

Antibacterial Effect of *Myrtus Communis* Hydro-Alcoholic Extract on Pathogenic Bacteria

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Article information	Abstract
<p>Article history: Received: 13 Sep 2011 Accepted: 26 Apr 2012 Available online: 29 Oct 2012 ZJRMS 2013; 15(6): 19-24</p> <p>Keywords: Myrtus communis Extract Antibacterial Pathogenic Bacteria</p> <p>*Corresponding author at: Department of Fisheries (Seafood Sciences), Faculty of Marine Sciences, Chabahar Maritime and Marine Sciences University, Chabahar, Iran E- mail: taheri@cmu.ac.ir</p>	<p>Background: Today, due to the changes in the form of the resistance of pathogenic bacteria, discovering new antimicrobial drugs is under study. So, the aim of this study is to evaluate the antimicrobial properties of the extract of the myrtle herb on some of pathogenic bacteria.</p> <p>Materials and Methods: Hydroalcoholic extract of the leaves of myrtle herb was evaluated at 4 concentrations including 10-80 mg/ml on four strains of pathogenic bacteria using penetrative dissemination method together with the measuring diameter of the growth inhibition zone; then the results were compared to four conventional antibiotics. The minimum inhibitory and bactericidal concentrations were studied using macro dilution method.</p> <p>Results: Treatment by the concentration of 80 mg/ml extract of this herb showed the greatest effect on the bacterium <i>Staphylococcus aureus</i> and <i>Vibrio cholera</i> serotype Ogawa which had a significant difference with all other treatments and standard antibiotics ($p < 0.05$). The extract showed no effect on the bacterium <i>Pseudomonas aeruginosa</i> and just concentration of 80 mg/ml showed a little effect on <i>E. coli</i> and other antibiotics had no significant effect except tetracycline which has little effect on this strain. Minimum inhibitory concentration was 0.2 mg/ml for bacterium <i>Staphylococcus aureus</i> (<i>S. aureus</i>) and the maximum for <i>E. coli</i> by 8 mg/ml.</p> <p>Conclusion: This study showed that under study bacteria were more resistant to the antibiotics and the extract of <i>Myrtus communis</i> leaves showed greatest antibacterial effect against <i>S. aureus</i> and <i>V. cholerae</i> serotype Ogawa.</p> <p>Copyright © 2013 Zahedan University of Medical Sciences. All rights reserved.</p>

Introduction

Today, environmental problems and high prices of some antibiotics have reinforced a tendency to replace them with less harmful substances. Among the various materials to replace antibiotics, products of plant origin have recently gotten a special place [1, 2]. Herbal medicines have been the only source of pain treatment during the centuries and now with the advancement of sciences and development in the application of synthetic drugs, medicinal plants are still used in large-scale [3]. This issue is very important for the application in drug therapy or side applications such as packaging of fisheries products.

However, due to the climatic variation and the vast area of Iran, wide spectrum of medicinal plants are found there which are the basis of Iran's traditional medicine [4-6]. Thus, in recent years, extensive researches have been conducted to evaluate the antimicrobial effect of the essential oils and extracts which shows the strength and ability of these compounds to inhibit the growth of a wide range of pathogenic microorganisms [7]. Myrtle (*Myrtus communis*) of the Myrtaceae family is a shrub, evergreen and aromatic herb with numerous stems and branches. This herb is found in the white Lab of Bakhtiari valley,

Khorasan River Valley, Sarab, Guilan-e-gharb, Kerman, Maharlou in Shiraz, Neyriz, Fasa, Mamasani and Bandar Abbas [5]. The dried leaves of this herb contain terpineolene, cineol, linalool, terpineole, linalyl acetate, tannins and flavonoids compounds and there are numerous reports about the anti-parasitic and anti-infective properties of the extract of this herb [6- 10]. Also its anti-virus effect has led to the production of anti-herpes simplex drug [11].

