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their potential as a functional feed (Lopez-Garcia *et al.*, 2001).

Although *Opuntia* cladodes have long been used in traditional Mexican medicine to treat type 2 diabetes mellitus (T2DM), their development as a functional food ingredient for managing various health conditions is more recent (Kashif *et al.*, 2022). Acarbose, a microbial secondary metabolite, is one example of a compound used in treating T2DM.

Despite extensive research on the beneficial properties of *Opuntia* plants, limited data exist regarding the anti- α -amylase and antibacterial effects of ethanolic extracts from *Opuntia cladodes*, particularly *O. streptacantha* and thornless *O. ficus-indica* cultivated in Libya. Therefore, the present study aims to determine the phytochemical and chemical composition of ethanolic cladode extracts (ECEs) from these species and to evaluate their antibacterial and anti- α -amylase activities through in vitro and in silico analyses.

Materials and Methods

Plant collection

Mature and young cladodes (*O. streptacantha* and *O. ficus-indica*) were collected between March and April 2023 in Al-Zawia, Libya (32°36'29.3"N 12°43'06.2"E, 32°46'12.1"N 12°44'52.2"E) and transported to the Center for Medical Research in Zawia. The spines were stripped (or plucked) off, and the cladodes were cleaned using distilled water before being finely chopped. The pieces were oven-dried at 55°C for 2 days until fully dehydrated, and then ground into a fine powder using an electric grinder (ARSHIA, Flugel Company, Germany).

Extraction procedure

For each extraction, 100 g of cladode powder was soaked in 400 ml of absolute ethanol at room temperature for 1 day. The mixture was then filtered through the Whatman No. 2 filter paper to separate the solid residues. The ECE was concentrated using a rotary evaporator set to 55°C. The resulting solid extracts were then collected and kept at -20°C until they were required for analysis.

Polyphenol quantification

Total polyphenolic content (TPC)

The Folin-Ciocalteu assay, modified from Lee *et al.* (2002), was used to detect the total polyphenolic content. Briefly, the mixture was formed by adding 125 μ l of both the 0.2 N Folin-Ciocalteu reagent and the cactus extract and 500 μ l of distilled water. After 3 minutes, 3 ml of distilled water and 1,250 μ l of 7% sodium carbonate were added. The mixture was then incubated for 3 hours in the dark at standard laboratory temperature for 3 hours. Absorbance was quantified at 760 nm using a spectrophotometer. TPC was quantified as milligrams of gallic acid equivalent per gram of dry weight (mg GAE/g DW) based on a gallic acid standard curve (0–100 μ g/ml).

Total flavonoid content (TFC)

The aluminum chloride method was used to assess TFC (Palacios *et al.*, 2011). Briefly, the process involved mixing 75 μ l of the 5% NaNO₂ solution with 250 μ l of the extract. The resulting solution was then reacted for 6 minutes at a standard laboratory temperature. Then, 150 μ l of aluminum chloride solution was added and incubated for 5 minutes, followed by the addition of 500 μ l of 1 M sodium hydroxide. Distilled water was added until the total volume reached 2,500 μ l. Absorbance was calculated at 430 nm. TFC was expressed as milligrams of catechin equivalent per gram of dry weight (mg CAT/g DW), based on a catechin standard curve (0–100 μ g/ml).

Tannin contents

The method used to determine the tannin content was modified by Muhammad (2019). In brief, 1.5 ml of concentrated hydrochloric acid (12M) was mixed with 300 μ l of the extract and 3 ml of 4% vanillin in methanol. The mixture was incubated for 15 minutes, and the absorbance was measured at 500 nm. Tannin content was quantified as catechin equivalent (mg CAT/g DW) per gram of dry weight (mg CAT/g DW) based on a catechin standard curve (0–200 μ g/ml).

Liquid chromatography/electrospray ionization/mass spectroscopy (LC-ESI-MS) analysis

For ECE, Ayaz *et al.* (2005) used a protocol to detect phenolic acid and flavonoids. In brief, 0.5 g of powder was mixed with 10 ml of absolute ethanol and agitated for 24 hours at room temperature for 24 hours. The samples were centrifuged (4,000 rpm, 25 minutes) and filtered through a 0.45 μ m Millipore membrane. Aliquots (5 μ l) were injected into the Liquid Chromatography-Mass Spectrometry system. Separation and detection were performed according to the protocol of Zourgui *et al.* (2020).

