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Original Article

Antioxidant and Antibacterial Activity of the Aqueous and Alcoholic Extracts of the Plant *Citrus maxima* Merr

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Abstract

Background: The resistance of pathogenic bacteria against synthetic drugs led scientists to conduct research on medicinal plants. The present study investigated the antioxidant and antibacterial activity of the aqueous, methanol, and ethanol alcoholic extracts of the plant *Citrus maxima* Merr. (Syn. *Citrus grandis*) against some human pathogenic bacteria. Then, the presence of secondary metabolites was evaluated in vitro, including alkaloid, saponin, and tannin.

Methods: The samples (i.e., root, stem, and seed) of *C. maxima* were collected at Babolsar, Mazandaran province, Iran. The agar well diffusion assay was used to determine antibacterial activity. In addition, several bacteria were applied based on the aim of the study, including *Streptococcus pyogenes* (PTCC-1447), *Bacillus subtilis* (PTCC-1156), *Bacillus cereus* (PTCC-1247), *Enterococcus faecalis* (PTCC-1185), *Micrococcus luteus* (ATCC-10987), and *Staphylococcus aureus* (PTCC-1189). Further, some Gram-negative bacteria were used, encompassing *Escherichia coli* (ATCC-25922), *Shigella boydi*(-), *Salmonella typhi* (PTCC-1609), *Pseudomonas aeruginosa* (PTCC-1181), *Enterobacter aerogenes* (PTCC-1221), and *Klebsiella pneumoniae* (PTCC-1139). Next, the minimum inhibitory and bactericidal concentrations were determined by the serial dilution method. Furthermore, free radical activity was identified by 2,2-diphenyl-1-picrylhydrazyl. Moreover, the total phenolic and total flavonoid contents were conducted by Folin-Ciocalteu and aluminum chloride methods, respectively. Finally, the phytochemical compounds were investigated as well.

Results: The highest sensitivity was observed on *M. luteus* against the root methanol extract. Additionally, the total phenolic content of root, seed, and leaf was determined as 98.22, 89.66, and 77.51 (mgGA/g), respectively. Similarly, the flavonoid content was determined as 3.52, 3.43, and 3.56 (mgQ/g), respectively. In addition, the IC50 of the root, seed, leaf, and ascorbic acid were calculated as 0.129, 0.135, 0.113, and 0.109 mg mL^{-1}, respectively. Eventually, the methanol extract of the leaf and root showed the presence of alkaloid, saponin, and tannin.

Conclusions: In general, *C. maxima* is suggested for producing natural drugs with antibiotic properties in the pharmaceutical industry due to the presence of secondary metabolites in its different parts.

Keywords: Antibacterial, *Citrus maxima*, Infectious bacteria, Phytochemical

Background

The antimicrobial activity of plants has long been considered as an effective mechanism for controlling pathogenic microorganisms (1). In addition, plants are a major source of antimicrobial compounds that have been used in the treatment of infectious diseases from ancient times (2). In recent years, the emergence of multi-drug resistant bacteria has become one of the main causes of the failure in treating infectious diseases (3). Thus, there is an urgent need to identify and introduce effective plant materials for the production of natural anti-infective agents with high biopotential with low side effects (4). It is estimated that ca. 25% of the total formulated drugs for the treatment of diseases have plant origin or analogues derived from the plants (5). According to the World Health Organization, 80% of the world's population relies on herb-derived drugs (6).

Pomelo, *Citrus maxima* Merr. (Syn. *Citrus grandis*) is a thin tree, which reaches 4.5-9 m in height, with angular branches, thin thorns, and ovate leaves with 10-20 cm in length. Further, it produces single flowers with broad petals, spherical fruits, and a large number of seeds in the fruit. It is reported that compounds with medicinal properties in *Citrus* spp. prevent heart and cancer diseases (7). For example, Coumarin from *Citrus grandis* is used for the treatment of blood circulation problems (8). The other reported cases are the antibacterial activity of the alcoholic and aqueous extracts of white and the color skin of *Citrus medica* (C. *medica*) n 10 human pathogenic bacteria and the phytochemical investigations of some

secondary metabolites (9). The other evidence is related to the investigation of the antibacterial and antioxidant activity of the root, stem, and seed of *C. medica* L extracts (10).