Today, the anti-parasitic and antimicrobial drug resistance is a major problem in the world which is due to the uncontrolled use of antimicrobial drugs. This resistance is so important that the theme of 2011 World Health Organization was "Resistance to antimicrobial drugs is a global threat". This resistance can be transmitted by microbes from one generation to another generation and even from a microbial species to another species through the creation of an antibiotic-resistant gene by them and ultimately the high levels of infection remained stable despite the administration of antibiotics. Until recently, several studies have been conducted on the antimicrobial properties of the leaf and stem extracts of myrtle against pathogenic bacteria and good results have

been obtained about its effects on *staphylococcus aureus* [12-4], *E. coli* [15-16], *Lactobacillus plantarum* [16], *Bacillus cereus* [12], *Listeria monocytogenes* and *Pseudomonas aeruginosa*, [17-18], *Klebsiella* and *Shigella* [17], though its antimicrobial effect against some strains of bacteria such as *E. coli* has been rejected in some studies[18].

Some gram-negative bacteria such as *E. coli* and *Pseudomonas aeruginosa* and some gram-positive bacteria such as *Staphylococcus aureus* have specific characteristics as far as bacterial infections are concerned and they are removed or isolated from the most of the clinical samples referred to the diagnostic laboratories [19, 20]. On the other hand, some reports on the cases of being infected with cholera by *Vibrio cholerae* which is a gram-negative bacterium are annually published and being infected with this bacterium has been observed in the southern districts of the country due to the consumption of contaminated raw or semi raw fishes and vegetables irrigated with sewage. Accordingly, given the importance of medicinal plants in the traditional medicine and very few side effects of these medicines on human and also due to the changes in the form of the resistance of pathogenic bacteria which requires monitoring of antibacterial effects substances in periodic intervals, the antimicrobial effects of hydroalcoholic extract of *Myrtus communis* leaves against the above-mentioned pathogenic bacteria have been evaluated in this study.

Materials and Methods

This study was conducted in 2011 at the Laboratory of Microbiology of Chabahar University of Maritime and Marine Sciences. Extraction was carried out with slight variations based on the study conducted by Sadeghi [21]. Accordingly, the leaves of myrtle were collected in spring from Zaranjan located in the district of Fasa and they were dried in the shade after washing. 600 grams of the ground leaf powder was mixed with 500 ml of distilled water and ethyl alcohol 96 at 1:1 ratio and was kept in a dark place for 48 hours. The contents of the Erlenmeyer flasks were stirred for 25 minutes once every 16 hours. After 48 hours, the contents of the Erlenmeyer flask were smoothed by the filter paper and smoothed fluid was extracted in the rotary evaporation apparatus under vacuum and 50°C temperature. The concentrated extract was poured in sterile Petri dish and dried in the oven with 40°C. Dried powders were collected and were prepared at concentrations of 10, 20, 40 and 80 mg using sterile distilled water. *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Vibrio cholerae* serotype Ogawa and *E. coli* Bacteria were prepared from the samples of patients referred to Imam Ali hospital of Chabahar city after doing specialized microbiologic tests for confirming the presence of microorganisms. Cultures were purred and stored at refrigeration temperature until the start of the study [22]. One day before experiments, a small portion of the mother culture was added to the Hinton broth medium.

Concentration of bacteria became equal to the McFarland standard tube No. 0.5 (108×1.5) after 24 h incubation in the logarithmic phase of growth, using a spectrophotometer. This suspension was considered as a reserve and diluted in a similar medium based on 1:100 ratio while taking on the same day (6 10×5/1) [23]. Susceptibility to the nosocomial microorganisms to the myrtle extract was conducted using penetrative dissemination method. Bacterial suspension was seeped on the medium using 20 ml sampler and was spread on the medium with a sterile cotton swap; then it was dried for 5 minutes and incubated for 15 minutes in order to equilibrate the moisture inside the plates.

Sterile crude discs with 6 mm diameter were placed on the surface of medium and 15 microliters of the myrtle solution was seeped in a specified concentration on the discs. Standard discs of streptomycin 10, gentamicin 10 and erythromycin 15 and tetracycline 30, produced by Iran Darou Co. were used as a standard.

