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Antibacterial activities of *Myrtus communis L* extract against multi-drug resistant *Klebsiella pneumonia* and *Pseudomonas aeruginosa*

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Abstract

In this study the antibacterial activities of *Myrtus communis L.* extract against Multi-Drug resistant *Klebsiella pneumonia* and *Pseudomonas aeruginosa* that isolates from the urinary tract infection by microtiterplate method are studied. All 12 strains of *Klebsiella pneumoniae* and *Pseudomonas aeruginosa* isolated from urine culture of hospitalized patients (Amir Al-Momenin Hospital, Zabol, south-eastern Iran) suffered from urinary tract infections during the years 2011- 2012. In this study, the extract of *Myrtus communis L* obtained by rotary and the minimum inhibitory concentrations were investigated to characterize the antimicrobial activities of this extract. Overall, *k. pneumoniae* was resistance to 3 of the agent including ceftazidime(33.3%), cefixime(58.3%), erythromycin(75%) and *P. aeruginosa* was resistance to 4 antibiotic that included ceftazidime(33.3%), tobramycin(8.3%), piperacillin(8.3%) and cefixime(83.3%). The highest MIC values of extract was found to be 20mg/ml against *K.pneumoniae* and ten of MIC value for *K.pneumoniae* was 10mg/ml and the highest MIC values of extract was found to be 20mg/ml againts *P. aeruginosa* and eight of MIC value for *Pseudomonas aeruginosa* was 10mg/ml. These results suggest that *M. communis L* leaves may be utilized as a potential source of bactericidal, but in vivo studies are required to support this.

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Introduction

Medicinal plants are very important one in human health, it will act as an antibactericide activity against the bacterial pathogens, this is followed from ancient times. *Myrtus communis* or common Myrtle (Myrtaceae) is an ever green shrub or small tree with evergreen leaves, 6-8 feet in height with small foliage and covered with a deep fissured bark. The Myrtle (*Myrtus*) is a genus of flowering plants with 1 or 2 species, native to Southern Europe and North Africa and widespread in the Mediterranean area. It is cultivated in North West India gardens for its fragrant flowers (Asif *et al*., 2011; Kirtikar *et al*., 1988). Myrtus species were reported as very rich in volatile oils(Satrani *et al*., 2006; Shikhiev *et al*., 1988) phenolic acids as gallic and ellagic acids,(Romani *et al*., 1999) flavonoids(Romani *et al*., 1999; Joseph *et al*., 1987) fatty acids (FA)(Cakir *et al*., 2004) tannins(Diaz *et al*., 1986) and anthocyanin pigments(Martin *et al*., 1990). Essential oils are gaining remarkable interest for their potential multipurpose use as antioxidant, antibacterial, and antiseptic agent (Alem *et al*., 2008; Gündüz *et al*., 2009); the essential oil obtained from the leaves was used in the past for the treatment of lung disorders.

klebsiella pneumoniae is among the most common gram negative bacteria encountered by physicians worldwide. It is an important gram-negative opportunistic pathogen causing primarily urinary tract infections (UTIs), respiratory infections and bacteraemia especially in immunocompromised individuals (Podschun and Ullmann, 1998). It is also a potential community acquired pathogen. Although the incidence of UTI has been reduced by the use of intermittent bladder catheterization, recurrence of UTI continues to be a troublesome problem in many patients.

Pseudomonas aeruginosa is a gram-negative bacterium that continues to be a major cause of opportunistic nosocomial infections, causing around 9–10% of hospital infections .Occasionally, *P. aeruginosa* can colonies human body sites, with a preference for moist areas, such as the perineum, axilla, ear, nasal mucosa and throat; as well as stools. The prevalence of colonization by *P. aeruginosa* in healthy subjects is usually low, but higher colonization rates can be encountered following hospitalization, especially amongst subjects treated with broad-spectrum antimicrobial agents (Rossolini and Mantengoli, 2005). In this study, the antibacterial and activities of extract *Myrtus communis L.* against Multi-Drug resistant *K.pneumonia* and *P. aeruginosa* that isolates from the urinary tract infection by micro titer plate method.

