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Evaluation of Antibacterial Activity of Essential Oil from Pistacia Khinjuk Stoks Gum of Iraqian Using Broth Microdilution

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Abstract: The aim at the current study was to determine that essential oils of mastic gum Pistacia khinjuk has some antibacterial activity. The essential oil was obtained with two methods (conventional and microwave method) for obtanining aqeous extract of gum essential oils with some biological activities from Pistacia khinjuk gum of Iraqıan. The analysis of highest essential oil yield of the gum for microwave method was in 200W for 90 min and the lowest yield in 500W for 60 min while in conventional method the highest yield was in 90 min and lowest was in 30 min. obtained from the study made in another laboratory in same university. Moreover, the analysis of physicochemical and phytochemical components of Pistacia khinjuk gum essentials oil by GC-MS in my colleaques' research in Natural and Applied Sciences field. The main compounds were (+)-α-Pinene, (±)-β-Pinene, D- Limonene and Camphene. The antibacterial activity of essential oil provided by two methods were compared against different species of bacteria. The results of the antimicrobial activity tests (minimal inhibitory concentration (MIC) and agar-disk diffusion) indicate that essential oils of mastic gum Pistacia khinjuk provided by both methods have higher activity against the tested strains compared to Gentamicin and Ampicilin, confirming its traditional uses. Moreover, mastic gum oil was found to inhibit both gram-positive; Staphylococcus aureus ATCC 29213, Enterococcus faecalis ATCC 29212, Listeria monocytogenes ATCC 15313 and Bacillus cereus ATCC 23456 and gram-negative bacteria; Salmonella enterica NTCT 13, E coli O157:H7 ATCC 35130, Klebsiella pneumonia ATCC 700603 with the exception of Chronobactersakazakii ATCC 29544.

Keywords: Essential Oil, Pistacia Khinjuk Stoks Gum of IRAQIAN, Antibacterial Activity, Standart Strain

1. Introduction

Pistacia khinjuk Stoks is an evergreen shrub or tree of the family Anarcadiaceae. This specie is native to Turkey, Syria, Iraq, Iran, Egypt, Afghanistan and Pakhistan [1]. It has been used for a long time as useful remedies for different diseases, for example, the fruits of P. atlantica, P. khinjuk, and P. terebinthus for their aphrodisiac activity and treatment of liver, kidney, heart, and respiratory system disorders, and the gum resin of P. lentiscus, P. atlantica, P. khinjuk, and P. terebinthus for their wound healing activity, and treatment of brain and gastrointestinal disorders [2]. The resin of P. khinjuk has been used to treat indigestion, and toothache and as a tonic and astringent in Iran Bakhtiari folk medicine. In medicine, a lot of research has been undertaken on the properties of mastic gum. For example, mastic gum has been used in clinical trials on patients with peptic ulcers. The same group of researchers [3] confirmed that mastic gum kills Helicobacter pylori, at concentrations as low as 0.06 mg/mL. The in vitro antimicrobial activity of P. lentiscus extract essential oil has also been tested on bacteria and fungi [3]. The chemical composition of the mastic oil and mastic gum

has recently been studied [4, 5], but as yet no antibacterial activity with method of microdilution on standart bacterial strains were documented. The assessment of antibacterial activity of essential oil of Pistacia khinjuk gum in three different dilution (0.1, 0.2 and 0.3 µL/mL) and two extraction methods (microwave and conventional methods) has been done. The aim at the current study was to determine that essential oils of mastic gum Pistacia khinjuk has further different activity against the tested standart strains.

2. Material and Method

The antibacterial activity of Pistacia khinjuk Stoks essential oil was tested against eight bacterium species: Gram-positive *Bacillus cereus* ATCC 23456, *Listeria monocytogenes* ATCC 15313, *Staphylococcus aureus* subsp. *aureus* ATCC 29213 and Enterococcus faecalis ATCC 29212, and Gram-negative *Escherichia coli* O157:H7 (ATCC 35130), *Salmonella enterica* subsp. *enterica* (NTCT 13), Klebsiella pneumonia ATCC 700603 and Chronobacter sakazakii ATCC 29544. The microorganisms were from the Microbiology Laboratory of the Kahramanmaras Sutcu Imam University Medicine Faculty, Turkey.

