



RESEARCH ARTICLE

Anticancer, antioxidant and antibacterial activities of extracts from *Scrophularia striata* and *Elaeagnus angustifolia*, growing in Ilam and Kurdistan provinces in Iran

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Abstract

Emerging evidence of the impact of plant compounds on growth inhibition of micropathogens and cancer cells has opened new areas to evaluate plants' treatment properties. Here, we aimed to investigate *in vitro* antioxidant, antibacterial and anticancer effects of the secondary metabolites isolated from different extracts produced by *Elaeagnus angustifolia* and *Scrophularia striata*. Antibacterial activity was evaluated against human and plant pathogenic bacteria by 3 methods of tubular dilution, well and disc diffusion. The anticancer effect of *E. angustifolia* extract was assessed by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay. Biochemical experiments showed the presence of compounds such as phenol, flavonoids, resins, quinones, steroids, terpenoids and alkaloids in extracts, with the highest antioxidant activity of the methanolic extracts. For both plants' strains, the disc method was more effective than the well diffusion method. The highest yields were obtained from Methanolic, ethanolic and hydroalcoholic extracts for *E. angustifolia* and aqueous extract for *Scrophularia striata*. The most sensitive bacteria for *E. angustifolia* were *Bacillus subtilis* and *Xanthomonas campestris* against pit extracts and *Clavibacter michiganensis* against pulp extracts. The most sensitive bacteria for *S. striata* were *Bacillus subtilis* and *Staphylococcus aureus*. Methanolic and aqueous solvents showed the maximum bacterial inhibitory and bactericidal activities in the Minimum inhibitory concentration (MIC) and the minimum bactericidal concentration (MBC) tests respectively. Additionally, *E. angustifolia* showed anticancer effects toward MCF7 breast cancer cells. These findings provided a better understanding of the widespread application of these plants as potential antioxidants, antibacterial and anticancer sources, and safe natural medicines in health maintenance and disease treatment.

Keywords

Elaeagnus angustifolia, *Scrophularia striata*, antioxidant, antibacterial, anticancer, *Xanthomonas campestris*, *Bacillus subtilis*

Introduction

Nearly a quarter of the world's drugs are botanically derived, either directly extracted from plants or synthesized based on herbal compounds. Due to the involvement of oxidative stress in a variety of disorders, aging and can-

cer, the evaluation of novel herbal antioxidant drugs to prevent or reduce the risk of diseases has received increasing attention (1, 2). Plant secondary metabolites such as phenolics and flavonoids, which are present in all parts of plants, have the potential to neutralize free radicals. Hence, due to the high prevalence of chronic diseases, using of plants to provide essential antioxidants, especially plants with high content of phenol and flavonoids is warranted (3, 4).

On the other hand, infectious diseases are one of the most important causes of mortality, and a large number of outbreaks are associated with these infections such as avian flu influenza A, tuberculosis, *Emergomyces canadensis* and diarrheal diseases, which require antibiotic treatment (5, 6). The application of synthetic antibiotics has led to the emergence of resistant bacteria, especially *Klebsiella*, *Escherichia coli*, *Staphylococcus aureus*, *S. epidermis*, *Sarajea* and *Salmonella* (7, 8), so antibiotic resistance is an account for the treatment failure of infectious diseases all over the world in recent years (6). Decreasing the clinical efficacy of many antibiotics with the emergence of increasing antibiotic resistance by microbial infectious agents has raised the screening of herbs with antimicrobial activity in the treatment of infectious diseases (9). Herbal medicines are widely prescribed due to their natural sources, fewer side effects, relatively low costs and more efficacies. Hence, researchers have focused on medicinal plants and traditional medicine to find more efficient drugs against microbial infections (10, 11). In recent research, aqueous and organic extracts of medicinal herbs were studied against the bacterial species including *Enterococcus faecalis*, *Bacillus subtilis*, resistant strains of *Staphylococcus aureus* and 2 sensitive species of *Escherichia coli*. They showed substantial antimicrobial properties, the highest antibacterial activity was related to *Thymus vulgaris* and *T. origanum* herbs (12, 13). Moreover, the effect of 45 species from 30 plant families that were applied in Iranian traditional medicine for the treatment of infectious diseases and burns were evaluated on different bacterial strains including *Bacillus cereus*, *B. pumilus*, *Escherichia coli*, *Klebsiella*, *Pseudomonas aeruginosa*, *P. fluorescens*, *Micrococcus luteus*, *Serratia*, *Staphylococcus aureus* and *S. epidermis*. The results indicated that all methanolic extracts of plants prepared by soaking (3 days at room temperature) show the effects on selected germs other than *Serratia* and *Bordetella* on disc plates on the microbes (14, 15).

One of the herbal remedies in traditional medicine is the *E. angustifolia* as a medication for joint pains and as epileptic seizures (16). Persian olive with the scientific name of *E. angustifolia*, which belongs to the family of *Elaeagnaceae*, contains flavones and polyphenols, as well as resin aromatic compounds (cinnamic acid, benzoic acid, phenyl ether benzoate and hydro benzoic acid) (17). *E. angustifolia* fruit is applied to treat amoebic diarrhea, and also the fruit and flowers of this plant are used in traditional medicine to treat nausea, vomiting, asthma, jaundice and abdominal distension (18). Until now, about 42 compounds with anti-inflammatory and anti-infectious properties are known in the *E. angustifolia* flowers such as limo-

nene hydrocarbons, squalene, monoterpene and tertiary hydrocarbons (19). The *E. angustifolia* fruit is sweet and astringent with a lot of malic acid, calcium malate, ascorbic acid and butyric acid. In addition to use in treatment of scurvy disease, it has a palliative effect on coughing (20). In addition, the aqueous extract of the kernel, exocarp and mesocarp has been shown to have an analgesic effect comparable to that of morphine (21). Overall, scientific studies on *E. angustifolia* have shown several pharmacological effects.

Scrophularia striata is a member of family *Scrophulariaceae*, which this family consists of 200 genera and 3000 species, that have long been applied in Iran as a medicinal herb for the treatment of various diseases such as rheumatics, allergy, eczema, goiter and chronic inflammatory disorders (22-24). Emerging studies have demonstrated the antibacterial, antioxidant and anticancer properties of *Scrophularia* (22, 25-27). However, people living in the province of Ilam have been experiencing with this plant for many years, the chemical composition of this plant and its medical application remain to be further determined. Regarding these proven antibacterial and antifungal advantages as well as curative properties of *E. angustifolia* and *S. striata*, here, we aimed to investigate the antioxidant and antibacterial effects of both *E. angustifolia* and *S. striata* extracts from two different provinces in Iran on some of important human and plant pathogenic bacteria. Moreover, the cytotoxicity effect of *E. angustifolia* on cancer cells was also evaluated. The results of this study would be useful to justify which components of these plants have more potential application in herbal medicine.

Materials and Methods

Plant's material and Sample preparation

Regarding the existing vegetation diversity of plants, the native *S. striata* samples were collected from Ilam province, the regions of Mimak, Saleh Abad, Mehran. To further confirmation, the plants were approved by the botanist of the Agricultural Research Center of Ilam province. *E. angustifolia* fruits were collected in the growing season from gardens around Kurdistan province and then confirmed with the identification keys. The aerial parts and fruits of samples were isolated and dried at the normal room shade temperature. Then the pit and pulp were separated from each other. To evaluate antibacterial and antioxidant properties as well as anticancer activity, dried plant specimens were powdered. In this study ethanol, methanol, hydroalcoholic, water and aqueous solvents were used for extraction as described by standard procedure (28). The extracts were condensed with a rotary machine (model LEV311, LabTech Italy) and dried with a freeze dryer (BETA1-8LDPLUS, German Christ company). To investigate the antibacterial effects of plant extracts, the ethanolic, methanolic and hydroalcoholic extracts with concentrations of 50, 100, 200, 400 mg of powdered extract in 1000 ml of dimethyl sulfoxide (DMSO) were prepared.

Source of microorganisms and cell line

Bacterial species, *Staphylococcus aureus*, *Bacillus cereus*, *B. subtilis*, *Enterococcus faecalis*, *Salmonella typhi*, *Xanthomonas campestris*, *Clavibacter michiganensis*, *Ralstonia solanacearum* all were procured from the Iranian Research Organization for Science and Technology as lyophilized. The human MCF7 breast cancer cell line was obtained from the Iranian Biological Research Center (IBRC).

Identification of different plant compounds with antioxidant properties

To determine the antioxidant properties of plant compounds, measurements of phenol, flavonoids, resins, quinones, steroids, terpenoids and alkaloids were performed according to method described by other researchers (29, 30). Briefly, phytochemical analyses of the extracts were performed as following;

Alkaloids: Some drops of Wagner's reagent were added to 1 ml of filtered methanolic and ethanolic extracts and the brownish-red precipitate pointed towards the presence of alkaloids.

Steroids (Liebermann-Burchard reaction): 1 ml of filtered methanolic and ethanolic extracts were mixed with 1 ml of a solution containing 4 ml acetic acid, 1 ml sulfuric acid and 2 ml chloroform. The formation of a blue-green ring was considered as evidence of the presence of steroids.

Flavonoids: 0.5 ml of each extract in methanol was added to 2 ml of 21% sulfuric acid solution and a yellow to orange coloration depicting the positive results for the presence of flavonoids.

Terpenoids: A volume of 200 μ l methanolic and ethanolic extracts was mixed with 1.0 ml of chloroform using vigorous shaking. Then, 1 ml of concentrated sulphuric acid was added. A reddish-brown or gray coloration of the mixture indicates the presence of terpenoids.

Phenolics: A mixture of 0.5 ml of filtered methanolic extracts and 3% (w/v) of ferric chloride was prepared. A formation of dark green to black coloration indicated the presence of tannin and phenol.

Antioxidant activity assay using 2,2-Diphenyl-1-picrylhydrazyl (DPPH)

To determine the anti-radical activity, DPPH radical scavenging assay was performed. Solutions were prepared as following: A) Methanolic solution of DPPH (Sigma, Germany) at a concentration of 0.0003 M, B) Concentrations of 0.2, 0.4, 0.6, 0.8 and 1 mg/ml of each extract in methanol and C) Concentrations of 0.2, 0.4, 0.6, 0.8 and 1 mg/ml of ascorbic acid in methanol. 2.5 ml of methanolic extract solution was mixed with 1 ml of DPPH 3×10^{-2} M solution and placed at room temperature under dark conditions for 30 min. After that, the absorbance was measured by a spectrophotometer (Lambda 45-UV / Visible model) at 517 nm wavelengths. To measure the anti-radical activity, the absorbance values of the 3 following samples were measured: As: Absorption of the treatment sample containing the extract solution and DPPH (2.5 ml of the extract solution +1 ml DPPH solution), Ac: Absorption of control sam-

ple (2.5 ml of DPPH solution + 1 ml methanol) and Ab: absorbent of blank (2.5 ml of extract solution + 1 ml of methanol). Then, the percentage of anti-radical activity (RSA) was calculated from the following formula:

$$\text{DPPH - RSA(\%)} = 100 \left(1 - \frac{A_s - A_b}{A_c} \right)$$

For comparison, ascorbic acid was used as a stable anti-radical compound, and ascorbic acid solutions with similar concentrations of extract were prepared and used for the extract treatment. Next, to compare the activity of the herb extracts, the activity level was determined. All evaluations were performed as 3 replications for each extract (31).

