

# Original Article

## Evaluation of antibacterial effect of *Myrtus communis* against *Acinetobacter baumannii* clinical strains

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### Abstract

Because of inappropriate use of antibiotics and prevalence of resistant bacteria, there is urgent need for antibacterial drugs that have fewer side effects than antibiotics. *Myrtus communis* is a medicinal plant which had many uses in traditional medicine. In this study, ethanol leave extract of this plant is tested on *Acinetobacter baumannii*. In the case of antimicrobial evaluation of plants, one of the effecting factors on effectiveness of the microbial inhibition is extraction techniques. In the presents study, the antibacterial activity of the Ethanol, Methanol, and Ethyl acetate extracts of *M. communis* plant was evaluated at seven different concentrations by broth microdilution method. The results of this study showed that the antimicrobial effect of *M. communis* extract is concentration dependent. Different extracts were obtained by the maceration method. Extracts of the plant exhibited antibacterial activity at varied levels against *A. baumannii*. Obtained results from our antibacterial experiments showed that all extracts have anti-bacterial activity against tested bacterial isolates According to the results, the ethyl acetate extracted fraction showed the highest level of activity at a MIC 400 mg/ml for *A. baumannii*. The results of this study indicate that, different extracts had growth inhibitory effect on *A. baumannii*. Therefore this plant has the potential to be evaluated as an alternative or adjunct to antibiotics to treat *Acinetobacter* infections.

**Keywords:** *Myrtus communis*, *Acinetobacter baumannii*, Antibacterial, Clinical strains

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## INTRODUCTION

Over the past 2 decades, there has been a profound interest in the field of herbal drug discovery and development. Recently, increasing drug resistance due to inappropriate use of common antimicrobial drugs thus difficulties in the treatment of infectious diseases becoming a global progressive problem (Shree et al. 2013). Isolation of bacterial agents less susceptible to regular antibiotics and resistant isolates during antibacterial therapy is rising worldwide problem which indicate the need for new safe drug resources (de Wouters et al. 2015). As a result, there has been a growing trend to use natural products with minimal cytotoxicity, biological compatible, cost-effective, and efficient alternatives for treatment of infectious diseases. In this field one of the great resources is medicinal plants. *M. communis* commonly known as Myrtle, is a perennial shrub widely distributed in Europe, Asia, Africa and America and is one of the important aromatic and medicinal species from Myrtaceae family (Haciseferogullari et al. 2012). The height of the plant varies between, 1.8–2.4 m in, and characterized by its branches, which form a close full head, thickly covered with ovate or lanceolate evergreen leaves. The plant have been used by locals as astringent, anti-microbial, for constipation, leaves are used as mouthwash, and as antimicrobial agent for the treatment of candidiasis (Aleksic & Knezevic, 2014). *M. communis* belongs to the Myrtaceae family, which comprises approximately 145 genera and over 5500 species. Myrtle is native to the Middle East and Asia and includes flowering plant with about 16 species (Feuillolay et al. 2016). In the case of antimicrobial evaluation of plant, one of the effecting factors on effectiveness of the microbial inhibition is extraction techniques (Deriu et al. 2007). Selective use of solvents in different extraction methods separated instinct amount of the plant metabolites.

Preparing plant extracts have several steps, it is important to emphasize that the quality of an extract is affected by several parameters such as the plant sections used as starting material, the type of solvent used for extraction and the extraction methodology (Maxia et al. 2011). The solvents commonly used for obtaining extracts are water, ethyl acetate, methanol and ethanol. During the past decade, *A. baumannii* exhibits a remarkable ability to rapidly develop antibiotic resistance. To extent that nowadays, treatment of *A. baumannii* infections because of resistant to antimicrobial agents of the bacterium is very difficult. The emergence and rapid spread of multidrug resistant (MDR) *A. baumannii* isolates is becoming a serious concern in global public health. Dissemination of MDR *A. baumannii* isolates in both hospitals and community not only leads to an increase in economic burden but it may also cause serious therapeutic problems. Given to increasing MDR among *A. baumannii* isolated from hospitalized patients, it is necessary that investigated the antibacterial activity of medicinal plants that can be used as a new source for antibiotic. The aim of this study was to investigate the antimicrobial activity of *M. communis* against *A. baumannii* clinical isolates.