Plates were incubated for 24 hours at 37°C and bacterial inhibition zone was measured and recorded using Vernier calipers. Experiments were conducted with three replications. The minimum growth inhibitory concentration and Minimum Lethal Concentration (MLC) of antimicrobials were determined using the tube dilution method [24, 25]. A series of nine test tubes were used for each concentration and each bacterium. There were 7 tubes for different dilutions, a positive control tube and a negative control tube. 9 ml nutrient broth was added to the test tubes and then was sterilized. 1000 microliters of the diluted extract was added to the first tube and after being homogenized, 1000 microliters of the homogenized fluid was added to the second tube and this operation was continued and 1000 microliters of homogenized solution (from 7th tube) was discarded. 10 microliters of the bacterial suspension was added to all tubes except the negative control tube based on McFarland tube 1.

All tubes were incubated for 24 h at 37 C and then the tubes were evaluated in terms of turbidity due to the bacterial growth. The last tube in which no turbidity was seen was considered as the minimum growth inhibitory concentration. The solutions inside all tubes with no growth turbidity were cultured using pour plate method for determining minimum bactericidal concentrations of the myrtle extract and the final concentration of the extract which was able to kill 99.9% of the live bacteria, was considered as the minimum bactericidal concentration of the microorganisms [26, 27].

Statistical analysis: Experiments were performed in three replications and Graphpad-Prism 7.00 software was used for the data analysis under analysis of variance test; and Duncan test was used to compare treatments at 95% confidence level.

Results

Table 1 shows the results of penetrating emissions test of the myrtle extract with different concentrations on under-study microorganisms. Accordingly, the concentration of 80 mg/ml of myrtle extract showed the

greatest effect on the *Staphylococcus aureus* bacterium and no significant difference was seen with all other treatments and control antibiotics and showed a higher efficacy ($p<0.05$). Lower concentrations also showed a significant difference with each other and all of them had significant differences with antibiotics except the concentration of 10 mg/ml that had no significant difference with gentamicin ($p<0.05$). Erythromycin and streptomycin did not show any effect on bacteria. Myrtle extract, at any concentration, did not show any effect on the bacterium *Pseudomonas aeruginosa* and the highest effect of control group of antibiotics was observed in gentamicin which had a significant difference with streptomycin ($p<0.05$).

The study conducted on the effects of myrtle extract on *E. coli* bacteria also showed that only the concentration of 80 mg/ml has the effect on this strain of bacteria and except for the very light effect of tetracycline on this strain, other control antibiotics did not have any effect. Concentration of 80 mg/ml showed the highest effect on the *Vibrio cholerae* Bacterium which had a significant difference with all other treatments ($p<0.05$).

Lower concentrations also showed a positive effect, all of them had a significant difference with each other ($p<0.05$). Tetracycline was the only antibiotic effective on this bacteria which had a significant difference with other concentrations except that concentrations of 10 and 20 mg/ml ($p<0.05$). Results of minimum inhibitory concentration of growth and minimum bactericidal concentrations after 24 h culture were shown in table 2.

According to the results of this table, myrtle extract had no effect on the bacterium *Pseudomonas aeruginosa*. Minimum inhibitory concentration belonged to the bacterium *Staphylococcus aureus* and the highest rate belonged to the *E. coli*. Similar results were obtained about the minimum bactericidal concentration.

Table 1. Comparison of the inhibition zone diameter (mm) of myrtle extracts and standard antibiotics on under study microorganisms

Type of bacteria	S. aureus	P. aeruginosa	E. coli	V. cholerae
Variable				
Extract concentration (mg/ml)				
10	11±0.3 ^d	-*	-	6.08±0.04 ^d
20	15.8±0.2 ^c	-	-	7.5±0.1 ^c
40	17.24±0.25 ^b	-	-	9.62±0.2 ^b
80	20.4±0.3 ^a	-	8.74±0.02 ^a	12.4±0.3 ^a
Antibiotic				
Gentamicin	10 ^d	11.29±0.02 ^a	-	-
Erythromycin	-	-	-	-
Tetracycline	7.6±0.1 ^e	-	6.2 ^b	6.86±0.04 ^{cd}
Streptomycin	-	9.9±0.1 ^b	-	-