Antibacterial assay

Agar-well diffusion method was used to evaluate antibacterial activity against human and animal pathogenic bacterial strains.

The aliquots of each extract (25 μ l) at different concentrations were added to 6-mm diameter wells cut into Mueller Hinton agar (MHA) plates infused with one of the test organisms. Solvent and Gentamycin (10 μ g) were used as negative and positive controls, respectively. Plates containing pathogenic bacteria were incubated for 24 hrs at 37 °C and 48 hrs at 28 °C plates for human and plant-containing pathogens bacterial respectively. After incubation, confluent bacterial growth was observed and the diameter of transparent halo around the wells was measured as inhibited bacterial growth. Moreover, the antibacterial activity of extracts was analyzed using the disc diffusion method. Antimicrobial properties were measured in 3 replications, and data analysis was performed using SAS software in a completely randomized design. Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of the extracts were determined using a modified broth microdilution method (32).

Anticancer activity assessment by MTT assay

MCF7 cells were cultured in respective growth medium, high glucose Dulbecco's modified Eagle's medium (DMEM, Gibco, Germany), supplemented with 10% fetal bovine serum (FBS, Gibco, Germany) and 100 U/ml penicillin, 100 μ g/ml streptomycin at 37 °C in 5% CO₂ in a humidified incubator. The media was replaced every three days. To evaluate the cell viability after *E. angustifolia* treatment, 70%-80% confluent MCF7 cells were detached with 0.05% trypsin/EDTA (Gibco, Germany) and washed with PBS and serum-free media. The cells were seeded at 10^4 cells for each well in 96-well flat-bottom plate. The cell media was replaced with 180 μ l of serum-free medium and 20 μ l of various concentrations of the *E. angustifolia* extract and the plate was incubated at 37 °C and 5% CO₂ for 24, 48, and 72 hr. Then, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay was performed according to the manufacturer's instructions (22). Briefly, the supernatant was removed, and each well was filled with 80 μ l of complete medium and 20 μ l of MTT solution (5 mg/ml). After 4 hrs, 80 μ l of the suspension were withdrawn and replaced with 100 μ l of DMSO in each well, and the absorb-

ance value was measured using multi-mode reader (Synergy HTX Multi-Mode Reader, Biotech) at 570 nm after 30 min of incubation in the dark. All tests were repeated 3 times and average values were calculated (22).

Statistical analysis

All test trials were performed in triplicate. Statistical data analyses were computed using SAS software in a completely randomized design and represented as mean values and standard deviations (mean \pm SD). The mean of Duncan's test at the $p < 0.05$ level was reported as repetitions average.

Results

Total content of *E. angustifolia* and *S. striata* extracts

The results from photochemical tests of *E. angustifolia* and *S. striata* extracts have been shown in Table 1. Based on these results, both the plants extracts contain flavonoid, phenol, resin, terpenoid and quinone metabolites, but have no steroids and alkaloids in pit extract.

DPPH radical scavenging (DPPH-inhibition percent) assay of *E. angustifolia* and *S. striata* extracts

Free radical scavenging activity of plant extracts was performed by DPPH assay as an easy, quick and reliable method to investigate the antioxidant activity of specific compounds or herbal extracts (33). In this evaluation, ascorbic

acid was used as a standard. Then, IC_{50} of each extract and ascorbic acid were calculated. The IC_{50} represents the concentration of the sample, which inhibits and scavenging of 50% of the DPPH free radicals. The values of inhibition of DPPH free radicals by various concentrations of plant extracts are presented in Table 2. IC_{50} values of *E. angustifolia* and *S. striata* extracts against ascorbic acid are shown in Fig. 1. The results indicated that the free radicals scavenging capacity of extracts increased with increasing the concentrations of herbal extracts. The most effective concentrations of the extracts for the calculation of IC_{50} are in the range of 0 to 0.2 mg/ml. The highest inhibition activities of DPPH-free radicals were recorded by the methanolic extract (0.137 mg/ml) and the pit extract (0.149 mg/ml) for *E. angustifolia* and *S. striata* respectively.

Antibacterial properties of *E. angustifolia* and *S. striata* extracts

Antibacterial activity of *E. angustifolia* extracts was examined by disc and well diffusion method, and the diameter of the growth inhibiting halo around the wells and discs was measured. Negative control groups containing 20 μ l of the used solvents as shown in Fig. 1, supplementary (a and b), did not exhibit an inhibitory effect on the target bacteria. Antibacterial effects of pit and pulp parts of *E. angustifolia* and aerial parts of *S. striata* were analyzed by disc and well methods. Regarding the significance of interaction between treatments, the mean comparison was done by

Table 1. The presence of secondary metabolites in the *E. angustifolia* and *S. striata* extracts.

Metabolite	<i>E. angustifolia</i> extracts				<i>S. striata</i> extracts	
	pit		pulp		Ethanol	Methanol
	Ethanol	Methanol	Ethanol	Methanol		
Phenol	+	+	+	+	+	+
Flavonoid	+	+	+	+	+	+
Resin	+	+	+	+	+	+
Quinone	+	+	+	+	+	+
Steroid	-	-	+	+	-	-
Terpenoid	-	-	+	+	+	+
Alkaloid	-	-	+	+	-	-

Table 2. Absorption of *E. angustifolia* and *S. striata* extracts specimens in the DPPH test As: Absorption of the treatment sample containing the extract solution and DPPH (2.5 ml of the extract solution +1 ml DPPH solution), Ac: Absorption of control sample (2.5 ml of DPPH solution + 1 ml methanol), and Ab: absorbent of blank (2.5 ml of extract solution + 1 ml of methanol).

Extract	Group	Absorbance and DPPH percent for extracts (mg/ml)						IC_{50}
		0.2	0.4	0.6	0.8	1.0		
<i>E. angustifolia</i> (pulp)	As	0.8657	0.6912	0.4832	0.3117	0.1880		
	Ab	0.0212	0.0718	0.0717	0.1112	0.1435	0.142 ^a	
	DPPH %	70.26	78.00	85.51	92.57	98.42		
<i>E. angustifolia</i> (Pit)	As	0.9688	0.7682	0.5200	0.3762	0.3125		
	Ab	0.0281	0.0428	0.0701	0.0825	0.1021	0.149 ^a	
	DPPH %	66.65	74.75	78.25	84	91		
<i>S. striata</i>	As	1.133	0.6815	0.4805	0.4905	0.3299		
	Ab	0.0652	0.0692	0.0787	0.0997	0.1423	0.160	
	DPPH %	62.40	78.44	82.33	62.39	93.39		

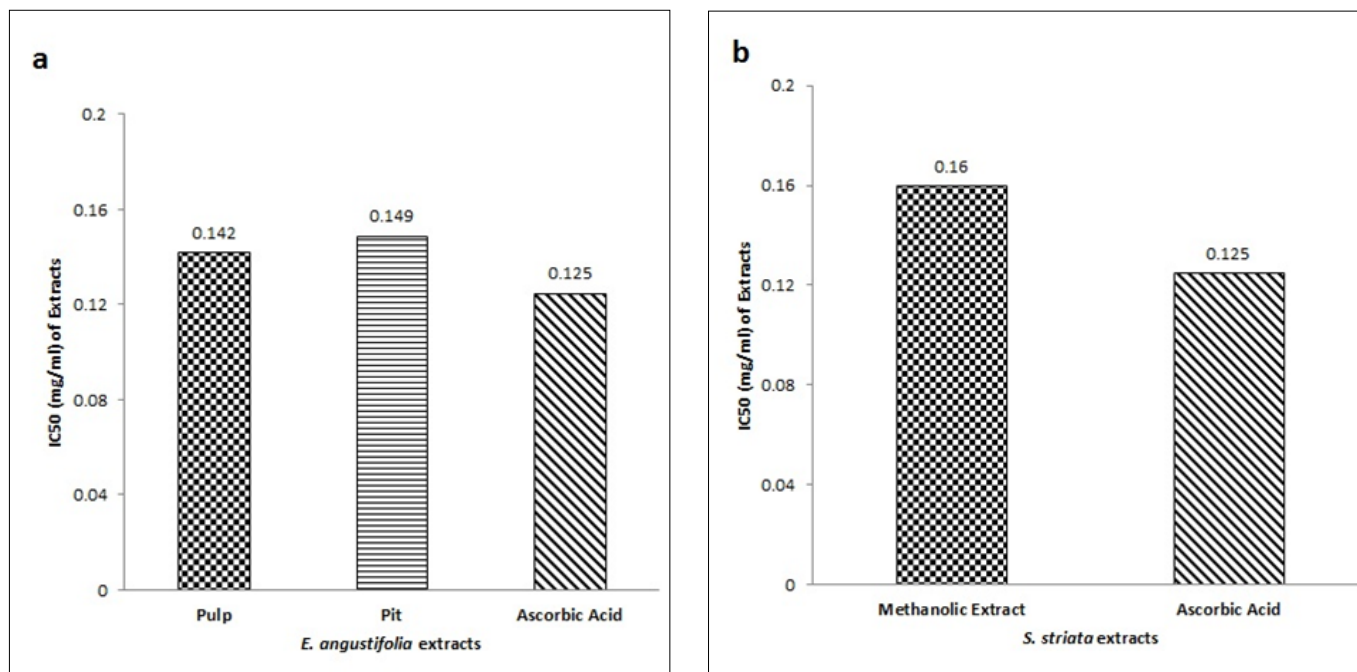


Fig. 1. The IC₅₀ values of extracts from *E. angustifolia* and *S. striata* as compared to ascorbic acid

Duncan method. Concentrations in antibacterial tests were included 50, 100, 200 and 400 mg/ml for each extract. Gentamicin antibiotic was used as a positive control (20 µg per disc) and bacterial halo was formed in bacterial culture medium (Fig. 2).