Phenolic and flavonoid compounds exhibit antioxidant activities by inactivating active oxygen species (11). The evidence indicates that the absorption of plant flavonoids in human beings reduces the risk of cardiovascular diseases (12). Another study reported the total phenolic and flavonoid contents of the color and the white skin of *C. medica* (9), along with the total phenolic and flavonoid contents of the root, stem, and seed of *C. medica* (10). Given the above-mentioned explanations, the aim of this study was to investigate the antibacterial and antioxidant activity of various extracts of *C. maxima* against 12 human pathogenic bacteria in vitro. Furthermore, the presence of alkaloid, saponin, and tannin was checked in the root, stem, and seed of the methanol extract.

Materials and Methods

Chemical Materials

Mueller-Hinton agar (MHA) and nutrient broth (NB) culture media, 2,2-diphenyl-1-picrylhydrazyl (DPPH), quercetin and gallic acid from Merck Company (Darmstadt, Germany), as well as gentamicin and ciprofloxacin antibiotics from Paten Tab Company (Tehran, Iran) were prepared based on the aim of the study.

Plant Extracts

The samples (i.e. root, stem, and seed) of *C. maxima* Merr. (Syn. *C. grandis*) were collected from Babolsar, Mazandaran province, Iran and then transferred to the Biotechnology Laboratory and dried under shadow at Bu-Ali Sina University. In addition, methanol (80%), ethanol (96%), and distilled water extracts were obtained according to the method by Fuselli et al (13). Next, 25 g of the dried powder was added to 250 mL of the solvent and shaken, and then the extracts were filtered and centrifuged at 10 000 rpm for 8 minutes after 8 hours. Finally, the obtained crude extract was transferred to an oven at 37 °C for complete drying (9), and the residue was stored in a dark glass bottle at -22°C freezer until further use (14).

Bacterial Strains

The antibacterial activity of *C. maxima* extracts was performed in vitro against Gram-positive and negative bacteria. The first group included *Streptococcus pyogenes* (PTCC-1447), *Bacillus subtilis* (PTCC-1156), *Bacillus cereus* (PTCC-1247), *Enterococcus faecalis* (PTCC-1185), *Micrococcus luteus* (ATCC 10987), and *Staphylococcus aureus* (PTCC-1189). Further, the second group encompassed *Escherichia coli* (ATCC-25922), *Shigella boydi*(-), *Salmonella typhi* (PTCC-1609), *Pseudomonas aeruginosa* (PTCC-1181), *Enterobacter aerogenes* (PTCC-1221), and *Klebsiella pneumoniae* (PTCC-1139). A bacterial colony was transferred to the MHA medium and incubated for 24 hours at 37°C, and then a loop of the bacterial colony was transferred to 1 mL of the NB medium and incubated at 37°C for 24 hours (15). Eventually, the turbidity of the suspension was adjusted to an equivalent 0.5 McFarland standard (ca. 1.5×10^8 CFU).

Agar Well Diffusion Assay

The antibacterial activity was used from crude extracts by agar well diffusion assay (16). Furthermore, ethanol (96%), methanol (80%), and distilled water extracts (200 mg mL^{-1}) were prepared from the roots, seeds, and leaves of *C. maxima*. Moreover, a volume of 250 mL of bacterial suspension (1.5×10^8 CFU) was poured onto the MHA medium. The wells (5 mm diameter) were created, and 50 µL of each extract solution was poured into individual wells and then the plates were incubated at 37°C for 24 hours (15). Gentamicin (10 µg) and ciprofloxacin (0.005 µg) were applied as positive controls (17). Then, the inhibitory zone around each well was measured (cm), and the results were analyzed by SAS software in triple.