## MATERIAL AND METHOD

Fresh leaves of *M. communis* were collected in the southern part of Iran, in April 2015, from a single collection site. The leaves were air-dried at the room temperature and stored in double-layer paper bags, until further analysis, protected from the direct light. The identity of the plant specimen was confirmed at the Department of Biology and deposited at the herbarium of the department of pharmacognosy, faculty of pharmacy, Tehran University of Medical Sciences.

**Microbial strains:** A total of 40 clinical isolates of *A. baumannii* were recovered from specimens

of patients suspected with *A. baumannii* infection who were hospitalized in Intensive Care Units (ICU) of Tehran hospitals between September 2014 and October 2015. All the clinical samples were transported to the Microbiology Research Laboratory in the Department of Microbiology, School of Medicine, Shahid Beheshti University of Medical Sciences and were processed immediately. Clinical samples were cultured on MacConkey and blood agar plates, Trypticase Soy Broth (TSB), and sub-cultured on chocolate agar for blood specimens, and chocolate agar for specimens other than urine. Identification was done based on culture characteristics and gram stain and biochemical tests, typical reaction of *A. baumannii* to glucose is positive and to oxidase, mannitol, maltose, Esculin, Indole, and H<sub>2</sub>S are negative. *A. baumannii* has also ALK/ALK reaction on Triple sugar iron (TSI) agar. Samples confirmed as *A. baumannii* were stored in Tryptic Soy Broth (TSB; Merck, Germany) containing 20% glycerol at -70°C and were subjected to further molecular identification.

#### **Isolation of the essential oil:**

Ethanol, methanol, ethyl acetate extracts of the samples were obtained by the maceration method. Maceration procedure used for preparation of extract: 50 mL ethanol 96% or methanol 96% for 20h was used for extraction of 1 g sample. WhatmanNo.1 (Camlab, UK) used for the mixture filtering and the filtrate was evaporated to dryness under vacuum at 38°C. Therefore, the dried extract was weighed and the product was calculated. Glycerin solution (Twenty percent) was used as solvent. 200 mL of prepared glycerin solvent was added to Fifty grams of fine powder of the leaves and heated for 30 minutes. Then, the extract was filtered by paper filters and centrifuged in 9000 gravity for 20 minutes.

#### **Determination of minimum inhibitory concentration:**

The minimum inhibitory concentration (MIC) were assessed for the test solutions by broth micro dilution method according to standard references Briefly, Mueller-Hinton broth used for overnight bacterial culture at 37°C; final density of McFarland set to 0.5 standard using a spectrophotometer at wave length of 625 nm. Then, they were dispensing to a microtiter plates (96-well) containing serial ten-fold dilutions of the essential oils. For positive control, piperacillin (1-8 µg/ml) was used and sterile water as negative control. The microplates were prepared by dispensing 100 µl of Mueller-Hinton broth for bacteria, into each well. A 100 µl from the stock solution of extracts was added into the first row of the plate. Then, serial dilutions were performed by using a micropipette. The obtained concentration range was from 100 to 0.8 mg/ml, and then added 10 µl of inocula to each well except for positive control. To ensure the accuracy of tests and also to prevent any possible bias these experiments were repeated 3 times. *Pseudomonas aeruginosa* ATCC 27853 were used as reference strains for susceptibility testing

#### **Statistical analysis**

Statistical analysis was carried out using SPSS, version 18.0 (SPSS Inc., Chicago, IL). A P value less than 0.05 was considered as statically significant.

