Open Veterinary Journal, (2024), Vol. 14(3): 769-778
ISSN: 2226-4485 (Print)
ISSN: 2218-6050 (Online)

Original Research
DOI: *10.5455/OVJ.2024.v14.i3.3*

Submitted: 05/08/2023 Accepted: 25/02/2024 Published: 31/03/2024

Antibacterial activity of flavonoid extracts from *Enteromorpha intestinalis* and *Caulerpa prolifera* against multidrug-resistant foodborne bacterial isolates

Rabia O. Alghazeer[1]*, Salah M. Azwai[2], Abdalla A. Elmansori[3], Khalid M. Alzintani[4], Sana Elghmasi[5], Asma Y. Alnajjar[6], Ftaim T. Gammoudi[2], Maamar Sidati[7], Samia T. Hawisa[8], Aboubaker M. Garbaj[9] and Ibrahim M. Eldaghayes[2]

[1]*Biochemistry Division, Chemistry Department, Faculty of Sciences, University of Tripoli, Tripoli, Libya*
[2]*Department of Microbiology and Parasitology, Faculty of Veterinary Medicine, University of Tripoli, Tripoli, Libya*
[3]*Botany Department, Faculty of Sciences, University of Tripoli, Tripoli, Libya*
[4]*Department of Botany, Faculty of Science, Al Zintan University, Zintan, Libya*
[5]*Department of Biochemistry, Faculty of Medicine, University of Tripoli, Tripoli, Libya*
[6]*Department of Soil and Water, Faculty of Agriculture, University of Tripoli, Tripoli, Libya*
[7]*Biotechnology Lab, Marin Biology Research Center, Tajura-East of Tripoli, Tripoli, Libya*
[8]*Department of Periodontology, Dental College, University of Tripoli, Tripoli, Libya*
[9]*Department of Food Hygiene, Faculty of Veterinary Medicine, University of Tripoli, Tripoli, Libya*

Abstract

Background: Food poisoning caused by bacterial agents is a worldwide problem, usually accompanied by unpleasant symptoms and may be severe leading to death. Natural compounds from marine algae namely flavonoids may play a role in the remedy of this condition.

Aim: This research aims to assess the potency of flavonoids extracted from *Enteromorpha intestinalis* and *Caulerpa prolifera* as antibacterial agents.

Methods: *Enteromorpha intestinalis* was collected from Western Libyan Coast and *C. prolifera* was collected from Farwa Island. The antimicrobial activity and determination of minimum inhibitory concentration of algal flavonoid-containing extracts was performed *in vitro* against some positive and negative Gram bacteria.

Results: Crude extract containing flavonoids from *E. intestinalis* was more effective than *C. prolifera* extract against *Staphylococcus aureus* with antimicrobial essay (25–28 + 1 and 14.5–37.5 + 0.5–1.5), MIC (50 and 50–250 µg/ml), MBC (75 and 75–250 µg/ml). In *Bacillus cereus,* the antimicrobial assay (19–24.5 + 0.5–1.5: 24 + 1), MIC (50–250 + 100 µg/ml), and MBC (250 and 125 µg/ml). On the other hand, flavonoids containing extract from *C. prolifera* were more effective than *E. intestinalis* against Enterohemorrhagic *Escherichia coli* O157 EHEC O157 (25–28 + 1: 14–18.5 + 0.5–1.5), MIC (100–250:100–500 µg/ml), and MBC (150–250 and 250–500 µg/ml). *Salmonella enterica* qualitatively combat by flavonoid from *E. intestinalis* (13.5–14 + 0.5–1: 10.5–13.5 + 0.5–1.5), MIC (100–250: 250 µg/ml), and MBC (100–250: 250 µg/ml). Flavonoids from *C. prolifera* (4 strains: 2 strains) were effective against *S. enterica.* Crude flavonoids from both algae were not effective against *Bacillus pumilus.*

Conclusion: Data from this study could conclude that flavonoid extracts from *E. intestinalis and C. prolifera* could be used against foodborne bacterial agents.

Keywords*: Enteromorpha intestinalis*, *Caulerpa prolifera,* Flavonoid extracts, Antibacterial activity, Foodborne pathogens.

Introduction

Foodborne illnesses are a global leading concern for human health, they are widespread and usually affect the digestive system and are manifested by mild symptoms including nausea, vomiting, or diarrhea; however, they may give rise to severe, invasive infections leading to death (Addis and Sisay, 2015; Pires *et al.*, 2015). Foodborne illness is due to the intake of food and water contaminated by different infectious microorganisms, as well as poisons or toxins, and caused by different infectious organisms including bacteria, viruses, and parasites or their toxins (Pires *et al.*, 2015). Chemicals

***Corresponding Author:** Rabia O. Alghazeer. Biochemistry Division, Chemistry Department, Faculty of Sciences, University of Tripoli, Tripoli, Libya. Email: *R.alghazeer@uot.edu.ly*

used in agriculture such as pesticides, plant growth regulators, and other contaminants such as copper, arsenic, and mercury, several lubricants, and sanitizing agents can foodborne illnesses (Rather *et al.*, 2017). Contamination of food can occur at any time during harvesting, processing, storage, and shipping as well as during food preparation; and the World Health Organization (WHO) estimates that about 1 in 10 persons has food poisoning. In addition, foodborne diseases cause 420,000 deaths every year globally, about 30% of them are children under the age of 5 and the rate of foodborne illnesses are in rising (WHO, 2015; Nazir *et al.*, 2023).

Different bacterial agents are linked to food poisoning including Gram-positive and Gram-negative bacteria, e.g., *Bacillus pumilus*, *Bacillus cereus*, *Staphylococcus aureus*, *Escherichia coli,* and *Salmonella enterica* (Fetsch *et al*, 2014; Shah *et al*, 2019). Bacteria commonly found on many uncooked foods such as *S. aureus*, *Salmonella*, *Clostridium perfringens*, *Campylobacte*r, *Listeria monocytogenes*, *Vibrio parahaemolyticus*, *Bacillus cereus*, and *Escherichia coli* causes most of the cases of food poisoning (Esther *et al.*, 2019). Antibiotics are used in the treatment of bacterial food poisoning, unfortunately, misuse of these drugs resulted in antibiotic-resistant bacterial strains (Zige and Anumudu, 2019). The emergence of multidrug resistant pathogens has been one of the most critical public health problems in the last decades and necessitates the search for new modes of treatment. The possible sources of antibacterial agents include natural products such as plants, fungi, and algae for example marine algae products.