Determination of Minimum Inhibitory Concentration and Minimum Bactericidal Concentration

The Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) of ethanol, methanol, and distilled water extracts were determined by the serial dilution method (15). The dilution series of 100, 50, 25, 12.5, 6.25, and 3.125 mg mL^{-1} were prepared for the MIC experiment. Additionally, a volume of 185 µL of the fresh culture medium was poured into each tube and then 200 µL of the extract was added to the first tube. Afterward, 200 µL of which was transferred to the second tube, and the process continued. Finally, 15 µL of bacterial suspension (1.5×10^8 CFU) was added to all tested tubes. Next, the tubes were incubated for 24 hours at 37°C. The lowest extract dilution with no growth was considered as MIC. To measure MBC, a volume of 5 µL of the plates with no human bacterial growth was added to the MHA culture medium. Eventually, the plates were incubated for 24 hours at 37°C, and the minimum concentration with no bacterial growth was considered as MBC.

Determination of Total Phenolic and Flavonoid Contents

The total phenolic content was performed according to the Folin-Ciocalteu method (18). In addition, the absorbance of samples was measured at 765 nm by a spectrophotometer as mg of the gallic acid per gram of dry extract weight (mgGA/g) after 15 minutes at 25°C in darkness. Then, the total flavonoid content was determined using the aluminum chloride method (19).

Finally, sample absorption was measured at 415 nm using a spectrophotometer as the mg of quercetin per gram of dry extract weight (mgQ/g) after 30 minutes at room temperature.

DPPH

The free radical activity was determined according to Stojičević et al (20). Different concentrations (i.e., 0.2, 0.4, 0.6, 0.8, and 1 mg mL^{-1}) of the root, seed, and leaf methanol extracts were prepared, and the ascorbic acid was used as the standard. Then, sample absorption was measured at 517 nm by a spectrophotometer after 30 minutes in darkness. The free radical scavenging activity (%) was calculated as follow:

RSA (%) = 100 (1 - (As - Ab)/Ac

As: Sample

Ab: Blank (methanol 99%)

Ac: Control

Identification of Phytochemical Compounds

To detect the presence of alkaloids, 0.5 g of methanol extract was dissolved in 5 mL HCl (1.0%) and kept in a warm distilled water bath for 5 minutes. Then, the solution was passed through the filter paper, and few drops of Mayer's reagent were added to it. The sediment or turbidity was considered as an indication of the presence of alkaloids (21,22). To detect the presence of tannin, 0.5 g of the methanol extract was dissolved in 5 mL distilled water and passed through a filter paper. Then, a few drops of $FeCl_3$ chloride (10%) were added as well. The observation of the black-green color indicated the presence of tannin (23). Further, 20 mL of distilled water was added to 0.25 g of the methanol extract and boiled to detect the presence of saponin. Then, it was passed through a filter paper, and 5 mL of it was mixed with 20 mL of distilled water and shaken. The stable foam on the paper represented the presence of saponin (23).

Statistical Analysis

The experiment was performed by a factorial test using a completely randomized design. The average comparisons were analyzed by the Duncan test at (P<0.01) with three replications by SPSS software, version 16.

Results

Antibacterial Activity

The diameter of the bacteria growth-inhibitory zone around the wells was measured as well. Table 1 presents the inhibitory effects of different alcoholic and aqueous extracts of the root, seed, and leaf of *Citrus maxima* against human pathogenic bacteria. Gentamicin and ciprofloxacin antibiotics were used as a positive control. Based on the results, ciprofloxacin showed the highest inhibitory effect on *Shigella boydii* (Table 1) while negative controls demonstrated no inhibitory effect on bacteria growth.

