

In vitro study of antioxidant, antibacterial, and cytotoxicity properties of *Cordia myxa* fruit extract

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ABSTRACT

Background and Objectives: Medicinal plants have recently received much interest because of the low production costs and fewer side effects associated with remedies made from them compared with chemical therapies. The current study investigated the antioxidant, antibacterial, and cytotoxicity properties of an ethanol extract of *Cordiamyxa* fruit (CMF) extract.

Materials and Methods: The antioxidant activity of CMF was determined by measuring electron-donating ability with a 1,1-diphenyl-2-picrylhydrazyl (DPPH) assay. The phenolic content was calculated as Gallic acid equivalents using the Folin-Ciocalteu assay. To evaluate the efficiency of CMF, five multidrug-resistant bacterial strains (*Salmonella enterica*, *Escherichia coli*, *Bacillus subtilis*, *Staphylococcus aureus*, and *Pseudomonas aeruginosa*) were tested using the agar diffusion method. Furthermore, the cytotoxic activity of CMF was determined using 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium (MTT) assay against a healthy fibroblast (L929) cell line.

Results: The CMF ethanol extract was revealed to have substantial phenol and flavonoid content (113.71 ± 0.04 mg gallic acid/g dried extract and 68.9 ± 0.002 mg quercetin/g dried extract, respectively) that showed the highest percentage of DPPH inhibition (86.45%), which was achieved by ethanol extract at the concentration of 60 $\mu\text{g/ml}$, with excellent antibacterial activity against *S. aureus*, *E. coli*, *S. enterica*, *B. subtilis*, and *P. aeruginosa* (17.5 ± 1.0 , 14.9 ± 1.0 , 13.3 ± 1.5 , 15.7 ± 1.0 , and 13.8 ± 1.5 mm IZ, respectively). In addition, no expressive antiproliferative effect was recorded in the assessment of cytotoxicity on L929 cells.

Conclusion: According to the current findings, CMF exhibits low cytotoxicity, antibacterial activity, and antioxidant properties *in vitro* and can be developed for pharmaceutical and medical uses in the future.

Keywords: *Cordiamyxa* fruit; Antioxidant activity; Cytotoxicity; Antibacterial

INTRODUCTION

Plants, in general, encompass a wide range of medicinally beneficial bioactivities, and they have acquired the ability to create structurally diverse compounds known as secondary metabolites over the

evolutionary process. These bioactive metabolites are considered to be potent bioactive agents for disease treatment as well as prospective sources for the discovery of new medications, food additives, flavors, and other industrially valuable products (1-4). Plants contain phenolics and flavonoids, which rep-

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resent a wide range of secondary metabolites. These compounds are created as a result of growth and development or in response to the environment (5). Phenolic and flavonoids are essential for plant growth and have the potential to improve human health by lowering the risk of chronic and degenerative disorders (6). Plant-derived phenolics and flavonoids, on the other hand, offer a number of beneficial biological effects related to antioxidant, anti-inflammatory, and antibacterial activities, and there is a lot of profit to be gained in the cosmetic, pharmaceutical, and food sectors from this group of compounds (7). In the human body, cellular processes eventually create dangerous molecules, such as reactive oxygen species and free radicals, which can damage living cells and cause a variety of clinical illnesses (8, 9). Numerous natural and synthetic antioxidants are efficient in decreasing free radical activity; nevertheless, consuming synthetic antioxidants has adverse effects on the human body (10). Natural products derived from medicinal plants may be viable sources of medicines to combat human pathogenic bacteria, especially with technological advancements such as nanobiotechnology (11-14). Thus, novel medications derived from natural sources are required to help humanity.

C. myxa is a flowering plant of the Boraginaceae family that may be found growing in subtropical as well as tropical areas of parts of Asia, Australia, and Africa (15, 16). *Cordia* species fruits are rich in vital minerals, carbohydrates, essential fatty acids, vitamins, and proteins, according to recent studies.

In Iraq, the *Cordia myxa* tree is known as "Bumber," (Fig. 1), and its fruit is used as an expectorant, demulcent, diuretic, and anti-diarrheal (17). Phytochemicals derived from the *Cordia* genus have been investigated for its antiviral and anti-inflammatory properties, tumor cell growth inhibitors, and free radical scavenging agents (18-20). Therefore, this study was undertaken to evaluate cytotoxic effects on normal fibroblast cells as well as their antioxidant and antibacterial activities of ethanol extracts of CMF grown in the Iraqi environment.

