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RESEARCH ARTICLE

The Chemical Composition and Anti-mycobacterial Activities of *Trachyspermum copticum* and *Pelargonium graveolens* Essential Oils

Jalil Kardan-Yamchi[1,2], Mohaddese Mahboubi[3], Hossein Kazemian[4,5], Gholamreza Hamzelou[6] and Mohammad M. Feizabadi[2,7,*]

[1]*Division of Microbiology, Department of Pathobiology, School of Public Health, Tehran University of Medical Sciences, Tehran, Iran;* [2]*Department of Medical Microbiology, School of Medicine, Tehran University of Medical Sciences, Tehran, Iran;* [3]*Medicinal Plants Research Department, Research and Development, TabibDaru Pharmaceutical Company, Kashan, Iran;* [4]*Department of Microbiology, Faculty of Medicine, Ilam University of Medical Sciences, Ilam, Iran;* [5]*Clinical Microbiology Research Center, Ilam University of Medical Sciences, Ilam, Iran;* [6]*Tehran Regional Reference Laboratory for Tuberculosis, Tehran, Iran;* [7]*Thoracic Research Center, Imam Khomeini Hospital Complex, Tehran University of Medical Sciences, Tehran, Iran*

Abstract: ***Background*:** Microbial resistance to antibiotics and their adverse effects related to these antibiotics are a matter of global public health in the 21th century. The emergence of drug-resistant strains, has gained the interest of the scientists to discover new antimicrobial agents from the essential oil of medicinal plants.

***Methods*:** Anti-mycobacterial effects of *Trachyspermum copticum* and *Pelargonium graveolens* essential oils were determined against multi-drug resistant clinical strains of *Mycobacterium tuberculosis*, *Mycobacterium kansasii*, *Mycobacterium fortuitum* and standard strain of *Mycobacterium tuberculosis* H37Rv by a Broth micro-dilution method. *Pelargonium graveolens* plant named Narmada was discovered by Kulkarni R.N *et al.* (Patent ID, USPP12425P2) and a formulation comprising thymol obtained from *Trachyspermum* is useful in the treatment of drug-resistant bacterial infections (Patent ID, US6824795B2). The chemical composition of hydro-distilled essential oils was determined by GC and GC-MS.

***Results*:** Minimum Inhibitory Concentration (MIC) values for *T. copticum* essential oil against tested isolates were ranged from 19.5 μg/mL to 78 μg/mL. The least minimum inhibitory concentration of *P. graveolens* extract against *M. Kansasii* and MDR-TB was 78 μg/ml.

***Conclusion*:** The results of the present research introduced *T. copticum* and *P. graveolens* essential oils as a remarkable natural anti-mycobacterial agent, but more pharmacological studies are required to evaluate their efficacy in animal models.

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1. INTRODUCTION

Due to the emergence of drug-resistant microorganisms [1, 2], scientists have focused on natural resources, such as plants, animals, and also microorganisms, in order to discover newer antimicrobi al agents [3, 4]. Medicinal plants, especially their essential oils, have been used as antimicrobial agents [5]. Among these essential oils, *Trachyspermum copticum* and *Pelargonium graveolens* essential oils have been introduced as antimicrobial agents as their traditional applications [4, 6]. *Pelargonium graveolens* plant named 'Narmada was discovered by Kulkarni *et al.* (Patent ID, USPP12425P2) [7] and a formulation comprising thymol obtained from *Trachyspermum*

*Address correspondence to this author at Department of Medical Microbiology, School of Medicine, Tehran University of Medical Sciences, Tehran, Iran; Thoracic Research Center, Imam Khomeini Hospital Complex, Tehran University of Medical Sciences, Tehran, Iran; Fax: 00982188955810; Tel: 09141904331; E-mail: mfeizabadi@tums.ac.ir

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is useful in the treatment of drug-resistant bacterial infections (Patent ID, US6824795B2) [8].

Trachyspermum copticum, a member of the Apiaceae family is native to the Middle East. The seeds and roots of *Trachyspermum copticum* have been used traditionally due to its diuretic and aphrodisiac activities [9]. The main component of *T. copticum* seeds is a brown colored essential oil with thymol as the main component. The essential oil exhibited antifungal and antibacterial effects [4, 10-13] against *Escherichia coli*, *Proteus vulgaris*, *Klebsiella pneumonia*, *Staphylococcus aureus*, *Bacillus subtilis*, *Bacillus megaterium* [10], *Streptococcus mutans* [12] and any other bacterial and fungal strains. Rather than the antimicrobial activity [14] of *T. copticum* essential oil, its antihypertensive, antispasmodic and broncho-dilating and hepatoprotective effects [15], diuretic activity [16], anti-inflammatory [17], antitussive [18] and gastroprotective effects have been demonstrated [19].

