Corchorus olitorius L. (Jute) leaf and seed extracts exerted high antibacterial activity against food and plant pathogenic bacteria

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Abstract

Aim of this study was to comparatively evaluate antibacterial activities of methanol (MetOH), acetone (Ace), petroleum ether (PE) and aqueous (dw) leaf (L), root (R), and seed (S) extracts of *Corchorus olitorius* L. on both food- and plant-borne pathogens, with DPPH radical scavenging activities (DRSA), and quantitative and qualitative constituent analysis. Leaf PE has the highest strain susceptibility on both food- and plant-borne pathogens. *Clavibacter michiganensis*, *Pseudomonas tomato*, and *Erwinia caratovora* were susceptible to nearly all the leaf and seed extracts. Very low minimum inhibitory concentration (8-128 μg mL^{-1}) and minimum bactericidal concentration (32-2048 μg mL^{-1}) were determined for both leaf and seed extracts against *C. michiganensis*. Total phenolic contents were correlated to DRSA. The phenolic compounds tested were higher in the leaf MetOH, cholorogenic acid being the most abundant one. Palmitic acid was determined in leaf PE and seed PE extracts. Results presented here demonstrate high antibacterial activity of *C. olitorius* leaf seed extracts against phytopathogens for the first time, and provide the most comprehensive data on the antibacterial activity screening against food-borne pathogens. Considering limitations in plant disease control, antibacterial activities of these extracts would be important in plant disease control.

Keywords: Jute, antibacterial activity, food-borne pathogens, phytopathogens, phenolic contents, fatty acid composition

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Introduction

Plants are sources of precursors or bioactive ingredients for pharmaceutics, cosmetics and pesticides. *Corchorus* L. (Malvaceae) is a genus of tropical flowering annual herbs. So, screening of plant extracts is important for the development of bioactive formulations. *Corchorus olitorius* (Jute) is a wild species of tropical areas of Africa and Asia (1). It is among the major sources of fiber, and has economic importance around the world for fiber production. In addition, it has edible leaves, thus, is widely cultivated and consumed in warm temperature areas including Cyprus and some Arabic countries. The common name for the edible vegetable is 'Molukhyia'. It has been reported to be used for the treatment of aches, dysentery, pectoral pains, fever, enteritis, and chronic cystitis in folklore medicine (2). Cardiovascular activities of the species of the genus *Corchorus* have extensively been studied, and attributed to cardiovascular glycosides and their derivatives (3). Anticonvulsant, hepatobiliary, renal, antiesterogenic, hematological, antimalarial, and antihistaminic activities were well reviewed in Khan et al. (3) as well as the related constituents. Anti-inflammatory and anti-pyretic effects of aqueous extract in rats were also demonstrated (2). Hydrophilic extract and lipophilic leaf extracts were comparatively evaluated for antioxidant capacities (4). Additionally, we previously demonstrated high cytotoxicity of methanolic seed extracts with an inhibitory 50 concentration of (IC$_{50}$) 17 μg mL^{-1}, as well as genotoxicity of leaf and seed extracts on ARH-77 human multiple myeloma cell line for the first time (5).

The need for new antibacterial agents and formulations has been increasing since the food- and plant-borne resistant strains is emerging. Antibacterial activity of *C. olitorius* leaf extracts, which were obtained by petroleum ether, methanol, and chloroform

extraction, against some food pathogenic bacteria have been previously demonstrated (6). Interestingly, ethanol extract of the leaves has also been to shown to synergize ciprofloxacin activity alone and ampicillin/cloxacilin combination activity on methicillin-resistant *Staphylococcus aureus* (7).

Bacterial plant diseases may be seed-borne and seed-transmitted, for which the control is limited due to the facts that agricultural use of antibiotics and copper based compounds is under strict control worldwide for sustainable agriculture and environmental issues. Screening of antibacterial and bactericidal potential of crude extracts of different plant species and organs has importance for eco-friendly control of plant pathogenic bacteria. Meanwhile, we have previously showed that *Urtica* spp. seed extracts exerted high antibacterial activity on food and plant pathogenic bacteria (8), and screened leaves, roots and seeds of *Nasturtium officinale* R.Br. (watercress) for antibacterial potential (9). In addition, we have demonstrated antibacterial activity of *Calendula officinalis* (pot marigold) flower and leaf extracts on plant pathogenic bacteria (10). Our cumulative knowledge on different plant extracts showed us that extracts of different parts of a plant, and the solvent used may exert varying potentials for biological activity. Besides, even resistant isolates of phytopathogenic strains may be interestingly susceptible to plant extracts due to possible natural and evolutionary defense mechanisms of plants. So, based on these we aimed to investigate the antibacterial potential of *C. olitorius* extracts on food- and plant-borne bacterial strains, and identify the compounds in extracts obtained from different parts of the plant. In this study, we also evaluated the extracts for their antioxidant potential.

Materials and Methods
Plant material and extraction
C. olitorius seeds were collected from Değirmenlik District of Nicosia (Cyprus) (5). Seeds were sowed and plants were grown in the Greenhouse under controlled conditions to obtain leaf and root samples. Authenticated voucher specimens of whole plants are stored at the Nezahat Gökyiğit Botanical Garden (İstanbul, Turkey) as herbarium materials (NGBB 3935). Extractions were performed as previously described with some modifications (5, 8). In brief, leaf, seed, and root materials were dried at dark. A blender was used to powder dry tissue. A hundred mL of pure methanol (MetOH) (34860, Merck, Germany), acetone (Ace) (100014, Merck), and petroleum ether (PE) (101769, Merck) was added for extractions of 10 g of plant powder. A hundred mL distilled water (dw) was added to 5 g powder for aqueous extractions. Methanol, acetone, and petroleum ether mixtures were continuously shaked at room temperature (dark) for 24 h. Aqueous mixtures were incubated at 70°C in a shaking water bath (dark) for 1 h. At the end of the incubation periods, mixtures were filtered by Whatman No. 40 filters. Thereafter, the samples were freeze-dried at -50°C and 0.50hPa (LyoPro 3000, Thermo Scientific Heto, Allerod, Denmark). The lyophilized extracts were weighed, and stored at -20°C for further experiments. Dry methanol, acetone, and petroleum ether

extracts were resolved in dimethyl sulfoxide (DMSO) (D8418, Sigma-Aldrich, St. Louis, MO, USA), whereas dry aqueous extracts were recovered in dw. Extract yields were calculated as:

Yield (%) = (g lyophilized material / 10 g powder) × 100

Leaf, root and seed methanol extracts had 15.3 ± 2.4 % (n=4), 9.8 ± 1.3 % (n=3), and 21.4 ± 2.2 % (n=4) yields, respectively. Leaf, root and seed acetone extracts had 12.4 ± 1.8 % (n=3), 8.9 ± 1.3 % (n=2), and 10.3 ± 1.6 % (n=3) yields, respectively. Petroleum ether extraction was less efficient in comparison to other solvents with 7.4 ± 0.5 % (n=2), 5.2 ± 0.5 % (n=2), and 8.8 ± 1 % (n=3) yields for leaves, roots, and seeds, respectively. Aqueous extractions had 10.2 ± 1.1 % (n=3), 14.6 ± 1.2 % (n=3), and 17.7 ± 1.5 % (n=3) yields, for leaves, roots, and seeds, respectively.

Antibacterial activities
Test strains and culture conditions
Bacterial strains of are given in Table 1. Food-borne strains were kindly supplied by the Department of Medical Microbiology, Faculty of Medicine, Baskent University (Ankara, Turkey). *E. coli*, *S. aureus*, *Enterobacter* spp., *K. pneumonia*, *Bacillus* spp., and *L. monocytogenes*, have worldwide industrial importance in terms of food spoilage. *Enterococcus* spp. can contaminate dairy products and meat during processing and storage. The cultures were stocked on Mueller Hinton (MH) (CM0405, Oxoid, Hampshire, UK) agar (CM0337, Oxoid, Hampshire, UK) at 4°C. Standard strains of phytopathogenic bacteria were purchased from the National Collection of Plant Pathogenic Bacteria (NCPPB) (UK). Isolates of the infected plants were obtained from the Department of Plant Protection, Çukurova University (Adana, Turkey). *C. michiganensis subsp. michiganensis* infects tomato causing wilt and canker disease, and is persistent on seeds. Resistant strains of *E. amylovora*, *Pseudomonas* spp., and *Xanthomonas campestris* take special attention on plant disease management. *X. vesicatoria* has a wide range of crop hosts. Phytopathogenic strains were stored on Nutrient Broth (NB) (1054430500, Merck, Darmstadt, Germany) agar supplemented with 1% (w/v) glucose at 4°C. Food-borne strains were inoculated to MH broth and incubated overnight at 37°C, whereas plant-borne strains were inoculated to NB broth, and incubated for 48 h at 27°C to obtain fresh cultures for the assays (8, 9).

Disc-diffusion assay
Extract concentrations were diluted to 22.5 mg mL^{-1} in distilled water, and filter sterilized by 0.45 μm filters (Millipore). Fresh cultures were adjusted to 0.5 McFarland standard turbid suspensions in 0.9% (w/v) saline solution. A hundred μL of food- and plant-borne bacterial suspensions were inoculated on MH or NB agar, respectively by spreading (12). Four hundred and fifty μg of extracts (20 μL) were loaded on 6 mm discs. The extract solution (22.5 mg mL^{-1}) contained 17.5% (v/v) of DMSO at most, and most of the extracts had less than 10%

(v/v) DMSO. Accordingly, we tested antibacterial potential of 20% (v/v) DMSO to eliminate activity of the solvent. Ampicillin (A1593, Sigma-Aldrich) tetracycline (87128, Sigma-Aldrich), gentamicin (G1914, Sigma-Aldrich) and sulbactam/cefoperazone (Pfizer, USA) mix were loaded onto discs as 10, 30, 10 and 50+50 µg, respectively, for positive controls. Food-borne bacterial strains were incubated at 37°C for 24 h, and plant-borne strains were incubated at 27°C for 48 h. The zones of inhibition (IZ) were measured (7 mm <) for each disc as the end-point of antibacterial activity. Median of the zones of inhibition obtained for each extract was also calculated. The results of the quadrate replicates were expressed as the mean ± standard errors of the mean (SEM).

Table 1. Bacterial strains tested and their antibiotic susceptibility profiles. Amp: ampicillin, Tet: tetracycline, Gent: gentamicin, Sul+-Cef: sulbactam and cefoperazone, MIC: minimum inhibitory concentration, and MBC: minimum bactericidal concentration.