MATERIALS AND METHODS

Plant material collection. All of the chemicals were purchased from Sigma-Aldrich Chemical Co. and were of analytical grade (St. Louis, MO, USA). *C. myxa* fruits were collected from *C. myxa* trees avail-

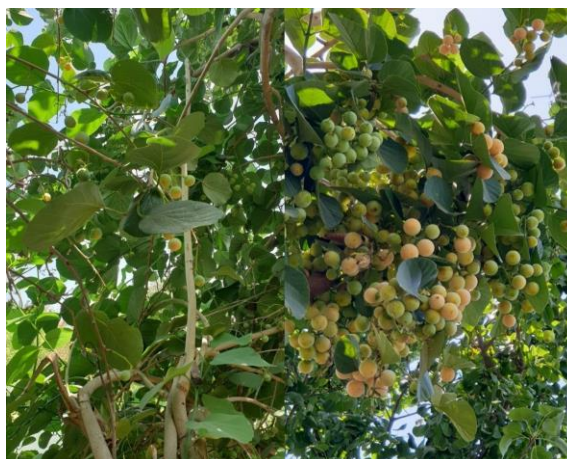


Fig. 1. Illustrates a part of the *C. myxa* tree bearing the fruit used in the current study

able in Zayouna gardens in Baghdad, Iraq, during the period from July to August 2021. The fruits were washed and checked carefully, and any physically or microbial damaged ones were excluded.

Preparation of extract. The plant extraction procedure of El-Massry et al., with some modifications, was performed (21). The harvested fruits were properly washed using tap water followed by deionized water. Eatable pieces of the crop were gathered, dried in shade, and ground into a fine powder. Then 100 g powder was blended with 250 ml ethanol in a conical flask using a magnetic stirrer for 3 hours at room temperature in the dark and filtered under suction (Whatman No. 1 Filter paper). Once again, the contents of the filter paper were moved to a conical flask and the operation was repeated. With a rotational evaporator, and temperature around 65°C the extract was pooled and dried. For yielding calculation, the resulting crude extract was weighed and stored at 4°C. A 70% aqueous solution of ethanol was used as a solvent for extraction. The fruit extract was dissolved in ethanol at a favorable concentration for subsequent experiments. All tests were conducted within 72 hours of extraction.

Quantification of total phenols and flavonoids. Total phenols were quantified using Folin–Ciocalteu reagent with gallic acid as a standard (22). The total phenolic content was expressed as milligrams of gallic acid equivalent to grams of dried plant material, and total flavonoids were determined using aluminum chloride colorimetric method (23). Total flavonoid

content was expressed as milligrams of quercetin equivalent to grams of dried plant material.

***In vitro* antioxidant activity evaluation of CMF extract: DPPH free radical scavenging assay.** The antioxidant activity of the *Cordia* fruit extract was measured using the DPPH free radical scavenging assay reported by Murthy et al. (24), with some modifications. CMF extract (1 mg/mL) and the control agent (ascorbic acid) were prepared as stock solutions, and serial dilutions with five separate concentrations were examined. Samples/standards of 10 μ L aliquots of CMF, followed by 390 μ L DPPH reagents, were loaded. The mixtures were then mixed thoroughly and kept in the dark at room temperature for 2 hours, and the absorbance was measured spectrophotometrically at 51517 nm (Schimadzu UV/Vis-240 IPC).

Results were expressed as a percentage (%) of inhibition (I.R.%) which was estimated using the following equation:

$$\text{radical scavenging activity} = (\text{Control OD} - \text{sample OD} / \text{Control OD}) \times 100. (1)$$

The results were presented as the concentration of extract needed to abolish 50% of the hydroxyl radical formed (IC₅₀ value).