Pelargonium graveolens (Geraniacea family) is cultivated for the production of essential oil. *P. graveolens* essential oil has been the focus of many studies [20-22]. Citronellol and geraniol have been reported as the main components of *P. graveolens* essential oil. The wound healing effects of *P. graveolens* essential oil [20] and its antimicrobial activity were reported [6, 22-25]. Although the antimicrobial activity of *P. graveolens* and *T. copticum* has been reported on different types of microorganisms, the antimycobacterial activity of these two essential oils has not been discussed in any study. Therefore, the antimycobacterial activity of *P. graveolens* and *T. copticum* essential oils was evaluated against three clinical isolates of mycobacteria, including multi-drug resistant (MDR) *Mycobacterium tuberculosis*, *Mycobacterium kansasii* and *Mycobacterium fortuitum* and also *Mycobacterium tuberculosis* H37Rv as a standard strain. Furthermore, due to the effect of different factors on the chemical composition of essential oils, this was analyzed by GC and GC-MS.

2. MATERIALS AND METHODS

2.1. Plant Essential Oils

The essential oils from full flowering aerial parts of *Pelargonium graveolens* (MC-ES20-NO7) and *Trachyspermum copticum* (MC-ES12-NO) seeds were prepared by the BarijEssence Pharmaceutical Company, Kashan, Iran *via* hydrodistillation method. The essential oils were in dark containers, and it had been recommended to keep in a cool and dark place.

2.2. Gas Chromatography (GC) and Gas Chromatography-mass Spectra (GC-MS)

GC and GC-MS instruments were used for the analysis of the chemical compositions of essential oils. The GC was conducted on Trace MS (Thermo Quest-Finnigan) with the capillary column of DB-5 (30 m × 0.25 mm, film thickness 0.25 μm) while for GC-MS analysis the Trace MS (Thermo Quest-Finnigan) coupled with 5973 network mass selective detector system. In two systems, the oven temperature was initiated at 60°C, held for 1 min, then raised upto 250°C at a rate of 3°C/min, held for 10 min. Helium was used as the carrier gas at a flow rate of 1.0 mL/min with a split ratio equal to 1/100 injector. The detector and injector temperatures were 250 and 230°C for GC and GC-MS, respectively. Components of essential oils were identified by the comparison with Retention Indices (RI) relative to a homologous series of n-alkanes and using libraries of Wiley 275.L and Wiley 7n.1, as well as a comparison of the fragmentation pattern of the mass spectra with data published in the literature [26].

2.3. Mycobacterial Strains

Antimycobacterial activity of these two essential oils was determined against 20 multidrug-resistant (MDR) *M. tuberculosis*, 2 *M. fortuitum* and 2 *M. kansasii* clinical isolates and also *M. tuberculosis* H37Rv as a standard strain. The mycobacterial strains isolated from suspected patients referred to the Tehran Regional Reference Laboratory for Tuberculosis. All isolates were subcultured on Lowenstein Jensen (LJ) media. The 1.0 McFarland bacterial suspension was prepared from fresh colonies in normal saline. Drug susceptibility testing against three first-line anti-tuberculosis drugs was performed using the proportion method explained already by WHO guidelines [27, 28]. Drug concentrations used as follows; rifampicin (40 μ g/mL), isoniazid (0.2 μ g/mL), and ethambutol (2 μg/mL).

2.4. Determination of Anti-mycobacterial Effects of Essential Oil by Micro-broth Dilution Assay

For evaluating the anti-mycobacterial assay, the MICs were determined by the broth microdilution method. First, 50 μl of Middlebrook 7H9 Broth

media (BD Difco, Bergen, United States) supplemented with OADC (oleic acid, albumin, dextrose, and catalase) (BD Difco, Bergen, United States) was added to all the wells in 96-well microplate. Then, 50 µl of media containing 1248 µg/mL of the essential oil was added to the first wells and mixed well. After that, two-fold serial dilutions were prepared by transferring 50 µl of suspension from a column to the next one except for a positive control well. Finally, all wells were inoculated (except negative control) by 50 µl of mycobacterial suspension diluted 1:50 in the same media to reach the 312 to 2.4 µg/mL dilutions of each essential oils. By the way, the last two columns were as negative and positive controls containing media with essential oil without bacterial suspension and only media with a bacterial suspension respectively. All tests were performed in duplicate. All the Microtiter plates were wrapped with cling film to prevent dehydration and incubated at 37°C for three weeks. The results were recorded on day 7 for *Mycobacterium fortuitum* and on day 21, for other slow-growing species. The first wells in ascending order without visible growth were determined as MICs.