The antibacterial assay was done by broth microdilution method (Clinical and Laboratory Standards Institute [CLSI], 2012) utilizing 96-well microtiter plates (Lp Italiana U Plate 96 well Sterile plate) to determine the minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC). The bacterial suspensions were adjusted with sterile saline solution until the concentration of 1.0×10^5 CFU/mL.; adjusted to turbidity equivalent to McFarland 0.5 with nephelometer. The inoculum was prepared daily and stored at 4°C until its utilization. The inoculum was cultivated in solid medium to verify the absence of contaminations, and for validation. *Pistacia khinjuk Stocks* essential oil was dissolved in a 5% dimethyl sulfoxide solution (Merck KGaA, Germany) containing 0.1% of polysorbate-80 (1 mg/mL) and added to mueller hinton broth medium with bacterial inoculum (1.0×10^4 CFU/well) to reach the desired concentrations. The microplates were incubated in a cultivator, for 24 h, at 37°C. The lowest concentration without visible of the microbial biomass growth under the optical microscope were defined as the concentrations that completely inhibited bacterial growth.

Minimum bactericidal concentration was determined by 2 µL serial subcultivation in microtiter plates containing 100 µL of broth per well and agar plates were incubated at 37 °C for 16-20 h. The test for each isolates was repeated three times. The lowest concentration without visible microbial biomass growth under optical microscope was defined as MBC, indicating the death of 99.5% of the original inoculum. The optical density for each well was measured and compared to a blank one (broth medium with diluted essential oil) and positive control. Gentamycin 10 µg (Oxoid™) and ampicillin 10 µg (Oxoid™) were utilized as positive controls (1 mg/mL in sterile saline solution). A solution of 5% dimethyl sulfoxide was utilized as negative control.

Turbidities were measured using one of the following; colorimeter (0.5 McFarland suspension), or the line method (CLSI/NCCLS 2005a). Suspensions targeted an inoculum density equivalent to approximately 1.0 × 108.

3. Statistical Analysis

The antimicrobial tests were carried out in duplicate and replicated three times. The results were expressed in values of arithmetical average ± standard deviation and analyzed by analysis of unidirectional variance (ANOVA), followed by Tukey's HSD (honestly significant difference) test with $\alpha = 0.05$ to determine statistical differences. The analysis was done by Statistical Package for the Social Sciences, v. 22.0.

4. Results

A more significant inhibition was seen with a higher oleoresin oil concentration 10µl and 12µl of P. khinjuk. The determination of MIC involves a semi-quantitative test procedure which gives an approximation of the least concentration of an antimicrobial that is needed to prevent microbial growth. The MIC assay method is widely used and is an accepted criterion for measuring the susceptibility of organisms to inhibitors. The researchers introduced that the broth microdilution method was a rapid quantitative determining of MIC based on measurement of the bioluminescent signal [6, 7]. Minimal inhibitory concentration (MIC) values were the lowest concentration of oils that completely inhibited microbial growth. The results were in micrograms per milliliter.

In our study, the MIC values regarding the antimicrobial activity of oil mastic gum extraction by conventional and microwave at 90 mintues of Pistacia khinjuk against Gram negative bacteria (Enterococcus faecalis, Salmonella enterica, Klebsiella pneumonia, E.coli) and Gram positive bacteria (S. aureus, Bacillus cereus and Listeria monocytogenes) were shown at Tables 1 and 2.

Furthermore, the oil mastic gum also exhibited an effect against the Gram-positive bacteria (S. aureus, Listeria monocytogenes and Bacillus cereus). However, this effect was more efficient (Listeria monocytogenes 0.9375µg/ml) than that presented against the Gram negative bacteria (Enterococcus faecalis, Salmonella enterica, Klebsiella pneumonia, and E. coli) (1.875µg/ml), since a higher MIC value was obtained with the Gram-positive bacteria. Differences in MIC values of bacteria may be related to differential susceptibility of bacterial cell wall, which is the functional barrier to minor differences present in outer membrane in the cell wall composition [8, 9].

The results were supported by results obtained from many other studies. One of them reported that the results arrived at from the minimal inhibitory concentration (MIC) of the leaves essential oils of Pistacia letiscus from Morocco are found to be active against Salmonella typhi, Escherichia coli, Enterobacter aerogenes, Klebsiella pneumonia and

Enterococcus faecalis at a minimal inhibitory concentration (MIC) of 0.08, 0.12, 0.07, 0.21 mg/mL and 0.68 mg/mL against Pseudomonas aeruginosa, Staphylococcus aureus, Staphylococcus intermedius and Bacillus sphericus the oil from the leaves was found to be more active; the oils showed MIC values of 1.01, 0.91, 1.02 mg/mL and1.56 mg/mL, respectively [10, 11].

Similar results were found studies conducted in different countries by different researchers. One of these studies found that the results indicated that the oil mastic gum of P. vera showed antibacterial activity, according to [12, 13], mainly against the Gram-negative bacteria (E. coli and Proteus spp).