The overuse and misuse of antimicrobials have led to an increase in pathogenic microorganisms with resistance to traditional antimicrobial compounds, which has led to an increased need for alternative therapeutic strategies. Therefore, recently, there has been increased interest in the development of novel antimicrobial compounds from various sources. Marine organisms such as seaweeds are rich sources of natural bioactive compounds with the potential for different uses applicable to human health that attracted huge interest in recent years. Studies have shown that seaweeds have various compounds with anti-inflammatory, antioxidant, and antimicrobial properties. Furthermore, various studies have shown that seaweeds are a potent source of antibacterial agents, for instance, algae contain compounds such as flavonoids (Tanna *et al*, 2019) which have antibacterial activity (Cushnie and Lamb, 2011; Farhadi *et al*, 2019). In addition, Seaweed or macroalgae have been shown to provide a great variety of metabolites and natural bioactive compounds with antimicrobial activity, such as polysaccharides, polyunsaturated fatty acids, phlorotannins, and other phenolic compounds, and carotenoids.

Microalgae showed a broad range of activity such as antimicrobial potential, as they can inhibit the growth of a broad spectrum of microbiaql agents, for example, Gram-negative, and Gram-positive bacteria (AL-Abdulameer, 2022). Thus, suggesting that microalgae could be an alternative approach in the treatment of emerging multidrug resistance pathogens and to avoid the improper use of antibiotics. Previous reports elucidated that particular algae namely, *Enteromorpha intestinalis* and *Caulerpa prolifera* have antibacterial activity (Zbakh *et al*, 2012). In addition, Swathi *et al.* (2022) showed that *Enteromorpha sp.* has excellent antioxidant and antibacterial properties. Furthermore, other types of microalgae have been explored for their potential to yield flavonoids with antibacterial properties; for example, *Spirulina* have been *reported to possess* significant antimicrobial and antioxidant activities (Abdel-Moneim *et al., 2022*). Therefore, previous evidence of the antibacterial potential of seaweeds necessitates further investigation to develop effective antibacterial drugs to combat infectious diseases.

Although marine organisms such as seaweed can be cultivated sustainably, and they are a source of polar molecules, such as pigments and phenolic compounds, which demonstrated antimicrobial potential; studies on the use of algae compounds such as flavonoids are scarce and the capability of flavonoids extracted from marine algae such as *E. intestinalis* and *C. prolifera* as antimicrobial was not well studied particularly in Libya. Previous studies showed that Libyan seaweeds have antimicrobial activities against a wide variety of microorganisms (Alghazeer *et al.*, 2013; Alshalmani *et al.*, 2014; Abdulrraziq and Salih, 2020). Building on that, it becomes essential to do more screening on some available seaweeds that have wide distribution on Libyan cost. Therefore, the aim of this study was to investigate the potency of flavonoids extracted from *E. intestinalis* and *C. prolifera* as antibacterial agents. Ethanolic extract was used to investigate their antimicrobial activity against bacterial strains isolated from meat, meat products, milk, and dairy products collected from different parts of Libya.

Materials and Methods

Algae collection and extraction

Enteromorpha intestinalis was collected in September 2015 from the Western Libyan Coast (SA 01: N 32°53′45.47 E 13°21′3.16; SA 02: N 32°53′46.23 E 13°20′50.90) and *C. prolifera* was collected from Farwa Island (Zuwara 90 km west of Tripoli) (S 1: 33° 5′24.04″N 11°42′52.74″E; S 2: 33° 5′57.39″N 11°42′45.94″E; S 3: 33° 6′33.54″N 11°41′40.94″E; S 4: 33° 5′58.12″N 11°41′35.15″E) during October 2015. The samples were identified at the Marine Plankton and Algae Department, Marine Biology Research Centre (MBRC), Tripoli (Fig. 1). Algae samples were cleaned, shade-dried for 7–14 days and then ground to powder in a kitchen-type blender. The flavonoid extraction was carried out as shown in Figure 2.

Fig. 1. Localization of the collection site of algae.

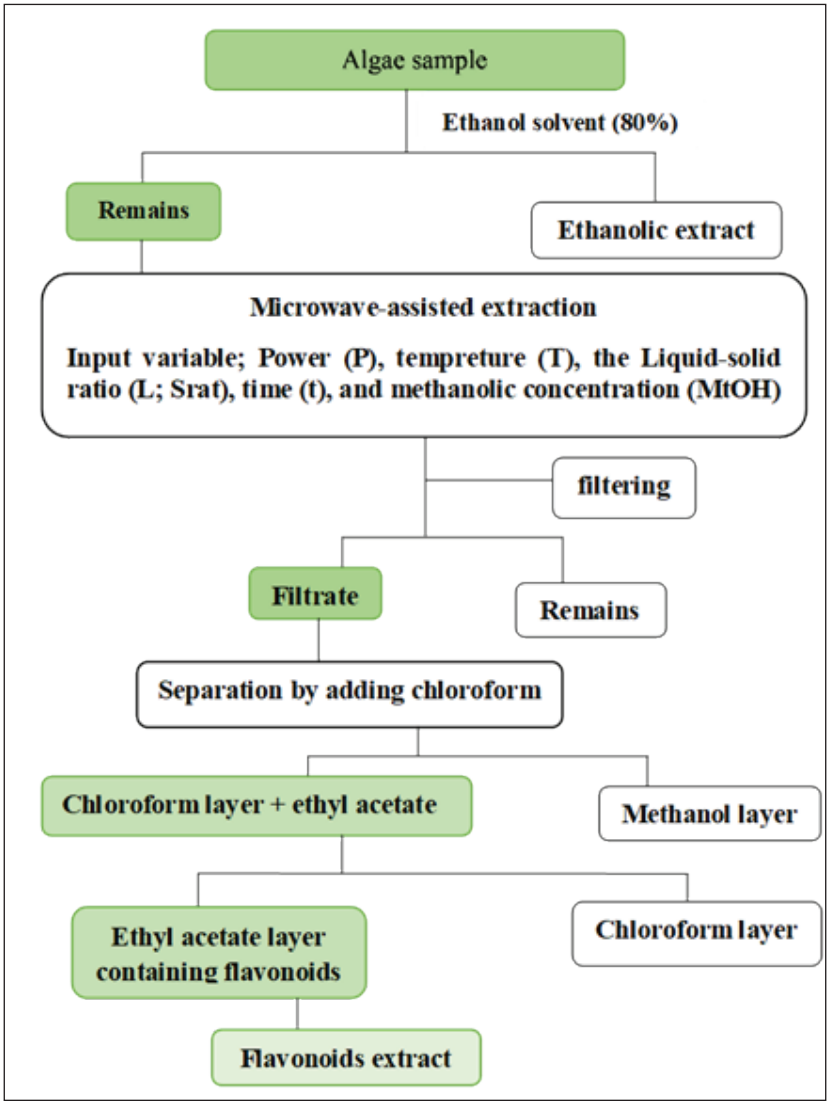

Fig. 2. Flavonoids extraction procedure.