Biological activities

Microbial strains and growth conditions

Five bacterial strains were used to assess antibacterial activity. Gram-positive bacteria were *Bacillus cereus* (BC7) and *S. aureus* (SA121), and Gram-negative bacteria were *Klebsiella pneumoniae* (Kp243), *E. coli* (EC321 colistin-resistant), and *E. coli* (EC47 colistin-sensitive). These isolates were obtained from the Food-borne Libyan type Bacterial Collection at the Department of Food Hygiene, Faculty of Veterinary Medicine, University of Tripoli, Libya. They were identified using standard microbiological techniques and confirmed by 16s mRNA sequencing (Garbaj *et al.*, 2016; Nass *et al.*, 2018; Nass *et al.*, 2019; Azwai *et al.*, 2024). Bacteria were cultured on MHA plates and incubated for 1 day at 37°C. Final inoculum concentrations of 10⁶ CFU/ml were used for Minimum inhibitory concentration and Minimum Bactericidal Concentration determination (Hsouna *et al.*, 2011).

The agar well diffusion method

The agar well diffusion assay (following the procedure of Hsouna *et al.*, 2011) was used to test the antibacterial

properties. A fresh bacterial suspension (106 CFU/ml) was inoculated onto an MHA plate using sterile swabs. After drying, 6 mm diameter wells were made using a sterile pasteur pipette, and 50 µl of each extract (150 mg/ml 10% DMSO) was added. DMSO (10%) was used as a negative control. First, the plates were preincubated at 4°C for 2 hours to facilitate diffusion and then incubated at 37°C for 24 hours. The inhibition zone diameter was measured to determine antibacterial activity.

Microdilution method for determining the MIC and MBC values

MIC values were determined using a microdilution assay for strains showing inhibition zones (Emad, 2012). Stock solutions of each extract (150 mg/ml in 10% DMSO) were serially diluted to obtain concentrations ranging from 1.17 to 150 mg/ml. Each well contained 100 µl of extract, 90 µl of Mueller–Hinton broth, and 10 µl of bacterial suspension (10⁶ CFU/ml). The positive controls consisted of the bacterial suspension, whereas the negative controls contained 10% DMSO without extract.

25 µl of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (0.5 mg/ml) was added to each well after incubation at 37°C for 24 hours to assess bacterial growth. No visible growth was observed in MIC at the lowest concentration. The MBC was determined as the lowest concentration showing no bacterial growth after subculturing on agar plates for 48 hours at 37°C (Daoud et al., 2015).

Statistical analysis

Data are expressed as mean ± SEM ($n = 3$). Statistical analyses were performed using the Statistical Package for the Social Sciences version 20. One-way analysis of variance followed by Duncan's and t-tests were used to determine significant differences ($\alpha \leq 0.05$).

Ethical approval

Not needed for this study.

Results

Phytochemical studies: extraction yield and total phenol, flavonoid, and tannin contents

Table 1 summarizes the extraction yields, TPC, TFC, and the tannin contents of ethanolic extracts for two species.

The total phenolic content of ethanolic extracts was expressed as GAE/g (mg), whereas the total flavonoid and tannin contents were expressed as CAT/g (mg).

The ECE of *O. streptacantha* extracts showed the highest phenolic acid, flavonoid, and tannin contents.

The obtained results indicate that the ECEs of Os and OF10 were not significantly different ($p \leq 0.05$) in terms of total phenolic content at a concentration of 112.00 mg GAE/g and 98.33 mg CAT/g. The flavonoid contents ranged from 51.67 mg CAT/g in the ECE of OF10 to 64.67 mg CAT/g in the ECE of Os, with no significant difference observed ($P \leq 0.05$). Tannin content ranged from 0.093 mg CAT/g in the ECE of OF10 to 0.408 mg CAT/g in the ECE of Os.

LC-ESI-MS analysis of *O. streptacantha* and thorn-less *O. ficus indica*

LC–ESI–MS analysis identified phenolic acids and flavonoids in the ethanolic extracts of *O. streptacantha* and thorn-less *O. ficus-indica* (Table 2). A total of 29 compounds were identified, including 8 phenolic acid compounds and 21 flavonoid molecules. Under ESI conditions, all analytes exhibited higher sensitivity in the negative mode. Among these phenolic acids, p-coumaric acid was the most abundant compound in the ethanolic extract, with the highest concentration in OF10 compared to Os (540.82 ppm and 5.1 ppm, respectively), followed by gallic acid with a concentration of 186.25 ppm registered in OF10, which was undetectable in Os. In terms of flavonoid contents, rutin exhibited the highest concentration in the ECE of OF10 (2,251.85 ppm) and was undetectable in the ECE of Os, followed by quercetin-3-o-rhamonosid (225.61 ppm), which was also undetectable in the ECE of Os. Apigenin was not detected in the ECE of Os.