Antibacterial effect of *E. angustifolia* and *S. striata* extracts in the disc method

According to Table S1 and S2 from supplementary data, by comparing the effect of different extracts on bacteria, all of the tested bacteria showed different sensitivities in different concentrations of the extracts. In the first 24 hrs of

measurement, *Bacillus subtilis* showed a higher sensitivity than other bacteria in presence of *E. angustifolia* extracts and with a significant difference relative to other bacteria located in group a, *C. michiganensis* in group b, *X. campestris* in group c, *X. campestris* in group d, *Enterococcus faecalis* and *Salmonella typhi* without significant different in group e, and *Bacillus cereus* and *S. aureus* in groups f to g respectively. Ethanolic, hydroalcoholic and methanolic solvents without significant differences were located in group a, and aqueous solvent was in group b. Moreover, pit extract with a significant difference relative to pulp ex-

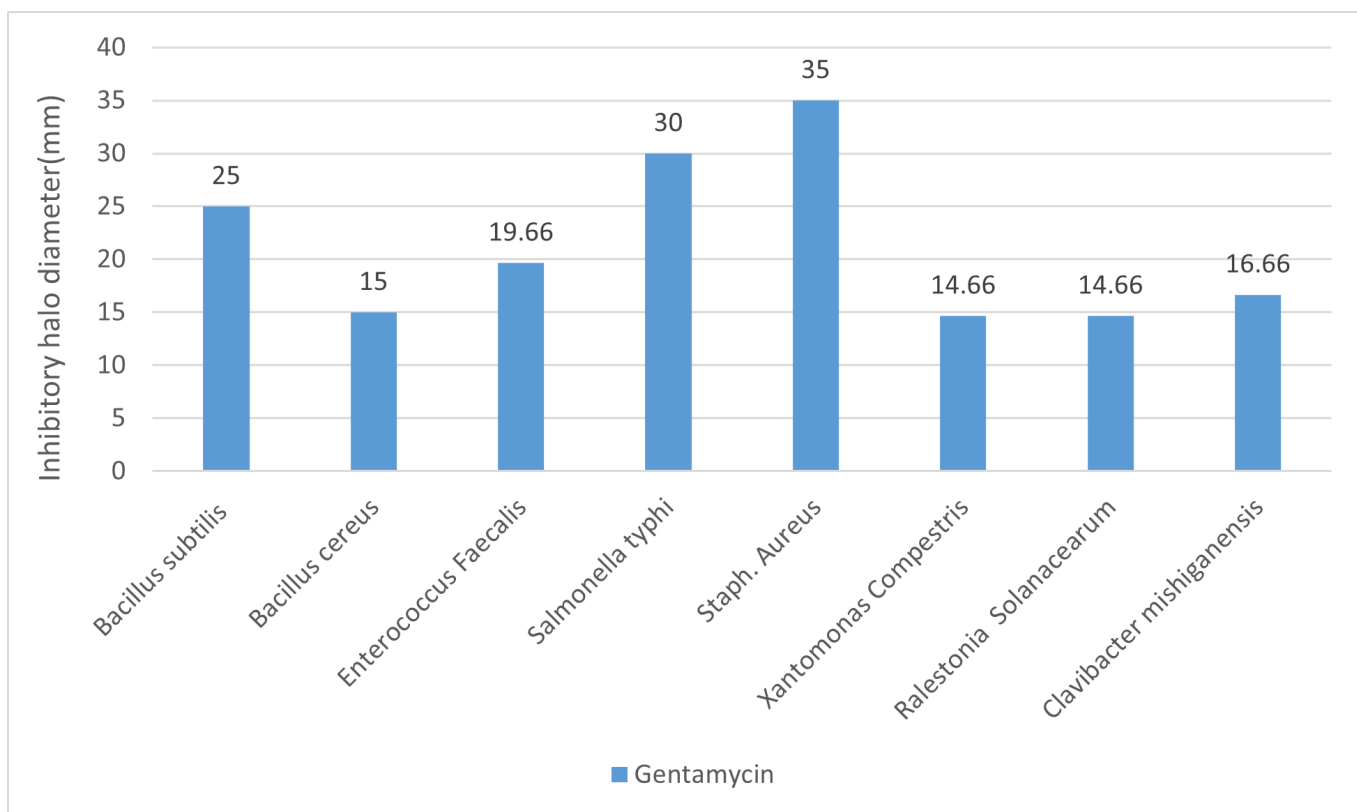
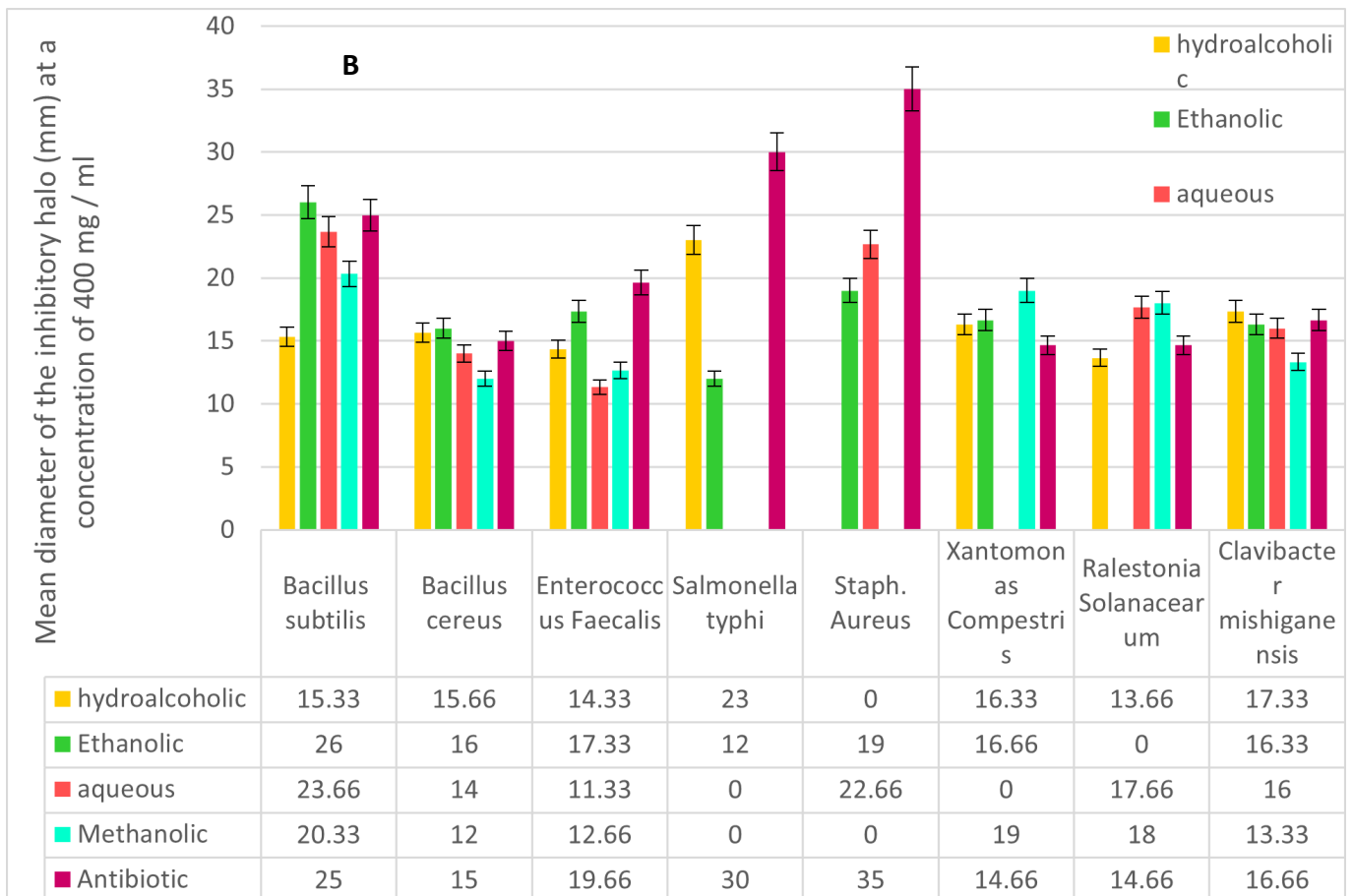
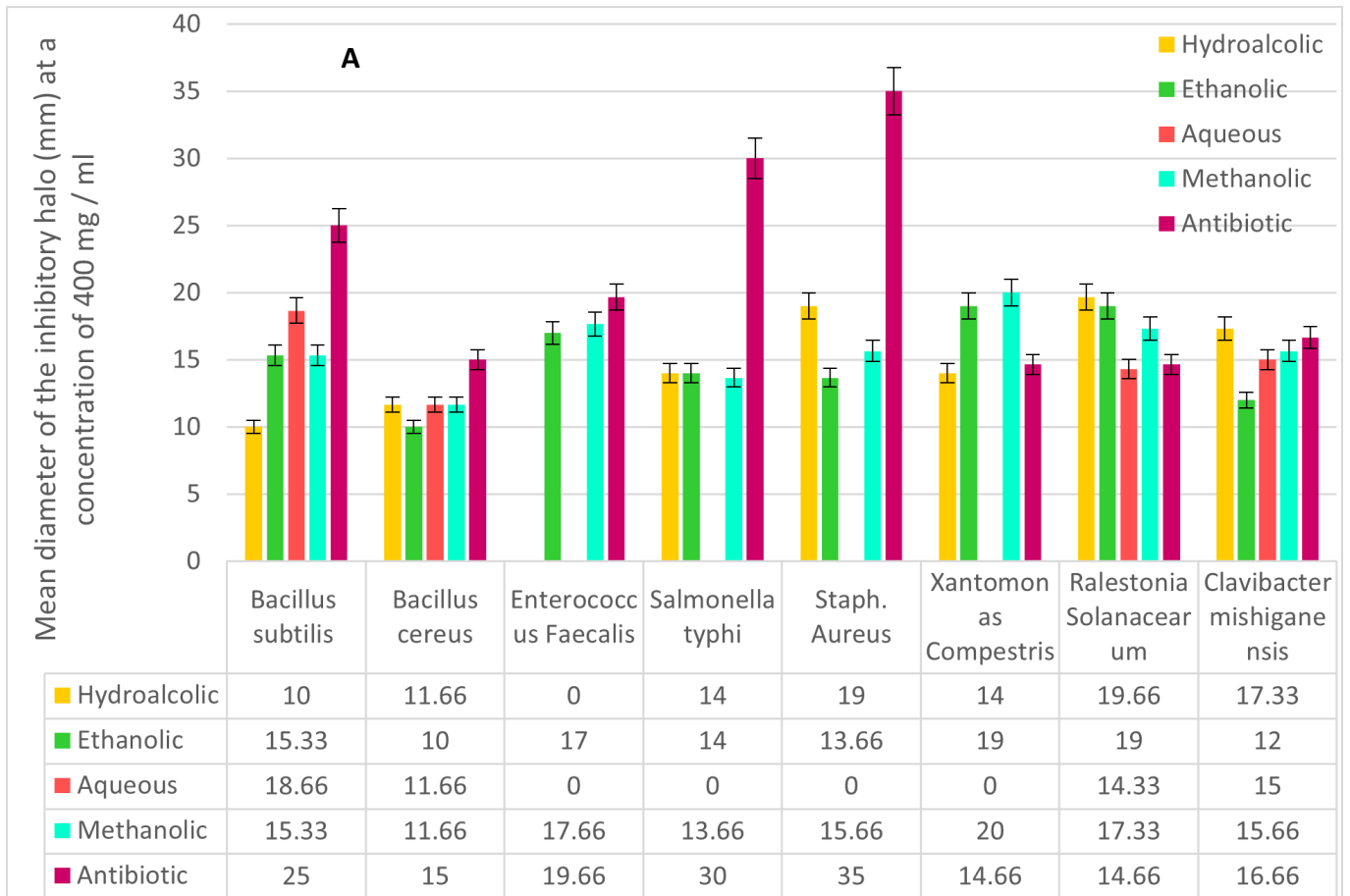


