

Antimicrobial activity of the aqueous extract of mint plant

Basheer A. Al-Sum, Abdullah A. Al-Arfaj

Department of Botany and Microbiology, College of Science, King Saud University

Email address:

basheeralsum@gmail.com(B. A. Al-Sum)

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Abstract: In the present study, an antimicrobial activity of the Aqueous extract of *Mentha species* was assessed using both well diffusion and microdilution method in multi-well micro-titer plates. Mint extract investigated for its antibacterial activity against seven selected pathogenic bacteria: *Bacillus fastidiosus*, *Staphylococcus aureus*, *Proteus mirabilis*, *Proteus vulgaris*, *Salmonella choleraesuis*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae* and *Serratia odorifera*. Ment extract at different concentrations (1:1, 1:5, 1:10, and 1:20) was active against all tested bacteria except for *S.aureus*, and the highest inhibitory effect was observed against *S. mutans* using the well diffusion method. Antibacterial activity of Aqueous extracts of selected commonly used Mint were screened against multi drug resistant bacteria ,which concludes that their extracts can be used against multi drug resistance bacteria capable of causing both nosocomial and community acquired infections.

Keywords: Mint, Aqueous Extracts, Antimicrobial Activity

1. Introduction

Antibiotics provide the main basis for the therapy of microbial infection. Since the discovery of these antibiotics and their uses as chemotherapeutic agents, there was a belief in the medical fraternity that this would lead to the eventual eradication of infection diseases (Rosina et al, 2009). However, overuse of antibiotics has become the major factor for the emergence and dissemination of multidrug resistant strains of several groups of micro-organisms (Gardiner, 2006). In the light of the evidence of rapid global spread of resistant clinical isolates, the need to find new antimicrobial agent is of paramount importance. However, the past record of rapid, wide spread emergence of resistance to newly introduced antimicrobial agents indicates that even new families of antimicrobial agents will have a short life expectancy (Coates et al, 2002). A wide variety of antibiotics are commonly used for the treatment of serious infections caused by aerobic Gram -ve bacteria (Tumah, 2005). The increased use of antibiotics has resulted in the development of resistant bacteria (Derrida, 2003). In recent years, misuse of antibiotics resulting in multi-drug resistance among bacteria has accelerated the search for drugs and dietary supplements effective against such multidrug resistant bacteria. It has been reported that in 1996, sales of botanical medicines increased by 37% over 1995 (Thongson et al, 2004). In this

connection, different parts of plants, herbs and spices have been used for many years for prevention of infections. These are easily available and can be used in domestic setting for self-medication. The present report gives an account of the antibacterial effect of different parts of *Mentha* plant.

2. Materials and Methods

2.1. Plant Material

Mint leaves were obtained commercially from a local garden in Riyadh, Saudi Arabia and identified by a botanical taxonomist at Botanical Survey, Department of Department of Botany and Microbiology, College of Science King Saud University. The leaves were washed first under running tap water, followed by sterilized distilled water and dried at room temperature in dark then grinded to powder using an electrical blender.

2.2. Preparation of Extracts

The leaves of the plants were air dried at room temperature for 3 weeks and grounded to coarse powder .15g of the powder was placed in 100ml of distilled water (cold water extract) in conical flask and The crude preparation was left overnight in the shaker at 35oC and then centrifuged at 2500rpm for 10 mins. The supernatant

containing the plant extract was then transferred to a preweighed beaker and the extract was concentrated by evaporating the solvent at 60°C. The crude extract was weighed and dissolved in a known volume of dimethyl sulphoxide, to obtain a final concentration of 200mg / ml. and sterilized by filtration through (0.45 µm) millipore filters. The Aqueous extracts were stored in sample bottles at 4°C prior to use (De and Ifeoma, 2002).

2.3. Microbial Cultures

Nine strains of bacteria were used as test microorganisms. All microorganisms were clinical isolates, obtained from the Microbiology Laboratory at Department of Department of Botany and Microbiology, College of Science King Saud University.

2.4. Standardization of Inoculum

Exactly 0.2ml of 24/hours old culture of each organism was dispensed into 20ml of sterile nutrient broth and was incubated for 3-5/hours to standardize the culture to 106cfu/ml (Collins et al, 1995). Antibacterial Testing: This was done using the agar wells diffusion method(s) of (Odeyemi and Fagbohun, 2005). 0.5ml of overnight broth culture of each clinical isolates containing 106 cfu/ml was aseptically transferred to the solidified nutrient agar and spread evenly on the agar surface using a sterile glass spreader. Four 6mm wells were bored unto the agar and filled with the Aqueous extracts (cold water extract) while the distill water serves as the control. The Petri dishes were incubated at 37°C for 18-24/hr and the inhibition zones were measured (mm).