Biological activities

MIC and MBC antibacterial activities of *O. cladode* extracts

The antibacterial activity of the *O. cladodes* extracts was assessed using the agar diffusion method. The microorganisms examined in this study are among the most significant human and animal pathogens known to cause contamination, food spoilage, and foodborne diseases (Eshamah et al., 2020).

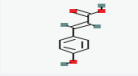
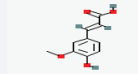
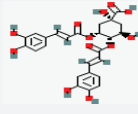
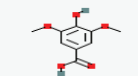
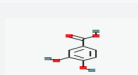
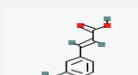
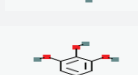
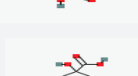
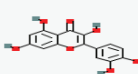


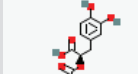
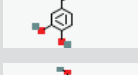
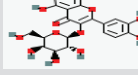
The inhibition zones ranged from 8.67 to 20.67 mm (Table 3). Significant differences in inhibition zones were observed between the ECEs ($p \leq 0.05$). Both extracts exhibited similar antibacterial activity against *S. aureus* (SA121) and *B. cereus* (BC7), with inhibition zones of 16.33 ± 2.85 mm and 10.00 ± 1.00 mm for *O. streptacantha*, and 20.67 ± 0.67 mm and 17.00 ± 1.53 mm for thorn-less *O. ficus-indica*, respectively.

Table 1. TPC, TFC, and tannin content extracted yields in ECE.

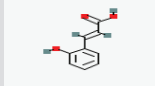
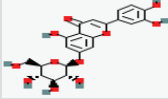
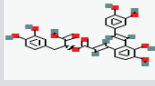
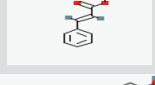
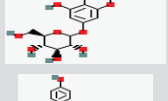
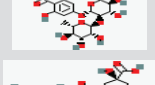
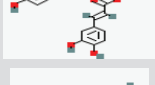
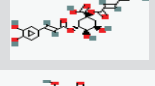

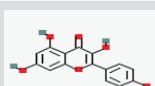
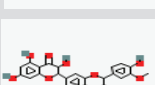
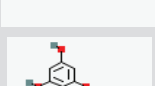
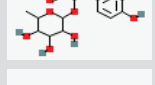
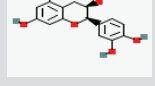
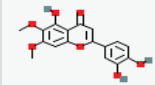
Type of extract	Yields %	TPC (mg GAE/g) *	TFC (mg CAT/g) **	Tannins content (mg CAT/g) **
ECE Os	2.51 ± 0.15	112.00 ± 0.58	64.67 ± 0.88	0.408 ± 0.046
ECE OF10	2.18 ± 0.28	98.33 ± 0.33	51.67 ± 0.33	0.093 ± 0.015

The data are expressed as mean ± SEM ($n = 3$); *Data in the same column with the different letters are significantly different at $p < 0.05$. ECE: ethanol cladode extract; ND: not determined; Os: *Opuntia streptacantha*; OF10: thorn-less *Opuntia ficus indica*.

Table 2. Chemical composition of ECEs for two types of Opuntia from Libya.

Group of the compound	ID	Name compounds	Chemical formula	Retention time	Chemical structure	Os (ppm)	OFI0 (ppm)
Phenolic acids	1	p-coumaric acid	C ₉ H ₈ O ₃	20..85		5.1	540.82
	2	Trans-Ferulic acid	C ₁₀ H ₁₀ O ₄	22.80		12.6	36.51
	3	3,4-di-O-caffeoyquinic acid	C ₂₅ H ₂₄ O ₁₂	16.55		0.61	38.54
	4	Syringic acid	C ₉ H ₁₀ O ₅	15.65		0.14	142.36 2
	5	Protocatchuic acid	C ₇ H ₆ O ₄	6.87		3.5	0.147
	6	Caffieac acid	C ₉ H ₈ O ₄	14.27		0.01	19.64
	7	Gallic acid	C ₇ H ₆ O ₅	4.033		N.D.	186.25
	8	Quinic acid	C ₇ H ₁₂ O ₆	2.07		12.5	2.84
Flavonoids	9	Quercetin	C ₁₅ H ₁₀ O ₇	33.49		4.25	N.D
	10	Apigenin	C ₁₅ H ₁₀ O ₅	34.11		N.D	78.725
	11	Hyperoside	C ₂₁ H ₂₀ O ₁₂	35.11		12.5	N.D.
	12	Rosmarinic acid	C ₁₈ H ₁₆ O ₈	26.858		N.D.	55.47
	13	Hyperoside (quercetin-3-o-galactoside)	C ₂₁ H ₂₀ O ₁₂	N.D.		N.D.	91.5
	14	Rutin	C ₂₇ H ₃₀ O ₁₆	N.D.		N.D.	2251.85