Fig. 2. The mean values of the halo diameter as inhibition zones of various bacterial strains in the presence of extract from (A) *E. angustifolia* and (B) *S. striata*

tract was located in group a. The highest inhibitory (mean diameter of the halo 26.00 ± 0.57 mm) was observed in the culture media of *Bacillus subtilis* containing 400 mg of pit extract from *E. angustifolia*. In the case of pulp extract, the highest inhibitory (mean diameter of the halo 22.33 ± 1.85

mm) was observed for *X. campestris* containing 200 mg of pulp extract. In presence of *S. striata* extract, in the first 24 hrs of measurement, *Staphylococcus aureus* showed a higher sensitivity than other bacteria and with a significant difference relative to other bacteria located in the group a.



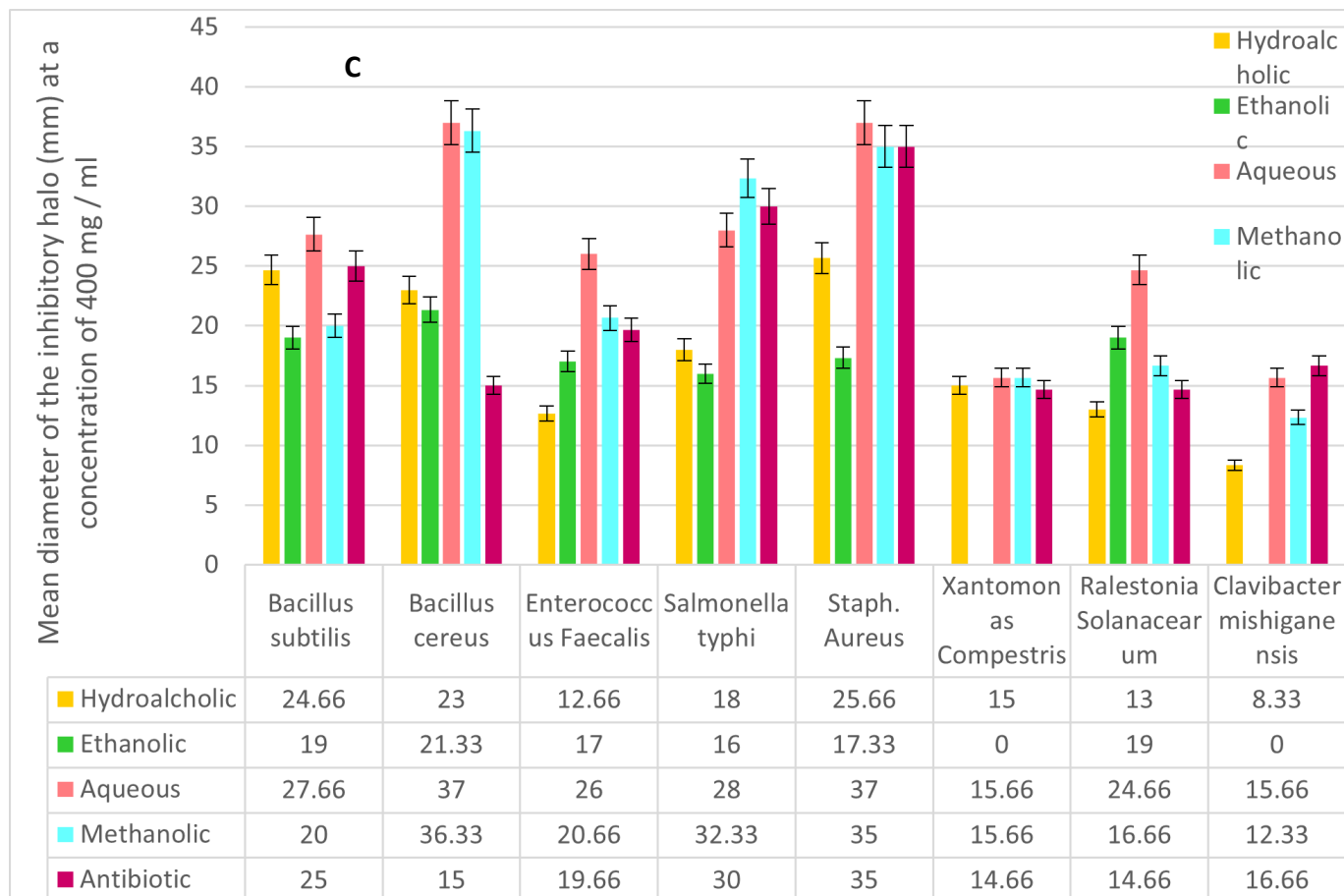


Fig. 3. The mean diameter of the inhibitory halo of hydroalcoholic, ethanolic, aqueous and methanolic extracts (400 mg) from (A) *E. angustifolia* pulp extract, (B) *E. angustifolia* pit extract and (C) *S. striata* extract against 8 bacterial strains (disc method)

Bacillus cereus, *B. subtilis*, *Salmonella typhi*, *Enterococcus faecalis*, *Ralstonia solanacearum*, *X. campestris*, *C. michiganensis* also with a significant difference in relation to each other were grouped in groups b to h respectively.

Among solvents, aqueous solvent was significantly different from the other solvents and located in the group a. The methanolic, hydroalcoholic and ethanol solvents with significant differences in relation to each other were located in group b to d. The highest inhibitory halo diameter (mean diameter of the halo 37.00 ± 1 mm) was observed in the culture media of *Staphylococcus aureus* and *Bacillus cereus* with a concentration of 400 mg/ml of aqueous extract and then the concentration of 400 mg/ml of methanolic extract in the plate of *Bacillus cereus* with a mean diameter of the halo 36.33 ± 1.33 mm. According to Fig. 3 a, regarding to *E. angustifolia* extracts, the mean diameter of inhibition halo of ethanolic and methanolic pulp extracts in disc method on *X. campestris*; hydroalcoholic, ethanolic and methanolic extracts on *Ralstonia solanacearum* and hydroalcoholic extract on *C. michiganensis* was significantly more than the mean diameter of the inhibitory zone of Gentamycin. The mean diameter of inhibition halo of pit ethanolic extract in disc method on *Bacillus subtilis*; hydroalcoholic and ethanolic on *B. cereus*; hydroalcoholic, ethanolic and methanolic extracts on *X. campestris*; aqueous extract on *R. solanacearum* and hydroalcoholic extract on *C. michiganensis* was greater than the mean diameter of the inhibitory zone of gentamycin (Fig. 3 b).

For *S. striata* extracts, the mean diameter of inhibi-

tion halo in aqueous extract on *Bacillus subtilis*, hydroalcoholic, ethanolic, methanolic and hydroalcoholic extracts on *Bacillus cereus*, aqueous and methanolic extracts on *Enterococcus faecalis*, methanolic extracts on *Salmonella typhi*, aqueous and methanolic extracts on *Staphylococcus aureus*, hydroalcoholic, aqueous and methanolic extracts on *X. campestris*, ethanolic, aqueous and methanolic extracts on *Ralstonia solanacearum* was greater than the mean diameter of the inhibitory zone of gentamycin (Fig. 3 c).