Bacterial isolates

Bacterial strains used in this study (*Table 1)* were isolated from meat, meat products, milk, and dairy products collected from different parts of Libya (Garbaj *et al.*, 2016; Naas *et al.*, 2018; Naas *et al.*, 2019; Garbaj *et al.*, 2022). All of the used isolates were identified using conventional microbiological techniques and biochemical tests and further confirmed by partial sequencing of 16S rDNA as described by Azwai *et al.* (2016). Isolation and identification were done at the Department of Microbiology and Parasitology, Faculty of Veterinary Medicine, University of Tripoli, Libya.

Determination of total flavonoid content

Total flavonoid content was estimated according to Zhishen *et al.* (1999) using a spectrophotometer (Jenway Model 6405). The assay was performed in triplicates, and the flavonoid content of the *E. intestinalis* and *C. prolifera* extracts was determined by interpolating the absorbance of the samples against a calibration curve constructed with rutin standard (1.25–20 mg/ml) and expressed as milligrams of rutin equivalent per gram of extract (mg RE/g).

Antimicrobial assay

The antimicrobial activity of algal flavonoid-containing extracts was performed *in vitro* using the hole-plate diffusion method (Saravana Kumar *et al.*, 2009) at a concentration of 2,000 µg/ml. The bacterial isolates were maintained in cryocare preservation (cryobeads) by storing at −80°C and were recovered for testing by growing them in Mueller–Hinton (MH) broth (Oxiod, England) for 24 hours at 37°C before use. The inoculum suspension containing approximately 1.5 × 10^8 CFU/ml of bacteria was used. Each extract was placed into an 8 mm diameter well and incubated at 37°C for 18 hours and methanol was used as a negative control. Diameters of inhibition zones (DIZs) were measured in millimeters and the results were recorded as the mean of triplicate experiments.

Determination of minimum inhibitory concentration (MIC)

The MIC is defined as the lowest concentration that inhibits the growth of bacteria within 24 hours. The MIC for the flavonoid-containing extracts was determined by the macrodilution agar method. In the macrodilution agar method, a two-fold serial dilution of the flavonoid-containing extracts was prepared in sterile freshly prepared MH broth and used as diluents to achieve a decreasing concentration ranging from 500–20 µg/ml. A sterile cork borer of 8 mm diameter was used to bore well in the pre-solidified MH plates, and 150µl volume of each dilution was added aseptically into the wells made in MH plates in triplicates that had bacteria seeded with the standardized inoculums (1.5 × 10^8 CFU/ml). All the test plates were incubated at 37°C and were observed after 24 hours for bacterial growth (Pundir and Bishnoi, 2011).

Determination of minimum bactericidal concentration (MBC)

MBC was defined as the lowest concentration that completely inhibited bacterial growth. For determination of MBC, each of the tested bacterial organisms was cultured on MH broth for 24 hours at 37°C. The content of the MIC tubes and the content of the preceding tubes in the serial dilutions (2, 3, and 4MIC) were subcultured into the MH broth. All bacterial plates were inoculated at 37°C for 24 hours after which they were observed. To confirm the results of MBC, 1 ml of the experimental suspensions was resub-cultured in the MH broth which was incubated at 37°C for 18–24 hours.

Statistical analysis

Data were expressed as means ± standard deviations (SD) of triplicate determinations. All statistical analyses were carried out using SPSS V.16 (Statistical Program for Social Sciences, SPSS Corporation, Chicago, IL). Statistical differences between extract

Table 1. List of bacterial isolates.

	Bacillus cereus and Bacillus pumilus	**Abbreviation**	***Staphylococcus aureus sub sp. aureus***	**Abbreviation**
Gram positive	*B. cereus* 4	(B1)	*S. aureus* 122	(S1)
	B. cereus 16	(B2)	*S. aureus* 128	(S2)
	B. cereus 72	(B3)	*S. aureus* 287	(S3)
	B. pumilus 124	(B4)	*S. aureus* 125	(S4)
			S. aureus 283	(S5)
Gram negative	*Escherichia coli EHEC (O157)*	Abbreviation	*Salmonella enterica sub sp. enterica*	Abbreviation
	EHEC O157 57	(E1)	*S. enterica* 17	(Sal1)
	EHEC O157 55	(E2)	*S. enterica* 18	(Sal2)
	EHEC O157 52	(E3)	*S. enterica* 19	(Sal3)
	EHEC O157 49	(E4)	*S. enterica* 29	(Sal4)

activities were determined using ANOVA followed by least significant difference testing. Differences were considered statistically significant when $p < 0.05$.

Ethical approval

Not needed for this study.

Results

Flavonoid contents in algae extracts

The flavonoid content in *E. intestinalis and C. prolifera* extracts is shown in Figure 3. There were significant differences in the flavonoid yield between the two algae extracts ($p < 0.05$); *C. prolifera* extract had a higher flavonoid yield (16.1%) compared to *E. intestinalis* extract (14.5%). Previous work on *E. intestinalis* resulted in 23 mg/100g dry alga (Sava and Sibru, 2010); however, there was no previous data available concerning flavonoids extraction from *C. prolifera* but a similar genus showed 250–750 mg/ml quercetin per gram of extract (Tanna *et al.*, 2018).