The antimicrobial effect of ethanol extract was investigated using *S. aureus* (ATCC29213), *E. coli* (ATCC 35218), *S. enterica* (ATCC 13076), *B. subtilis* (ATCC 6633), and *P. aeruginosa* (ATCC 27853). For this purpose, a defined aliquot (1 mL) of a standard stock suspension of microorganisms containing 10⁶ CFU/mL was diluted with 100 mL of Mueller–Hinton agar medium and kept at 45°C. The Mueller–Hinton agar medium was divided into aliquots (20 mL) and placed in a previously sanitized plate of agar medium and allowed to set at room temperature. Then, 4 chambers 10mm in diameter were created in each of these plates utilizing a sterilized cork borer (No. 4). Different volumes of ethanol extract (25 g/mL, 50 mg/mL, and 100 mg/mL) were put into the holes inside the agar discs and left untouched at room temperature for 2 hours to diffuse. Then, the plates were incubated at 37°C overnight. After that, the plates were examined for the presence of microbial (bacterial) growth, and the inhibition zone diameter was determined.

Cytotoxic effects of CMF extract on normal fibroblast cell line. Fibroblast cell line (L929) cells were cultured on DMEM culture medium supplied with 10% FBS and 1% penicillin/streptomycin and

then incubated at 37°C in a humidified incubator with 5% CO₂. Cells were inoculated at a density of 1 × 10⁴ cells per well in a 96-well flat-bottom microtiter plate and were permitted to adhere for 24 hours at 37°C in a CO₂ incubator.

The media was withdrawn, freshly prepared medium (without serum) was replaced, and cells were incubated with various concentrations of the extract to reach the final concentrations of 0.25, 0.5, and 1 mg/mL for varied durations (24, 48, and 72 hours of incubation). After that, 10 mL of MTT stock solution (5 mg/mL in phosphate buffer solution) was added to each well, and the plate was incubated in a CO₂ incubator for 4 hours at 37°C. The produced formazan crystals were then solubilized by adding 100 μ L of DMSO per well and then left for 30 minutes at 37°C in a CO₂ incubator. Eventually, using an ELISA plate reader set to 570 nm (26), the strength of the dissolved formazan crystals (purple color) was measured.

Statistical analysis. Statistical package for social scientists (SPSS) was utilized to analyze data. The mean \pm standard deviation of triplicate measurements and one-way variance analysis (ANOVA) was used to conduct data analysis. The significance of difference was set at $p \leq 0.05$.

RESULTS

Quantification of total phenols and flavonoids. The CMF ethanol extract showed high phenol and flavonoid contents (113.71 \pm 0.04 mg gallic acid/g dried extract and 68.9 \pm 0.002 mg quercetin/g dried extract, respectively).

Phenols and flavonoids have great importance among phytochemicals, as they play an important role in immune defense activities.

***In vitro* antioxidant activity.** Generally, plant- or fruit/vegetable-derived foodstuffs are rich in phenols and exhibit an excellent antioxidant capacity, particularly phenols that can give the hydrogen atoms in their hydroxyl groups (27). Herein, the CMF ethanol extract was also evaluated for antioxidant activity. The antioxidant activity of *C. myxa* fruit ethanol extract was determined by the free radical scavenging diphenyl- β -picrylhydrazyl assay (DPPH). The DPPH is the first method to assess the antioxidant possibility of a component, extract, or another biological source

(28). Ethanol extract of CMF displayed an antioxidant activity comparable to that of standard ascorbic acid at various concentrations tested, i.e. 2, 4, 6, 8, and 10 mg/mL (Table 1 and Fig. 2). The highest ability of inhibition caused by DPPH (86.45%) was reached in ethanol extract and found at the concentration of 10 mg/ml. For all measured concentrations, there was a dose-dependent increase in the percentage of antioxidant activity.

The antibacterial activity of CMF ethanol fruit extract was tested using the disc diffusion method. For each of the tested concentrations (25, 50, and 100 mg/mL), CMF exhibited antibacterial activity against *S. aureus*, *E. coli*, *S. enterica*, *B. subtilis*, and *P. aeruginosa*. Kanamycin was used as antibiotic positive control (Table 2). The results revealed that the crude sample has antibacterial activity against all pathogenic microorganisms in a concentration-dependent manner. The ethanol extract of *C. myxa* demonstrated a better zone of inhibition against *S. aureus* (17.5 1.0

mm at 100 mg/ml concentration), while against *E. coli*, the inhibition zone was 14.9 1.0 mm. Furthermore, *S. enterica*, *B. subtilis*, and *P. aeruginosa* were all significantly suppressed by ethanol extract with inhibited zones of 13.3 1.5, 15.7 1.0, and 13.8 1.5 mm, respectively.