Food-borne	Strain		Inhibition zones (mm ± SEM)				
		Gram	Amp (10 µg)	Tet (30 µg)	Gent (10 µg)	Sul+Cef (50+50 µg)	MIC (MBC) (µg mL^{-1})
Escherichia coli	ATCC 25922	(-)	18.3±0.3	27.5±0.3	25.8±0.6	34.5±0.5	16 (32) Amp
Enterococcus gallinarum	CDC-NJ-4	(+)	26.5±0.7	11.3±0.3	23.5±2.0	23.0±0.9	32 (64) Amp
Enterococcus faecalis	ATCC 29212	(+)	28.0±1.0	17.0±0.7	19.3±1.3	18.3±0.6	16 (8) Amp
Streptococcus pyogenes	ATCC 19615	(+)	25.0±0.0	11.50.3	22.0±0.0	21.8±0.8	8 (Amp)
Staphylococcus aureus	ATCC 29213	(+)	15.5±1.0	29.3±2.5	22.8±0.5	31.0±0.9	4 Sul+Cef
Listeria monocytogenes	ATCC 7644	(+)	27.8±1.1	31.0±0.6	30.0±0.4	31.0±0.6	<0.125 Amp
Pseudomonas aeruginosa	ATCC 27853	(-)	18.5±0.3	33.5±0.3	26.8±0.8	28.8±0.5	16 Sul+Cef
Klebsiella pneumoniae	Clinical isolate, Baskent University Hospitals	(-)	-	17.5±0.3	25.8±0.3	29.0±0.4	0.5 Sul+Cef
Proteus vulgaris	Clinical isolate, Baskent University Hospitals	(-)	-	19.0±0.7	25.8±0.5	31.3±0.3	0.5 Sul+Cef
Shigella spp.	Clinical isolate, Refik Saydam Hygiene Institute	(-)	30.0±0.8	32.5±0.3	29.8±0.5	30.8±1.0	0.5 Amp
Bacillus pumilus	*Zea mays* isolate M1 (11)	(+)	33.0±1.4	32.5±0.3	32.0±0.4	31.5±0.3	0.25 (0.5) Amp
Plant-borne							
Clavibacter michiganensis subsp. michiganensis	Erd-Cmm; *L. esculentum* isolate; ErdemLi, Mersin	(+)	-	65.5±0.5	34.9±0.3	58.8±0.4	1 (2) (Tet)
Pseudomonas tomato	Erd-Pst; *L. esculentum* isolate; ErdemLi, Mersin	(-)	-	47.3±0.5	38.3±0.3	38.0±0.6	0.25 (0.25) (Tet)
Pseudomonas corrugata	NCPPB No. 2445	(-)	-	37.3±1.9	17.8±0.3	17.80.3	2 (Tet)
Pseudomonas viridiflava	NCPPB No. 1382	(-)	-	34.3±1.9	15.8±0.3	13.7±0.6	1 (Tet)
Xanthomonas vesicotoria	Krs-Xav; *C. annum* isolate; Karaisalı, Adana	(-)	9.3±0.6	50.5±1.2	34.3±0.5	43.0±0.0	2 (Tet)
Xanthomonas perforans	NCPPB No.4321	(-)	-	41.8±0.3	22.5±0.5	36±0.8	2 (Tet)
Xanthomonas gardneri	NCPPB No.4323	(-)	8.0±0.0	45.8±0.3	21.8±0.8	39.3±0.5	0.5 (Tet)
Erwinia caratovora	Khs-Ecc; *L. esculentum* isolate; Kocahasanlı, Mersin	(-)	-	44.3±0.5	31.5±0.9	30.5±0.5	0.25 (Tet)
Erwinia amylovara	Poz-Ea; *P. communis* L. isolate Pozantı, Adana	(-)	15.0±1.1	40.3±0.8	25.3±0.3	40.5±0.3	1 (4) (Tet)
Erwinia persicinus	NCPPB No.3774	(-)	-	46.5±1.2	49.5±0.5	19.0±0.7	<0.0625 (Tet)
Agrobacterium tumefaciens	NCPPB No. 2437:	(-)	-	32.3±1.4	17.8±0.3	19.0±1.1	2 (16) (Tet)

Ampicillin, tetracycline, gentamicin, and sulbactam/cefoperazone mix were loaded onto discs and tested by micro-well dilution assay as positive controls.

Micro-well dilution assay

The lowest concentration to inhibit 90% of bacterial growth is defined as the minimal inhibitory concentration (MIC). MIC were determined for the extracts and strains with a cut-off 7mm ≤ IZ. Assays were performed as previously described (8, 12). In brief, 2-fold serial dilutions of extracts in MH or NB (4 - 2048 μg mL^{-1}) were performed in 96-well plates. Ampicillin, tetracycline, gentamicin, and sulbactam/cefoperazone mix were tested as positive controls. Medium and cell control columns were also included. A hundred μL inoculation volume with 5% fresh bacterial cultures (0.5 McFarland) were added to each well. Plates were incubated at previously mentioned conditions. For the confirmation of MIC, 5 μL was sampled from the wells that have no visible growth and streaked on MHA. The minimum bactericidal concentration (MBC), which is defined as the minimum concentration of complete inhibition of bacterial growth on MHA, was used as the end-point for the comparative evaluation of bactericidal potential.

DPPH radical scavenging assay (DRSA)

Extracts were analyzed for free radical scavenging potential by use of free DPPH• (1,1-diphenyl-2-picrylhydrazine) (D9132, Sigma-Aldrich) radical (13). The extract solutions were 2-fold serially diluted in a concentration range of 3.125 - 1600 μg mL^{-1}. Assays were performed in 4 mL reaction volume with 3:1 ratio of extract to DPPH• (in 200 μM MetOH). After 30 min of incubation at room temperature (dark), the absorbance was measured at 517 nm for the quantitative analysis of the inhibition of formation of the reduced form and subsequent decoloration. DPPH• radical scavenging activity (DRSA) was calculated as:

$$DRSA\ (\%) = [(A_{control} - A_{sample}) / A_{control})] \times 100$$

Where $A_{control}$ defines the absorbance of the assay medium without extract, and A_{sample} defines the absorbance of the sample assays with extracts or standards. DRSA plot against sample concentration were constructed by the Microsoft Excel, and inhibitory concentration 50 (IC_{50}) were calculated from the equations of trend lines of plots with $R^2 > 0.9$. Butylated hydroxytoluene (BHT) (W218405, Sigma-Aldrich), α-tocopherol (α-Toc) (258024, Sigma-Aldrich), and L-ascorbic acid (AscA) (A92902, Sigma-Aldrich) were tested as positive controls. The results of the triplicates were expressed as the mean ± SEM.

Analysis of total phenolics (TP) in the extracts

The Folin-Ciocalteu (1.09001, Merck) method was used to determine amount of total phenolics in the extracts as previously described (8, 14, 15). The absorbance of the assay mixture, which contained 0.05 g mL^{-1} extract, was measured at 765 nm, and the amount of total phenolics were represented as mg gallic acid (G7384, Sigma-Aldrich) equivalents (GAE) per g extract using the calibration curve ($R^2 > 0.9$). The results of the triplicates were expressed as the mean ± SEM.

Quantitative and qualitative content analysis

High performance liquid chromatography coupled with mass spectrometer (HPLC-TOF-MS) and gas chromatography analysis were performed at the Department of Chemistry, Çankırı University (Çankırı, Turkey).

For the analysis of phenolic compounds in the MetOH, Ace, and dw leaf and seed extracts, HPLC-TOF-MS (Agilent Technologies 1260 Infinity LC, 6210 TOF-MS) was used. In brief, 10 μL 200 ppm samples were injected to ZORBAX SB-C18 column (4.6x100 mm, 3.5 μm) through a 0.45 μm filter at 35°C column temperature. Standard solutions of 23 phenolic compounds were injected, and for the construction of calibration curves. Molecular weight and retention time analysis in comparison to standards was used to determine phenolic content of the extracts.