Antimicrobial assay

The antibacterial assays were done using a well agar diffusion test and measuring the inhibition zone. Figure **4** shows the effect of *E. intestinalis and C. prolifera* extracts on the growth of tested bacterial

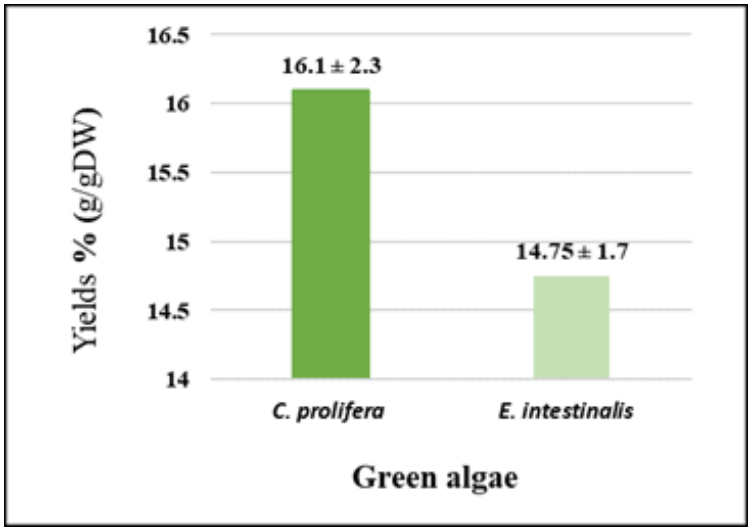

Fig. 3. Flavonoid contents yield in *E. intestinalis* and *C. prolifera* extracts.

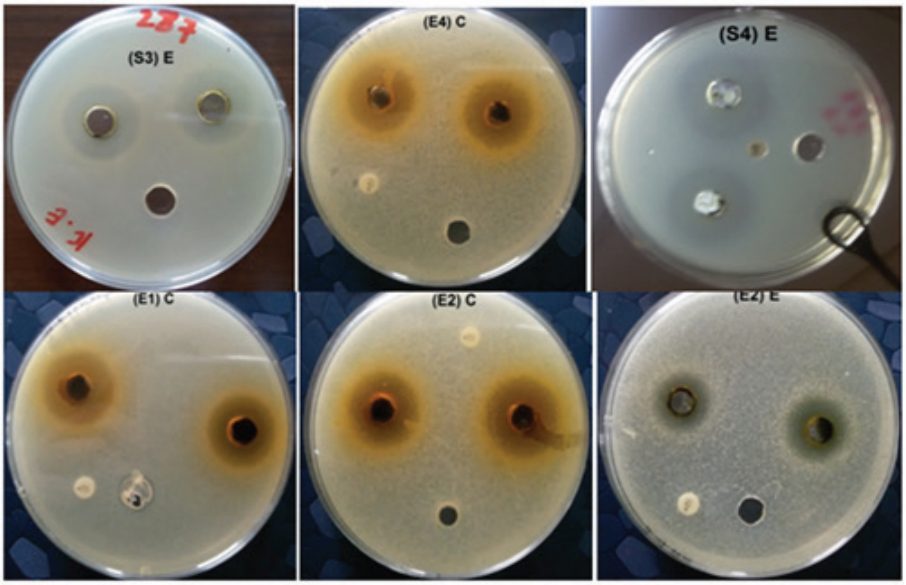

Fig. 4. Growth inhibition effect of algal extracts against some tested bacterial strains. **E1**: EHEC *O157* 57; **E4**: EHEC *O157* 49; **S3:** S.aureus 287; **S4**: S.aureus 125. **E:** Favonoids containing extracts from ***E. intestinalis*** and **C:** flavonoids containing extracts from ***C. prolifera***.

isolates [*S. aureus* and *E. coli EHEC (O157)*]. There was an inhibition of the growth of bacterial strains tested using both algal extracts.

As shown in Table 2, the highest antibacterial activity was seen using flavonoid-containing extract from *E. intestinalis* against *B. cereus* strain B3 (37.5 ± 0.5); however, the antibacterial activity was undetected on three bacteria strains (*S. enteric* strains Sal1 and Sal2 and *B. pumilus* strain B4). There were varied results from flavonoid containing extract of *E. intestinalis* on *S. aureus* strain S1–S4 (25–28 ± 1), *S. enteric* strain Sal3 and Sal4 (13.5–14 ± 0.5–1), *B. cereus* strain B1 and B2 (14.5–21.5 ± 0.5–1.5) and EHEC O157 strain E1-E4 (19–24.5 ± 0.5–1.5) with highest activity on *S. aureus* strains S1–S4.

The flavonoids containing extracts from *C. prolifera* resulted in antibacterial activity against *S. aureus* strain S1–S4 (15–27 ± 1), *S. enteric* strain Sal3–4 (10.5–13.5 ± 0.5–1.5), *B. cereus* strain B2 and B3 (24 ± 1) as well as EHEC O157 strain E1-E4 (19–24.5 ± 0.5–1.5). However, the antibacterial effect of flavonoids containing extract from *C. prolifera* was undetected on *B. cereus* strain B1 (Table 2).

MIC

MIC results of flavonoids containing extract are shown in Figure 5. Results revealed that *S. aureus* growth was inhibited at 50–250 µg/ml, and the least inhibitory concentration (50 µg/ml) was dominated by *E. intestinalis* extract against S1–S5 isolates, while *C. prolifera* against S4. On the other hand, *C. prolifera* extract inhibited the growth of strains *S*1–S3 and S5 at a concentration of 250 µg/ml.

The growth of *B. cereus* was inhibited at 50–250 µg/ml of extract and the lowest concentration was shown from *E. intestinalis* extract which was 100 and 50 µg/ml against isolate B2 and B3 respectively. *Caulerpa prolifera* extract inhibited strain B2 and B3 at 100 µg/ml. Growth of *E. coli* was inhibited at a concentration of 100–500 µg/ml. The lowest concentration from *C. prolifera* extract against E1, E3, and E4 strains was found to be at 100 µg/ml. While, *E. intestinalis* extract inhibited strain E1, E2, and E4 at 100 µg/ml and strain E3 at 500 µg/ml. Furthermore, *S. enterica* was inhibited at a concentration of 100–250 µg/ml of algal extracts. The lowest concentration (100 µg/ml) was from *E. intestinalis* extract against strain Sal4 and 250

Table 2. *In vitro* antimicrobial activity of the algal flavonoids containing extracts against bacteria isolated from food products.