2.5. Minimum Inhibition Concentration (MIC) of the Extract

The (MIC) was defined as the lowest concentration that completely incubated the growth of microorganisms for 24 hours (Thongson et al, 2004). The MIC of the extracts was also done using the agar well diffusion technique. Two fold dilution series was prepared to achieve a decreasing concentration range of 200 to 12.5% (V/V). A 0.5ml volume of each solution was added aseptically into the wells of Mueller Hinton agar plates that were already seeded with standardized inoculum (106 cfu/ml) of the bacterial isolates. The plates were incubated at 37°C for 24/hr. The lowest concentration of the extracts showing a clear zone of inhibition was considered as the (MIC).

3. Results

Table 3 indicates the antibiotic susceptibility of the bacterial isolates used for this research. Four antibiotics of choice Tetracycline (Tet), chloramphenicol (Chlo), Rifampin (Rifam) and Ampicillin (Amp) were used. *Staphylococcus aureus* was resistant to (Rifampin and Ampicillin) , *E. coli* was resistant to (chloramphenicol, Rifampin and Ampicillin), *Klebsiella pneumoniae* was

resistant to (chloramphenicol, Rifampin, Ampicillin and Tetracycline), *Serratia odorifera* was resistant to (chloramphenicol, Rifampin, Ampicillin and Tetracycline), *Salmonella choleraesuis* was resistant to (Rifampin, Ampicillin and Tetracycline), *Bacillus fastidiosu* was resistant to (chloramphenicol, Rifampin, Ampicillin and Tetracycline), *Proteus vulgaris* was resistant to (chloramphenicol, Rifampin, Ampicillin and Tetracycline), *Proteus mirabilis* was resistant to (chloramphenicol, Rifampin, Ampicillin and Tetracycline), *Pseudomonas aeruginosa* was resistant to (chloramphenicol, Rifampin and Ampicillin).

Table 1. Diameter of zone of inhibition (mm) of Antimicrobial extracted from *Mentha* plant against Clinical Bacterial Isolates

Antibiotic Resistant Isolates	Mint extract (mm)	Control			
		1	2	3	4
<i>Bacillus fastidiosus</i>	24	13	12	8	11
<i>Staphylococcus aureus</i>	18	15	19	7	10
<i>Proteus mirabilis</i>	15	12	12	7	11
<i>Proteus vulgaris</i>	15	12	12	8	11
<i>Salmonella choleraesuis</i>	15	11	20	7	8
<i>Escherichia coli</i>	14	18	11	8	12
<i>Pseudomonas aeruginosa</i>	13	14	15	5	12
<i>Klebsiella pneumoniae</i>	8	10	9	5	8
<i>Serratia odorifera</i>	0	9	7	0	9

1: Tetracycline, 2: chloramphenicol, 3: Ampicillin, 4: Rifampin

Table 2. Minimum Inhibitory Concentration (MIC) of Antimicrobial extracted from *Mentha* plant against Clinical Bacterial Isolates

Antibiotic Resistant Isolates	Mint extract (µg / ml)	Control			
		1	2	3	4
<i>Bacillus fastidiosus</i>	8	128	256	128	256
<i>Staphylococcus aureus</i>	16	64	2	128	128
<i>Proteus mirabilis</i>	32	128	128	128	512
<i>Proteus vulgaris</i>	32	128	128	128	512
<i>Salmonella choleraesuis</i>	32	128	1	256	128
<i>Escherichia coli</i>	64	32	128	64	128
<i>Pseudomonas aeruginosa</i>	128	32	128	256	256
<i>Klebsiella pneumoniae</i>	256	256	256	512	256
<i>Serratia odorifera</i>	512	256	256	512	256