* There was no inhibition zone; treatment of any columns indicated significant difference with dissimilar letters

Table 2. Comparison of the minimum growth inhibitory concentration and minimum bactericidal concentrations of the myrtle leaf extract on microorganisms

Type of bacteria	MIC (mg/ml)	MLC (mg/ml)
Staphylococcus aureus	0.2	2
Pseudomonas aeruginosa	-	-
E. coli	8	40
Vibrio cholerae	2	20

*The extract had no effect

Discussion

In the present study, hydroalcoholic extract of the myrtle leaves showed the greatest effect on *Staphylococcus aureus* which had significant difference with the standard antibiotics. The positive effect of the myrtle extract on *Vibrio cholerae* serotype Ogawa was well. In this study on these two strains of bacteria, concentration of the used extract also caused a significant difference in the results. Myrtle extract showed a very little effect on *E. coli* only at the highest concentration but *Pseudomonas aeruginosa*, was completely resistant to the extract.

For centuries in Iran, several herbs have been used to treat diseases. Different generations' trust in the treatment with the traditional medicine demonstrates the positive impact of this type of treatment. On the other hand, the increasing resistance of pathogenic bacteria and continuous change of the resistance form of these microorganisms have led to the major challenges in the use of common antibiotic drugs which requires the search for the new compounds with anti-bacterial properties.

Myrtle is an aromatic and medicinal herb for which antibacterial, antifungal, antiviral, antioxidant and anti-mutagenicity properties have been reported [28-32]. Positive properties of the myrtle extract on hospital bacteria have been reported. In a study, effects of several plants, including myrtle against *Streptococcus pneumoniae*, *Haemophilus influenzae* and *Moraxella catarrhalis* isolated from the patients in hospitals were evaluated and the results showed that the myrtle extract creates 30, 50 and 22 mm growth inhibition zone in the diffusion penetration test; therefore the use of myrtle extract for the treatment of sinusitis and bronchitis was proposed [33].

The positive effect of the myrtle extract on *S. aureus* in this study was consistent with the results obtained by Salvagnini. These researchers were studied the effect of the oil and ethanolic extract of myrtle on several strains of bacteria and reported that the ethanolic extract of myrtle has a positive effect on *S. aureus* with 12 mm inhibition zone. But the oil has shown a greater effect [29]. Alem also reported the antibacterial effects of the myrtle extract on *S. aureus* isolated from human samples with 0.5 mg/ml of the minimum inhibitory concentration [14]. Ghلامhsynyan Najjar et al. who had observed a very positive effect of the chloroform, ethyl acetate and methanol extracts of the myrtle leaves on *S. aureus* acknowledged that antimicrobial effects of myrtle extract on this bacterial strain is partly related to the stimulation of free radicals and the concentration of the extract has an

effect on antibacterial activity [34]. In the study of control antibiotics were less effective than different concentrations of the myrtle extract on *Pseudomonas aeruginosa*.

Fewer efficacies of these antibiotics can confirm the resistance of the mentioned strains of bacteria and on the other hand, various studies have shown the effects of myrtle extract on *P. aeruginosa* [6, 35].