Bacterial isolate	Number of isolate	*E. intestinalis*	*C. prolifera*
		DIZ (mm)	
S. aureus	S1	25 ± 1^{b}	15 ± 1^{d}
	S2	28 ± 1^{b}	18 ± 1^{d}
	S3	26 ± 1^{b}	15 ± 1^{d}
	S4	25 ± 1^{b}	27 ± 1^{b}
	S5	25 ± 1^{b}	15 ± 1^{d}
S. enterica	Sal1	-	12.5 ± 0.5^{c}
	Sal2	-	13.5 ± 1.5^{c}
	Sal3	13.5 ± 0.5^{c}	10.5 ± 0.5^{d}
	Sal4	14 ± 1^{c}	12.5 ± 1.5^{c}
B. cereus	B1	14.5 ± 1.5^{d}	-
	B2	21.5 ± 1.5^{c}	24 ± 1^{c}
	B3	37.5 ± 0.5^{a}	24 ± 1^{c}
B. pumilus	B4	-	-
EHEC O157	E1	16 ± 1^{b}	22 ± 1^{a}
	E2	14 ± 1.5^{c}	19 ± 1^{b}
	E3	18.5 ± 0.5^{b}	21.5 ± 1.5^{a}
	E4	16 ± 1^{b}	24.5 ± 0.5^{a}

Letters (a–d) indicate statistically significant differences between groups ($p < 0.05$). **DIZ**: diameter of inhibition zone; Data are expressed as the mean ± standard deviation (SD) of three replicates. **S1**: *S. aureus* 122; **S2**: *S. aureus* 128; **S3**: *S. aureus* 287; **S4**: *S. aureus* 125; **S5**: *S. aureus* 283. **Sal 1**: *S. enterica* 17; **Sal2**: *S. enterica* 18; **Sal3**: *S. enterica* 19; **Sal4**: *S. enterica* 29. **B1**: *B. cereus* 4; **B2**: *B. cereus* 16; **B3**: *B. cereus* 72; **B4**: *B. pumilus* 124. **E1**: EHEC *O157* 57; **E2**: EHEC *O157* 55; **E3**: EHEC *O157* 52; **E4**: EHEC *O157* 49.

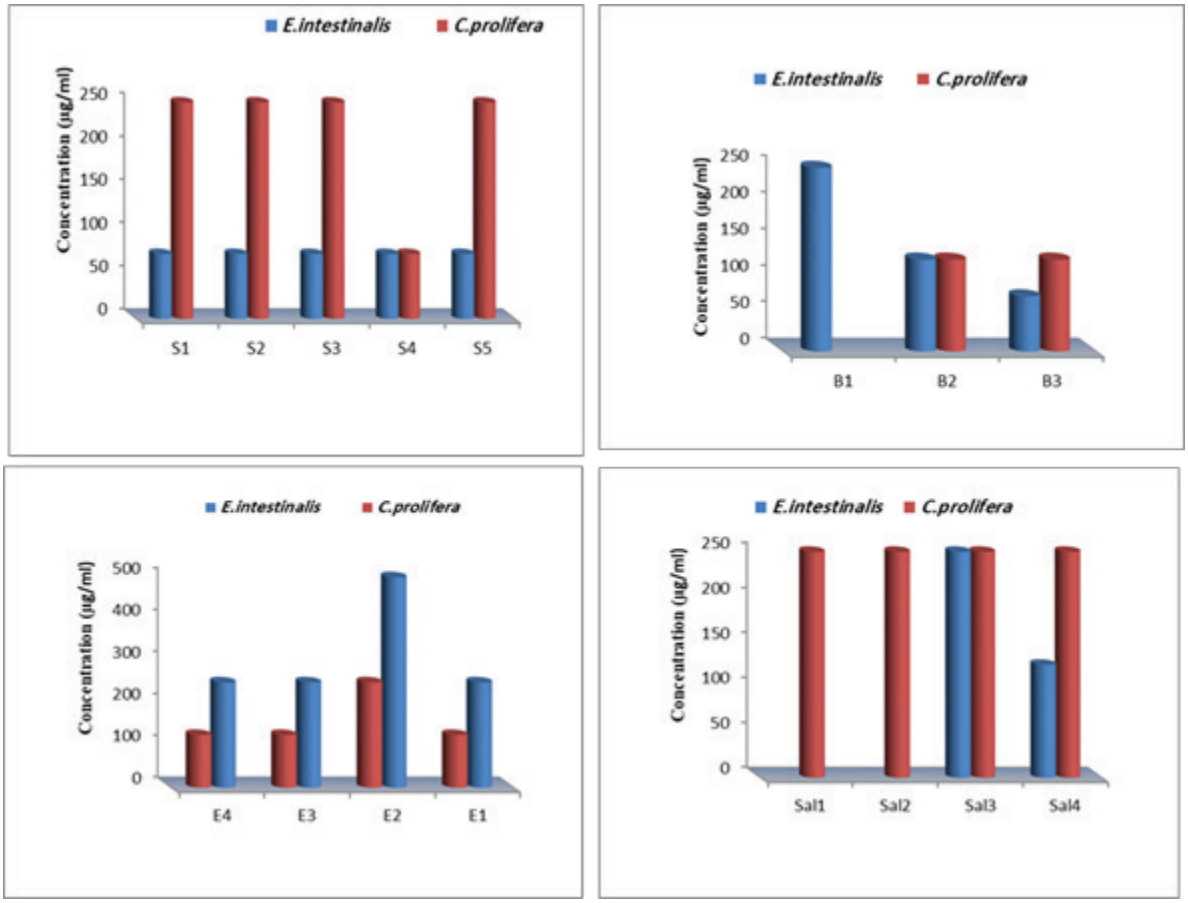

Fig. 5. Minimal inhibitory concentration. MIC of the algal extracts against *Staphylococcus aureus* (S1: *S. aureus* 122; S2: *S. aureus* 128; S3: *S. aureus* 287), *Bacillus cereus* strains (B1: *B. cereus* 4; B2: *B. cereus* 16; B3: *B. cereus* 72), *Salmonella enterica* strains (Sal 1: *S. enterica* 17; Sal 2: *S. enterica* 18; Sal 3: *S. enterica* 19; Sal 4: *S. enterica* 29), and *Escherichia coli* EHEC *0157* strains (E1: *E. coli O157* EHEC 57; E2: *E. coli O157* EHEC 55; E3: *E. coli O157* EHEC 52; E4: *E. coli O157* EHEC 49).

µg/ml against strain Sal3. Furthermore, extract from *C. prolifera* inhibited strain Sal1–4 at 250 µg/ml.

MBC

The MBC of *E. intestinalis* and *C. prolifera* flavonoid-containing extracts are shown in Figure 6. *S. aureus* has not grown at 75–250 µg/ml. The lowest concentration (75 µg/ml) was dominated by flavonoid-containing extract *(E. intestinalis)* against strain S1–S3. In similar strains, flavonoid-containing extract (*C. prolifera)* was not grown at 250 µg/ml.