1: Tetracycline, 2: chloramphenicol, 3: Ampicillin, 4: Rifampin

Table 3. Antibiotic Resistant of Clinical Bacterial Isolates

Antibiotic Resistant Isolates	Tetracycline	chloramphenicol	Rifampin	Ampicillin
<i>Bacillus fastidiosus</i>	R	R	R	R
<i>Staphylococcus aureus</i>	S	S	R	R
<i>Proteus mirabilis</i>	R	R	R	R
<i>Proteus vulgaris</i>	R	R	R	R
<i>Salmonella choleraesuis</i>	R	S	R	R
<i>Escherichia coli</i>	S	R	R	R
<i>Pseudomonas aeruginosa</i>	S	R	R	R
<i>Klebsiella pneumoniae</i>	R	R	R	R
<i>Serratia odorifera</i>	R	R	R	R

Table 2 showed the susceptibility pattern of the Aqueous extract of Mint against the bacterial isolates. The extract of Mint was the most effective extract showing the most

antibacterial activity against all the isolates tested *Bacillus fastidiosus*, *Staphylococcus aureus*, *Proteus mirabilis*, *Proteus vulgaris*, *Salmonella choleraesuis*, *Escherichia coli*, *Pseudomonas aeruginosa* and *Klebsiella pneumoniae*, with inhibition zones (mm) of 24, 18, 15, 15, 15, 14, 13, 8 respectively. The extract was effective on all the test isolates except *Serratia odorifera*.

Table 1 The highest inhibitory effect was observed against *Bacillus fastidiosus* (zone of inhibition: 24 mm) while the weakest activity was demonstrated against *Staphylococcus aureus*, *Proteus mirabilis*, *Proteus vulgaris*, *Salmonella choleraesuis*, *Escherichia coli*, *Pseudomonas aeruginosa* and *Klebsiella pneumoniae* (zone of inhibition: 18, 15, 15, 15, 14, 13 and 8 mm) respectively. In view of the results obtained by the well diffusion method, the minimal inhibitory concentration (MIC) of Mint extract was determined by broth microdilution assay (Table 2). The highest (MIC) value (8, 16, 32, 32 and 32 µg/ml) was observed against *Bacillus fastidiosus*, *Staphylococcus aureus*, *Proteus mirabilis*, *Proteus vulgaris* and *Salmonella choleraesuis* respectively, while *Escherichia coli*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae* and *Serratia odorifera* ranked next (MIC 64, 128, 256 and 512 µg/ml) respectively. The standard drug Tetracycline was active against all reference bacteria (zone of inhibition range: 9– 18 mm; MIC range: 32–256 µg/ml), Chloramphenicol was active against all reference bacteria (zone of inhibition range: 7– 20 mm; MIC range: 1–256 µg/ml), Ampicillin was active against all reference bacteria (zone of inhibition range: 0– 8 mm; MIC range: 64–512 µg/ml) and Rifampin was active against all reference bacteria (zone of inhibition range: 8– 12 mm; MIC range: 128–512 µg/ml).

4. Discussion

Plants have formed the basis of sophisticated traditional medicine system and natural products make excellent leads for new drug development (Newman *et al.*, 2007). In addition to these properties, it has also been used as appetite stimulant, a treatment for gastrointestinal infection and to lower blood sugar in diabetics. Its use for the treatment of certain types of cancer and viral infections has also been reported (Abascal *et al.*, 2003). Its active constituents are 5-a-stigmasta-7, 25-dien-3-b-ol, elasterol and lanosterol which may be responsible for its antibacterial activity. Leaf extracts of *M. charantia* showed broad spectrum antimicrobial activity since various water, ethanol and methanol extracts of the leaves have exhibited antibacterial activities against *E. coli*, *Staphylococcus*, *Pseudomonas*, *Salmonella*, *Streptobacillus*. Besides, extract of the entire plant has shown antiprotozoal activity against *Entamoeba histolytica* and its fruit extract has demonstrated antibacterial properties against *Helicobacter pylori*, the bacteria causing stomach ulcer. It has been documented in the literature that *Mentha piperita* is used internally as a tea, tincture, oil or extracts, and applied externally as a rub or

liniment. Herbalists consider it as an astringent, antiseptic, antipuritic, antispasmodic, anticatarrhal, antimicrobial, rubefacient, stimulant and emmenagogue (Gislene *et al.*, 2000). The varying degree of sensitivity of the bacterial strains may be due to the intrinsic tolerance of the bacterial and the nature and combinations of phytochemicals present in the extracts as observed by Suree and Pana (2005).

5. Conclusion

In the present study, the isolated compound demonstrated promising antimicrobial activities against the most prevalent microorganisms in oral infections. The use of this plant in the treatment of sore throat, mouth or throat irritation is validated, scientifically supported by the results obtained in this work.

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