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Antibacterial Activity of Some Iranian Medicinal Plants Against Important Human Pathogens

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ABSTRACT

In order to validate antibacterial properties of five Iranian medicinal plants with respect to traditional uses, we have screened antimicrobial activity of these plants against ten important human pathogenic bacteria. A cross-sectional study was performed Place and Duration of Study: Study performed in Islamic Azad University, Kerman, Iran and Zabol University, Zabol, Iran during 2012 to early 2013. The antimicrobial effect of ethanol, aqueous, Ethyl acetate and chloroform extracts of *Marrubium vulgare*, *Calotorpis procera*, *Myrtus communis* L., *Piper nigrum* and *Cuminum cyminum* L. on pathogenic bacteria namely, *Pseudomonas aeroginosa*, *Shigella shinga*, *Klebsiella pneumoniae*, *Salmonella typhi*, *Proteus mirabilis*, *Serratia marcescens*, *Bacillus cereus*, *Enterobacter cloacae*, *Staphylococcus saprophyticus* and *Staphylococcus aureus* were determined using broth microdiluation method. The results revealed that ethanol and Ethyl acetate was the best extractive solvent in contrast with other solvents for separation of effective components and observation of maximum antimicrobial properties of these plants ($p<0.05$). Furthermore, *P. nigrum* and *C. cyminum* L. were a potent antimicrobial activity respectively against gram-positive (*Bacillus cereus*) and gram-negative (*Proteus mirabilis*, *Serratia marcescens*, *Pseudomonas aeroginosa*, *Enterobacter cloacae*) bacteria. The present studies confirm the use of mixes of *P. nigrum* and *C. cyminum* L. crude ethanol and Ethyl extracts as widespread antimicrobial agent. Further research is required to evaluate the practical values of therapeutic applications.

Key words: Medicinal plants, antibacterial, human pathogens

INTRODUCTION

Pathogenic bacteria are the most serious threats to human health in the world. One of the traditional methods for treatment of patients infected with pathogenic bacteria is the application of plant crude extracts or plant products (Capasso, 2003). Medicinal plants play a major role in all the traditional system of medicine and contain a rich source of natural products. Most of which have been used for human welfare, especially to cure disease caused by pathogenic microorganisms without any side effects. Antibacterial materials in the plant crude extracts suppress one or more