#### **Results**

In present study, antibacterial activity of chloroform, methanol, and ethyl acetate of *M. communis* have been investigated in vitro against *A. baumannii*. Minimal inhibition concentration assays were performed to determine the concentration at which the extracts are effective. In this regard, we evaluate the antibacterial potency of extract

at different concentrations (12.5, 6.25, 3.125, 1.56, 0.78, 0.39 mg/ml) and also based on percentage; percent of bacterial colonies that grows inhibited. According to results, all of the extracts showed antibacterial activity against *A. baumannii*. As shown in Table 2, the minimum inhibitory concentration values of methanol extract against the bacterial strains used were lower than those of the chloroform extract. In fact, MIC order is concentration dependent, along with increasing extract concentration, the bactericidal effect of extract improved. Most effective inhibitory activity of extract belong to highest *M. communis* concentration (12.50% mg/ml). In this study the ethyl acetate extracts showed the highest ( $P<0.05$ ) antibacterial activity. Obtained results from the antibacterial screening tests were shown in Table. As clearly depicted in Table 2 the growth of *A. baumannii* were more inhibited by the ethyl acetate extracts than two other extracts. Methanol and ethyl acetate

extracts exerted higher ( $P<0.05$ ) antibacterial activity than the chloroform extracts. At each distinct concentration, chloroform extract show weakest antibacterial effect on the tested bacteria. By comparing number amount of inhibited colonies by each extract, we found that at MIC 400 mg/ml ethyl acetate extract have maximum inhibitory effects (62.5%) on bacterial colonies. Minimum inhibitory effect belong to methanolic extract at MIC 50 mg/ml. In other hand, at MIC 50 mg/ml The methanolic and chloroform extracts of *M. communis* leaves exhibited similar inhibitory effect. Minimum Bactericidal Concentration (MBC), in regard to extract concentration is also depicted in the table 1. As can be seen MBC order is concentration dependent. Along with increasing extract concentration, the bactericidal effect of ethanolic extract fortified. Highest bactericidal effect of extract belongs to highest *M. communis* concentration (12.50% mg/ml).

**Table 1: Comparing minimum inhibitory concentration (MIC) of *M. communis* extracts**

MIC <sub>90</sub> (mg/ml)	MIC <sub>50</sub> mg/ml	MIC <sub>90</sub> mg/ml	<i>M. communis</i> extracts
Methanol	100-800	200	100
Ethyl acetate	20-800	200	50
Choloroform	20-400	100	50

**Table 2: Comparing inhibitory effect of *M. communis* extracts with on *A. baumannii* colonies**

MICmg/ml	Different types of <i>M. communis</i> extracts		
	Ethyl acetate NO (%)	Methanol No (%)	Choloroform No (%)
MIC=400	25 (62.5)	20 (50)	25 (62.5)
MIC=200	5 (12.5)	11 (27.5)	6 (15)
MIC=100	5 (12.5)	5 (12.5)	5 (12.5)
MIC=50	5 (12.5)	4 (10)	4 (10)

**Table 3: Comparing MBC of different extracts of *M. communis***

MICmg/ml	Different types of <i>M. communis</i> extracts		
	Ethyl acetate NO (%)	Methanol No (%)	Choloroform No (%)
MBC=800	25 (62.5)	20 (50)	25 (62.5)
MBC=400	5 (12.5)	11 (27.5)	6 (15)
MBC=200	5 (12.5)	5 (12.5)	5 (12.5)
MBC=100	5 (12.5)	4 (10)	4 (10)