3. RESULTS

3.1. Essential Oil Composition

Analysis of the chemical composition of *Trachyspermum copticum* essential oil showed the presence of 26 compounds that comprised 99.34% of total oil compositions. Thymol (50.7%), p-cymene (27.3%) and γ-terpinene (18.6%) were the main components of *T. copticum* essential oil (supplementary Table **1**). Analysis of the *Pelargonium graveolens* essential oil exhibited 52 components that present 99.4% of oil composition. β-Citronellol (39.4%), geraniol (23.7%), and nerol (11.7%) were the main components of essential oil, followed by trans-menthan-3-one (2.9%), p-menthone (2.7%), citronellyl-n-butyrate (2.6%), phenyl ethyl alcohol (2.4%), geranyl acetate (1.5%), and rose oxide (1.5%) (supplementary Table **2**).

3.2. Drug Resistance Pattern of Isolates

The collected twenty MDR isolates resistant to both rifampicin and isoniazid selected for further analyses. 11 out of the 20 MDR isolates were also resistant to ethambutol (55%). The targeted 2 *M. fortuitum* and 2 *M. kansasii* isolates were resistant to all tested drugs. Drug susceptibility testing results for testing isolates are shown in Table **1**.

Table 1. Drug resistance pattern of mycobacterial isolates.

Strains	Rifampicin	Isoniazid	Ethambutol
H37Rv	S	S	S
MTB1	R	R	S
MTB2	R	R	R
MTB3	R	R	S
MTB4	R	R	R
MTB5	R	R	R
MTB6	R	R	R
MTB7	R	R	S
MTB8	R	R	R
MTB9	R	R	S
MTB10	R	R	S
MTB11	R	R	R
MTB12	R	R	R
MTB13	R	R	S
MTB14	R	R	R
MTB15	R	R	S
MTB16	R	R	S
MTB17	R	R	R
MTB18	R	R	R
MTB19	R	R	S
MTB20	R	R	R
M. kansasii 1	R	R	R
M. kansasii 2	R	R	R
M. fortuitum 1	R	R	R
M. fortuitum 2	R	R	R

MTB; *Mycobacterium tuberculosis*, R; resistant, S; susceptible.

3.3. Anti-mycobacterial Activity of Essential Oils

The results of the broth micro-dilution assay showed *T. copticum* and *P. graveolens* essential oils effectively inhibited the growth of MDR *M. tuberculosis*, *M. fortuitum*, *M. kansasii* and also *M. tuberculosis* H37Rv. The Mycobacterial species showed more sensitivity to *T. copticum* than

P. graveolens. The MIC values of *T. copticum* essential oil against mycobacterial species were found between 19.5 µg/mL to 78 µg/mL (Table **2**). Sensitivity to *P. graveolens* essential oil was ranging from 78 µg/mL to 156 µg/mL (Table **3**).

Table 2. Antimycobacterial activity of *Trachyspermum copticum* essential oil.

Mycobacterial Species	MIC (No. of Isolates)			
	19.5 µg/mL	39 µg/mL	78 µg/mL	156 µg/mL
H37Rv MTB	1	-	-	-
MDR-MTB	10	8	2	-
M. kansasii	-	2	-	-
M. fortuitum	-	1	1	-

MIC; minimum inhibitory concentration, MTB; *M. tuberculosis*, MDR-MTB; multi-drug resistant *M. tuberculosis*.

Table 3. Antimycobacterial activity of *Pelargonium graveolens* essential oil.

Mycobacterial Species	MICs (No. of Isolates)			
	19.5 µg/mL	39 µg/mL	78 µg/mL	156 µg/mL
H37Rv MTB	-	-	1	-
MDR-MTB	-	-	11	9
M. kansasii	-	-	1	1
M. fortuitum	-	-	-	2

MIC; minimum inhibitory concentration, MTB; *M. tuberculosis*, MDR-MTB; multi-drug resistant *M. tuberculosis.*

4. DISCUSSION

Plant extracts as a potential source of various compounds with different biological activities can provide new agents in drug development [1]. Many works of literature demonstrated the antimicrobial activity of different parts of various plants [1, 3, 6, 29, 30]. *T. copticum* and *P. graveolens* were two of these medicinal plants that their antimicrobial characteristics were assayed in previous studies [4, 6, 23]. Herein, the anti-mycobacterial activity of these two plant essential oils was the subject of this study.

The antifungal activity of *T. copticum* and its inhibitory effects against Gram positive and Gram negative bacteria, such as *Staphylococcus* spp., *Enterococcus* spp., *Escherichia coli*, *Pseudomonas aeruginosa* were confirmed. [4, 31]. But there was not any study on antimycobacterial activity of this plant. In this study, we found that the essential oil from *Trachyspermum copticum* effectively inhibited the growth of tested mycobacterial species by a broth microdilution method. Our results showed that MIC of this essential oil against *M. tuberculosis* H37Rv, MDR *M. tuberculosis*, *M. kansasii* and *M. fortuitum* strains was remarkable, as noted in Table **2**. The 50% of MDR-MTB, both the *M. kansasii* and 1 of the *M. fortuitum* strains were susceptible as H37Rv standard strains with a concentration of 19.5 µg/ml. As Thymol (50.7%), p-cymene (27.3%), and γ-terpinene (18.6%) were found as the main components of *T. copticum* essential oil (supplementary Table **1**), the antimycobacterial effect of total essential oil of this plant on tested isolates may be related to these components.