factors that are essential for microbial survival (Hoffmann, 2003). It has been shown that the antibacterial activities of higher plants are more potential source of novel antibiotic prototypes (Daniel, 2006). The interest in plants with antimicrobial properties has been revived because of drug resistance associated with the use of antibiotics. Nowadays, several plant crude extracts have been studied for their potential antimicrobial activity or for funding new antibacterial agents (Tajkarimi *et al*., 2010; Dorman and Deans, 2000; Indu *et al*., 2006). *Cuminum cyminum* L. (Cumin, Apiaceae) is an aromatic plant which is used in Iranian ancient medicine for treating toothache, diarrhea and epilepsy (Mirshekari *et al*., 2008). It has been shown that *Cuminum cyminum* L. has a broad antibiotic spectrum against both gram-positive and gram negative bacteria (Sowbhagya, 2011; Pajohi *et al*., 2011; Bettaieb *et al*., 2011; Wanner *et al*., 2010; Derakhshan *et al*., 2010). Some researchers have noted that cumin can be an emerging alternative antimicrobial agent for human applications (Jazani *et al*., 2008). *Marrubium vulgare* (*M. vulgare*, Lamiaceae), another medicinal plant species, found wild in many regions of Iran and It has been shown that the plant possesses stimulant, expectorant, diaphoretic and diuretic properties (Firuzi *et al*., 2010; Salama *et al*., 2012). It is helpful for bronchial asthma and nonproductive cough and in some studies showed antimicrobial activity (Zarai *et al*., 2011; De Oliveira *et al*., 2011; Boudjelal *et al*., 2012; Meyre-Silva and Cechinel-Filho, 2010; Ahmed *et al*., 2010; Rigano *et al*., 2007; Hayet *et al*., 2007). *Calotropis procera* (Sodom apple, Asclepiadaceae) is a shrub about 6 m high and is distributed in tropical and subtropical regions of Asia and Africa (Verma *et al*., 2010). It is generally used to treat common diseases such as fever, rheumatism, indigestion, ecozema and bacterial infections (Verma *et al*., 2010; Lima-Filho *et al*., 2010). Piper nigrum (Black pepper, piperaceae) is a flowering vine and native to India and its phytochemical screening shows that it contains 4% alkaloids in the berry with some antimicrobial activity (Awoyinka *et al*., 2006; Ghori and Ahmad, 2009; Karsha and Lakshmi, 2010). Myrtus communis L. (Myriaceae) is a perennial shrub and widely distributed in the Mediterranean area and Iran. Its leaves contain tannins, flavonoids such as quercetin, catechin and myricetin derivative and volatile oil. The present study was carried out to determine the in vitro potential antibacterial agent of five Iranian medicinal plants (*M. vulgare*, *C. procera*, *M. communis* L., *P. nigrum* and *C. cyminum* L.) belonging to five different botanical families against ten bacterial species which are known to cause pneumonia or wound infection (*Klebsiella pneumonia*, *Proteus mirabilis*), hemoragic diarrhea (*Shigella shinga*), typhoid fever or food borne illness (*Salmonella typhi*, *Bacillus cereus*) and urinary or respiratory tract infections (*Pseudomonas aeroginosa*, *Serratia marcescens*, *Enterobacter cloacae*, *Staphylococcus aureus*, *Staphylococcus saprophyticus*) in humans.

MATERIALS AND METHODS

Plant material: The leaf of *M. vulgare*, *C. procera*, *M. communis* L., fruit of *P. nigrum* and seed of *C. cyminum* L. were collected in the region of Iran (Zahedan and Kerman, south-eastern, Iran) and plant in Kerman Azad University herbarium received approval and dried at room temperature. Samples were crashed and transferred into glass container and preserved until extraction procedure was performed in the laboratory. Twenty grams of ground powders from each plant were soaked in 60 mL organic solvents i.e., ethanol (95% v/v), chloroform, ethyl acetate and aqueous with occasionally shaking. After one day of dissolving process, materials were filtered through a Whatman no. 1 filter paper. Then the filtrates were evaporated using a rotary evaporator. At last, 0.97 g of dried extracts was obtained and then stored at 4°C in air tight screw-cap tube (Hanafy and Hatem, 1991).

Bacterial strains and culture conditions: Bacterial strains were obtained from standard laboratory of the Veterinary department in Islamic Azad University, Kerman, Iran. To evaluate the antibacterial activity of the plant crude extracts, ten bacterial strains including seven gram-negative bacteria: [*Pseudomonas aeroginosa* (ATCC9027), *Shigella dysenteriae* (ATCC13313), *Klebsiella pneumonia* (ATCC13183), *Salmonella typhi* (ATCC1006), *Proteus mirabilis* (ATCC49565), *Serratia marcescens* (ATCC21074), *Enterobacter cloacae* (ATCC13047)] and three strains of gram-positive bacteria: [*Bacillus cereus* (ATCC4010), *Staphylococcus saprophyticus* (ATCC15305) and *Staphylococcus aureus* (ATCC6538)] were selected. The typed cultures of bacteria were sub-cultured on Nutrient agar (Oxoid) and stored at 4°C until needed.

Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC): The broth micro dilution method was used to determine MIC and MBC (Wiegand *et al.*, 2008). All tests were performed in Mueller Hinton broth supplemented with Tween 80 at a final concentration of 0.5% (v/v). Briefly, serial doubling dilutions of the extract were prepared in a 96-well microtiter plate ranged from 0.3 to 10.00 mg mL^{-1}. All selected plant extracts were prepared by dissolving firstly 10 mg of dry extract in 2 mL of DMSO (indicator solution) and then preparing further dilutions ranging from 0.3 to 10 mg mL^{-1}. Ten microliter of the indicator solution and 10 μL of the Mueller Hinton Broth were added to each well. Finally, 10 μL of bacterial suspension (10^6 CFU mL^{-1}) was added to each well to achieve a concentration of 10^4 CFU mL^{-1}. The plates were wrapped loosely with cling film to ensure that the bacteria did not get dehydrated. The plated were prepared in triplicates and then they were placed in an incubator at 37°C for 18-24 h. The color change was then assessed visually. The lowest concentration at which the color change occurred was taken as the MIC value. The average of 3 values was calculated to provide the MIC and MBC values for the tested extract. The MIC is defined as the lowest concentration of the extract at which the microorganism does not demonstrate the visible growth. The microorganism growth was indicated by turbidity. The MBC was defined as the lowest concentration of the extract at which the incubated microorganism was completely killed.

Agar well diffusion assay: Antibacterial activity of plant crude extracts was tested using the agar well diffusion method. The test inoculums (0.5 McFarland's turbidity) were spread onto Muller-Hinton agar by using a sterile cotton swab. The wells were made by a sterile well puncture and 20 μL of the extracts were added to each well and incubated at 37°C for 24 h. The presence of zones of inhibition was regarded as the presence of antimicrobial action. The average diameter of zone of inhibition was measured in millimeter. Erythromycin was included as a positive control in each assay.

Statistical analysis: The results were expressed as mean and ranked in order of importance as percent (%). The data were subjected to one-way analysis of variance (ANOVA), using the SPSS-17 software. A p-value less than 0.05 were regarded as significant.

RESULTS AND DISCUSSION

The antimicrobial activity of several plant extracts was quantitatively assessed on the bacterial growth in the medium. All plant crude extracts showed inhibitory activity against gram-positive and gram-negative bacteria with varying magnitudes and these effects were in dose dependent manners. Among the four solvents, ethyl acetate and ethanol extracts represented higher inhibitory effects against all the bacterial strains than other solvents (Table 1, 2). All plant crude extracts

Table 1: Antibacterial effects of plant crude extracts against gram-positive pathogenic bacteria

	MIC (mg mL^{-1}) and MBC (mg mL^{-1}) of herbal extracts				
Standard bacteria	*C. procera* MIC/MBC	*M. vulgare* L. MIC/MBC	*C. cyminum* L. MIC/MBC	*M. communs* L. MIC/MBC	*P. nigrum* MIC/MBC
Bacillus cereus	2.5/5[a]	5/10[a]	2.5/5[a]	1.25/2.5[a]	1.25/2.5[a]
(ATCC4010)	2.5/5[b]	2.5/5[b]	2.5/5[b]	2.5/5[b]	2.5/2.5[b]
	1.25/2.5[c]	1.25/1.25[c]	1.25/2.5[c]	1.25/1.25[c]	0.62/1.25[c]
	5/10[d]	10/20[d]	5/10[d]	5/10[d]	2.5/5[d]
Staphylococcus saprophyticus	5/10[a]	1.25/2.5[a]	2.5/5[a]	2.5/5[a]	2.5/5[a]
(ATCC15305)	5/10[b]	5/10[b]	5/10[b]	5/10[b]	2.5/2.5[b]
	2.5/5[c]	1.25/1.25[c]	1.25/1.25[c]	1.25/2.5[c]	1.25/2.5[c]
	10/20[d]	10/10[d]	5/10[d]	5/10[d]	5/10[d]
Staphylococcus aureus	2.5/5[a]	2.5/ 5[a]	2.5/5[a]	1.25/2.5[a]	1.25/2.5[a]
(ATCC6538)	5/10[b]	5/10[b]	1.25/2.5[b]	2.5/5[b]	2.5/5[b]
	2.5/5[c]	1.25/2.5[c]	1.25/1.25[c]	1.25/1.25[c]	1.25/2.5[c]
	10/20[d]	10/20[d]	5/10[d]	5/10[d]	5/10[d]