Table 1. *Average of Minimal Inhibitory Concentration (MIC) for Pistacia khinjuk essential oil extraction by conventional method at 90 minutes.*

No	Microorganisms	MIC (µg/ml) Dilutions (µg/ml)											
		1	2	3	4	5	6	7	8	9	10	11	12
1	Staphylococcusaureus	7.5	3.75	0	0	0	0	0	0	0	0	0	0
2	Bacillus cereus	0	3.75	0	0	0	0	0	0	0	0	0	0
3	Enterococcus faecalis	7.5	3.75	1.875	0	0	0	0	0	0	0	0	0
4	Salmonella enterica	7.5	3.75	0	0	0	0	0	0	0	0	0	0
5	Klebsiella pneumonia	7.5	3.75	0	0	0	0	0	0	0	0	0	0
6	Chronobacter sakazakii	0	0	0	0	0	0	0	0	0	0	0	0
7	E.coli O157:H7	7.5	3.75	0	0	0	0	0	0	0	0	0	0
8	Listeria monocytogenes	7.5	3.75	1.875	0	0	0	0	0	0	0	0	0

Note= Dilutions are 1: 7.5, 2:3.75, 3:1.875, 4:0.9375, 5:0.46, 6:0.23, 7:0.11, 8:0.05, 9:0.002, 10:0.012, 11:0.005

Table 2. *Average of Minimal Inhibitory Concentration (MIC) for Pistacia khinjuk essential Oil extraction by microwave method 200 W at 90 minutes.*

No	Microorganisms	MIC (µg/ml) Dilutions (µg/ml)											
		1	2	3	4	5	6	7	8	9	10	11	12
1	Staphylococcusaureus	7.5	3.75	0	0	0	0	0	0	0	0	0	0
2	Bacillus cereus	7.5	3.75	0	0	0	0	0	0	0	0	0	0
3	Enterococcus faecalis	7.5	3.75	1.875	0	0	0	0	0	0	0	0	0
4	Salmonella enterica	7.5	3.75	1.875	0	0	0	0	0	0	0	0	0
5	Klebsiella pneumonia	7.5	3.75	0	0	0	0	0	0	0	0	0	0
6	Chronobacter sakazakii	0	0	0	0	0	0	0	0	0	0	0	0
7	E.coli O157:H7	7.5	3.75	0	0	0	0	0	0	0	0	0	0
8	Listeria monocytogenes	7.5	0	1.875	0	0	0	0	0	0	0	0	0

Note= Dilutions are 1: 7.5, 2:3.75, 3:1.875, 4:0.9375, 5:0.46, 6:0.23, 7:0.11, 8:0.05, 9:0.002, 10:0.012, 11:0.005

In this study, the MIC values of the oil indicate that several components (+)-αPinene (77.48%) followed by (±)-β-Pinene (17.88%), D- Limonene (1.456%), Camphene (1.85%); the major component was α-pinene. Essential oils rich in β-pinene demonstrated potential antibacterial activity.

Similar results are in the studies conducted in different countries by different researchers. It is evident that carvacrol is the most valuable constituent, followed by camphene, limonene, R-pinene, borneol and R-terpineol. Therefore, high efficacy of P.vera oil against the microorganisms can be due to carvacrol, camphene, and limonene, respectively [12].

P. khinjuk stoks contain the compounds of; 22 Spathulenol, 58 Myricetin-3-glucoside, 59 Myricetin-3-galactoside, 60 Myricetin-3-rutinoside. When the antibacterial activity studied with extract/essential oil isolated component: Chloroform, ethyl acetate, ethyl alcohol, and diethyl ether extracts in recent studies with microdilution method; they found activity against bacteria including Bacillus subtilis, Enterococcus faecalis, Staphylococcus aureus Staphylococcus epidermidis, Escherichia coli, and Klebsiella pneumoniae (MIC = 0.02–0.5 mg/mL) and fungi including Candida albicans and Saccharomyces cerevisiae (MIC = 0.06–0.4 mg/mL). Chloroform extract inhibited growth of fungi more than others [2].

5. Conclusion

Phytochemical studies provided evidence for traditional applications of these species. With respect to phytochemical assays, triterpenes found in the resin and monoterpens are the most abundant composition of the essential oil from different parts of these species. Essential oil constituents might be valuable chemotaxonomic marker to ascertain different Pistacia chemotypes. Considering the therapeutic effect of isolated components, it can be concluded that terpenoids including mono, di-, and triterpenoids are associated with anti-inflammatory and antimicrobial effects. High amounts of natural phenols and flavonoids are related to potent antioxidant and anticancer activities [14]. The results of the antimicrobial activity tests minimal inhibitory concentration (MIC) indicate that essential oil of mastic gum Pistacia Khinjuk exhibited higher activity against the tested strains and confirm its traditional uses [15]. The findings indicated that α-pinene, verbenone, R-terpineol, linalool, carvacrol

and flavones are major compounds related to antibacterial activity [1].

Conflict of Interest Declaration

The author(s) declare(s) that there is no conflict of interest.

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