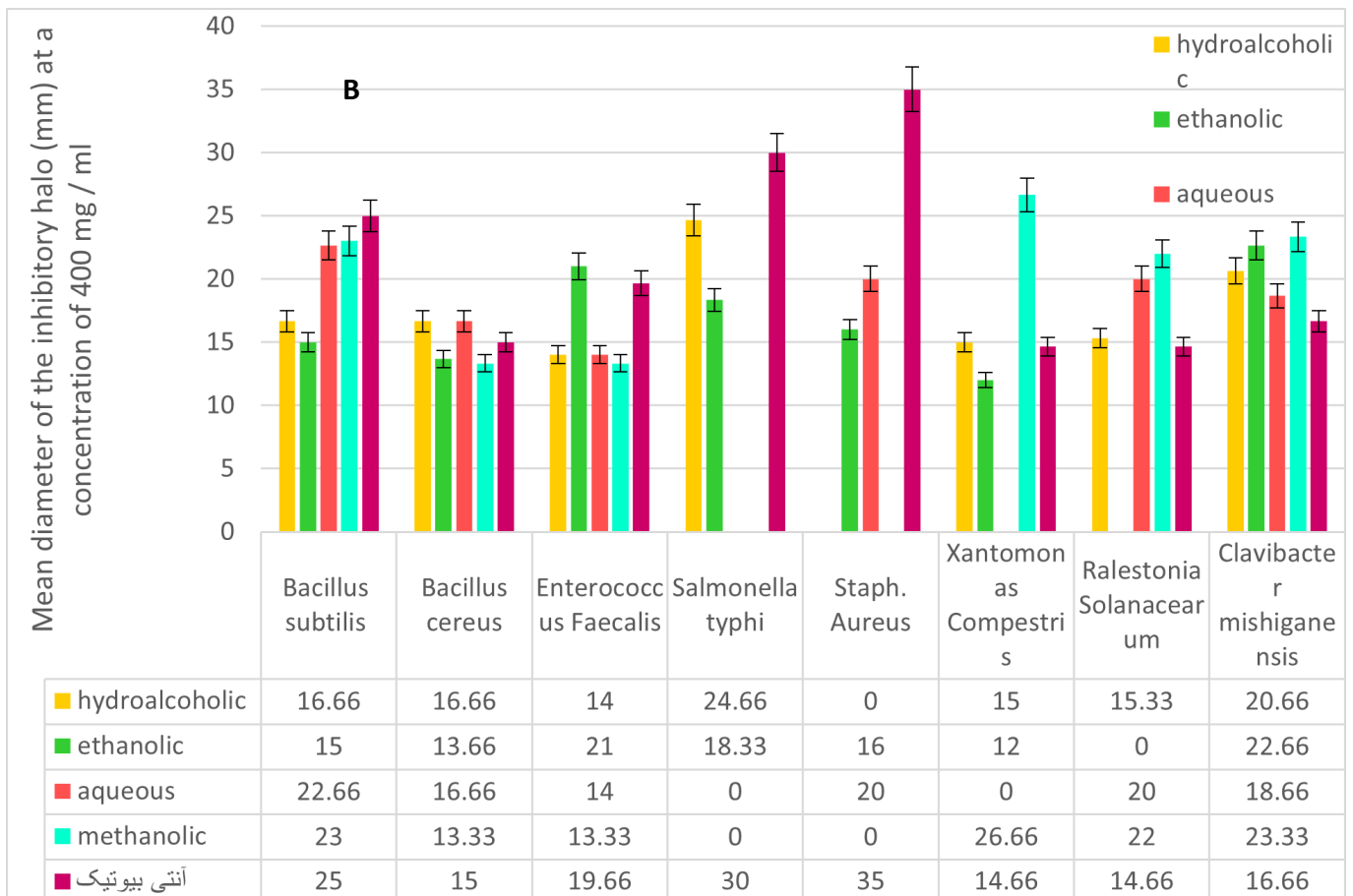
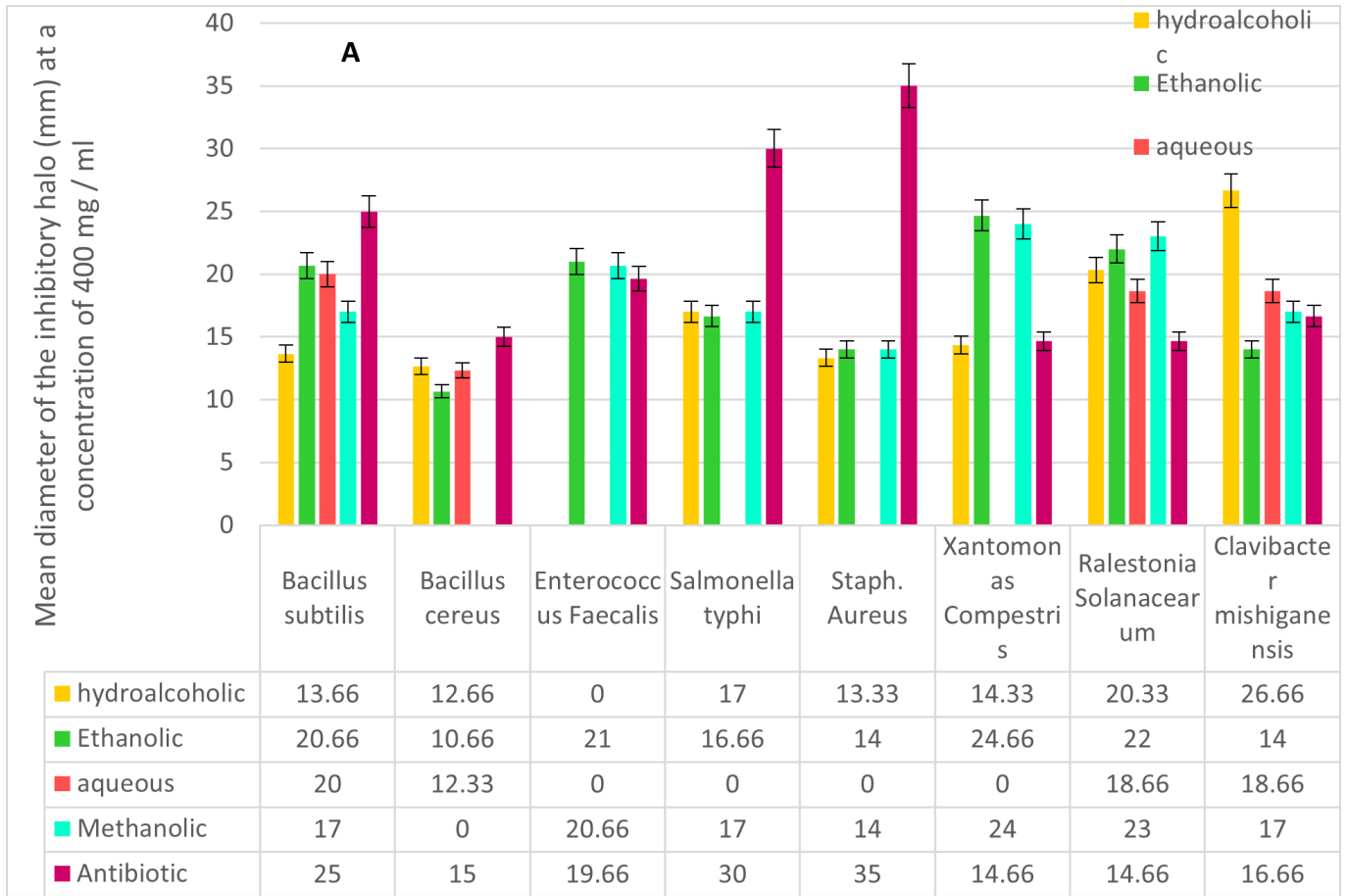
Antibacterial effect of *E. angustifolia* and *S. striata* extracts in the well method

According to comparing the effect of different extracts on bacteria in the well method, the tested bacteria showed different sensitivities in different concentrations of the extracts. In the first 24 hrs of measurement, in the presence of *E. angustifolia* extracts, *C. michiganensis* was more sensitive than other bacteria and with a significant difference than other bacteria were located in group a. Subsequently, *Bacillus cereus*, *B. subtilis*, *Ralstonia solanacearum*, *X. campestris*, *Enterococcus faecalis*, *Salmonella typhi* and *S. aureus* with significant differences were located in groups b to c. Among solvents, methanolic solvent was significantly different from the other solvents and was located in the group a. Ethanolic and hydroalcoholic solvents without significant different relative to each other were located in group b, and aqueous solvent in group c.

According to Fig. 4 a, the mean diameter of inhibition halo of the aqueous extracts on *Bacillus subtilis*; hydroalcoholic, ethanolic, methanolic and hydroalcoholic

extracts on *Bacillus cereus*; aqueous and methanolic extracts on *Enterococcus faecalis*; methanolic extract on *Salmonella typhi*; and aqueous extract on *Staphylococcus aureus*; aqueous and methanolic extracts on *X. campestris*; ethanolic, aqueous and methanolic extracts on *Ralstonia*

solanacearum were higher than the mean diameter of growth inhibition zone by gentamicin antibiotic on the same bacteria. Among the tissues, the pit extract with significant difference was located in group a. The highest inhibition halo for 400 mg/ml of pit extract was observed on



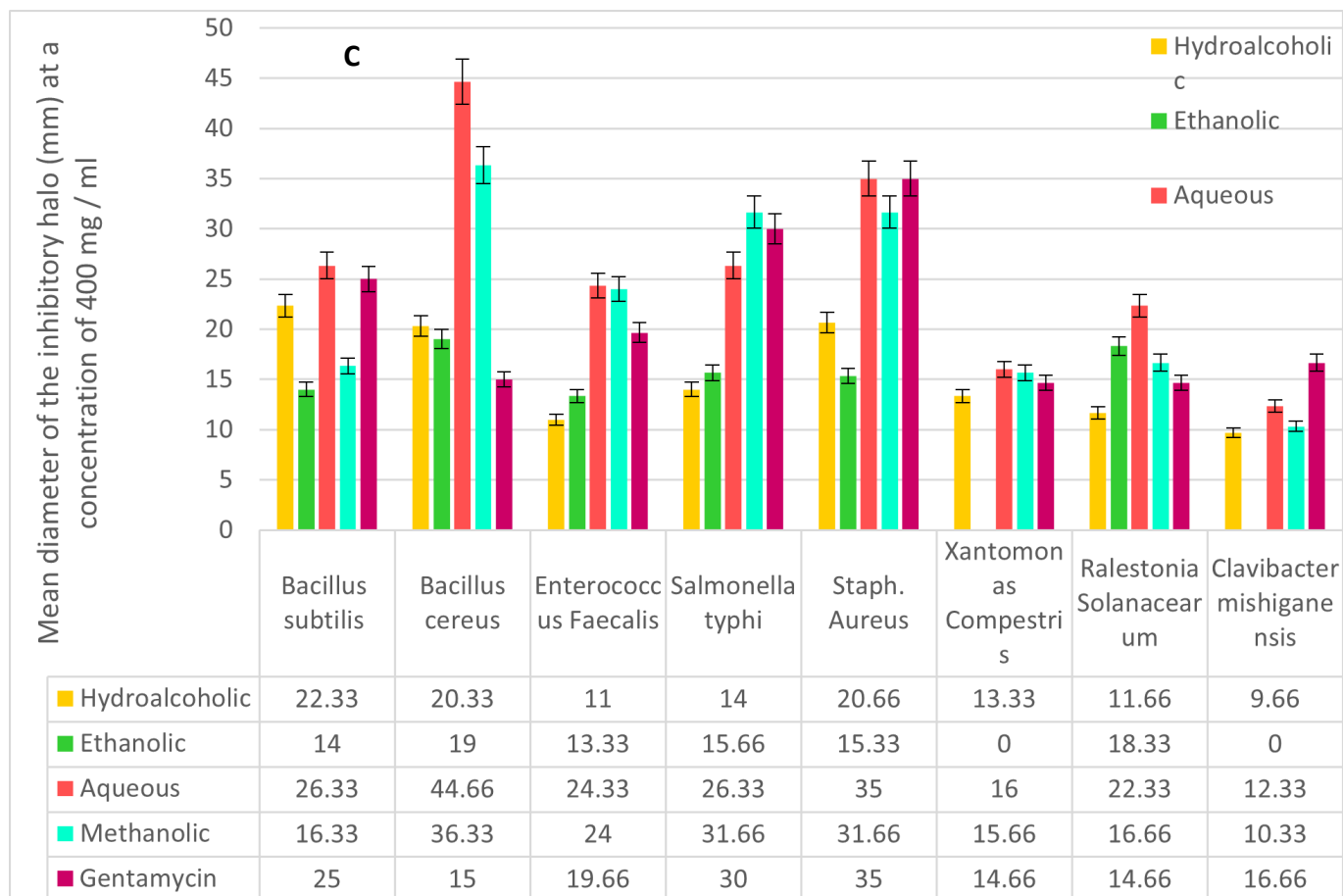


Fig. 4. The mean diameter of the inhibitory halo of hydroalcoholic, ethanolic, aqueous and methanolic extracts (400 mg) from (A) *E. angustifolia* pulp extract, (B) *E. angustifolia* pit extract and (C) *S. striata* extract against 8 bacterial strains (well method)

X. Campestris (diameter 26.66 mm). The highest inhibition halo for pulp hydroalcoholic extract (400 mg/ml) was observed on *C. michiganensis* (diameter 27.66 mm). As shown in Fig. 4 b, the mean diameter of inhibition halo of the pulp extracts (well method) ethanolic and methanolic extract on *Enterococcus faecalis*, hydroalcoholic, methanolic on *Salmonella typhi*; ethanolic, methanolic and hydroalcoholic on *S. aureus*; ethanolic and methanolic on *X. campestris*; ethanolic, methanolic, hydroalcoholic and aqueous on *Ralstonia solanacearum* were higher than the mean diameter of growth inhibition zone by antibiotic gentamicin. Regarding *S. striata* extract, in the first 24 hrs of measurement, *S. aureus* and *Bacillus cereus* were more sensitive than other bacteria and with a significant difference than other bacteria located in group a. *Salmonella typhi* and *Bacillus subtilis* were located in group b and c respectively. *Enterococcus faecalis* and *Ralstonia solanacearum* showed similar sensitivity and without significant differences were located in group d. *X. campestris*, *C. michiganensis*, with significantly different relative to each other were located in e to f groups respectively.