[a]Ethanol extract, [b]Chloroform extract, [c]Ethyl acetate extract and [d]Aqueous extract

Table 2: Antibacterial effects of plant crude extracts against gram-negative pathogenic bacteria

	MIC (mg mL^{-1}) and MBC (mg mL^{-1}) of herbal extracts				
Standard bacteria	*C. procera* MIC/MBC	*M. vulgare* L. MIC/MBC	*C. cyminum* L. MIC/MBC	*M. communs* L. MIC/MBC	*P. nigrum* MIC/MBC
Klebsiellas pneumonia	1.25/2.5[a]	5/10[a]	1.25/ 2.5[a]	0.62/1.25[a]	1.25/2.5[a]
(ATCC13183)	2.5/2.5[b]	5/5[b]	2.5/5[b]	1.25/2.5[b]	1.25/1.25[b]
	2.5/5[c]	2.5/2.5[c]	0.62/1.25[c]	2.5/5[c]	0.62/1.25[c]
Salmonella typhi	5/10[d]	5/10[d]	2.5 / 5[d]	5/10[d]	2.5/5[d]
(ATCC1006)	2.5/5[a]	2.5/5[a]	0.62/1.25[a]	2.5/5[a]	2.5 / 5[a]
	5 /10[b]	2.5/5[b]	1.25/1.25[b]	5/10[b]	2.5 /2.5[b]
Shigella dysenteriae	1.25/2.5[c]	1.25/1.25[c]	0.3/0.3[c]	2.5/5[c]	1.25/2.5[c]
(ATCC13313)	5/10[d]	5/10[d]	2.5/5[d]	5/10[d]	5/10[d]
	2.5/5[a]	2.5/5[a]	1.25/2.5[a]	2.5/5[a]	1.25/2.5[a]
	2.5/5[b]	2.5/5[b]	5/10[b]	2.5/2.5[b]	2.5/5[b]
Proteus mirabilis	1.25/2.5[c]	1.25/2.5[c]	2.5/5[c]	1.25/2.5[c]	1.25/1.25[c]
(ATCC49565)	2.5/2.5[d]	5/10[d]	5/10[d]	5/10[d]	5/10[d]
	0.62/1.25[a]	0.62/1.25[a]	2.5/5[a]	2.5/5[a]	1.25/1.25[a]
	0.62/0.62[b]	1.25/2.5[b]	2.5/5[b]	1.25/2.5[b]	1.25/2.5[b]
Pseudomonas mirabilis	0.3/0.62[c]	0.62/0.62[c]	1.25/2.5[c]	1.25/1.25[c]	0.62/0.62[c]
(ATCC49565)	2.5/5[d]	2.5/5[d]	2.5/2.5[d]	5/10[d]	2.5/5[d]
	1.25/2.5[a]	0.62/1.25[a]	0.3/0.62[a]	1.25/2.5[a]	2.5/5[a]
	2.5/2.5[b]	0.62/1.25[b]	0.62/0.62[b]	2.5/2.5[b]	2.5/2.5[b]
Serratia marcescens	0.62/1.25[c]	0.62/0.62[c]	0.3/0.62[c]	0.62/1.25[c]	1.25/2.5[c]
(ATCC21074)	2.5/5[d]	2.5/5[d]	1.25/1.25[d]	2.5/5[d]	2.5/5[d d]
	1.25/2.5[a]	0.3/0.62[a]	1.25/2.5[a]	2.5/5[a]	2.5/5[a]
	1.25/2.5[b]	1.25/1.25[b]	2.5/5[b]	5/5[b]	2.5/5[b]
Enterobactor cloacae	0.62/0.62[c]	0.3/0.3[c]	1.25/1.25[c]	1.25/1.25[c]	1.25/2.5[c]
(ATCC13047)	5/10[d]	5/10[d]	2.5/2.5[d]	10/20[d]	5/10[d]
	1.25/2.5[a]	5/10[a]	1.25/2.5[a]	5/10[a]	0.62/1.25[a]
	2.5/5[b]	5/10[b]	2.5/5[b]	5/10[b]	1.25/2.5[b]
	1.25/2.5[c]	2.5/2.5[c]	0.62/1.25[c]	2.5/2.5[c]	0.3/0.62[c]
	2.5/5[d]	10/20[d]	2.5/5[d]	5/10[d]	5/5[d]