Fatty acids in petroleum ether extracts were determined by Agilent 7890A Gas Chromatograph, equipped with 5975C inert MSD with Triple-Axis Detector (USA). HP-5MS (5% Phenyl Methyl Siloxane; 30m×250 μm, 0.25 μm) (Agilent) column was used. One microliter of sample was injected to the column. The injector and flame ionization detector were at 250°C. The column temperature program was started from 120°C for 4 min, then ramp to 200°C with the heating rate of 3°C min^{-1}, hold for 10 min, and a final temperature increase to 280°C with at a rate of 15°C min^{-1} and hold for 30 min.

Statistical analysis

Statistical analyses were performed using SPSS 17 software (SPSS Inc., USA). All data are expressed as mean ± standard error of the means (SEM). Mean difference between the inhibition zones of extracts for a strain or mean difference between the inhibition zones of an extract obtained by different strains, IC_{50} values, and total phenolic contents were statistically evaluated using one-way ANOVA analysis at the 0.05 level and post hoc Tukey analyses were carried out to find groups whose mean differences were significant.

Results

Leaf and seed extracts exerted high antibacterial activity against food and plant pathogenic bacteria tested

Table 2 demonstrates disc-diffusion results. Solvent control (20% DMSO) had no effect on the strains tested. Highest strain susceptibility among food-borne strains was obtained with PE extract of leaves i.e., 8 strains were susceptible to extracts out of 11 strains tested. Leaf Ace and seed PE and Ace extracts were effective 7 food-borne strains. Antibacterial activity of root extracts were lower than leaf and seed extracts in terms of both number of susceptible strains and the inhibition zones obtained with the susceptible strains (p<0.05). Median of the IZ were 12.5 (range 8 - 13.5), 12.1 (range 8.3 - 13.3), 11.8 (range 11.4 -12.8), and 11.5 (range 10.6 - 14.5) for seed PE, leaf PE, seed Ac, and leaf Ac, respectively. Highest antibacterial activity (14.5 ± 0.5 mm) was obtained on *L. monocytogenes* with leaf Ace extract (p<0.05). Interestingly, all leaf and seed extracts as well as root MetOH and Ace extracts were effective against *L. mono-*

Table 2. Results of disc diffusion assay. MetOH: methanol, PE: petroleum ether, Ace: acetone, and dw: distilled water.

Food-borne	G	Leaf			Seed				Root			
		MetOH	PE	Ace	MetOH	PE	Ace	dw	MetOH	PE	Ace	dw
E. coli	(-)	-	-	-	-	-	-	-	-	-	-	-
E. gallinarum	(+)	-	9.8±0.5	11.5±0.4	-	12.3±0.5	12.3±0.5	-	-	-	-	-
E. faecalis	(+)	10.3±0.3	9.3±0.5	11.7±0.2	13.0±0.0	12.8±0.6	11.8±0.3	-	8.5±0.3	-	-	-
S. pyogenes	(+)	7.5±0.3	-	11.2±0.2	12.0±0.0	13.5±0.3	11.5±0.2	-	8.3±0.3	-	-	-
S. aureus	(+)	-	13.0±0.0	-	-	-	-	-	-	-	-	-
L. monocytogenes	(+)	11.5±0.3	13.3±0.5	14.5±0.1	13.3±0.2	12.5±0.3	12.5±0.6	8.5±0.3	7.0±0.0	-	7.0±0.0	-
P. aeruginosa	(-)	10.0±0.0	12.8±0.6	13.5±0.2	11.80.5	8.0±0.7	11.8±0.3	-	-	-	-	-
K. pneumoniae	(-)	-	-	-	-	-	-	-	-	-	-	-
P. vulgaris	(-)	-	11.3±0.3	-	-	-	-	-	-	-	-	-
Shigella spp.	(-)	-	12.8±0.5	10.6±0.2	12.8±0.3	13.0±0.4	11.4±0.7	7.8±0.3	8.0±0.4	-	7.0±0.0	-
B. pumilus	(+)	-	8.3±0.3	10.9±0.4	13.3±0.5	11.8±0.5	12.8±0.7	7.3±0.1	7.3±0.3	7.7±0.3	7.0±0.0	-

Plant-borne	G	Leaf			Seed				Root			
		MetOH	PE	Ace	MetOH	PE	Ace	dw	MetOH	PE	Ace	dw
C. michiganensis	(+)	-	19.5±0.6	19.8±0.3	25.3±1.0	19.5±0.3	18.8±0.3	8.3±0.3	-	7.7±0.3	-	-
P. tomato	(-)	10.3±0.3	11.8±0.3	10.2±0.4	11.3±0.3	13.5±0.9	-	8.8±0.4	-	-	-	-
P. corrugata	(-)	-	9.7±0.3	-	-	11.4±0.3	-	8.4±0.3	-	-	-	-
P. viridiflava	(-)	-	-	-	-	-	-	8.0±0.0	-	-	-	-
X. vesicotoria	(-)	8.5±0.5	7.5±0.3	-	8.4±0.8	-	-	8.5±0.3				
X. perforans	(-)	9.0±0.7	10.5±0.3	8.5±0.5	-	10.0±0.0	11.0±0.0	7.8±0.5	-	-	8.6±0.3	-
X. gardneri	(-)	-	-	-	-	-	-	8.8±0.5	-	-	-	-
E. amylovara	(-)	-	9.0±0.0	-	-	-	-	7.0±0.0	11.4±0.3	11±0.4	11±0.4	-
E. caratovora	(-)	10.5±0.5	12.8±0.3	8.4±0.3	11.5±0.5	13.3±0.8	-	8.3±0.3	-	-	-	-
E. persicinus	(-)	-	-	-	-	-	-	-	-	-	-	-
A. tumefaciens	(-)	-	-	-	-	-	-	7.8±0.3	-	-	-	-

cytogenes in a range of 11.5 - 14.5 mm. None of the extracts had effective against *E. coli* and *K. pneumoniae*, and *P. vulgaris* was susceptible only to leaf PE extract. Aqueous extract of roots had no effect on any of the food-borne and phytopathogenic strains tested. Aqueous extract of seeds were effective against 10 of the 11 phytopathogenic strains tested, though the IZ were 7.0 - 8.8 mm (median: 8.4 mm). On the other hand, leaf PE extract was effective against 7 strains in a range of 7.5 - 19.5 mm (median: 10.5). Seven extracts tested had antibacterial activity against *C. michiganensis*, and all the leaf and seed extracts except for the leaf MetOH had antibacterial activity with IZ in a range of 8.3 - 25.3 mm (median: 19.5 mm). *P. tomato* and *E. caratovora* were also susceptible to all leaf and seed extracts except for the seed Ace. None of the extracts had effective against *E. persicinus*, and *A. tımefaciens* was susceptible only to aqueous seed extract.