Pelargonium graveolens was the other medicinal plant tested in our study. Several studies indicated that the essential oil of this plant able to prevent the growth of microorganisms especially many bacterial species *in vitro* [6, 23, 32]. However, the antimycobacterial properties of this plant were not the subject of any study. Broth microdilution results showed, *P. graveolens* essential oil significantly inhibited the growth of mycobacterial species. As pointed in Table **3**, 11 (55%) MDR-TB and 1 *M. kansasii* strains were inhibited by a concentration of 78 µg/mL of *P. graveolens* essential oil like H37Rv strain. According to GC and GC-MS results, the antimycobacterial effect of *Pelargonium graveolens* may be due to compounds including β -Citronellol (39.4%), geraniol (23.7%), and nerol (11.7%) as were the main components of essential oil (supplementary Table **2**).

Our findings are considerable in comparing with other plant extracts against mycobacterial species. Maham *et al.* showed the least concentrations of ethanolic extracts of *Mentha spicata* and *Mentha piperita* that inhibit the *Mycobacterium Bovis* growth were 0.39 mg/mlL and 100 mg/ml respectively [33]. The antimycobacterial activity of methanolic extracts of *Allium ascalonicum L.* (Shallot) against three non-tuberculosis mycobacteria including *M. fortuitum* ATCC 684, *M. smegmatis* ATCC 19420 and *M. phlei* ATCC 19240 was ranged from 50 mg/ml to 200 mg/ml in Igbokwe *et al.* experiment. But this extract was not effective against *M. abscessus* [34]. An MDR-TB strain was inhibited by *Z. multiflora* extract at a concentration of 78 µg/mL showed by Kazemian

et al. However, 156 µg/mL was reported as a minimum inhibitory concentration of *S. rechingeri* and *S. khuzestanica* extracts against MDR-TB [35].

According to the literature, about one-third of the world's population is at risk contaminated by *M. tuberculosis* [36]. Furthermore, *M. tuberculosis* strains that resistant to first-line and second-line drugs are emerging and spreading worldwide, becoming a great concern [37]. Various plants are used for the treatment of tuberculosis and non-tuberculosis mycobacteria disease in different areas [34, 38]. The hallmark of the present study is the considerable activity of tested essential oils against MDR-TB strains. Resistance to rifampin and isoniazid were observed in these cases, but their growth inhibited considerably by studied extracts *in vitro*. By the global distribution and pathogenicity of mycobacterial species in healthy and immunocompromised individuals and development of drug resistance isolates, the inhibitory effect of these two essential oils at low concentrations (between 39 µg/mL to 156 µg/mL) can be more important [39-41].

CONCLUSION

In conclusion, tested essential oils from two different medicinal plants showed considerable inhibitory activities against mycobacterial clinical species. Our results concerning a low inhibitory concentration of essential oils support the idea that these plants can be as good candidates in herbal medicine and had antimycobacterial compounds could be a source of new antibiotic compounds and potentially beneficial in chemotherapeutic development. As the first study was done on antimycobacterial effects of *Trachyspermum copticum* and *Pelargonium graveolens* essential oils, further studies should be done on their biological activities and more isolates and confirm the mechanism of the main compounds responsible for the antimycobacterial effect.

CURRENT AND FUTURE DEVELOPMENTS

Tuberculosis is an infectious disease caused by M. tuberculosis. Drug-resistant TB is caused by MTB strains that are resistant to at least one first-line anti-TB drug and Treating of drug-resistant TB is complicated and inappropriate management can have life-threatening results. Furthermore, non-tuberculous mycobacteria have become emergent pathogens and are resistant to a large number of antibiotics such as antituberculosis agents. Thus, new approaches to tackle the drug-resistant mycobacterial infections are the urgent need. The current study described the antimycobacterial activity of Trachyspermum copticum and Pelargonium graveolens essential oils. In future, further investigations are needed to evaluate the efficacy of these essential oils and application in chemotherapeutic development.

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

Not applicable.

HUMAN AND ANIMAL RIGHTS

No animals/humans were used for studies that are base of this research.

CONSENT FOR PUBLICATION

Not applicable.

AVAILABILITY OF DATA AND MATERIALS

The authors confirm that the data supporting the findings of this research are available within the article.

FUNDING

None.

CONFLICT OF INTEREST

The authors declare no conflict of interest, financial or otherwise.

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SUPPLEMENTARY MATERIAL

Supplementary material is available on the publisher's website along with the published article.

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