[a]Ethanol extract, [b]Chloroform extract, [c]Ethyl acetate extract, [d]Aqueous extract

Table 3: Antimicrobial activity of the plant crude extracts as mean of inhibition diameter zone against gram-positive pathogenic bacteria (mm) (30 Al disc-1)

	MIC (mg mL^{-1}) and MBC (mg mL^{-1}) of herbal extracts				
Standard Bacteria	*C. procera* MIC/MBC	*M. vulgare* L. MIC/MBC	*C. cyminum* L. MIC/MBC	*M. communs* L. MIC/MBC	*P. nigrum* MIC/MBC
Bacillus cereus	10 mm[a]	8 mm[a]	10 mm[a]	12 mm[a]	11 mm[a]
(ATCC4010)	10 mm[b]	10 mm[b]	10 mm[b]	10 mm[b]	11 mm[b]
	12 mm[c]	11 mm[c]	12 mm[c]	12 mm[c]	13 mm[c]
	7 mm[d]	6 mm[d]	6 mm	7 mm[d]	10 mm[d]
Staphylococcus saprophyticus	7 mm[a]	12 mm[a]	10 mm[a]	10 mm[a]	9 mm[a]
(ATCC15305)	7 mm[b]	7 mm[b]	8 mm[b]	6 mm[b]	9 mm[b]
	9 mm[c]	12 mm[c]	12 mm[c]	12 mm[c]	11 mm[c]
	5 mm[d]	5 mm[d]	7 mm[d]	7 mm[d]	7 mm[d]
Staphylococcus aureus	10 mm[a]	10 mm[a]	8 mm[a]	12 mm[a]	11 mm[a]
(ATCC6538)	7 mm[b]	7 mm[b]	12 mm[b]	9 mm[b]	9 mm[b]
	10 mm[c]	12 mm[c]	12 mm[c]	12 mm[c]	11 mm[c]
	5 mm[d]	5 mm[d]	6 mm[d]	7 mm[d]	7 mm[d]

[a]Ethanol extract, [b]Chloroform extract, [c]Ethyl acetate extract, [d]Aqueous extract

except of ethyl acetate extract of *P. nigrum* exhibited same antibacterial activity against *Bacillus cereus* (Table 1). Moreover, all plant crude extracts showed relatively same antibacterial activity against *Staphylococcus saprophyticus* and *Staphylococcus aureus* (Table 1). Maximum inhibition of gram-negative bacteria was observed with the ethyl acetate extract of *P. nigrum* against *Bacillus cereus* (13 mm) (Table 3). The least MIC and MBC value for gram-negative bacteria were observed by the ethyl acetate the crude extract of *M. communis* L. against *Proteus mirabilis* and *C. cyminum* L. against *Serratia marcescens* (0.3 and 0.3 mg mL^{-1}). In general, aqueous extracts of all plants showed the highest MIC and MBC values (Table 2). Ethyl acetate crude extract of *M. communis* L. had a maximum inhibitory effect on *Pseudomonas aeroginosa* and *Serratia marcescens* (Table 2). The crude extract of *C. cyminum* L. had a minimum inhibitory effect on *Salmonella typhi* and *Shigella shinga* (Table 2). The crude extract of *M. communis* L. had a minimum inhibitory effect on *Salmonella typhi* and *Serratia marcescens* (Table 2). Extracts of *P. nigrum* had a minimum inhibitory effect on *Salmonella typhi, Serratia marcescens* and *Shigella shinga* (Table 2). Totally, the crude extract of *C. procera* showed relatively same antibacterial activity against all bacteria and *Salmonella typhi* was the more resistant bacteria for all plant crude extracts (Table 2). Maximum inhibitory effects of plant extracts on the gram-negative bacteria were observed with the ethyl acetate extract of *M. communis* L. against *P. mirabilis* (16 mm) (Table 4).