For example Alem reported the effect of myrtle extract on *Pseudomonas aeruginosa* isolated from human cases with a minimum inhibitory concentration of 120 mg/l [14]. But the myrtle extract used in this study had no effect on bacteria which could be due to the mutations in the bacterial strains which are still sensitive to antibiotics but are resistant to the myrtle extract, although differences in the extracted essential oil and even used concentrations could be another reason for the lack of conformity with the present findings. In a study conducted by Amensour, leaf and stem extract of myrtle was examined on 15 strains of bacteria which cause food borne diseases. They reported that the methanol and ethanol extracts of the myrtle leaves and branches have respectively antimicrobial properties on *Listeria monocytogenes*, *Pseudomonas aeruginosa* and *Staphylococcus aureus* and the diameters of the inhibition zone of ethanol extract in these bacteria are respectively 30, 23 and 37 mm and minimum bactericidal concentration for *Staphylococcus aureus* was reported less than 0.075 mg/ml. Also the highest antibacterial activity was against gram-positive bacteria [18]. Comparison of the results of this study shows that the extract has no effect on *Pseudomonas aeruginosa* and the inhibition zone of *Staphylococcus aureus* was about 20 mm in the best condition and the minimum bactericidal concentration for *Staphylococcus aureus* was 2 mg/ml.

The comparison shows the difference between pathogenic bacteria and food born disease agents and may be another proof for the resistance of bacteria studied in this study. In the studies conducted on the effects of extracts on *E. coli*, most of the reports show that the myrtle extract has no effect on these bacterial strains [18, 29]. In a study conducted in Iran in the past, the positive effect of alcoholic extract of the myrtle herb on *E. coli* had been reported. Also according to the report of Ghasemi Pirbalouti et al. although the methanol extract showed no effect on the diffusion penetrating activity, but the minimum lethal concentration for *E. coli* was 10 mg/ml [6, 35]. Comparison of the results of this study revealed that myrtle extract, even in the highest concentration, has little effect on the diffusion penetration of *E. coli* and minimum lethal concentration was determined as 40 mg/ml. The resistance of this strain of pathogenic bacteria could be a reason for this finding. It has been generally reported that the gram-negative bacteria are more resistant to the extracts and essential oils of this herbs, because hydrophilic structure of the cell walls of the gram-positive bacteria is mainly composed of polysaccharides lipoprotein which prevents the penetration of hydrophobic oil and also prevents accumulation of its compounds in the cell membrane of

the bacteria [36- 38]. Due to this reason, a gram-positive bacterium is more susceptible to the extract than gram-negative bacteria.

No report on the effects of myrtle extract against the bacteria causing cholera (i.e. *Vibrio cholera*) has been reported yet. The results of this study show the positive effect of the hydroalcoholic extract of the myrtle on this strain of bacteria; serotype Ogawa which is much better than that of tetracycline which is the only antibiotic effective on this bacterium. In a study conducted by Rahbar et al. *Vibrio cholerae* serotype Inaba and Ogawa were sensitive to Tetra Doxycycline, erythromycin and ampicillin [39]. It can be said that the less efficacy of antibiotics in this study can be due to the resistance of this strain of pathogenic bacteria. Several studies have reported the antibacterial activity of essential oil extracted from the herb. In recent years, many of these researchers came to this understanding that the presence of phenolic compounds in the leaves of myrtle herb is due to the presence of Flavenols (Quercetin glycosides and myricetin) and derivatives of Galoyl including Galoyl glycosides, Alajytanyn, the Galvyl-quinic acid [40] and they attributes the antibacterial activity of these compounds to the presence of polyphenols [41].

Many studies have shown that the mechanism of this effect is due to the influence on the cell wall and reported that the cell wall and cell membrane are affected and their permeability is changed and lead to the release of intracellular contents which can be accompanied with the disrupt in the membrane function such as electron transfer, enzyme activity or nutrient absorption [18].

In sum, it can be said that the results of this study indicate the antibacterial effect of hydroalcoholic extract of the leaves of myrtle herb on some pathogenic bacteria, especially *Staphylococcus aureus* and *Vibrio cholerae* which can be an alternative for the treatment of diseases resulted from these bacterial strains that the resistance form of which has been changed as an herbal antibiotic with side effects fewer than conventional antibiotics. Also working on other extraction materials and methods such as extracting by methanol and ethyl acetate may indicate different functions. Also clinical confirmation and pharmacological standardization is required before their provision as the anti-bacterial drug.

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Authors' Contributions

All authors had equal role in design, work, statistical analysis and manuscript writing.

Conflict of Interest

The authors declare no conflict of interest.

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