Among solvents, aqueous solvent was significantly different from the other solvents and was located in the group a, methanolic, hydroalcoholic and ethanol solvents with significant different relative to each other located in group b to d. According to Fig. 4 c, the mean diameter of inhibition halo of the aqueous extracts on *Bacillus subtilis*, hydroalcoholic, ethanolic, methanolic and hydroalcoholic extracts on *Bacillus cereus*, aqueous and methanolic ex-

tracts on *Enterococcus faecalis*, methanolic extract on *Salmonella typhi* and aqueous extract on *Staphylococcus aureus*, aqueous and methanolic extracts on *X. campestris*, ethanolic, aqueous and methanolic extracts on *Ralstonia solanacearum* were higher than the mean diameter of growth inhibition zone by antibiotic gentamicin on the same bacteria.

Results of *E. angustifolia* extracts on MIC and MBC

The results of minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of each *E. angustifolia* extract on human and animal bacteria (after 24 hr incubation at 37 °C) and for plant bacteria (after 48 hr incubation at 28 °C) were recorded. Negative control (the nutrient broth medium culture containing plant extracts) has no effect and the positive control (the nutrient broth

Table 3. The results of Kruskal-Wallis statistical test (*E. angustifolia*).

Traits	Chi-squared statistics		df	Coverage of statistics level	
	MIC	MBC			
Tissue	0.407	0.231	2	0.891	0.816
Bacteria	1.612	4.629	7	0.705	0.978
Solvent	1.049	4.393	3	0.222	0.789
Concentration	180.35	85.81	3	0.000	0.000

medium culture containing the bacterial suspension), showed a good growth. In this evaluation, to determine the MIC and MBC of hydroalcoholic, methanolic, ethanolic and hydroalcoholic extracts from *E. angustifolia*, the concentration 600 mg /ml of different extracts were used in tube dilution method (Table S3, Supplementary data).

Different extracts had inhibitory effects on most bacteria. However, these effects were in high dilution, the lowest inhibitory concentration in the pulp methanolic extract was observed on *Ralstonia solanacearum*.

In the case of pulp extract, in all bacteria, except the hydroalcoholic and aqueous on *E. faecalis* and the aqueous extract on *S. typhi* and *X. campestris*, inhibitory effects were observed. The bactericidal effect of ethanolic extracts on *Bacillus subtilis*, ethanolic and methanolic extracts on *Enterococcus faecalis*, hydroalcoholic, methanol, ethanol and aqueous extracts on *S. aureus* and *Ralstonia solanacearum*, hydroalcoholic, ethanolic and methanolic extracts on *X. campestris*; hydroalcoholic and methanolic extracts on *C. michiganensis* were observed. The lowest concentrations were observed for the inhibitory effect of pulp methanolic extract on *R. solanacearum* with a dilution of one thousandth. In the case of pit extract, in all bacteria, except aqueous and methanolic extracts on *S. typhi*, aqueous extract on *X. campestris*; ethanolic extract on *R. solanacearum*, inhibitory effect was observed. Bactericidal effect in all bacteria except hydroalcoholic and ethanolic extracts on *Bacillus subtilis* and *R. solanacearum*; hydroalcoholic, methanolic and ethanolic extracts on *B. cereus*;

Table 4. The results of Kruskal-Wallis statistical test (*S. striata*).

Traits	Chi-squared statistics		df	Coverage of statistics level	
	MIC	MBC			
Bacteria	1.612	4.629	7	0.705	0.978
Solvent	1.049	4.393	3	0.222	0.789
Concentration	180.35	85.81	3	0.000	0.000

hydroalcoholic, methanolic and aqueous extract on *E. faecalis*, methanolic and aqueous extracts on *S. typhi*; aqueous extracts on *X. campestris* were observed. To determine the existence of significant differences between levels of inhibition on different bacteria, a non-parametric test (Kruskal–Wallis) was used for statistical analysis of MIC and MBC results. The Kruskal-Wallis test shows whether these ranks are so different from each other that cannot be said they have been extracted from a common statistical society and show the importance of average rankings. Table 4S supplementary data, shows the number of subjects in each group and the average rank of each of the variables, which the last column is the average of the rank columns. The higher the rank, the more quantitative and qualitative it is. In MIC, *Ralstonia solanacearum* was more inhibited and *Enterococcus faecalis* had the least amount of inhibition. In MBC, *R. solanacearum* and *C. michiganensis* were in first place and *Bacillus cereus* showed the lowest MBC. Among solvents, in the MIC test, the methanol solvent showed the highest inhibitory activity and in the MBC test, aqueous solvent had the highest bactericidal activity. Table 3 shows

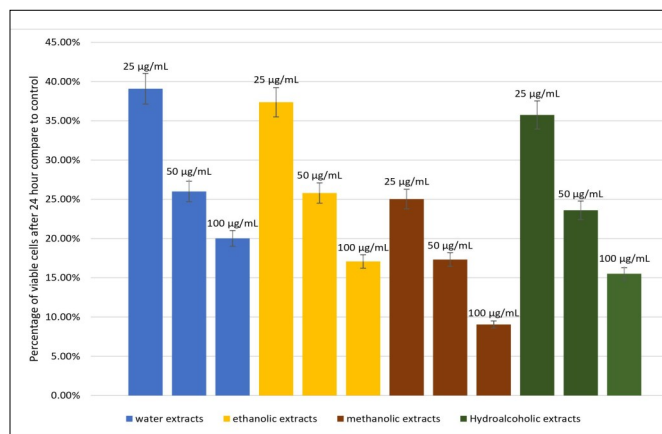


Fig. 5. The antiproliferative activities of *E. angustifolia* extracts on the MCF 7 cell lines. % of viable cells after 24 hrs compare to control

the number of statistics and the contents of the test main result and is used to determine whether the difference in mean values is significant or not. Here, because the decision criterion is greater than 0.05, there is no reason to reject zero assumption. Therefore, it can be admitted that there is no significant relationship between the bacteria and solvents with the level of inhibitory and fatal effects. Also, the significance level of the P-value test can be seen in the concentration grouping variable with the value of zero, which indicates that the assumption that the averages are equal and that there is no difference is rejected and the concentration variable has a significant difference compared to the effects of fatal and inhibitory effects.

Results of *S. striata* extract on MIC and MBC

The results of MIC and MBC of each *S. striata* extract on human and animal bacteria (after 24 hr incubation at 37 °C) and for plant bacteria (after 48 hr incubation at 28 °C) were recorded. In this evaluation, to determine the MIC and MBC of hydroalcoholic, methanolic, ethanolic and hydroalcoholic extracts from *S. striata*, the concentration 600 mg /ml of different extracts were used in tube dilution method (Table S5 supplementary data). Different extracts had inhibitory effects on most bacteria. However, these effects were in high dilution, the lowest MIC in the aqueous extract was observed on *Bacillus cereus* and *S. aureus* and the lowest MIC in aqueous extract on *Bacillus cereus* and *S. aureus* was observed at a dilution of 1000.

In MIC, *Ralstonia solanacearum* had more inhibitory effect and *Enterococcus faecalis* had the least inhibitory effect. In MBC, *C. michiganensis* and *R. solanacearum* were in first place and *Bacillus cereus* bacterium showed the lowest MBC. Among solvents, in the MIC test, the methanol solvent had the most effect on inhibitory activity and in the MBC test, aqueous solvent had the highest bactericidal activity Table S6 supplementary data. The result of Kruskal–Wallis statistical test is shown in Table 4.

3.4 Anticancer activity of *E. angustifolia* on MCF7 breast cancer cells

Cytotoxic activity of water, ethanolic, hydroalcoholic, and methanolic extracts from *E. angustifolia* were evaluated by treating the MCF7 breast cancer cells and then, the measurement of cell viability by MTT assay. The cell viability of the MCF7 breast cancer cells were significantly reduced by

E. angustifolia water, ethanol, hydroalcoholic and methanol extracts in 25, 50 and 100 µg/ml concentrations after 24 hrs, more efficiently by methanolic extract (Fig. 5). The results indicated that *E. angustifolia* has potential antiproliferative and anticancer activities. All samples were compared to control.

Discussion

Regarding the role of antioxidants in decreasing the risk and complication of various diseases through neutralizing the oxidative stress, using of medicinal plants which are rich in natural antioxidants can prevent an oxidative stress by neutralizing the activity of free radicals. Due to the similar effects of *E. angustifolia* extracts and methanolic extract from *S. striata* with ascorbic acid as observed in our study, and also considering the use of this vitamin in pharmacy and medicine, the extracts of these plants can be used as candidates for the natural replacement of Vitamin C in medical therapeutic and preventive approaches. Extensive studies have been conducted on the antioxidant properties of medicinal plants. In general, the inhibitory effect of the extracts on DPPH free radicals is dependent on the type of extraction solvent and their polarity, separation methods, the purity of the active compounds and the measurement method (34). The study on the antioxidant activity of various extracts of *Achillea filipendulina* and the amount of phenol and flavonoids of these extracts, there was a direct correlation between phenolic and flavonoid content of plant extracts and antioxidant activity (35). So that the higher amounts of phenolic compounds led to more radical scavenging and more inhibitory activity than lipid peroxidation (35). In the present study, an antioxidant activity was observed in the extracts of the tested plants. The antioxidant activity of pit extract of *E. angustifolia* and *S. striata* extract showed that the highest percentage of DPPH inhibition was belonged to methanolic extract, which was statistically more than ascorbic acid. In line with our finding, Saboonchian *et al.* reported the presence of flavonoids and phenol in *E. angustifolia*, which probably have valuable medicinal properties (36). Another study showed that flavonoids are one of the most important components of this plant that have anticoagulant and anti-inflammatory effects. About nine flavonoids, such as: catechin, epicatechin, galactocatechin, campral, quercetin, luteolin, isotamine and isoramentine, galactopyrarenose are identified in this plant (37). Fruits and leaves of *E. angustifolia* contain significant amounts of flavonoids, terpenoids, cardiac glycosides, cytostrol and carvacrol and have analgesic and anti-inflammatory effects (38, 39). Polyphenols are the main components of the *E. angustifolia* with antioxidant activity and plays an important role in scavenging and neutralizing the free radicals and suppressing single and triplet oxygen as well as the breakdown of peroxides (40). In fact, plants containing flavonoid have a strong antioxidant property (41, 42). According to observed antioxidant activity of *E. angustifolia* related to phenol and flavonoids, this plant is a good candidate for food and drug industry. Some evidences showed the antioxidant and anticancer properties of *S. striata* (25, 26, 28, 43).