Materials and methods

A cross-sectional study was carried out based on reports of bacteria isolates from the urinary tract infection of Amir- Hospital- Zabol-Iran from 2011-2012. All samples that were collected aseptically from the 12 patients were plated right after the collection. Identification of all causative microorganisms was performed by standard microbiologic methods. Susceptibility testing was performed using disk diffusion method. The results were interpreted according to the guide lines of the Clinical and Laboratory Standards Institute (CLSI, 2006).

Plant material

The leaf of *Myrtus communis* L was purchased from Municipal market at Zahdan-Iran during February, 2012 and kept in sterilized screw-cap glass container. Sample was crashed and transferred into glass container and preserved it until extraction procedure in the laboratory .Twenty gram of grinded powders from each plant was soaked in 60 ml organic solvents i.e. ethanol (95 %v/v) with occasionally shaking. After one day of dissolving materials were filtered through a Whatman no. 1 filter paper. Then the filtrates were evaporated using rotary evaporator. At last, 0.97 g of dried extracts was obtained and then stored at 4^{o}C in air tight screw-cap tube.

Minimum Inhibitory Concentration (MIC) of extract

The broth microdilution method was used to determine MIC. 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 micro titer plate ranged from 10 mg/ml- 0.3 mg/ml. To each well, 10 µl of indicator solution (prepared by dissolving a 10-mg extract in 2 ml of DMSO) and 10 µl of Mueller Hinton Broth were added. Finally, 10 µl of bacterial suspension (10^6 CFU/ml) was added to each well to achieve a concentration of 10^4 CFU/ml. The plates were wrapped loosely with cling film to ensure that the bacteria did not get dehydrated. The plates were prepared in triplicates, and then they were placed in an incubator at 37°C for 18–24 hours. 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 providing the MIC 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.

Result

Overall, *k. pneumoniae* was resistance to 3 of the agent including ceftazidime(33.3%) ,cefixime(58.3%), erythromycin(75%) and *P. aeruginosa* was resistance to 4 antibiotic that included ceftazidime(33.3%), tobramycin(8.3%) , piperacillin(8.3%) and cefixime(83.3%) . The highest MIC values of extract was found to be 20mg/ml against *K.pneumoniae* and ten of MIC value for *K.pneumoniae* was 10mg/ml and the highest MIC values of extract was found to be 20mg/ml against *P. aeruginosa* and eight of MIC value for *Pseudomonas aeruginosa* was 10mg/ml.

Table 1. Antimicrobial susceptibility, MIC plant extract for *k.* pneumonia (mg/ml).

Bacterial Cod	MIC for plant extract (mg/ml)	Resistance pattern
1	10	A_1, A_2
2	10	A_1, A_2
3	10	A_1
4	5	-
5	10	A_1
6	20	A_1, A_2, A_3
7	10	A_1, A_2, A_3
8	10	A_1, A_2, A_3
9	20	-
10	10	A_1, A_2, A_3
11	10	A_1
12	10	A_2

A_1= Erythromycin, A_2= cefixime, A3= Ceftazidime.

Table 2. Antimicrobial susceptibility, MIC plant extract for *P. aeruginosa* (mg/ml).

MIC	piperacillin	tobramycin	cefixime	ceftazidime	Bacterial cods
5mg/ml	S	S	R	S	1
5mg/ml	S	S	R	S	2
20mg/ml	S	R	R	R	3
10mg/ml	I	S	I	R	4
10mg/ml	I	S	R	I	5
1.25mg/ml	S	S	R	S	6
10mg/ml	S	S	I	S	7
10mg/ml	I	S	R	I	8
10mg/ml	S	S	R	I	9
10mg/ml	R	S	R	R	10
10mg/ml	S	S	R	R	11
10mg/ml	S	I	R	S	12