The methanol extract of the *C. maxima* root exhibited the most potent activity against *M. luteus*. Conversely, the root aqueous extract revealed no inhibitory effect on the growth of *M. luteus, S. boydi*, and *P. aeruginosa.* Furthermore, the methanol and ethanol extracts of the root

Table 1. Antibacterial Activity of Methanol, Ethanol, and Aqueous Extracts Obtained From the Roots, Leaves, and Seeds of *Citrus maxima* Against Human Pathogenic Bacteria

Bacteria	Leaf			Root			Seed			Gentamicin	Ciprofloxacin
	Methanol	Ethanol	Aqueous	Methanol	Ethanol	Aqueous	Methanol	Ethanol	Aqueous		
B. subtilis	14±0.57	13.67±0.33	11.67±0.33	14±1.73	11.67±0.88	9.33±0.33	13±1.52	15.67±1.2	14±0.57	29±0.57	29.5±0.33
B. cereus	17.33±0.33	12.67±0.88	11.33±1.2	8.33±0.88	11±1.53	9.67±0.33	11.33±0.33	12.67±0.33	11.33±1.45	19.66±0.33	28.5±0.66
E. faecalis	13±0.33	12±0.58	10±0.88	14±0.66	114.5±0.33	11±0.33	15±.088	12±0.88	9±0.66	19±0.33	22±0.33
S. aureus	21.67±0.88	20.67±0.33	14.33±0.33	17.67±0.33	15.33±2.4	10±0.58	6.67±0.33	12.67±0.66	13±0.57	20±1	28.5±0.66
S. pyogenes	7.33±0.33	4.67±2.33	8.33±0.33	0±0.00	0 ±0.00	8±1	0 ±0.00	0 ±0.00	0 ±0.00	20±0.57	31.5±0.33
M. luteus	24.33±0.88	17.33±0.33	15±0.57	26.67±1.76	13.67±0.33	0±0.00	13.67±0.66	14.33±1.2	11.67±1.45	22±0.33	30±1
S. typhi	12±0.57	12±0.57	9.67±0.33	13±0.58	14±1	15.67±0.88	10.33±0.33	9.33±1.2	8.33±0.88	29.5±1	33±0.57
K. pneumoniae	14±0.33	13.5±0.57	10±0	13.5±0.88	12±0.88	9.5±0.33	12±0	11.5±0.33	10±0.88	18±.033	24±088
Sh. boydii	11±0.57	9.33±0.33	8.67±0.88	13.33±0.33	10.33±0.88	0±0.00	12.67±0.33	11.67±0.88	11±0.57	19±0.57	37.5±0.66
P. aeruginosa	11±0.57	10.67±0.88	10.67±0.88	15.33±1.2	1.67±0.67	0±0.00	0 ±0.00	0 ±0.00	0 ±0.00	20±0.33	24.5±0.66
E. coli	21±0.57	12.67±0.88	9.67±1.76	19.67±0.88	15.67±1.2	12.33±0.33	7.33±0.33	11.33±0.66	9±0.57	19.5±1	24.5±0.57
E. aerogenes	15±0.57	12.33±0.66	6±3.21	22±0.58	20.67±0.88	9.33±1.33	8.33±0.33	11.33±0.33	12.67±0.88	11±0.33	28±0.33

Note. B. subtilis: Bacillus subtilis; B. cereus: Bacillus cereus; E. faecalis: Enterococcus faecalis; S. aureus: Staphylococcus aureus; S. pyogenes: Streptococcus pyogenes; M. luteus; Micrococcus luteus; S. typhi: Salmonella Typhi; K. pneumoniae: Klebsiella pneumoniae; Sh. boydii: Shigella boydii; P. aeruginosa: Pseudomonas aeruginosa; E. coli: Escherichia coli; E. aerogenes: Enterobacter aerogenes.

exerted no inhibitory effect on the growth of *S. pyogenes*. However, the root methanol extract showed better antibacterial activities against *M. luteus* and *E. aerogenes*. Moreover, the methanol extract of the leaves exhibited better inhibitory effects on *S. aureus, M. luteus, E. coli,* and *E. aerogenes* compared to gentamicin (Table 1). Based on the findings, the ethanol extracts of the *C. maxima* seed demonstrated the highest diameter of the inhibitory zone against *B. subtilis*. Similarly, the ethanol and aqueous extracts of the seed showed further inhibitory effects on *E. aerogenes* when compared to gentamicin. The ethanol extract of the seed was more effective in comparison to methanol and aqueous extracts. However, *P. aeruginosa* and *S. pyogenes* bacteria represented resistance against different extracts of seeds.