B. cereus was not grown at 75–250 µg/ml, while the lowest concentration (75 µg/ml) resulted from flavonoid-containing extract of *E. intestinalis* against strain B3. Other results from flavonoid-containing extract of *E. intestinalis* were 125 and 250 µg/ml against strains B2 and B1. Flavonoid-containing extract from *C. prolifera* inhibited the growth of strains B2 and B3 completely at a concentration of 125 µg/ml.

There was no growth of *E. coli* at 150–500 µg/ml of algal extracts. The lowest concentration (150 µg/ml) was from flavonoid-containing extract of *C. prolifera* against strains E1, E3s and E4. Other results from flavonoid-containing extract *(C. prolifera)* at 250 µg/ml against strain E2. On the other hand, flavonoid-containing extract *(E. intestinalis)* was able to kill strains E1, E2s, and E4 at 250 µg/ml and strain E3 at 500 µg/ml. In addition, *S. enterica* was killed at 100–500 µg/ml, the lowest concentration (100 µg/ml) was from flavonoid-containing extract (*E. intestinalis)* towards strain Sal4 and 250 µg/ml against strain Sal3 and flavonoid from *C. prolifera* killed strain Sal1-4 at 250 µg/ml.

Discussion

Foodborne diseases are of great concern worldwide and are caused by different pathogenic microorganisms including bacteria. The emergence of multidrug-resistant bacteria leads to searching for new antibacterial agents from natural products including algae. Therefore, the capability of algae extracts obtained from Libyan costs

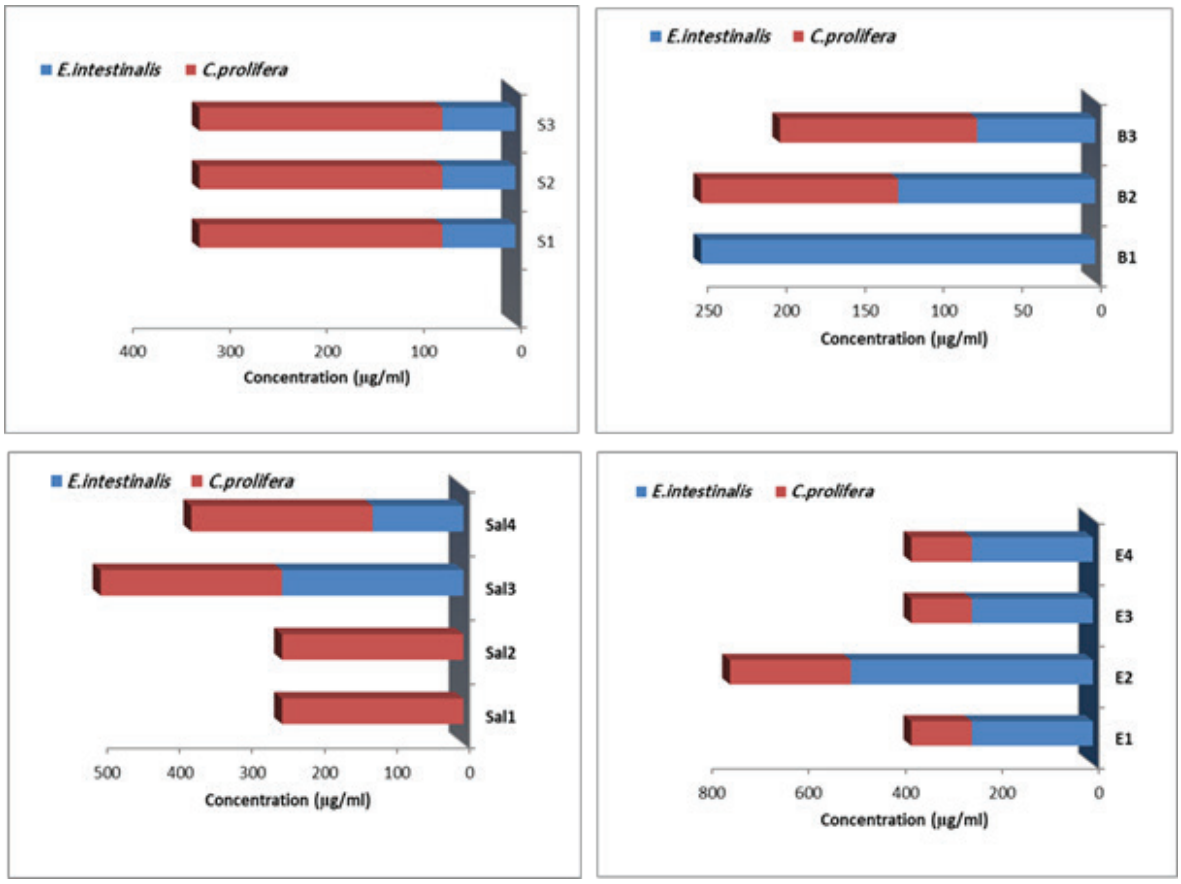

Fig. 6. Minimal bactericidal of the algal flavonoids containing extracts. MBC (B) of the algal flavonoids containing extracts against *Staphylococcus aureus* (S1: *S. aureus* 122; S2: *S. aureus* 128; S3: *S. aureus* 287), *Bacillus cereus* strains (B1: *B. cereus* 4; B2: *B. cereus* 16; B3: *B. cereus* 72), *Salmonella enterica* strains (Sal 1: *S. enterica* 17; Sal 2: *S. enterica* 18; Sal 3: *S. enterica* 19; Sal 4: *S. enterica* 29), and *Escherichia coli* EHEC *0157* strains (E1: *E. coli O157* EHEC 57; E2: *E. coli O157* EHEC 55; E3: *E. coli O157* EHEC 52; E4: *E. coli O157* EHEC 49).

(*E. intestinalis* and *C. prolifera*) to inhibit the growth of some foodborne pathogens was assessed in this study.

This study revealed that algal extract (*E. intestinalis* and *C. prolifera*) obtained from Libyan costs had antibacterial activity against tested bacterial strains. The antibacterial effect on different bacterial isolates was variable indicated by producing variable zones of inhibition against studied bacterial isolates.