Discussion

In the study, result showed that *k. pneumonia* was resistance to 3 of the agent including ceftazidime (33.3%), cefixime(58.3%), erythromycin(75%). The study of Zamani showed that the most effective antibiotics against the isolates were tobramycin (79.05%), ceftazidime (79.05%), ceftizoxime (78.09%), ciprofloxacin (76.19%), ceftriaxone (76.24%) and amikacin (74.29%)(Zamani *et al*., 2013). In the study *P. aeruginosa* was resistance to 4 antibiotic that included ceftazidime (33.3%), tobramycin (8.3%), piperacillin(8.3%) and cefixime(83.3%). According to study of Rahimi, antibiotic susceptibility of *P. aeruginosa* test showed that resistance rates to imipenem, meropenem, gentamicin, amikacin, ciprofloxacin, and ceftazidime were 35%, 35%, 14%, 9%, 23% and 15%, respectively(Rahimi *et al*., 2012).

The highest MIC values of extract were found to be 20mg/ml against *K.pneumoniae*. and the highest MIC values of extract were found to be 20mg/ml In the other hand P. *aeruginosa* and eight of MIC value for *P. aeruginosa* was 10mg/ml. According to study of Alem, the Minimum Bactericidal Concentration of Myrtle for most tested microorganisms was similar to the Minimum Inhibitory Concentration. i.e. 0.5 mg/ml. for *S. aureus*, 2.5 mg/ml for *P. mirabilis* and *P. vulgaris*, 15 mg/ ml for *Klebssiela* and *S. typhi*, 20 mg/ml for *P. aeruginosa*.

And the MBC of Myrtle for the two relatively least sensitive species, *Shigella* and *E. coli* was 40 mg/ml and 45 mg/ml of media, respectively (Alem *et al*., 2008). According to study of Bonjar, the least MIC, as 0.62 mg/ml to *Myrtus communis* seeds against *S. aureus*, *Bacillus cereus* and *B. bronchiseptica*, (Bonjar, 2004).

The oils of *M. communis* had well to excellent antimicrobial activities against *Escherichia coli*, *Staphylococcus aureus* and *Candida albicans* (Yadegarinia *et al*., 2006). The study of Gholamhoseinian showed that ethyl acetate extracts of *M. communis* which inhibited the *Staphyloc occus aureus* growth at 5 µg/ml concentration (.Gholamhoseinian *et al*., 2009). Based on the another study, dilution 1/2 of essential oil *Myrtus communis* showed deadly effect against *E.coli, B. subtilis, B.licheniformis, C.albicans* and *S. cerevisiae*(Rasooli *et al*., 2002). Mansouri evaluated the antibacterial activity of methanol crude extract of *M. communis* against 10 laboratory strains of microorganisms, including 6 Gram positive (*Staphylococcus aureus, Micrococcus luteus, Streptococcus pneumoniae, S. pyogenes, S. agalactiae* and *Listeria monocytogenes*) and 4 Gram negative bacteria (*Escherichia coli, Proteus vulgaris, Pseudomonas aeruginosa* and *Campylobacter jejuni*). The crude extract inhibited the growth of all tested bacteria except *C. jejuni* (Mansouri *et al*., 2001). Akin also assayed antimicrobial activity of *M. communis* against seven pathogen bacteria (*Staphylococcus aureus*, *Listeria monocytogenes*, *Enterococcus durans*, *Salmonella typhi*, *Escherichia. coli*, *Pseudomonas aeruginosa* and *Bacillus subtilis*) (Akin *et al*., 2010). According to study of Saeidi, the least MIC value for gram-negative bacteria was observed by the ethyl acetate the crude extract of M.communis against Proteus mirabilis (0.3 mg/ml) (Saeidi *et al*., 2013). In another study *M. communis* showed inhibitory activity against *Morganella morgana* with levels of MIC and MBC were observed ranges from 2.5 and 5 mg/ml in radius respectively (Bokaeian *et al*., 2013). The results indicate the potential antimicrobial properties of extract and hence a hope to see the emergence of antimicrobial compounds from natural sources in the near future.

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