Determination of Minimum Inhibitory Concentration and Minimum Bactericidal Concentration

As shown in Table 2, the root methanol extract against *M. luteus* and the leaf methanol extract on *E. coli* and *M. luteus* exhibited MIC of 3.125 mg mL^{-1}. The seed methanol extract showed a MIC of 6.25 mg mL^{-1} on *M. luteus*. The leaf methanol extract on *M. luteus* demonstrated an MBC of 6.25 mg mL^{-1}. Accordingly, the methanol extract was more potent compared to ethanol and aqueous extracts. Based on the results, *S. pyogenes* presented resistance in different extracts of *C. maxima* in the serial dilution test. The aqueous extract of *C. maxima* had no effect on *E. faecalis* and *K. pneumonia.*

Determination of Total Phenolic Content and Flavonoid

Table 3 provides the results of the total phenolic and flavonoid content of the root, seed, and leaf methanol extracts of *C. maxima*. The total phenolic content of the root, seed, and leaf was determined as 98.22, 89.66, and 77.51 (mgGA/g), respectively. Additionally, the flavonoid content was measured as 3.52, 3.43, and 3.56 (mgQ/g), respectively. Finally, the methanol extracts of *C. maxima* showed a significant difference in phenolic content.

Antioxidant Activity

The antioxidant activity of the root, seed, and leaf methanol extract using DPPH is presented in Table 4. The ascorbic acid was used as the standard, and it was observed that the amount of the free radical inhibition of DPPH increased by increasing extract concentration. The IC_{50} of the root, seed, leaf, and ascorbic acid was measured

Table 2. MIC and MBC (mg mL^{-1}) of *C. maxima* Different Extracts Against Human Pathogenic Bacteria

		Bacteria	*B. subtilis*	*B. cereus*	*S. aureus*	*M. luteus*	*E. aerogenes*	*S. typhi*	*P. aeruginosa*	*E. coli*	*S. pyogenes*	*Sh. boydii*	*E. faecalis*	*K. pneumoniae*
Leaf	M	MIC	50	12.5	12.5	3.125	50	50	50	3.125	-	-	25	50
		MBC	100	50	25	6.25	50	100	-	50	-	-	50	100
	E	MIC	50	50	50	50	50	100	100	50	-	50	50	100
		MBC	100	100	-	100	50	100	-	50	-	100	50	100
	A	MIC	50	100	50	100	-	100	100	-	-	-	-	-
		MBC	-	-	-	-	-	100	-	-	-	-	-	-
Seed	M	MIC	12.5	25	50	6.25	-	50	-	-	-	100	50	50
		MBC	50	100	100	25	-	100	-	-	-	-	100	100
	E	MIC	50	50	50	25	100	-	-	50	-	50	100	100
		MBC	100	100	100	50	100	-	-	100	-	-	-	-
	A	MIC	50	100	100	50	100	-	-	-	-	100	-	-
		MBC	100	100	-	100	-	-	-	-	-	-	-	-
Root	M	MIC	50	-	50	3.125	50	50	100	50	-	50	50	100
		MBC	-	-	50	25	50	25	100	50	-	100	100	100
	E	MIC	100	100	100	100	100	100	50	25	-	100	100	100
		MBC	100	100	-	-	100	-	100	50	-	-	100	-
	A	MIC	-	-	100	100	100	100	100	100	-	100	-	-
		MBC	-	-	-	-	-	-	-	100	-	-	-	-