Extract from *C. prolifera* was more effective against *EHEC O157*. There was a variable effect of the tested algal extract on *S. enterica* strains. While *C. prolifera* extract exerted an antibacterial effect against the 4 tested strains of *S. enterica* (Sal 1–4), The *E. intestinalis* only affected 2 strains (Sal 3 and Sal 4). *E. intestinalis* had a stronger effect against *B. cereus* than *C. prolifera* which affected only 2 strains of *B. cereus* (B2 and B3). However, both *E. intestinalis* and *C. prolifera* extracts were ineffective against *B. pumilus* isolates.

Data indicated that the algal extract (*E. intestinalis* and *C. prolifera*) used had antibacterial activity against both Gram-positive and Gram-negative bacterial isolates used in this study with variable effects on certain strains, similar results were reported in previous studies (Slem *et al.*, 2015). In addition, antimicrobial properties of 70% methanol and DMSO extracts from *Gracilaria corticata* and *Gracilaria edulis* were found to be effective against *B. subtilis (*Arulkumar *et al.*, 2018).

The observed antibacterial activity of both algal extracts (*E. intestinalis* and *C. prolifera*) used in this study could be attributed to the active compounds including flavonoids (Alghazeer *et al.*2022). It has been shown that flavonoids from different sources have antibacterial activity, flavonoids can reduce colony-forming unit (CFU) numbers of bacteria in time–kill and MBC assays (Cushnie and Lamb, 2011). In addition, other mechanisms of action have been investigated and related to robinetin, myricetin, apigenin, rutin, galangin, 2,4,2′-trihydroxy-5′-methylchalcone and lonchocarpol A (Cushnie and

Lamb, 2005). Furthermore, flavonoid derivative compounds such as morin and rutin can be considered in combination as antibacterial agents. Morin inhibits DNA synthesis, and this effect is promoted by rutin (Arima *et al.*, 2002). Moreover, flavonoids have been shown to have antioxidant activity (Ioku *et al.*, 2001).
Separation and further characterization of such compounds are necessary for fully understanding the different properties of these algae and for future use of these natural sources in novel drug preparation and application of these biologically important compounds to combat increasing multiple antibiotic resistance, as well as, these compounds require further analysis to determine whether the detected activity is selective. In addition, algae-derived flavonoids can be utilized as food wrap to prevent foodborne pathogens from invading food products leading to food poisoning (Han and Wang, 2017).

Conclusion

Flavonoids from *E. intestinalis* were more effective than *C. prolifera* against *S. aureus* and *B. cereus*. On the other hand, flavonoids extracted from *C. prolifera* were more effective against *E. coli O157* EHEC. *S. enterica* can be qualitatively combated by flavonoids from *E. intestinalis* and quantitatively by flavonoids from *C. prolifera.* Both flavonoid extracts from different algae species were not effective against *B. pumilus.*
Further research work should be carried out to study other species of seaweeds from Libyan coasts to provide complete data on the antimicrobial potential of seaweeds along the long coast of Libya. In addition, more work needs to be done for the characterization of the bioactive compounds present in the extract. It is also necessary to perform separation, purification, and characterization of biologically active compounds for the synthesis of novel antibiotics using chromatographic and spectroscopic techniques. Further and more in-depth studies are required to explore the mechanism of action and identify the specific compounds responsible for the antimicrobial effect. As many natural products with potential antibacterial activity have turned out to be toxic to cells, therefore, it is important to carry out cytotoxicity studies for algae extract used in this study to investigate whether they are safe or toxic.

Acknowledgment

None.

Authors contribution

All authors contributed to this study. All authors read and approved the final manuscript.

Conflict of interest

All authors declare that there is no conflict of interest.

Funding

None.

Data availability

All data are provided in the manuscript.

References

AL-Abdulameer, S. 2022. The wide spectrum activity of chlorella and spirulina extracts on the viability of pathogenic and environmental bacteria in Baghdad, Iraq. Egyptian J. Aquat. Biol. Fisheries 26(6), 1–11.

Abdel-Moneim, A.E., El-Saadony, M.T., Shehata, A.M., Saad, A.M., Aldhumri, S.A., Ouda, S.M. and Mesalam, N.M. 2022. Antioxidant and antimicrobial activities of *Spirulina platensis* extracts and biogenic selenium nanoparticles against selected pathogenic bacteria and fungi. Saudi J. Biol. Sci. 29(2), 1197–1209.

Abdulrraziq, A.A. and Salih, S.M. 2020. Biological effect of posidonia oceanica seaweed on some pathogenic microbes. Al-Mukhtar J. Sci. 35(4), 339–346.

Addis, M. and Sisay, D. 2015. A review on major food borne bacterial illnesses. J. Trop. Dis. Public Health. 3(176), 1–7.

Alghazeer, R., Whida, F., Abduelrhman, E., Gammoudi, F. and Azwai, S. 2013. Screening of antibacterial activity in marine green, red and brown macroalgae from the western coast of Libya. Nat. Sci. 5, 7–14.

Alghazeer R., El Fatah H., Azwai S., Elghmasi S., Sidati M., El Fituri A., Althaluti E., Gammoudi F., Yudiati E., Talouz N., Shamlan G., AL-Farga A., Alansari W.S., Eskandrani A.A. 2022. Nutritional and nonnutritional content of underexploited edible seaweeds. Aquacul. Nutr. 2022, 8422414.

Arima, H., Ashida, H. and Danno, G. 2002. Rutin-enhanced antibacterial activities of flavonoids against *Bacillus cereus* and *Salmonella enteritidis*. Biosci. Biotechnol. Biochem. 66(5), 1009–1014.

Arulkumar, A., Rosemary, T., Paramasivam, S. and Rajendran, R.B.J.B. 2018. Phytochemical composition, *in vitro* antioxidant, antibacterial potential and GC-MS analysis of red seaweeds (Gracilaria corticata and Gracilaria edulis) from Palk Bay, India. Biocatal. Agricult. Biotechnol. 15, 63–71.

Azwai, S., Alfallani, E., Abolghait, S., Garbaj, A., Naas, H., Moawad, A., Gammoudi, F., Rayes, H., Barbieri, I. and Eldaghayes, I. 2016. Isolation and molecular identification of *Vibrio* spp. by sequencing of 16S rDNA from seafood, meat and meat products in Libya. Open Vet. J. 6(1), 36–43.

Cushnie, T.T. and Lamb, A.J. 2005. Antimicrobial activity of flavonoids. Int. J. Antimicrob. Agents. 26(5), 343–356.

Cushnie, T.T. and Lamb, A.J. 2011. Recent advances in understanding the antibacterial properties of flavonoids. Int. J. Antimicrobe. Agents. 38(2), 99–107.