Continued

Group of the compound	ID	Name compounds	Chemical formula	Retention time	Chemical structure	Os (ppm)	OFI0 (ppm)
Flavonoids	15	o-coumaric acid	C ₉ H ₈ O ₃	N.D.		N.D.	33.84
	16	Luteolin-7-o-glucoside	C ₂₁ H ₂₀ O ₁₁	25.029		N.D.	8.12
	17	Salvialonic acid	C ₂₆ H ₂₂ O ₁₀	27.72		0.14	N.D.
	18	Cinnamic acid	C ₉ H ₈ O ₂	31.72		12.41	85.21
	19	Apegenin-7-O-glucoside	C ₂₁ H ₂₀ O ₁₀	26.19		0.66	61.87
	20	Naringin	C ₂₇ H ₃₂ O ₁₄	25.60		2.36	N.D.
	21	4,5-di-O-caffeoyquinic acid	C ₂₅ H ₂₄ O ₁₂	N.D.		N.D.	10.58
	22	1,3-di-O-caffeoyquinic acid	C ₂₅ H ₂₄ O ₁₂	26.56		0.12	N.D.
	23	Cirsilineol	C ₁₈ H ₁₆ O ₇	38.10		0.52	8.125
	24	Acacetin	C ₁₆ H ₁₂ O ₅	39.66		0.14	N.D.
	25	Kampherol	C ₁₅ H ₁₀ O ₆	32.413		N.D.	25.684
	26	Silymarin	C ₂₅ H ₂₂ O ₁₀	N.D.		N.D.	12.78
	27	Quercetrin (quercetin-3-o-rhamnosid)	C ₂₁ H ₂₀ O ₁₁	N.D.		N.D.	225.61
	28	Epicatechin	C ₁₅ H ₁₄ O ₆	N.D.		N.D.	9.54
29	Cirsiliol	C ₁₇ H ₁₄ O ₇	35.881		N.D.	14.902	

These results were comparable to the positive control (chloramphenicol: 18.33 ± 3.67 mm and 21.67 ± 0.67 mm, respectively).

For gram-negative bacteria (*K. pneumonia*), the ECE of *O. streptacantha* produced an inhibition zone of 8.67 ± 1.76 mm, whereas the extract of thornless *O. ficus-indica* produced a markedly higher activity (16.01 ± 1.00 mm). However, both antibiotics were less effective than the reference antibiotic (28.33 ± 0.88 mm). In contrast, the ECE of *O. streptacantha* showed a strong antibacterial effect on *E. coli*, with an inhibition zone of 19.33 ± 0.67 mm, which was slightly higher than that of chloramphenicol (18.00 ± 0.00 mm) and greater than that of *O. ficus-indica* (14.67 ± 0.33 mm).

Additionally, the MIC and MBC values were determined to further evaluate the antimicrobial potential of the extracts (Table 3). The ECE of *O. streptacantha* exhibited the lowest MIC (43.73 mg/mL) against *S. aureus* (SA121), *K. pneumonia* (Kp243), and *E. coli* (EC47 colistin resistant). Similarly, the ECE exhibited the lowest MIC value (43.73 mg/ml) against *B. cereus* (BC7) and *E. coli* (EC321 colistin sensitive).

Structure-function relationship study of MCR-1

In this study, a molecular docking approach was employed to investigate how the compounds identified in thorn-less *O. ficus-indica* contribute to antibacterial activity against colistin-resistant bacteria, using eugenol and tetrandrine as reference inhibitors.

Mutations in chromosomal genes, such as pmrAB, phoPQ, and mgrB, have been linked to resistance to colistin (Luo *et al.*, 2017; Abiola *et al.*, 2014). The MCR-1 enzyme (pdb code: 5GRR) contains two distinct domains. The first is an N-terminal transmembrane domain with five putative α -helices, while the second is a C-terminal $\alpha/\beta/\alpha$ sandwich domain that faces the periplasmic space, which contains the active site and mobile loop regions (Son *et al.*, 2019). The MCR-1 active site is organized around two zinc ions (Zn1 and Zn2) coordinated by conserved residues (Glu246, Thr285, His395, Asp465, His466, and His478), which are essential for PEA-transferase activity and colistin resistance. The catalytic residues in the active site of MCR-1 include Thr285, which acts as a nucleophile; Glu246, which activates Thr285 and protonates the leaving group; and His395, which protonates the dephosphorylated lipid A (Fig. 1).