According to micro-well dilution assay (Table 3) lowest MICs were determined with seed PE extract. In particular, a MIC of 64 µg mL^{-1} and a MBC of 2048 µg mL^{-1} were deter-mined for *S. pyogenes*. In addition, MICs were 256 (MBC 256 µg mL^{-1}), and 512 µg mL^{-1} (MBC 2048 µg mL^{-1}) for *Shigella* spp. and *E. feacalis*, respectively. Similarly, 512 µg mL^{-1} MIC was determined for leaf PE and Ace extracts against *L. mono-cytogenes*, *B. pumilus*, and *E. gallinarum*. Among leaf extracts, MBCs of 1024 and 512 µg mL^{-1} were determined for PE extract against *L. monocytogenes* and *B. pumilus*, respectively. In con-cordance to disc diffusion results low MICs (8 - 128µg mL^{-1}) and MBCs (32 - 2048 µg mL^{-1}) were determined for both leaf and seed extracts against *C. michiganensis*. Lowest MIC of 8 µg mL^{-1} with MBC of 32 µg mL^{-1} were obtained with seed PE ex-tract. 256 µg mL^{-1} MIC of leaf PE and Ace were effective against *X. vesicotoria* and *X. perforans*, respectively.

Methanol and acetone extracts had high DPPH radical scavenging activity (DRSA)

Free radical scavenging activity of the plant extracts have been performed using DPPH radical. Results (Table 4) demonstrated that MetOH and Ace extracts of roots had significantly higher

Table 3. Results of micro-well dilution assay. MetOH: methanol, PE: petroleum ether, Ace: acetone, and dw: distilled water.

Food-borne	G	Leaf MetOH	Leaf PE	Leaf Ace	Seed MetOH	Seed PE	Seed Ace	Seed dw	Root MetOH	Root PE	Root Ace	Root dw
E. coli	(-)	-	-	-	-	-	-	-	-	-	-	-
E. gallinarum	(+)	-	1024	512	-	1024 (1024)	2048	-	-	-	-	-
E. faecalis	(+)	2048<	2048<	1024	2048	512 (2048)	2048	-	2048	-	-	-
S. pyogenes	(+)	2048<	-	2048	2048<	64 (2048)	2048	-	2048<	-	-	-
S. aureus	(+)	-	2048	-	-	-	-	-	-	-	-	-
L. monocytogenes	(-)	2048<	512 (1024)	2048	2048<	2048<	2048<	1024	2048<	-	2048<	-
P. aeruginosa	(-)	2048<	2048<	1024	2048	2048<	2048<	-	-	-	-	-
K. pneumoniae	(-)	-	-	-	-	-	-	-	-	-	-	-
P. vulgaris	(-)	-	2048	-	-	-	-	-	-	-	-	-
Shigella spp.	(+)	-	-	1024	1024 (2048)	256 (256)	2048<	2048<	2048<	-	2048<	-
B. pumilus	(+)	2048<	512 (512)	1024	2048	1024	2048	2048<	2048<	2048<	2048<	-

Plant-borne	G	Leaf MetOH	Leaf PE	Leaf Ace	Seed MetOH	Seed PE	Seed Ace	Seed dw	Root MetOH	Root PE	Root Ace	Root dw
C. michiganensis	(+)	-	128 (256)	128 (2048)	64 (128)	8 (32)	16 (32)	2048<	-	2048<	-	-
P. tomato	(-)	2048<	2048 (2048)	2048	2048<	2048<	-	2048<	-	-	-	-
P. corrugata	(-)	-	2048<	-	-	2048<	-	2048<	-	-	-	-
P. viridiflava	(-)	-	-	-	-	-	-	2048<	-	-	2048<	-
X. vesicotoria	(-)	1024	256 (2048)	-	512	-	-	2048<				
X. perforans	(-)	2048<	2048<	256	-	2048<	1024	2048<	2048<	-	2048<	2048<
X. gardneri	(-)	-	-	-	-	-	-	2048<	-	-	-	-
E. amylovara	(-)	-	2048<	-	-	-	-	2048<	2048	2048	2048	-
E. caratovora	(-)	2048<	2048<	2048<	2048<	2048	-	2048<	-	-	-	-
E. persicinus	(-)	-	-	-	-	-	-	-	-	-	-	-
A. tumefaciens	(-)	-	-	-	-	-	-	2048<	-	-	-	-

radical scavenging activity with IC$_{50}$ of 8.2 (p<0.05) and 11.0 (p<0.05) μg mL^{-1} than other extracts. IC$_{50}$ of root MetOH extract was significantly indifferent from IC$_{50}$ of α-Toc. Similarly, IC$_{50}$ of root Ace extract was significantly indifferent from IC$_{50}$ of AscA and BHT. Leaf MetOH had the highest DRSA (IC$_{50}$: 22.7 μg mL^{-1}) among leaf extracts tested (p<0.05). Seed extracts had lower DRSA (1000 μg mL^{-1} < IC$_{50}$) in comparison to leaf and root extracts (8.2 < IC$_{50}$ < 787.3 μg mL^{-1}) (p<0.05).

The leaf and root methanol extracts had high total phenolic content

Total phenolic contents of the extracts are demonstrated on Table 4. Root Ace extract had the highest total phenolic content (95.72 mg GAE g^{-1} extract) among all extracts tested (p<0.05). In addition, total phenolic contents of the leaf and root methanol extracts were significantly higher than other extracts (p<0.05).