Esther, K., Nelson, M. and Ephantus, K. 2019. Bacterial agents causing food poisoning among patients attending Thika Level 5 Hospital, Kiambu County, Kenya. Open J. Med. Microbiol. 9, 168–177.

Farhadi, F., Khameneh, B., Iranshahi, M. and Iranshahy, M. 2019. Antibacterial activity of flavonoids and their structure–activity relationship: an update review. Phytother. Res. 33(1), 13–40.

Fetsch, A., Contzen, M., Hartelt, K., Kleiser, A., Maassen, S., Rau, J., Kraushaar, B., Layer, F. and Strommenger, B. 2014. *Staphylococcus aureus* food-poisoning outbreak associated with the consumption of ice-cream. Int. J. Food Microbiol. 187, 1–6.

Garbaj, A.M., Awad, E.M., Azwai, S.M., Abolghait, S.K., Naas, H.T., Moawad, A.A., Gammoudi, F.T., Barbieri, I. and Eldaghayes, I.M. 2016. Enterohemorrhagic *Escherichia coli* O157 in milk and dairy products from Libya: isolation and molecular identification by partial sequencing of 16S rDNA. Vet. World 9(11), 1184–1189.

Garbaj, A.M., Gawella, T.B.B., Sherif, J.A., Naas, H.T., Eshamah, H.L., Azwai, S.M., Gammoudi, F.T., Abolghait, S.K., Moawad, A.A., Barbieri, I. and Eldaghayes, I.M. 2022. Occurrence and antibiogram of multidrug-resistant *Salmonella enterica* isolated from dairy products in Libya. Vet. World. 15(5), 1185–1190.

Han, Y. and Wang, L. 2017. Sodium alginate/carboxymethyl cellulose films containing pyrogallic acid: physical and antibacterial properties. J. Sci. Food Agricult. 97(4), 1295–1301.

Ioku, K., Aoyama, Y., Tokuno, A., Terao, J., Nakatani, N. and Takei, Y. 2001. Various cooking methods and the flavonoid content in onion. J. Nutr. Sci. Vitaminol. 47(1), 78–83.

Naas, H.T., Edarhoby, R.A., Garbaj, A.M., Azwai, S.M., Abolghait, S.K., Gammoudi, F.T., Moawad, A.A., Barbieri, I. and Eldaghayes, I.M. 2019. Occurrence, characterization, and antibiogram of *Staphylococcus aureus* in meat, meat products and some seafood from Libyan retail markets. Vet. World. 12, 925–931.

Naas, H.T., Zurghani, M.M., Garbaj, A.M., Azwai, S.M., Eshamah, H.L., Gammoudi, F.T., Abolghait, S.K., Moawad, A.A., Barbieri, I. and Eldaghayes, I.M. 2018. *Bacillus cereus* as an emerging public health concern in Libya: isolation and antibiogram from food of animal origin. Libyan J. Med. Sci. 2, 61–65.

Nazir, A., Ochani, S., Nazir, A., Fatima, B., Ochani, K., Hasibuzzaman, M.A. and Ullah, K. 2023. Rising trends of foodborne illnesses in the U.S.: short communication. Ann. Med. Surg. (Lond). 85(5), 2280–2281.

Pires, S.M., Fischer-Walker, C.L., Lanata, C.F., Devleesschauwer, B., Hall, A.J., Kirk, M.D., Duarte, A.S.R., Black, R.E. and Angulo, F.J. 2015. Aetiology-specific estimates of the global and regional incidence and mortality of diarrhoeal diseases commonly transmitted through food. PLoS One 10(12), e0142927.

Pundir, R. and Bishnoi, S. 2011. Antimicrobial activity of Mitragyna parvifolia barks and Butea monosperma leaves extracts against human pathogenic microbial strains. Int. J. Drug Develop. Res. 3, 141–147.

Rather, I.A., Koh, W.Y., Paek, W.K. and Lim, J. 2017. The sources of chemical contaminants in food and their health implications. Front. Pharmacol. 8, 830.

Saravana Kumar, A., Venkateshwaran, K., Vanitha, J., Ganesh, M., Vasudevan, M. and Sivakumar, T. 2009. Evaluation of antibacterial activity, phenol and flavonoid contents of Thespesia Populnea flower extracts. Pakistan J. Pharmaceut. Sci. 22, 282-286.

Sava, C. and Sibru, R. 2010. Analytical study of the determination of flavonoids in Black Sea algae. Ovidius Univ. Ann. Chem. 21, 29–34.

Shah, M.M., Miringu, G., Wada, A., Kaneko, S. and Ichinose, Y. 2019. Case report: *Bacillus pumilus*–caused Bacteremia in a patient with food poisoning. Am. J. Trop. Med. 100(3), 688–690.

Swathi, N., Kumar, A.G., Parthasarathy, V. and Sankarganesh, P. 2022. Isolation of *Enteromorpha* species and analyzing its crude extract for the determination of *in vitro* antioxidant and antibacterial activities. Biomass Convers Biorefin. 23, 1–10.

Tanna, B., Choudhary, B. and Mishra, A. 2018. Metabolite profiling, antioxidant, scavenging and anti-proliferative activities of selected tropical green seaweeds reveal the nutraceutical potential of Caulerpa spp. Algal. Res. 36, 96–105.

Tanna, B., Brahmbhatt, H.R. and Mishra, A. 2019. Phenolic, flavonoid, and amino acid compositions reveal that selected tropical seaweeds have the potential to be functional food ingredients. J. Food Process. Preserv. 43(12), e14266.

WHO. 2015. WHO estimates of the global burden of foodborne diseases: World Health Organization. Geneva, Switzerland: World Health Organization.

Zbakh, H., Chiheb, H., Bouziane, H., Sánchez, V. and Riadi, H. 2012. Antibacterial activity of benthic marine algae extracts from the Mediterranean Coast of Morocco. J. Microbiol. Biotechnol. Food Sci. 2, 219–228.

Zhishen, J., Mengcheng, T. and Jianming, W. 1999. The determination of flavonoid contents in mulberry and their scavenging effects on superoxide radicals. Food Chem. 64(4), 555–559.

Zige, D.V. and Anumudu, C.K. 2019. Prevalence and multi-drug resistance pattern of food poisoning enteric bacteria associated with diarrhoea patients. Am. J. Biomed. Life Sci. 7(3), 63–67.