The docking analysis of the compounds identified in the ethanolic extract of thornless *O. ficus indica* was compared with the docking profile of two standard inhibitors (eugenol and tetrandrine) reported in a previous study as MRC-1 inhibitors (Wang *et al.*, 2018; Yi *et al.*, 2022). These two molecules were docked using the same parameters as the docking of our compounds. The results showed that the binding led to the affinity of the corresponding compounds in the active sites of MRC-1. The binding energies of the identified compounds in (OF10), eugenol, and tetrandrine are presented in Table 4.

In summary, the binding affinities of the identified compounds in thorn-less *O. ficus-indica* (ranged from -6.4 to -8.0 kcal/mol) were comparable to those of the reference compounds eugenol and tetrandrine (binding energy -6.2 and -7.3 kcal/mol, respectively). To further analyze these results, the binding interactions of all the identified compounds in thornless *O. ficus-indica* against MRC-1 were compared. The analysis highlighted two compounds with particularly interesting interaction profiles: rutin and luteolin-7-O-glucoside (Fig. 2). Rutin and Luteolin-7-o-glucoside were anchored in the active site of MRC-1 through electrostatic, hydrophobic, and hydrogen interactions. These compounds also interact with the catalytic residue Thr285. Specifically, rutin forms three hydrogen bonds with Thr285 (catalytic amino acid) and two additional hydrogen bonds with Lys 333 and Gly 396. Two hydrophobic bonds Pi-Pi were found with Ala 286, Met 392, and His 395 (catalytic residue). Carbone hydrogen bonds with His 395 (catalytic residue) and Pro 397 were observed. One pi-donner hydrogen bond was established with Ser 330. On the other hand, the luteolin-7-o-glucoside MRC1 complex is stabilized by 4 hydrogens interaction with Lys 348, Arg 402, Ser 330, and Thr 285 (catalytic residue). The electrostatic bond-type pi-anion was formed by Glu 363. Pi-sigma hydrophobic interactions were observed with Met 392, and carbon-hydrogen bonds were observed with His 395.

Discussion

Phenolic compounds are small molecules characterized by the presence of one or more phenol groups in their chemical structure. This group of compounds can be divided into smaller groups, including flavonoids, tannins, and phenolic acids, which are found in plant extracts (Ponnusamy *et al.*, 2011; Čavar Zeljković *et al.*, 2021). Plants produce a wide variety of polyphenols and many other compounds that are used to protect against various pathogens and UV radiation. Numerous factors, including physiological-biochemical, molecular-genetic, and environmental factors, influence polyphenol biosynthesis and accumulation in plants. The solubility of these compounds also depends on the type of solvent used (Ksouri *et al.*, 2008; Del Socorro Sánchez Correa *et al.*, 2023).

The ECEs of Os and OF10, collected from Libya, exhibited high polyphenol levels (112 and 98.33 mg GAE/g, respectively), and prior research on *O. ficus-indica* fruit extract also documented its total phenolic content, which showed a value of 59.48 GAE/100 g dry weight (Medina-Pérez *et al.*, 2019).

The flavonoid concentrations of the examined samples were higher than those reported by Dib *et al.* (2013) (11.86 mg CAT/g of DW) (Dib *et al.*, 2013). The total tannin contents of the two ECEs of Os and OF10 were 0.093 and 0.408 mg CAT/g, respectively. The tannin contents in Os extracts were significantly higher than

Table 3. Antibacterial activities MIC and MBC for *Opuntia* species cladode extracts.

Bacteria strains	Inhibition zone diameters (mm)		Concentration (mg/ml)	
	Extract	Chloramphenicol	MIC	MBC
ECE Os				
<i>K. pneumonia</i>	8.67 ^a ± 1.76	28.33 ^c ± 0.88	43.73	ND
<i>Bacillus cereus</i>	16.33 ^a ± 2.85	21.67 ^a ± 1.67	56.25	ND
<i>Staph. Aureus</i>	10.00 ^a ± 1.00	18.33 ^b ± 3.76	43.73	ND
<i>E.coli resistant to colistin</i>	-----	18.00 ^b ± 0.58	43.73	ND
<i>E.coli sensitive to colistin</i>	19.33 ^b ± 0.67	18.00 ^b ± 0.00	56.25	ND
ECE OF10				
<i>K. pneumonia</i>	16.00 ^b ± 1.00	28.33 ^c ± 0.88	56.25	ND
<i>Bacillus cereus</i>	20.67 ^a ± 0.67	21.67 ^a ± 1.67	43.73	ND
<i>Staph. Aureus</i>	17.00 ^{ab} ± 1.53	18.33 ^b ± 3.76	56.25	ND
<i>E.coli resistant to colistin</i>	12.33 ^a ± 0.33	18.00 ^b ± 0.58	43.73	ND
<i>E.coli sensitive to colistin</i>	14.67 ^a ± 0.33	18.00 ^b ± 0.00	56.25	ND

The data are expressed as mean ± SEM ($n = 3$); ^{a-c}Data in the same column with the different letters are significantly different at $p < 0.05$. ECE: ethanol cladode extract; ND: not determined; Os: *Opuntia streptacantha*; OF10: thorn-less *Opuntia ficus indica*.