Our findings further confirmed the antioxidant activity of extracts derived from *S. striata*.

Despite extensive use of antibiotics and their semi-synthetic analogues, large groups of bacteria such as methicillin-resistant *Staphylococcus aureus*, multi-drug-resistant *Mycobacterium tuberculosis*, carbapenem-resistant Enterobacteriaceae gut bacteria and vancomycin-resistant *Enterococcus* have become resistance to them.

Hence, the discovery of new materials that have sustained activity against harmful microorganisms is one of the current goals of the researchers, and many researches were done to detect and produce the novel antimicrobial compounds (44). Various factors such as plant extract concentration, extraction method; type of solvent and the culture medium can affect the antimicrobial properties of the plant extracts. On the other hand, different microorganisms may have different responses to the antimicrobial effects of the plant extracts and also based on extraction methods and solvents, plant extracts can exhibit different antimicrobial effects on specific species of microorganisms (45, 46). The moisture content of the plant, the temperature and the size of the powdered particles are effective on extraction (47). In this study, in order to obtain the best extracts with the highest inhibitory content, different extracts examined and the results indicated that the type of solvent was very effective, so that in the *S. striata* plant, the best performance belong to aqueous solvent. Methanol as a solvent can extract the alkaloids, whereas the ethanolic solvent does not have this ability. Considering these and this fact that the alkaloid composition has antimicrobial properties, the better activity of methanolic extract in the evaluation of the antibacterial and anticancer properties of *E. angustifolia* can be influenced by the presence of alkaloids in the obtained extracts. The antimicrobial activity of the extracts can also be related to the presence of phenolic and tannin compounds that damage the cell membranes of microorganisms (48).

The antimicrobial results of plant extracts indicate the acceptable potential of these plants as a drug against microorganisms caused diseases. In this study, different extracts showed different antibacterial and anticancer activities and in some cases the diameter of the inhibition halo was equal to or even greater than the diameter of the inhibition halo by gentamicin antibiotic as reference antibiotic. Given that, with the increase of concentration of extracts, the comparison of the halo diameter in most of the microorganisms was similar, it can assume that the plant extracts have approximately the same type and number of effective compounds in the control of bacteria. Antimicrobial activity was observed in ethanolic, methanolic and hydroalcoholic extracts of the *E. angustifolia* and pit extract showed more antibacterial activity. By analyzing the antibacterial properties of *S. striata*, it was observed that in both disc and well methods, the highest efficiency was related to aqueous extract and the most sensitive bacteria were *Staphylococcus aureus* and *Bacillus cereus*. Regarding this fact that the herbal extracts are effective on both gram positive and negative bacteria, it can be argued that their effective compounds act by interfering in

mechanisms that are common in both of them. In the dilution test, some extracts did not exhibit MBC, probably due to the presence of bactericidal and sporicidal (in the case of bacillus) compounds in extracts (49).

Here, 3 methods of wells diffusion, disk diffusion and tube dilution were used to study the efficiency of these methods. The results of 2 well and disc diffusion methods showed that the disc method is significantly more efficient than other methods.

Regarding the MIC and MBC values, it seems that the aqueous extract of this plant is one of the herbal compounds affecting the bacteria and indicates that the active ingredient of this plant is better extracted in the aqueous phase. The results of this study showed that the effective compounds of plants are very likely related to extract type. Among solvents, in the MIC test, methanolic solvent had the most effect on inhibitory activity and in the MBC test, the aqueous solvent had the highest activity for both plants extract.

Our results were in line to those reported by others that confirmed the antibacterial effect of *S. striata* extract on *Staphylococcus aureus* and *Pseudomonas aspergilos*, which was equivalent to betadine as well as *S. striata* extract can use as an antiseptic agent in the treatment of external infections (50, 51).

Conclusion

Overall, our data from the evaluation of the antioxidant and antibacterial activities of *Elaeagnus angustifolia* and *Scrophularia striata* extracts against eight pathogenic bacterial strains, strongly suggest that both of these plants may utilize as antioxidant and antibacterial sources. However, these abilities need to be further explored on other types of bacteria specially through *in vivo* studies in pre-clinical animal setting.

In summary, the presence of Biochemical compounds such as phenol, flavonoids, resins, quinones, steroids, terpenoids and alkaloids in *E. angustifolia* and *S. striata* extracts were confirmed, and the highest free radical inhibitory activity of DPPH was related to the methanolic extracts. Our findings showed that for both plants strain the disc method is more effective than well diffusion method. The most sensitive bacteria for *E. angustifolia* were *Bacillus subtilis* and *X. campestris* against the pit extracts in disc method and *X. campestris* and *C. michiganensis* against pit and pulp extracts respectively as well as this plants extracts have anticancer properties. The most sensitive bacteria for *Scrophularia striata* were *Bacillus subtilis* and *Staphylococcus aureus*. Among the solvents in the MIC test, the methanolic solvents had the highest bacterial inhibitory activity, and in the MBC test, aqueous solvents had the highest bactericidal activity. To further verify the general anticancer activity of these plants, analysis of the effects of their extract on different cancer cell lines and *in vivo* animal studies are needed.

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Authors contributions

KhP. contributed to the study design and supervised the study. ZN. collected the plant samples, performed experiments, analyzed the data and prepared the original draft of the manuscript. MN. contributed to writing and editing the manuscript. SN. performed cell culture experiment. All authors have read and agreed to the published version of the manuscript.

Compliance with ethical standards

Conflict of interest: The authors declare that they have no conflict of interest.

Ethical issues: None.

Supplementary data

Supplementary Table S1. The comparison of the antibacterial effects of *E. angustifolia* extracts on bacteria by disc Method

Supplementary Table S2. The comparison of the antibacterial effects of *S. striata* extract on bacteria by disc method

Supplementary Table S3. The results of *E. angustifolia* extracts on MIC and MBC

Supplementary Table S4. The number of subjects in each group and the average rank of variables (*E. angustifolia*).

Supplementary Table S5. The results of *S. striata* extracts on MIC and MBC.

Supplementary Table S6. The number of subjects in each group and the average rank of the variables (*S. striata*).

Supplementary Fig. 1S. Growth inhibiting halo around the wells in the well-diffusion method for (a) *E. angustifolia* and (b) *S. striata* extracts.

References

1. Noguchi N, Niki E. Phenolic antioxidants: A rationale for design and evaluation of novel antioxidant drug for atherosclerosis. *Free Radic Biol Med.* 2000;28(10):1538-46. [https://doi.org/10.1016/S0891-5849\(00\)00256-2](https://doi.org/10.1016/S0891-5849(00)00256-2)
2. Alok S, Jain SK, Verma A, Kumar M, Mahor A, Sabharwal M. Herbal antioxidant in clinical practice: A review. *Asian Pacific Journal of Tropical Biomedicine.* 2014;4(1):78-84. [https://doi.org/10.1016/S2221-1691\(14\)60213-6](https://doi.org/10.1016/S2221-1691(14)60213-6)
3. Mathew S, Abraham TE. *In vitro* antioxidant activity and scavenging effects of *Cinnamomum verum* leaf extract assayed by different methodologies. *Food Chem Toxicol.* 2006;44(2):198-206. <https://doi.org/10.1016/j.fct.2005.06.013>
4. Mathew S, Abraham TE. Studies on the antioxidant activities of cinnamon (*Cinnamomum verum*) bark extracts, through various *in vitro* models. *Food Chem.* 2006;94(4):520-28. <https://doi.org/10.1016/j.foodchem.2004.11.043>
5. Michaud C. Global burden of infectious diseases. *Encyclopedia of Microbiology.* 2009:444. <https://doi.org/10.1016/B978-012373944-5.00185-1>