Our findings are same with previous reports that showed, the ethanol and ethyl acetate were better solvents for extraction of antimicrobial active substances than water (Ahmed *et al*., 2010). It has been reported that the major phytochemical compound of the crude extract of *P. nigrum* is piperine (Fan *et al*., 2011; Reshmi *et al*., 2010). Our results demonstrate that same component in the crude extract of *P. nigrum* and *M. communis* L. has a similar antibacterial effect on the gram-positive bacteria, especially *Enterobacter cloacae*. The study of Karuna, showed that the methanolic extract of *P. nigrum* fruit was more effective than others against *Staphylococcus aureus* (20 mm) and *Bacillus subtilis* (18 mm) (Karuna and Archita, 2012). Keskin and coworkers have been shown that acetone extract of *P. nigrum* has an inhibitory effect on all tested microorganisms

Table 4: Antimicrobial activity of the plant crude extracts as mean of inhibition diameter zone against gram-negative pathogenic bacteria (mm) (30 Al disc-1)

	MIC (mg mL^{-1}) and MBC (mg mL^{-1}) of herbal extracts				
Standard bacteria	*P. nigrum* MIC/MBC	*C. cyminum* L. MIC/MBC	*M. communis* L. MIC/MBC	*M. vulgare* L. MIC/MBC	*C. procera* MIC/MBC
Klebsiellas pneumonia	12 mm[a]	7 mm[a]	12 mm[a]	13 mm[a]	10 mm[a]
(ATCC13183)	9 mm[b]	6 mm[b]	10 mm[b]	8 mm[b]	10 mm[b]
	9 mm[c]	10 mm[c]	13 mm[c]	10 mm[c]	13 mm[c]
	7 mm[d]	7 mm[d]	10 mm[d]	6 mm[d]	9 mm[d]
Salmonella typhi	12 mm[a]	10 mm[a]	11 mm[a]	9 mm[a]	12 mm[a]
(ATCC1006)	7 mm[b]	10 mm[b]	9 mm[b]	6 mm[b]	12 mm[b]
	10 mm[c]	12 mm[c]	11 mm[c]	9 mm[c]	10 mm[c]
	7 mm[d]	8 mm[d]	6 mm[d]	5 mm[d]	6 mm[d]
Shigella dysenteriae	10 mm[a]	9 mm[a]	12 mm[a]	8 mm[a]	8 mm[a]
(ATCC13313)	10 mm[b]	12 mm[b]	12 mm[b]	8 mm[b]	8 mm[b]
	13 mm[c]	12 mm[c]	14 mm[c]	10 mm[c]	10 mm[c]
	9 mm[d]	7 mm[d]	11 mm[d]	7 mm[d]	7 mm[d]
Proteus mirabilis	10 mm[a]	10 mm[a]	14 mm[a]	12 mm[a]	13 mm[a]
(ATCC49565)	10 mm[b]	10 mm[b]	12 mm[b]	12 mm[b]	12 mm[b]
	13 mm[c]	13 mm[c]	16 mm[c]	15 mm[c]	14 mm[c]
	9 mm[d]	9 mm[d]	12 mm	10 mm[d]	11 mm[d]
Pseudomonas aeroginosa	11 mm[a]	11 mm[a]	13 mm[a]	14 mm[a]	12 mm[a]
(ATCC9027)	11 mm[b]	9 mm[b]	13 mm[b]	11 mm[b]	10 mm[b]
	13 mm[c]	13 mm[c]	13 mm[c]	15 mm[c]	14 mm[c]
	9 mm[d]	9 mm[d]	11 mm[d]	9 mm[d]	9 mm[d]
Serratia marcescens	10 mm[a]	12 mm[a]	12 mm[a]	12 mm[a]	9 mm[a]
(ATCC21074)	9 mm[b]	12 mm[b]	10 mm[b]	10 mm[b]	6 mm[b]
	12 mm[c]	14 mm[c]	12 mm[c]	14 mm[c]	11 mm[c]
	9 mm[d]	7 mm[d]	10 mm[d]	8 mm[d]	5 mm[d]
Enterobacter cloacae	10 mm[a]	8 mm[a]	11 mm[a]	7 mm[a]	14 mm[a]
(ATCC13047)	9 mm[b]	8 mm[b]	10 mm[b]	7 mm[b]	10 mm[b]
	11 mm[c]	11 mm[c]	13 mm[c]	10 mm[c]	15 mm[c]
	9 mm[d]	6 mm[d]	9 mm[d]	6 mm[d]	8 mm[d]