Quantitative and qualitative content analyses were only performed for extracts having high antibacterial activity i.e., leaf and seed extracts. Twenty three phenolic compounds were quantitatively analyzed in the MetOH, Ace, and aqueous extracts (Table 5). The phenolic compounds tested were mostly abundant in the leaf MetOH extract. Among these compounds, cholorogenic acid was the most abundant one. 767 μg g^{-1} cholorogenic acid was determined in leaf MetOH extract, and it was determined in other extracts except for the seed MetOH extract. 42 and 125 μg g^{-1} 4-hydroxybenzoic acid was determined in methanol extract of leaf and aqueous extract of seed, respectively. Vanillic acid was 11, 70, and 59 μg g^{-1} in methanol extracts of leaf and seed, and acetone extract of the seed, respectively.

Most abundant FA in the seed PE extract was hexadecanoic acid

Results of the fatty acid (FA) content analysis of PE extracts of seeds are given on Table 5. Most abundant FA (35.8%) in the

Table 4. Inhibitory concentration 50 (IC_{50}) of free radical scavenging activity of and total phenolic contents the extracts. SEM were obtained from 3 replicate experiments. GAE: Gallic acid equivalents

Extract		IC_{50} (µg mL^{-1} ± SEM)	Total phenolic contents (mg GAE g^{-1} extract ± SEM)
Leaf	Methanol	22.7 ± 1.8	78.1 ± 1.0
	Petroleum ether	787.3 ± 84.9	6.0 ± 1.7
	Acetone	122.7 ± 12.5	18.6 ± 1.0
Seed	Methanol	1040.8 ± 11.0	2.2 ± 0.5
	Petroleum ether	3443.5 ± 303.9	8.9 ± 3.1
	Acetone	993.1 ± 13.0	1.4 ± 0.1
	Aqueous	5381.7 ± 264.2	6.0 ± 0.6
Root	Methanol	11.0 ± 2.3	82.72 ± 7.6
	Petroleum ether	219.2 ± 23.9	4.6 ± 0.4
	Acetone	8.2 ± 1.7	95.7 ± 0.5
	Aqueous	64.2 ± 3.3	9.6 ± 2.2
Controls	α-Tocopherol	12.6 ± 0.5	-
	Ascorbic acid	7.6 ± 0.9	-
	Butylated hydroxytoluene	5.3 ± 0.5	-

Table 5. Quantitative analysis of phenolics of selected extracts with high antibacterial activity by HPLC-TOF. MetOH: methanol, Ace: acetone, and dw: distilled water.

| Compound	t_R* (min)	Leaf MetOH (µg g^{-1})	Leaf Ace (µg g^{-1})	Seed MetOH (µg g^{-1})	Seed Ace (µg g^{-1})	Seed dw (µg g^{-1})
Gallic acid	2.55	2.87	0.00	5.41	0.00	8.51
Gentisic acid	4.27	0.89	0.16	0.00	0.13	2.64
Catechin	6.29	1.01	0.00	0.00	0.00	2.68
Chlorogenic acid	6.30	766.84	15.91	0.00	1.28	5.01
4-Hydroxybenzoic acid	6.67	42.23	0.76	0.00	0.00	124.83
Protocatechuic acid	6.80	0.00	0.00	0.00	0.00	0.00
Caffeic acid	7.64	6.21	0.41	0.00	0.00	15.13
Vanillic acid	7.77	10.86	0.00	69.70	59.10	0.00
4-Hydroxybenzaldehyde	8.96	0.00	0.00	0.00	0.00	0.00
Rutin	9.90	0.00	0.00	0.00	0.00	0.00
Chcoric acid	10.02	0.00	0.00	0.00	0.00	0.00
p-Coumaric acid	10.20	0.00	0.00	0.00	0.00	0.00
Ellagic acid	10.03	0.00	0.00	0.00	0.00	0.00
Ferulic acid	10.93	0.00	0.00	0.00	0.00	0.00
Hesperidin	11.88	0.00	0.00	0.00	0.00	0.00
Apigenin 7-glucoside	11.91	0.00	0.00	0.00	0.00	0.00
Rosmarinic acid	12.54	0.00	0.06	0.65	0.31	0.86
Protocatechuic acid ethyl ester	13.48	0.00	0.00	0.00	0.00	0.00
Salicylic acid	13.62	0.00	0.00	2.23	0.00	11.92
Resveratrol	14.16	0.00	0.00	0.00	0.00	0.00
Quercetin	15.46	5.00	0.15	0.52	0.34	0.90
Naringenin	17.53	0.00	0.00	0.00	0.00	0.00
Kamperol	17.98	0.00	0.00	0.00	0.00	0.00
Total value		835.91	17.45	78.51	61.15	172.48