6. Watkins K. Emerging infectious diseases: a review. *Current Emergency and Hospital Medicine Reports*. 2018;6(3):86-93. <https://doi.org/10.1007/s40138-018-0162-9>
7. Mangione-Smith R, Elliott MN, Stivers T, McDonald LL, Heritage J. Ruling out the need for antibiotics: are we sending the right message? *Arch Pediatr Adolesc Med*. 2006;160(9):945-52. <https://doi.org/10.1001/archpedi.160.9.945>
8. Fair RJ, Tor Y. Antibiotics and bacterial resistance in the 21st century. *Perspect Medicin Chem*. 2014;6:PMC. S14459. <https://doi.org/10.4137/PMC.S14459>
9. Lanteri C, Mende K, Kortepeter M. Emerging infectious diseases and antimicrobial resistance (EIDAR). *Military medicine*. 2019;184(Supplement_2):59-65. <https://doi.org/10.1093/milmed/usz081>
10. Kabra SK, Lodha R. Antibiotics for preventing complications in children with measles. *Cochrane database of systematic reviews*. 2013(8). <https://doi.org/10.1002/14651858.CD001477.pub4>
11. Kumar SR S, Rao KVB. *In-vitro* antimicrobial activity of marine actinobacteria against multidrug resistance *Staphylococcus aureus*. *Asian Pacific journal of tropical biomedicine*. 2012;2(10):787-92. [https://doi.org/10.1016/S2221-1691\(12\)60230-5](https://doi.org/10.1016/S2221-1691(12)60230-5)
12. Essawi T, Srour M. Screening of some Palestinian medicinal plants for antibacterial activity. *J Ethnopharmacol*. 2000;70(3):343-49. [https://doi.org/10.1016/S0378-8741\(99\)00187-7](https://doi.org/10.1016/S0378-8741(99)00187-7)
13. Antih J, Houdkova M, Urbanova K, Kokoska L. Antibacterial activity of *Thymus vulgaris* L. essential oil vapours and their GC/MS analysis using solid-phase microextraction and syringe headspace Sampling Techniques. *Molecules*. 2021;26(21):6553. <https://doi.org/10.3390/molecules26216553>
14. Bonjar S. Evaluation of antibacterial properties of some medicinal plants used in Iran. *J Ethnopharmacol*. 2004;94(2-3):301-15. <https://doi.org/10.1016/j.jep.2004.06.007>
15. Soroushzhadeh SMA, Khiveh A, Gerayelimalek V. Role of Iranian traditional medicine in the prevention of respiratory infectious diseases. *Iranian Journal of Medical Sciences*. 2016;41(3 Suppl):S56.
16. Hosseinzadeh H, Ramezani M, Namjo N. Muscle relaxant activity of *Elaeagnus angustifolia* L. fruit seeds in mice. *J Ethnopharmacol*. 2003;84(2-3):275-78. [https://doi.org/10.1016/S0378-8741\(02\)00331-8](https://doi.org/10.1016/S0378-8741(02)00331-8)
17. Bucur L, Negreanu-Pîrjol T, Giurginca M, Istudor V. Some new *Elaeagnus angustifolia* L. extracts and the pharmaceutical products' antioxidant activities determined by the chemiluminescence method. *Rev Roum Chim*. 2008;53:961-64.
18. Wang Q, Ruan X, Huang J-h, Xu N-y, Yan Q-c. Intra-specific genetic relationship analyses of *Elaeagnus angustifolia* based on RP-HPLC biochemical markers. *Journal of Zhejiang University Science B*. 2006;7(4):272-78. <https://doi.org/10.1631/jzus.2006.B0272>
19. Ielpo M, Basile A, Miranda R, Moscattello V, Nappo C, Sorbo S et al. Immunopharmacological properties of flavonoids. *Fitoterapia*. 2000;71:S101-S9. [https://doi.org/10.1016/S0367-326X\(00\)00184-2](https://doi.org/10.1016/S0367-326X(00)00184-2)
20. Goncharova N, Rashkes YV, Isamukhamedov AS, Glushenkova A. Oxygenated fatty acids of the seeds of *Elaeagnus angustifolia*. *Chemistry of Natural Compounds*. 1994;30(6):661-65. <https://doi.org/10.1007/BF00630597>
21. Hosseinzadeh H, Namjo N, Ramezani M. Muscle relaxant activity of *Elaeagnus angustifolia* L. fruit seeds in mice. 2002. [https://doi.org/10.1016/S0378-8741\(02\)00331-8](https://doi.org/10.1016/S0378-8741(02)00331-8)
22. Havasian MR, Panahi J, Pakzad I, Davoudian A, Jalilian A, Azodi MZ. Study of inhibitory effect of alcoholic and aqueous extract of *Scrophularia striata* (tashne dari) on *Candida albicans* *in vitro*. *Pejouhesh dar Pezeshki*. 2013;36(5):19-23.
23. Sharafati Chaleshtori R, Sharafati Chaleshtori F, Sharafati Chaleshtori A, Ashrafi K. Antimicrobial effects and evaluation of total phenols flavonoids and flavonols contents of ethanolic extracts of *Scrophularia striata*. *Journal of Shahrekord Univeristy of Medical Sciences*. 2010;11.
24. Díaz-Miranda D. Genera of Scrophulariaceae in the Paramos of the Venezuelan Andes. *Brittonia*. 1988:235-39. <https://doi.org/10.2307/2807013>
25. Safavi F, Meighani H, Ebrahimi P, Ghoran SH. Antioxidant and antibacterial activity of *Scrophularia striata*. *Res Pharm Sci*. 2012;7(5):852.
26. Ardeshiri-Lagimi A, Barzegar M, Rezaei-Tavirani M, Hashemi M, Heidari-Kashal S, Moghaddamnia S et al. Effects of *Scrophularia striata* extract on human fibroblast cells. *Medical Sciences Journal of Islamic Azad University*. 2009;19(3).
27. Rezaie-Tavirani M, Mortazavi SA, Barzegar M, Moghadamnia SH, Rezaee MB. Study of anti cancer property of *Scrophularia striata* extract on the human astrocytoma cell line (1321). *Iranian Journal of Pharmaceutical Research: IJPR*. 2010;9(4):403.
28. Zamanian-Azodi M, Ardeshiryajimi A, Ahmadi N, BagherRezaee M, AziziJalilian F, Khodarahmi R. Antibacterial effects of *Scrophularia striata* seed aqueous extract on *Staphylococcus aureus*. *Journal of Paramedical Sciences (JPS)*. 2013;4(1):2008-4978.
29. Akter K, Barnes EC, Loa-Kum-Cheung WL, Yin P, Kichu M, Brophy JJ et al. Antimicrobial and antioxidant activity and chemical characterisation of *Erythrina stricta* Roxb.(Fabaceae). *J Ethnopharmacol*. 2016;185:171-81. <https://doi.org/10.1016/j.jep.2016.03.011>
30. Mujeeb F, Bajpai P, Pathak N. Phytochemical evaluation, antimicrobial activity and determination of bioactive components from leaves of *Aegle marmelos*. *BioMed research international*. 2014;2014. <https://doi.org/10.1155/2014/497606>
31. Stoilova I, Krastanov A, Stoyanova A, Denev P, Gargova S. Antioxidant activity of a ginger extract (*Zingiber officinale*). *Food Chem*. 2007;102(3):764-70. <https://doi.org/10.1016/j.foodchem.2006.06.023>
32. Nitiema LW, Savadogo A, Simpore J, Dianou D, Traore AS. *In vitro* antimicrobial activity of some phenolic compounds (coumarin and quercetin) against gastroenteritis bacterial strains. *Int J Microbiol Res*. 2012;3(3):183-87.
33. Kedare SB, Singh R. Genesis and development of DPPH method of antioxidant assay. *Journal of Food Science and Technology*. 2011;48(4):412-22. <https://doi.org/10.1007/s13197-011-0251-1>
34. Tsuda T, Watanabe M, Ohshima K, Norinobu S, Choi S-W, Kawakishi S et al. Antioxidative activity of the anthocyanin pigments cyanidin 3-O-. beta.-D-glucoside and cyanidin. *J Agric Food Chem*. 1994;42(11):2407-10. <https://doi.org/10.1021/jf00047a009>
35. Kiyandpour V, Fakhari A, Asghari B, Yousefzadi M. Chemical composition and antibacterial activity of the essential oil of *Achillea filipendulina* (Asteraceae). *Planta Med*. 2011;77(12):PE49. <https://doi.org/10.1055/s-0031-1282380>
36. Saboonchian F, Jamei R, Sarghein SH. Phenolic and flavonoid content of *Elaeagnus angustifolia* L. (leaf and flower). *Avicenna Journal of Phytomedicine*. 2014;4(4):231.
37. Wolniak M, Tomczykowa M, Tomczyk M, Gudej J, Wawer I. Antioxidant activity of extracts and flavonoids from *Bidens tripartita*. *Acta Pol Pharm*. 2007;64(5):441-47.
38. Zeng F, Wang W, Zhan Y, Xin Y. Establishment of the callus and cell suspension culture of *Elaeagnus angustifolia* for the production of condensed tannins. *African Journal of Biotechnology*. 2009;8(19).

39. Zangeneh N, Barzegar H, Mehrnia MA, Noshad M, Hojjati M. Effect of oleaster (*Elaeagnus angustifolia*) flour on gluten free cake properties. Iranian Food Science and Technology Research Journal. 17(1):69-81.
40. Zheng W, Wang SY. Antioxidant activity and phenolic compounds in selected herbs. J Agric Food Chem. 2001;49(11):5165-70. <https://doi.org/10.1021/jf010697n>
41. Badami S, Gupta MK, Suresh B. Antioxidant activity of the ethanolic extract of *Striga orobanchioides*. J Ethnopharmacol. 2003;85(2-3):227-30. [https://doi.org/10.1016/S0378-8741\(03\)00021-7](https://doi.org/10.1016/S0378-8741(03)00021-7)
42. Adedapo AA, Jimoh FO, Afolayan AJ, Masika PJ. Antioxidant activities and phenolic contents of the methanol extracts of the stems of *Acokanthera oppositifolia* and *Adenia gummifera*. BMC Complement Altern Med. 2008;8(1):54. <https://doi.org/10.1186/1472-6882-8-54>
43. Mahboubi M, Kazempour N, Nazar ARB. Total phenolic, total flavonoids, antioxidant and antimicrobial activities of *Scrophularia striata* Boiss extracts. Jundishapur Journal of Natural Pharmaceutical Products. 2013;8(1):15. <https://doi.org/10.17795/jjnpp-7621>
44. Sheena N, Ajith T, Mathew A, Janardhanan K. Antibacterial activity of three macrofungi, *Ganoderma lucidum*, *Navesporus floccosa* and *Phellinus rimosus* occurring in South India. Pharm Biol. 2003;41(8):564-67. <https://doi.org/10.1080/13880200390501226>
45. Singh A, Singh R, Bhunia A, Singh N. Efficacy of plant essential oils as antimicrobial agents against *Listeria monocytogenes* in hotdogs. LWT-Food Science and Technology. 2003;36(8):787-94. [https://doi.org/10.1016/S0023-6438\(03\)00112-9](https://doi.org/10.1016/S0023-6438(03)00112-9)
46. Nostro A, Germano M, D'angelo V, Marino A, Cannatelli M. Extraction methods and bioautography for evaluation of medicinal plant antimicrobial activity. Lett Appl Microbiol. 2000;30(5):379-84. <https://doi.org/10.1046/j.1472-765x.2000.00731.x>
47. Makanjuola SA. Influence of particle size and extraction solvent on antioxidant properties of extracts of tea, ginger and tea-ginger blend. Food science and nutrition. 2017;5(6):1179-85. <https://doi.org/10.1002/fsn3.509>
48. Tiwari P, Kaur M, Kaur H (Editors). Phytochemical screening and Extraction: A Review 2011.
49. Mashouf RY, Hashemi SH, Bijarchi M. Bacterial agents of meningitis in children and detection of their antibiotic resistance patterns in Hamadan, Western Iran. Pak J Biol Sci. 2006;9(7):1293-98. <https://doi.org/10.3923/pjbs.2006.1293.1298>
50. Abbasi N, Abdi M, Azizi Jalilian F, Seifmanesh M. Antimicrobial effect of extracts of *Scrophularia striata* on *Staphylococcus aureus* and *Pseudomonas aeruginosa* and comparison with selective effective antibiotics. Ilam: Ilam University of Medical Sciences. 2004.
51. Rahmati RM, Zamanian AM, Salimpour F. Herbal remedies and medicine; introducing some iranian plants. 2013.

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