[a]Ethanol extract, [b]Chloroform extract, [c]Ethyl acetate extract, [d]Aqueous extract

except for *C. xerosis* (Keskin and Toroglu, 2011). Antimicrobial activity of *P. nigrum* is deferent dependent on plant collection site, bacterial strains and extracts (Erturk, 2006; Indu *et al*., 2006; Karsha and Lakshmi, 2010). The main components of *C. cyminum* oil were *p*-mentha-1,4-dien-7-al, cumin aldehyde, *ç*-terpinene and *γ*-pinene (Iacobellis *et al*., 2005; Jalali-Heravi *et al*., 2007). Linalool, Myrtenyl acetate and Myrtenol were shown to be the major components of oil in the leave of *M. communis* that exhibited antimicrobial activity against gram positive, gram negative bacteria and fungus with MICs in the range of 0.5-32, 8-64 and 0.03-16 μL mL^{-1}, respectively (Djenane *et al*., 2011; Gemeda *et al*., 2008; Zomorodian *et al*., 2013). Similarly, our results demonstrated that the same components in *P. nigrum* and *M. communis* L. and *C. cyminum* have antibacterial effects on gram-negative bacteria, especially *Proteus mirabilis, Serratia marcescens* and *Pseudomonas aeroginosa*. Different effects of the extracts on the bacteria may be due to the presence of lipoproteins and lipopolysaccharides in the wall of the gram-negative bacteria which form a barrier for entering the hydrophobic compounds in to the cell (Wang and Chen, 2009; Mazutti *et al*., 2008; Bachir and Benali, 2012). The bacteriocidal activity of CP could be due to

the presence of calactin, mudarin and protein which are called calotropain (Kareem *et al*., 2010; Nagariya *et al*., 2010). The ethanol and methanol extracts of *C. procera* latex did not show any activities against *Salmonella typhi* and *Shigella boydii* (Shivaji *et al*., 2012; Yesmin *et al*., 2008). From the above experiment we conclude that *P. nigrum* and *M. communis* L. Mixed extracts suggest significant growth inhibiting effects on both gram-positive and gram-negative bacteria. The efficacy of CCL and PN against these bacteria may provide a scientific ground for the application of the herb in the prevention and treatment of them which have developed resistance to antibiotics.

CONCLUSION

Thus on the basis of the results it is inferred that the extract of *P. nigrum* and *M. communis* L. whole plant had adequate *in-vitro* antibacterial. Further phytochemical studies are needed to identify active constituents responsible for the observed activity. The results of this study present the herb as a good candidate to explore new alternative antibacterial agents to combat pathogenic microorganisms.

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