*t_R represents retention time
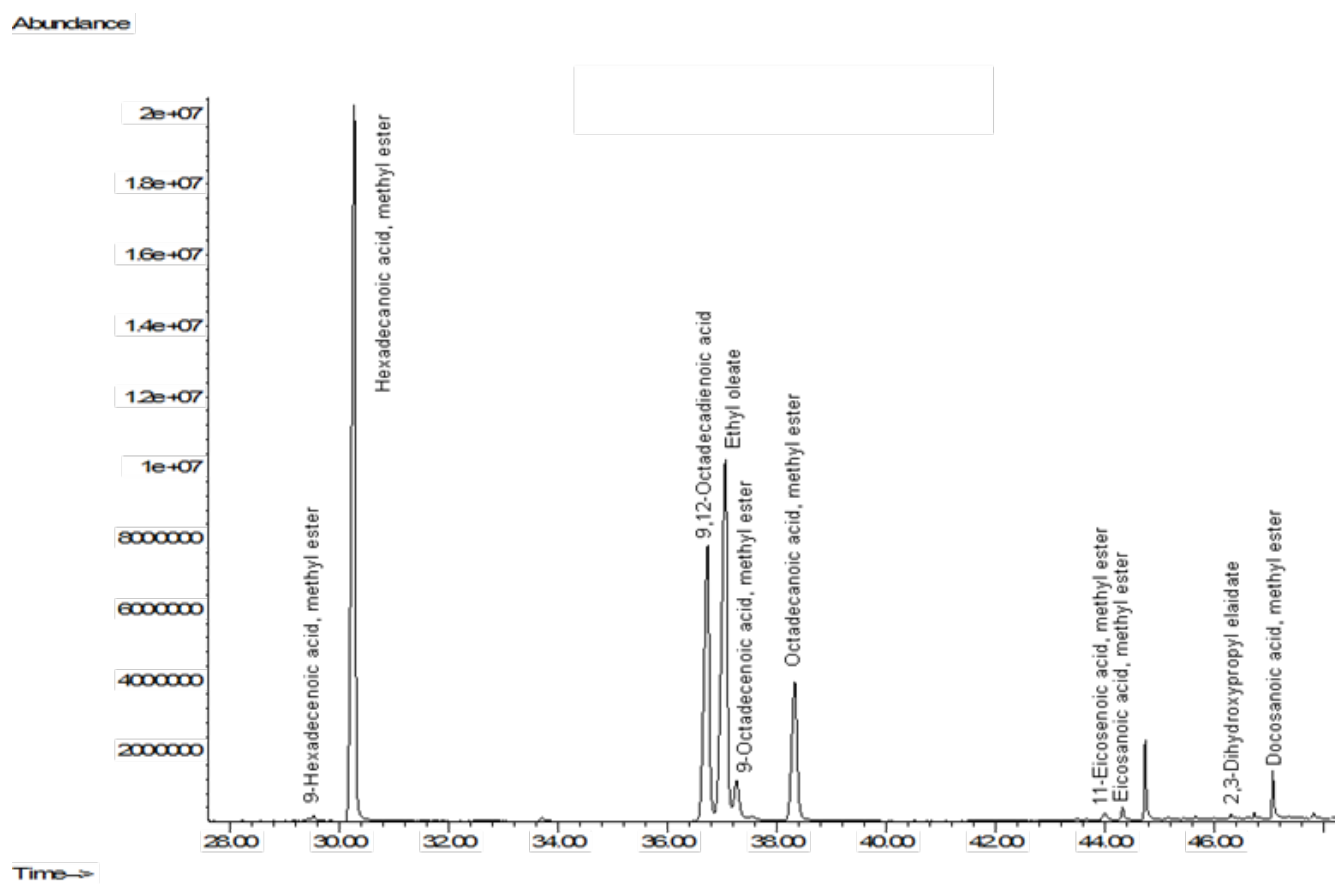
Figure 1. Fatty acid chromatogram of seed petroleum ether extract.

seed PE extract was hexadecanoic acid (palmitic acid), methyl ester. ethyl oleate (26.6%) and 9,12-octadecadienoic acid (linolenic acid) (18.7%) were other abundant FA components of the seed PE extracts . In comparison to standards, only hexadecanoic acid, methyl ester peak was determined in leaf PE extract.

Discussion

Antibacterial activity of the extracts differed

Compounds that are added and/or used during food processing to prevent food spoilage are discussed in terms of human health and their environmental contamination by industrial waste water, and studies on control of pathogens and toxin producing bacteria in foods by use of plant extracts have provided promising results on the issue (16, 17). In addition, improvement of meat quality and retardation of lipid oxidation of food during storage have also been discussed recently (18). Previously, a classification for antibacterial activity of plant extracts has been reported (19). Accordingly, inhibition of growth with a MIC below 500 µg mL^{-1} is classified as "strong inhibition". Inhibition with a MIC value in between 600 µg mL^{-1} and 1500 µg mL^{-1} is defined as "moderate inhibition", whereas a MIC higher than 1600 µg mL^{-1} is defined as "low inhibition". Highest strain susceptibility among food-borne strains was obtained with PE

extract of leaves (Table 2). Strong inhibitions were determined with seed PE extract against *S. pyogenes* and *Shigella* spp (Table 3). Similar to seed PE extracts, leaf PE extracts exerted strong inhibitory effect against *L. monocytogenes* and *B. pumilus*, and *E. gallinarum*. Antibacterial activity of a compound may be due to interaction with cytoplasmic membrane, nucleic acids, ribosomes, and specific groups (thiol, amino, sulphydryl) of enzymes of cell membrane or cytoplasm. Strong biocides have multiple target sites within the microbial cell and the overall damage to these target sites results in this bactericidal effect. Outer membrane of Gram-negative bacteria act as permeability barriers and are more resistant to various antibacterial compounds (20).

Control of bacterial diseases of crops is an important agricultural problem. Use of bactericidal agents are limited and legally restricted because of the ecological and environmental concerns, as well as sustainable agriculture. In addition, contaminated and/or infected seeds cause spread of phytopathogenic bacteria at long distances. Considering plant extracts to protect and disinfect seeds for the control of seed-borne and seed-transmitted bacterial diseases is a newly emerging eco-friendly approach. The aerobic Gram-positive *C. michiganensis* subsp. *michiganensis* causes considerable crop loss

in contaminated lands as well as at long distances due to seed transmission. Very strong antibacterial activity against *C. michiganensis* with MIC as low as 8 μg mL^{-1} were obtained with seed and leaf extracts except for the leaf MetOH. Seed extracts also exerted strong bactericidal effect. Due to the fact that resistant strains of *E. amylovora*, *Pseudomonas* spp., and *Xanthomonas campestris* have been isolated and identified (21), these species take special attention on plant disease management. Amongst, the leaf and seed extracts were the most effective against *X. vesicotoria*. *X. vesicatoria* has a wide range of crop hosts, the species of the *Solanacea* in particular, causing spot disease. Antibacterial activity of leaf acetone extract against *X. perforans* was also noticeable. In a previous study (22), tomato and pepper seeds were infected with *C. michiganensis*, *X. vesicotoria*, and *P. tomato*, and carvacrol, *S. spicigera* essential oil, and thymol were shown to exert higher antibacterial activity than streptomycin for the elimination of bacterial growth on seeds. *In vivo* studies in plant disease models are important to elucidate potential antibacterial activity, though, there are limited studies. Balestra et al. (23) performed *in vivo* tests of garlic and common fig extracts in tomato disease models. They inoculated plants with *C. michiganensis*, *P. tomato*, and *X. vesicotoria* and found dissemination of the infection 15 days after inoculation with extract application. Nonetheless, it is worth to mention that MIC and *in vivo* assay concentrations of the extracts in these studies were higher than ours.