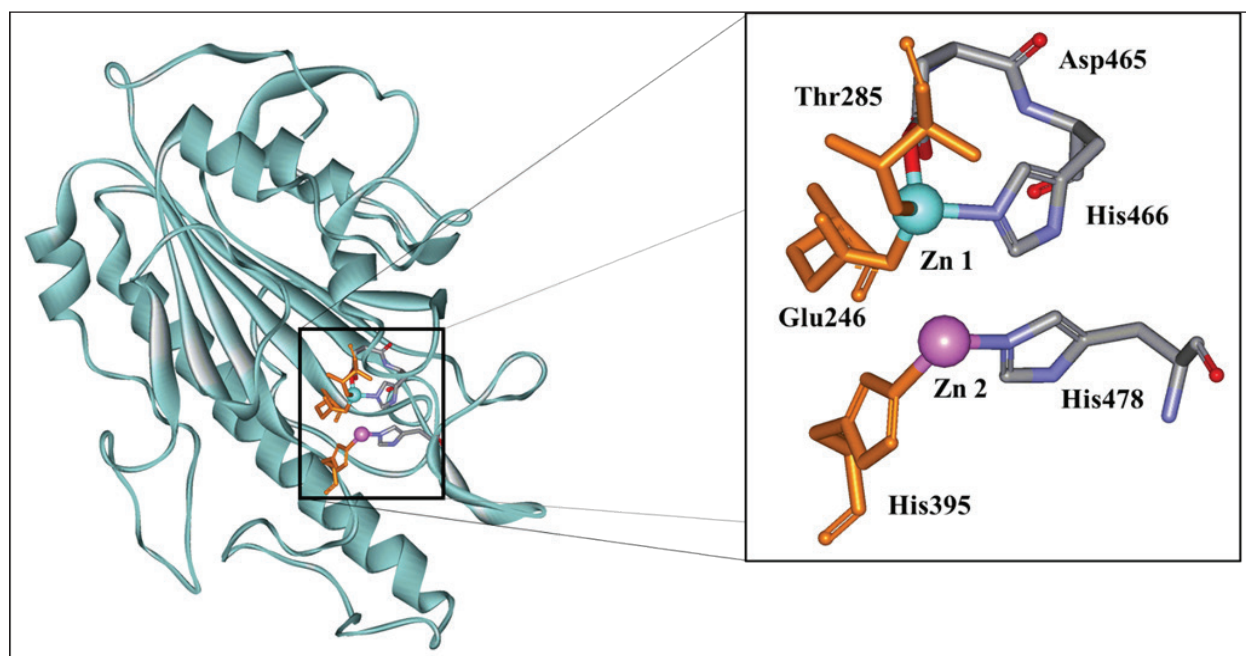


Fig. 1. MRC1 3D structure (pdb code: 5GRR) and Zn 1 and Zn 2 binding residues (catalytic residues shown in orange).

those in the OF10 ECE. These findings are similar to the results found by Haile *et al.* (2016) in the same species grown in Ethiopia. In fact, the flavonoid and tannin contents from the ECE showed the same pattern, with the Os extract having the highest contents and the OF10 extract having the lowest contents. The tannin content was the lowest, with a value of 1 mg CAT/g. LCMS/MS analysis for phenolic and flavonoid compounds in the ethanolic extracts for Os and OF10 showed 29 compounds. The 29 compounds in the

ethanolic extracts included 8 phenolic acids and 21 flavonoids. The concentrations of the identified compounds ranged from 0.01 to 2251.85 ppm. Caffeic acid had the lowest concentration (0.01 ppm) in the ECE of Os. Rutin in the ECE of OF10 had the highest concentration (2251.85 ppm), as reported previously (Rosiak *et al.*, 2023; Wang *et al.*, 2023). Regarding the flavonoid compounds present in the ECE of Os, hyperoside was the most abundant component in the extracts, with a concentration of 12.5 ppm. Similar