## Discussion

Inappropriate use of conventional antibiotics, produced problems including side effects, antimicrobial resistance and environmental problems (Guilhelmelli et al. 2013). These challenges reinforced a tendency to replace synthetic antibiotics with natural alternative agents. Accordingly, extensive research has been carried out in order to evaluate the antimicrobial effect of the herbal extracts (Perez et al. 2007). Obtained results from our antibacterial experiments showed that all extracts have anti-bacterial activity against tested bacterial isolates. In this regard, several studies proved that myrtle possess significant activity against tested bacteria. Mansouri et al. (2001) investigated the antibacterial activity of the crude extracts and fractionated Constituents of *M. communis*. In the study antibacterial activity of methanol crude extract of myrtle was evaluated against several strains of microorganisms, including 6 Gram positive as well as 4 Gram negative bacteria. According to the results, crude extract prevented the growth of tested bacteria except *C. jejuni*. This antibacterial activity may be attributed to the presence of several specific chemical compounds. The dried leaves of this herb contain linalyl, terpineole, cineol, linalool, terpinolene, tannins and flavonoid compounds. For example, cineole as a main myrtle essential oils components have a significant bactericidal effect against some Gram positive and Gram negative bacteria (Taamalli et al. 2014). The increasing prevalence of antimicrobial drug-resistant microorganisms recovered from hospitalized patients is a major concern worldwide. Many strains of *Staphylococcus aureus* and many strains of Gram negative bacteria display multi-drug resistance (Yalcin et al. 2014). According to the results of present study, the ethyl acetate extracted fraction showed the highest level of activity at a MIC 400 mg/ml for *A. baumannii*. It may due to the higher extraction of effective substances which

may be responsible for antimicrobial activities of the plant as compared to the methanolic and chloroform extracts. Other extracts either showed inhibition against *A. Baumannii* but it has lower antibacterial potency than Ethyl acetate extract. Bokaeian et al. (2013) assessed antibacterial potency of ethanol extracts of *M. communis* against *Morganella morganii* isolation of urinary tract infections. The result show that levels of MBC and MIC were obtained varies from 2.5 and 5 mg/ml in radius respectively. The plant extracts showed inhibitory activity against *Morganella morganii* with varying magnitudes and these effects were dose dependent manner. Tuba Mert et al, evaluate antimicrobial and cytotoxic activities of n-hexane, methanol, ethanol, ethyl acetate and water extracts of Myrtle leaves against *Staphylococcus aureus*, *Escherichia coli*, and *Staphylococcus epidermidis*. In that study the growth of *Escherichia coli* was only inhibited by the methanol extract. None of the tested extracts showed activity against *Enterobacter cloacae*, *Enterococcus faecalis* and *Candida albicans* (Mert, 2008). Annalisa Casaburi et al, investigate the antimicrobial activity of hydro-alcoholic extract of *M. communis* L. in vitro and in situ against some meat spoilage biotypes of *Pseudomonas fragi* and *Brochothrix thermosphacta*. MIC and MLC values vary between 12.5–50 and 25–100 mg DM/ml, for *B. thermosphacta* and *P. fragi* strains, respectively. Results from In situ experiments showed that except for *Pseudomonas* spp, the microbial population significantly decreased in ground meat with added 5 % of freeze-dried *M. communis* extract. High antibacterial activity of methanol, ethanol, and ethyl acetate leaf and berry myrtle extracts was observed when it tested against foodborne pathogens (Tumen et al. 2012). The methanolic leaf extract of *M. Communis* showed antibacterial activity against all tested bacteria, even *P. aeruginosa* and *L. Monocytogenes*. When tested against isolated strains from burns, predominantly *S. aureus* and *P. aeruginosa*, aqueous leaves extracts of Myrtle

gave an excellent inhibitory effect on bacterial growth and their effects were situated within the limits of antibiotic effects. Different antimicrobial mechanisms are involved in the antibacterial activity of essential oils and extracts (Zanetti et al. 2010). It is likely that their antimicrobial activity is not due to a single mechanism. The mode of myrtle extract and essential oil activity affect predominantly cell wall and membrane component. These extracts mainly affect the permeability of bacterial cell wall and cell membrane, leading to the release of cell contents thus cell death because of disruption in the membrane function such as nutrient absorption electron transfer or enzyme activity (Nassar et al. 2010; Janahiraman et al. 2015). *A. baumannii* have recently got attention as an important hospital pathogen, specifically for nosocomial infections. These bacteria have started to gain resistance to widely used antibiotics and there is a significant increase in the methicillin-resistant *A. baumannii* infections. Extensive literature survey revealed that *M. communis* has a broad traditional use for wide range of infections. Many of the traditional uses have been validated by scientific research. In this regard, potential of *M. communis* in preventing and treating different diseases require further studies to be carried out.

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