Phenolics, aliphatic and aromatic alcohols mainly act as cytoplasmic membrane disrupters and proton conductors. Damage to the membrane results in physical disruption of the membrane and cytoplasmic leakage, dissipation of the proton motive force, and inhibition of membrane-associated enzyme activity (20). Quantitative analysis of the phenolic compounds in the MetOH, Ace, and dw leaf and seed extracts were determined (Table 5). Amongst, cholorogenic acid was the most abundant one. Previously, the antibacterial activity of chlorogenic acid on gram-positive and gram-negative bacteria was determined (MIC: 20-80 μg mL^{-1}). In this study it was shown to bind to the outer membrane, disrupt the membrane, exhaust the intracellular potential, and release cytoplasmic macromolecules (24). Aqueous leaf extracts of *C. olitorius* was reported to constitute of monophenols such as vanillic acid, caffeic acid, ferulic acid and flavonoids such as kaempferol, luteolin, rutin and quercetin. Jute fiber is a lignocellulosic fiber, composed mainly of 58-63% cellulose, 20-24% hemicellulose, and 12-15% lignin (25). Depending on the presence of mineral acids, hemicellulose residues can be hydrolyzed and the hydrolysates may contain aromatic acids derived from lignin such as ferulic, gallic, 4-hydroxybenzoic, syringic, and vanillic acids. These organic acids were tested for their activity on *E. coli* LY01. With the exception of ferulic acid, they all caused damage on membrane integrity at high concentrations. In addition, ferulic, 4-hydroxybenzoic and gallic acid were shown to inhibit fermentation. Gallic acid which is found in leaf and seed MetOH and seed dw extracts, showed to exert antimicrobial activity against both cariogenic and periodontopathic bacteria, and

considered useful antimicrobial agents against oral pathogens (26). Caffeic and p-coumaric acids have a significant role in and effect on nutrient uptake, activities of many enzymes, protein synthesis, and photosynthetic pathways in plants. In a recent study of Stojković et al. (17) in situ time dependent inhibitory effect of caffeic acid on *S. aureus* was demonstrated by inoculating chicken soup.

Free fatty acids have roles in host defenses against potential pathogenic or opportunistic microorganisms. FAs possess detergent properties which cause membrane disruption and impairment of energy generation, may affect the expression virulence factors, and saturated and unsaturated fatty acids can prevent initial bacterial adhesion and subsequent biofilm formation (27). Palmitic acid (C16:0), was the most abundant FA in both leaf and seed extracts. Antibacterial activity of palmitic acid against gram-positive and gram-negative bacteria have been previously evaluated (28). Other abundant FAs were longer unsaturated fatty acids, ethyl oleate (C20:2) and linolenic acid (C18:2). In fact, unsaturated FFAs tend to have greater potency than saturated fatty acids with the same length carbon chain (28).

Radical scavenging activity and total phenol contents were different in the extracts

Solvents used to obtain extracts have different polarity. So, plant secondary metabolites having different structure and polarity are expected to be extracted from the plant material, and in concordance to have distinct biological properties.

Free radicals are mainly scavenged by the phenolics found in plant cells (29). The phenol ring delocalizes the unpaired electron, exert a chain-breaking function, and chelate metal ions. Three classes of phenolics are derived from the basic structure of phenol (hydroxybenzene) i.e. non-flavonoids, flavonoids and tannins. There are many reports demonstrating a positive correlation between antioxidant capacity and total phenolic content of plant extracts (30). We have evaluated antioxidant potential of jute extracts. We have also correlated the DPPH radical scavenging activity with total phenol content of the extracts, and found a correlation coefficient of 0.71 (graph not shown). MetOH and Ace extracts of roots had the highest radical scavenging activity, statistically indifferent from the IC$_{50}$ of well-defined antioxidant AscA, BHT, and α-Toc. Seed extracts had significantly lower DRSA in comparison to leaf and root extracts. In concordance, root Ace extract had the highest TP content among all extracts tested. Total phenolic contents of the leaf and root methanol extracts were significantly higher in comparison to seed extracts. In a study of Dewanjee et al. (31) it was found that the supplementation of *C. olitorius* leaves reduced the Cd induced toxicity of mice hepatocytes. The results were correlated to free radical scavenging activity, Cd clearance through chelation, and other antioxidant mechanisms. Free radical mediated upregulation of mitochondrial proteins were also demonstrated.

Possible applications of plant extracts in agro-industry

The results of the present study provides a comprehensive evaluation of antibacterial activity of leaf, root and seed extracts of *C. olitorius*. In particular, this report demonstrates antibacterial activity of the seed extract for the first time, with comprehensive screening of the leaf and root extracts. Likewise, high antibacterial potential of *C. olitorius* extracts on phytopathogenic bacteria is reported for the first time. Chlorogenic acid and palmitic acid are the abundant phenolic and the fatty aid contents of the extracts with high antibacterial activity, respectively. Increasing cellular permeabilization by disrupting plasma membrane, blocking the nutrient flow, and leakage of intracellular components may be possible mechanisms of antibacterial activity. Bioactive volatile organic compounds of plants, allelochemicals, affect seed germination, growth and development of other plants in an ecologically relevant context. On the other hand, seed pre-treatments with organic and inorganic compounds and plant growth regulators may decrease germination time, increase germination rate and uniformity, enhance antioxidant response, and increase abiotic stress tolerance. We previously observed decreased mean germination time, increased germination efficiency, increased fresh weight, and root to shoot ratio with pretreatment of tomato seeds with *C. olitorius* seed extracts at a biologically relevant concentrations (8-64 μg mL^{-1}) (32). In a recent study (33), we have also demonstrated the protective effects of seed methanol extract (100 μg mL^{-1}) on tomato seedlings under copper stress as evidenced by reduced malondialdehyde, endogenous H_2O_2 levels, and DNA damage, together with enhanced catalase activity. So, seed extracts have priming and protective effect against metal toxicity at their antibacterial concentrations. Considering our and others studies on metal toxicity, extracts also have a potential use with copper based compounds used in agricultural practices. Plant originated, non-toxic, eco-friendly antibacterial compounds can be an alternative approach to plant disease management. Conclusively, data of the present study has agricultural application potential to overcome limitations in plant disease control in an environmentally friendly way.

Conflict of Interest

The authors report no conflicts of interest.

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