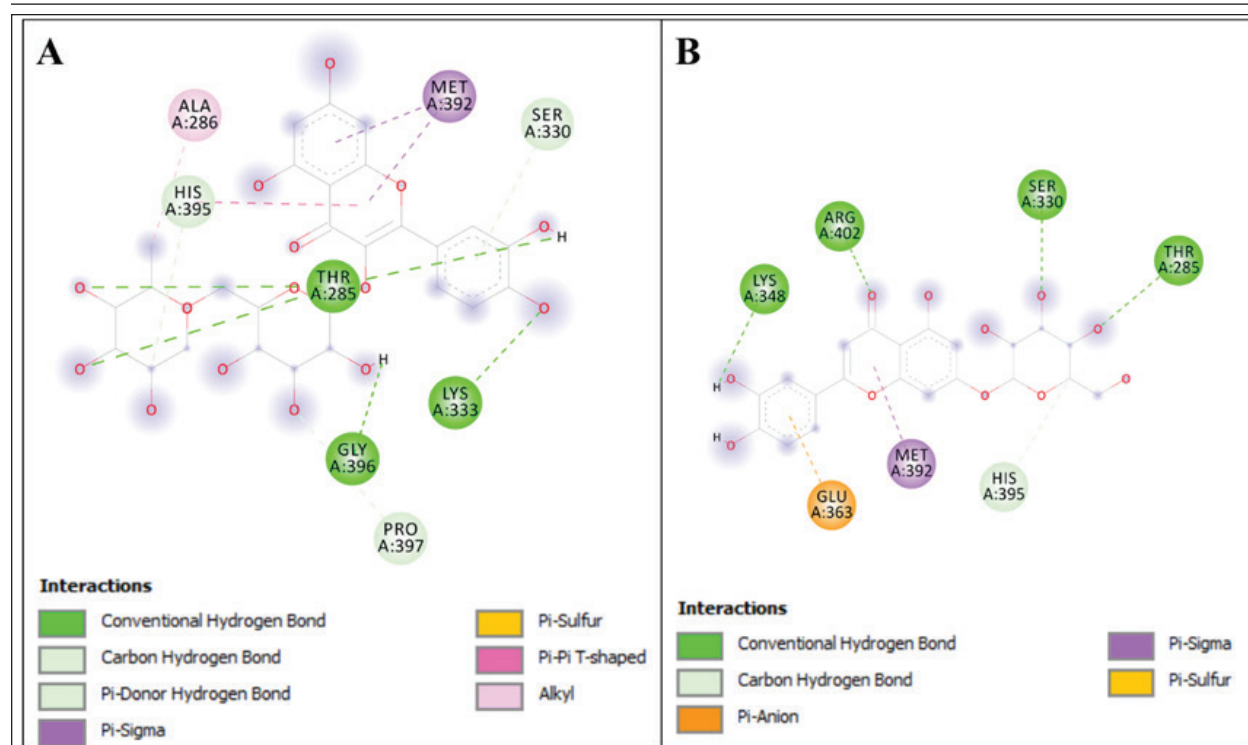


Fig. 2. Interaction profile of rutin (A) and luteolin-7-o-glucoside (B) against MRC1 (pdb code: 5GRR).

Table 4. The binding energies of specific compound from the ECE of OF10, Eugenol and Tetrandrine with MRC1.

Compound	Energy binding MRC1
Gallic acid	-6.7
Epicatechin	-6.4
Hyperoside (quercetin-3-o-galactoside)	-6.8
Rutin	-6.9
o-coumaric acid	-7.3
Luteolin-7-o-glucoside	-7.3
Kampherol	-6.8
Silymarin	-8.0
Quercetrin (quercetin-3-o-rhamonosid)	-7.0
Rosmarinic acid	-7.0
Cirsiliol	-7.1
Eugenol	-6.2
Tetrandrine	-7.3

results were achieved by Dib *et al.* (2013), Boutakiout *et al.* (2018), and Wit *et al.* (2013). On the other hand, the OF10 extract showed different results, where rutin was the most abundant flavonoid compound, with a concentration of 2251.85 ppm. These results agree with those of Belhadj Slimen and his group in 2019,

who found that rutin had the highest concentration in the ECEs (31.927 mg/kg) (Belhadj Slimen *et al.*, 2019). Transferulic acid was the main phenolic acid identified in the ECE of Os, while p-coumaric acid was the predominant phenolic acid in the ethanolic extract of OF10. The differences in the phenolic and flavonoid contents and concentrations between the two species agree with the results reported by De Santiago *et al.* (2018a, b). They stated that differences in environmental conditions, the original, maturity stage, harvesting season, species type, and post-harvest treatments influence the detection and amounts of these compounds (Park *et al.*, 2004; Djeridane *et al.*, 2006). In the investigation of antibacterial activity, *Opuntia species* extracts from Libya showed efficacy against gram-positive and gram-negative germs. The antibacterial activity of *Opuntia* extracts may be attributed to their high polyphenol content, particularly trans ferulic acid, which has been studied for its bacteriostatic activity against *E. coli*, *Bacillus subtilis*, and *S. aureus*. Ferulic acid also inhibited *S. aureus* and *E. coli* (Bhattacharya *et al.*, 2016; Pinheiro *et al.*, 2021). Another previous study on the antibacterial activity of ethanolic extracts of *O. stricta* cladodes (Affi *et al.*, 2021) reported an inhibition zone against gram-positive and gram-negative bacteria. Similar results were reported for the ethanolic extract of *O. ficus indica* L. mill cladode, the inhibition zone, which produces against gram-positive (*S. aureus*) and gram-negative bacteria (*E. coli*) (Blando *et al.*, 2019).