Note. MIC: Minimum inhibitory concentration; MBC; Minimum bactericidal concentration; M: Methanol; E: Ethanol; A: Aqueous. *B. subtilis: Bacillus subtilis; B. cereus: Bacillus cereus; S. aureus: Staphylococcus aureus; Micrococcus luteus; M. luteus; E. aerogenes: Enterobacter aerogenes; S. typhi: Salmonella Typhi; P. aeruginosa: Pseudomonas aeruginosa; E. coli: Escherichia coli; S. pyogenes: Streptococcus pyogenes; Sh. boydii: Shigella boydii; E. faecalis: Enterococcus faecalis; K. pneumoniae: Klebsiella pneumoniae.*

Table 3. Total Phenolic and Flavonoid Contents of the Root, Seed, and Leaf Methanol Extract of *Citrus maxima*

	Root	Seed	Leaf
Phenol (mgGA/g)	98.22[a]	89.66[b]	77.51[c]
Flavonoid (mgQ/g)	3.52[a]	3.43[a]	3.56[a]

Note. The same letters are not significantly different at $P<0.01$.

Table 4. Antioxidant Activity (IC50: mg mL^{-1}) of *Citrus maxima* Different Extracts and Inhibition Percentage of DPPH

	Inhibition Percentage of DPPH (mg mL^{-1})					IC50
	0.2	0.4	0.6	0.8	1	
Root	77.38	86.56	94.15	97.92	97.39	0.129[a]
Seed	74.06	80.2	87.41	93.85	96.65	0.135[a]
Leaf	87.93	94.21	96.34	98.02	98.94	0.113[b]
Ascorbic acid	91.3	92.43	97.41	98.56	99.67	0.109[b]

Note. DPPH: Determination of 2,2-diphenyl-1-picrylhydrazyl; IC50: Changing 50% inhibitory concentration. Same letters are not significantly different at $P<0.01$.

as 0.129, 0.135, 0.113, and 0.109 mg mL^{-1}, respectively. Based on the obtained data, a significant difference was observed between the IC50 values of the root and the seed methanol extract with ascorbic acid.

Investigation of Phytochemical Compounds

The presence of alkaloid, saponin, and tannin were tested in the methanol extract. The leaf and root methanol extract showed the presence of alkaloid, saponin, and tannin. Based on the results, alkaloid was only observed in the methanol extract of the seed (Table 5).

Discussion

Due to the resistance of microbes, especially the pathogenic bacteria against synthetic antibiotics which cause infectious diseases, medicinal plants with antimicrobial effects are suggested to be used (24). In most cases, the extracts had higher inhibitory activity, especially on gram-positive bacteria. Once a bacteriostatic agent can be selected as a drug, its MBC value is three times higher than its MIC value (25). There has been no research on the antimicrobial activity of the root, seed, and leaf extracts of *C. maxima*. Therefore, our results were compared to the, reports related to the plant extracts which are close to this species.

According to the results of Mokbel and Suganuma (8), the methanol (80%) extract of *C. grandis* on *S. aureus* demonstrated a better inhibitory effect, which is similar to our results. In their study, Kabra et al (26) reported that *P. aeruginosa* was more susceptible whereas resistance was observed against different extracts from the seeds in the present study. In another study (9), the highest inhibitory activity was related to the methanol extract of *C. medica* color skin against *B. cereus* (24±0.57 mm) while the root methanol extract of *C. maxima* exhibited the most potent activity against *M. luteus* (26.67±1.76 mm). Based on the results of Shojaemehr et al (10), the methanol extract of the root showed the highest inhibitory activity against *M. luteus,* which is in line with our results. The MIC of the leaf methanol extract from *C. medica* against *E. coli* was obtained in 6.25 mg mL^{-1} (10) although, in our study, the MIC of the *C. maxima* methanol extract was observed on *M. luteus* in 6.25 mg mL^{-1}. Differences in the degree of bacteria growth sensitivity against the plant extract are related to the intrinsic tolerance of microorganisms, the type of solvent, and the present of antimicrobial compounds in plant extracts including alkaloids, tannins, saponins, phenols, glycosides, and flavonoids, and the like (27).