In vitro cytotoxicity test: MTT assay. The cell viability assay was used to determine the cytotoxic effect of CMF extract on a healthy fibroblast cell line (L929), which was assessed through mitochondrial-dependent reduction of yellow MTT to purple formazan. Cell growth rates on the CMFE at the concentrations 0.25, 0.5, and 1 mg/mL for varied durations (24, 48, and 72 hours) showed minimal cytotoxicity (Fig. 3).

DISCUSSION

Generally, plant- or fruit/vegetable-derived food-stuffs are rich in phenols and exhibit an excellent antioxidant capacity, particularly phenols that can give the hydrogen atoms in their hydroxyl groups (29). Herein, the CMF ethanol extract was also evaluated for antioxidant activity. The antioxidant activity of *C. myxa* fruit ethanol extract was determined by the free radical scavenging diphenyl-β-picrylhydrazyl assay (DPPH). The DPPH is the first method to assess the antioxidant possibility of a component, extract, or another biological source (30). The presence of phenols and flavonoids compounds was revealed in the phytochemical quantification of CMF. This finding

Table 1. Evaluation of antioxidant activity at different concentrations using DPPH assay, ascorbic acid (standard), and *C. myxa* extract (DPPH % inhibition)

Conc. (mg/ml)	DPPH % in ethanolic extract	DPPH % (standard)
2	31.15 ± 1.29	53.42 ± 1.01
4	38.12 ± 0.98	65.44 ± 1.62
6	49.25 ± 1.42	79.43 ± 0.68
8	72.32 ± 0.92	97.16 ± 1.42
10	86.45 ± 0.62	99.28 ± 1.68

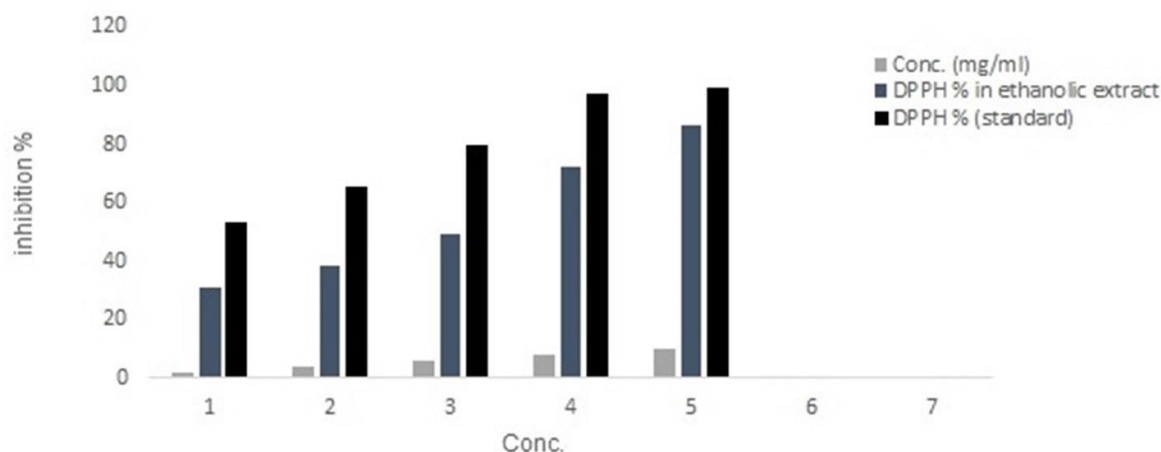


Fig. 2. DPPH free radical scavenging activity, % inhibition for standard drug, and phenolic extract tested at various concentrations