Furthermore, the ECE of OF10 exhibited antibacterial activity against *E. coli* colistin resistance. Molecular docking is a powerful computational approach for predicting the binding configurations and affinities of potential drug candidates. (Elkady *et al.*, 2022). The molecular docking approach was used in our investigation to provide deeper insights into the antibacterial potential of the compounds identified exclusively in the ethanolic cladodes extract of OF10. These compounds were docked as ligands into key enzymes implicated in colistin resistance mechanisms. To the best of our knowledge, recent studies have reported the synergistic effect of eugenol (Wang *et al.*, 2018) and tetrandrine (Yi *et al.*, 2022) with colistin against clinically isolated colistin-resistant *E. coli* strains. Interestingly, the docking result shows that eugenol and tetrandrine interact with several active site amino acids but not with the catalytic residue, whereas Rutin and Luteoline-7-o-glucoside interact with the catalytic residues Trp 286 and His 395. The results of this study also agreed with those of Selma, W. B and colleagues tested the ability of essential oil from Tunisian *Thymus algeriensis* to kill gram-negative bacterial isolates that had previously shown resistance to colistin in vitro. Additionally, they confirmed the enhanced effect (synergism) achieved when this essential oil and colistin were administered together against the colistin-resistant *E. coli* strains carrying the *mcr-1* gene. Furthermore, a docking study showed that carvacrol exhibited high binding free energies against MCR-1, a phosphoethanolamine transferase catalytic domain (PDB ID: 5GRR). These findings allowed them to confirm the therapeutic strategy to use carvacrol alone or as an antibiotic adjuvant to colistin against gram-negative bacteria resistant to colistin (Ben Selma *et al.*, 2024). Similarly, our findings support the potential use of rutin and luteolin-7-o-glucoside alone or synergistically with colistin as an effective strategy against gram-negative bacteria exhibiting resistance to colistin. This suggestion is supported by Yi *et al.* (2024), who found that rutin is effective against multidrug-resistant *E. coli* (Yi *et al.*, 2024). Wu *et al.* (2023) also found that Luteoline-7-o-glucoside is effective against drug-resistant intestinal microbiota, including *E. coli* (Wu *et al.*, 2023).

Conclusion

The study's findings demonstrated that the *O. streptacantha* extract contains higher levels of polyphenols than the thornless *O. ficus indica* extract. Both extracts exhibited antibacterial activity against gram-positive and gram-negative bacteria; however, only the ECE of thornless *O. ficus indica* showed antibacterial activity against *E. coli* resistant to colistin. The structure-function relationship analysis showed that the bioactive compounds responsible for this antibacterial activity against colistin-resistant *E. coli* are rutin and luteolin-7-o-glucoside. These findings

highlight the potential role of *Opuntia species* as valuable natural resources for treating some bacterial infections and other animal health issues. Nevertheless, further in-depth studies on the isolation and individual characterization of the active compounds are required to support their potential application in the development of agrifood and/or pharmaceutical preservatives.

Acknowledgments

The authors would like to thank the Tunisian Ministry of Higher Education and Scientific Research for their support in this project. The authors would also like to thank the Research Laboratory BMA "Biodiversity, Molecules, Application" at the Higher Institute of Applied Biology of Medenine, University of Gabes (Tunisia) for their involvement in supporting this project. The authors would also like to thank the Faculty of Veterinary Medicine at the University of Tripoli (Libya), the National Medical Research Center, Zawia (Libya), and the Laboratory of Plant Biotechnology at the Faculty of Sciences, University of Sfax, Tunisia for their support.

Conflict of interest

The authors have no conflicts of interest to disclose.

Funding

This study received no external funding.

Authors' contributions

B.K. drafted the manuscript. LZ, SMA, and AAM revised and edited the first draft of the manuscript. TA, SMA, AMG, and IME participated in preparing and critically checking this manuscript. ID, AAM, FTG, and KA performed the methodology. All authors have read, revised, and approved the published version of the manuscript.

Data availability

Data are available upon request from the corresponding author.

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