An antioxidant is a substance that can prevent or delay the oxidative damage to the target molecule and is a major factor in the neutralization of free radicals. In addition, antioxidants reduce the risk of developing cardiovascular diseases and prevent the progression of cancers (28). The results of an empirical study demonstrated that polyphenols play an important role in the prevention of cardiovascular disease, cancer, osteoporosis, diabetes, and neurological diseases (29).

Likewise, Mokbel and Suganuma (8) reported the percentage of the inhibition of the free radicals of the *C. maxima* methanol extract as 74.5%. The IC_{50} value of the *C. medica* hexane extract was measured as 0.147 mg mL^{-1}, which is consistent with the findings of the present study (30). The IC_{50} of the methanol extract of the color and white skin and ascorbic acid were calculated as 0.1505, 0.1738, and 0.1095 mg mL^{-1}. On other hand, the total phenolic content and flavonoid content were reported as 109.5 and 105.6 (mgGA/g), as well as 3.53 and 3.02 (mgQ/g) from color and white skin, respectively (9), which is proximately similar to our results. Similarly, Ghasemi et al (31) measured the total phenolic content and flavonoid content from the methanol extract of 13 Iranian citrus species in the range of 66.5-396.8 (mgGA/g) and 0.3-31.1 (mgQ/g), respectively, which contradicts the results of the present study. Further, the total phenolic content and total flavonoid content from the root, seed, and leaf methanol extract of *C. medica* were determined as 106.1, 103.8, and 102.7 (mgGA/g), as well as 3.24, 3.02, and 3.96 (mgQ/g), respectively (10), which is inconsistent with the result of our study.

Table 5. The Presence of Alkaloid, Saponin, and Tannin in *Citrus maxima* Methanol Extract

	Alkaloid	Saponin	Tannin
Root	+	+	+
Leaf	+	+	+
Seed	+	-	-

Furthermore, Wu et al (32) demonstrated the presence of alkaloids in the root acetone extract of *C. maxima*. The presence of tannin, alkaloids, and saponin was reported in the leaf diethyl ether extract of *C. maxima* (33). According to the results of Reddy et al (34), the presence of alkaloids, saponins, and tannins was observed in the leaf methanol extract of *C. aurantium*. In another study, Mishra et al (35) indicated the presence of tannin and saponin in the skin methanol extract of *C. limetta*. Similarly, another study confirmed the presence of saponin and alkaloids from the skin ethanol extract of *C. reticula, C. sinensis,* and *C. maxima* (36). Moreover, Shojaemehr and Alamholo (9) reported the presence of alkaloids while the absence of saponin and tannin from the color skin methanol extract of *C. medica*, which is similar to our findings. In their study, Sheikhlar et al (37) showed the presence of tannin and alkaloid while the absence of saponin in the skin methanol extract of *C. limon*. As reported in (9), the leaf methanol extract of *C. medica* revealed the presence of alkaloid, saponin, and tannins. Finally, the difference in the presence and the absence of secondary metabolites in plants rely on the type of species, extract type, solvent, and testing methods (38).

Conclusions

Overall, the highest sensitivity was observed on *M. luteus* against the root methanol extract of *C. maxima*. Based on the findings, the antibacterial activity of the *C. maxima* extract on some human pathogenic bacteria was stabilized, and the results of this research showed better antiradical and antioxidant activity. In addition, the phytochemical analysis of this plant extract represented the presence of some secondary metabolites. Accordingly, it is suggested to create antimicrobial drugs in medicinal and pharmaceutical sciences based on the chemical composition analysis from the extract of this ethnomedicinal plant.

Conflict of Interests

The authors declare that they have no conflict of interests.

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Ethical Approval

Not applicable

Authors' Contribution

In this study, the scientific and professional advice, and also writing and submitting the article was done by MA. In addition, the most laboratory tests and statistical analysis of MS and editing the article and advice from JS were done. Eventually, all laboratory tests were performed at the Buali Sina University, Hamadan, Iran.

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