Table 2. Antimicrobial activity of CMF crude extract

Microbes	Zone of inhibition (diameter in mm)					
	Ethanol extract (mg/mL)			Kanamycin (mg/ml)		
	25	50	100	25	50	100
<i>S. aureus</i>	13.8 ± 1.0	15.6 ± 1.5	17.5 ± 1.0	21.4 ± 2.1	26.0 ± 2.5	33.0 ± 2.0
<i>E. coli</i>	12.4 ± 1.5	12.6 ± 1.5	14.9 ± 1.0	18.8 ± 2.5	27.5 ± 1.0	34.2 ± 2.5
<i>S. enteric</i>	11.6 ± 0.5	12.4 ± 1.0	13.3 ± 1.5	16.4 ± 1.5	24.8 ± 2.5	30.5 ± 1.0
<i>B. subtilis</i>	10.8 ± 0.5	14.4 ± 0.1	15.7 ± 1.0	14.3 ± 1.0	27.6 ± 2.0	33.0 ± 2.0
<i>P. aeruginosa</i>	12.1 ± 0.5	13.3 ± 1.0	13.8 ± 1.5	17.4 ± 1.2	25.7 ± 1.0	28.0 ± 5.0

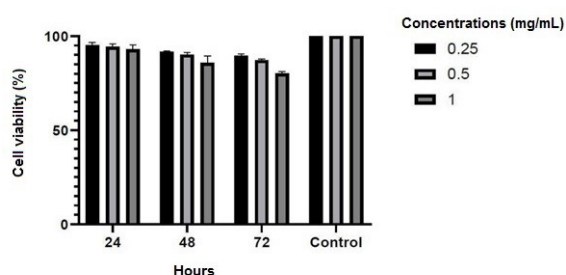


Fig. 3. Cytotoxic effect of CMF extract on L929 cell line assessed by mitochondrial activity (% Mean ± SD). Cells were treated for 24, 48, and 72 h with different CMF concentrations (0.25, 0.5, and 1 mg/mL). No treatment was given to the control group (100% cell viability). L929: healthy fibroblast cell line

was consistent with previous studies that found significant levels of Total Phenols and Flavonoids in CMF fruit extract (ethanol extract), as well as high antioxidant activity as measured by DPPH radical scavenging experiments. (31, 32). In the case of antimicrobial activities, this study was showed positive results of CMF along *S. aureus*, *E. coli*, *S. enterica*, *B. subtilis*, and *P. aeruginosa*, which was corresponded to the previous study was reported antibacterial activities of CMF fruit extracted by ethanol, which revealed antimicrobial activities against *S. aureus* and *E. coli* (33). These results suggest that the ethanol extract of CMF can be utilized to inhibit foodborne pathogens such *S. aureus*, *E. coli*, *S. enterica*, *B. subtilis*, and *P. aeruginosa*. The current results are consistent with previously published findings on the antibacterial activity of *Cordia* plant extracts. Al-Hamdani et al. showed that *Cordia myxa* extracts (aqueous and alcoholic) produced concentration-dependent inhibition zones against *Pseudomonas fluorescens*, *Salmonella*, *Shigella*, and *E. coli* (34). These findings are consistent with the outcomes of antioxidant activities, and

this could be used to continue the hunt for the active substance in the extract of CMF which is ineffective against all these strains. The results suggested that fruit extract could be used to reduce microbial infections caused by *S. aureus*, *E. coli*, *S. enterica*, *B. subtilis*, *P. aeruginosa*, *A. brasiliensis*, and *S. cerevisiae*, which are among the most common causative agents of various infections because of their bioactive components.

Plant extracts are naturally occurring substances with chemically complex compositions that are responsible for the biological action of the extracts and can be used alone or in combination. Due to their powerful antioxidant effects, flavonoids are the most well-known phenolic compounds. Adsorption to and disintegration of microbial membranes, ion deprivation, enzyme interaction, and contact with membrane transporters are among ways that phenolic chemicals have antibacterial activity (35).

It is indeed feasible that CMF exhibited negligible cytotoxicity with normal cells at different doses (0.25, 0.5, and 1 mg/mL) for different time periods (24, 48, and 72 hours); however, increasing the concentration of extract could be cytotoxic. As a result, more research will be undertaken to determine the response of normal cells as well as cell lines when using higher quantities of the extract.

CONCLUSION

The presented study reports that *C. myxa* fruit contains different phytochemical compounds in ethanol extract analyzed by GC-MS profile. The crude extract demonstrated high *in vitro* antioxidant activity compared to standard (ascorbic acid) by inhibiting DPPH, and antimicrobial activities on various investigated pathogenic microbes which may be revealed

to the phytochemicals included in the CMF extract. In the current study, the *in vitro* experiments provide significant evidence that CMF is a potential source of antioxidants and antimicrobial activity, indicating its use as a value-adding functional component.

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