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In-vitro evaluation of the antibacterial activity of the essential oils of *Micromeria barbata*, *Eucalyptus globulus* and *Juniperus excelsa* against strains of *Mycobacterium tuberculosis* (including MDR), *Mycobacterium kansasii* and *Mycobacterium gordonae*

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ABSTRACT

Background: *Mycobacterium* spp. are responsible for several diseases, particularly in immunocompromised populations. The spreading of the resistance to antimycobacterial drugs is a significant problem to the public health and requires to find out a new and innovative alternative for the treatment of drug resistant mycobacterial strains. In this study, the antimycobacterial activity of *Micromeria barbata*, *Eucalyptus globulus* and *Juniperus excelsa* essential oils extracted from Lebanese plants was investigated against selected *Mycobacterium* spp. strains.

Methods: Several dilutions of the three aforementioned essential oils were studied for antimycobacterial activity against four *Mycobacterium* spp. strains: *Mycobacterium tuberculosis* subsp. *tuberculosis* (ATCC® 27294™), multidrug-resistant *M. tuberculosis* (CMUL 157), *Mycobacterium kansasii* Hauduroy (ATCC® 12478™) and *Mycobacterium gordonae* Bojalil et al. (ATCC® 14470™).

Results: Even with high dilutions, all tested essential oils showed a high antimycobacterial activity against targeted strains. Our data showed that *M. barbata*, *E. globulus* and *J. excelsa* essential oils totally inhibit the mycobacterial growth whatever the tested strains for the dilution 1/250, 1/100 and 1/250, respectively.

Conclusion: To our knowledge, this is the first study regarding the antimycobacterial activity of essential oils in Lebanon. Our data show promising results, and encourage to investigate more on these medicinal plants, especially *M. barbata*.

Introduction

Tuberculosis (TB) is an ancient scourge that has affected mankind throughout known history and human prehistory [1]. In 2017, 10 million (range, 9 million–11.1 million) people around the world became sick with TB disease. There were 1.3 million TB-related deaths worldwide (including 0.3 million among people with HIV). Moreover, about 558,000 and 458,000 new cases developed rifampicin and multidrug resistant (MDR) TB worldwide in 2017, respectively [2].

Despite the implementation of directly observed treatment short course strategy, approximately 20% of TB cases are now resistant to at least one major anti-TB drug and around 5% of all cases of TB are classed as MDR or extensively drug-resistant (XDR) [3,4]. The spread of MDR and XDR *Mycobacterium tuberculosis* strains threatens to disrupt decades of progress in controlling TB [5]. While some new antimycobacterial drugs such as bedaquiline and delamanid with the potential to treat some drug-resistant strains are becoming approved by Food and Drug Administration (FDA), recent studies showed the rapid emergence of resistant strains [6–8]. Hence, the

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treatment of drug-resistant TB remains a big challenge due to the need to consider, when approaching it, the context of individual and collective health and the drug resistance.

In fact, essential oils are considered as good potential antibiotic alternatives and holistic medicine for similar purposes [9–11]. These natural compounds are now widely used as traditional medicines in pharmaceutical, food, and cosmetic products because these oils effectively inhibit the growth of microbes with lower side effects than synthetic antimicrobial agents in humans. Recently, several reports described the anti-TB activity of medicinal plants and natural products [12–15]. However, to our knowledge, no data are currently available regarding the anti-tubercular activity of *Micromeria barbata* essential oil. Therefore, the main aim of this study was to evaluate the antimycobacterial activity of *M. barbata* essential oil. Moreover, other essential oils extracted from two Lebanese plants *Eucalyptus globulus* and *Juniperus excelsa* were also assessed for the first time in Lebanon.

Materials and methods

The samples of the essential oils

The original essential oils of 3 medicinal plants were used: *M. barbata, E. globulus* and *J. excelsa*. All plants were collected from the region of Dinniyeh, North Lebanon. The essential oils were obtained by hydrodistillation technique using the Clevenger-type apparatus in the laboratory of the chamber of commerce, industry and agriculture in Tripoli. The percentage yield (2%) of the oils was calculated on moisture-free basis. All extracted oils were stored in a sterile container in the dark at 4 °C till analysis.

Selection of mycobacterial strains

4 strains of *Mycobacterium* spp. were included in this study. The first was *M. tuberculosis* subsp. *tuberculosis* (ATCC® 27294™) sensitive to rifampicin, isoniazid, ethambutol, and pyrazinamide. The second was also a clinical strain of *M. tuberculosis* (CMUL 157) isolated from pulmonary secretions of an infected Lebanese patient, and resistant to first line TB drugs (rifampicin, isoniazid, ethambutol, and pyrazinamide). The third and fourth were nontuberculous mycobacteria American Type Culture Collection (ATCC) reference strains: (i) *Mycobacterium kansasii* Hauduroy (ATCC® 12478™) resistant to streptomycin and p-aminosalicylic acid, and (ii) *Mycobacterium gordonae* Bojalil et al. (ATCC® 14470™) susceptible to all atypical antimycobacterial drugs. This part of the study was carried out in the Laboratoire Microbiologie Santé et Environnement at Lebanese University.

Preparation of the mycobacterial suspension

After adding 20–30 sterile glass beads in a sterile conical tube (50 ml), fresh mycobacterial colonies were transferred by using sterile swab consistent with mycobacteria growing on Lowenstein–Jensen media. After vortexing for 1 min, 0.5 ml of Middlebrook 7H9 broth with glycerol (Becton, Dickinson®, USA) was added to the aggregates followed by 1 min of vortexing. Afterwards, 5 additional ml of Middlebrook 7H9 broth with glycerol were added. The suspension was homogenized by vortexing the tube for 1 min (solution A). From this suspension, a new suspension was prepared by adding few ml of solution A in 100 ml Middlebrook 7H9 broth with glycerol in order to adjust by optical density a suspension with a turbidity equivalent to a BCG suspension (Sanofi Pasteur MSD, France) titrated to $2-8 \times 10^5$ CFU/ml (Solution B). OADC (125 μl/ml) and PANTA (25 μl/ml) were supplemented to the solution B. PANTA (Becton, Dickinson, USA) is a mixture of antibiotics including polymyxin B, amphotericin B, nalidixic acid, trimethoprim and azlocillin.

Study of the antimycobacterial activity of essential oils

In order to evaluate the antimycobacterial activity of essential oils, two consecutive steps were performed using a liquid medium (Middlebrook 7H9 broth) for 7 days, and a solid medium (Lowenstein–Jensen) for 3 weeks, respectively. Unfortunately, the presence of essential oils prevents the direct detection of mycobacterial growth on Middlebrook 7H9 broth. In order to overcome this issue, after incubation in the liquid medium, a subculture is made on Lowenstein–Jensen medium in order to detect mycobacterial colonies. In fact, the time required to obtain reliable drug susceptibility testing results with Mycobacteria Growth Indicator Tube (MGIT) medium is between 3 to 12 days [16]. However, the use of a strict standardized high inoculum concentration ($2-8 \times 10^5$ CFU/ml) allowed us to successfully perform our experiments.

Firstly, because of the immiscibility of the essential oils to water and thus to the culture medium an emulsification was performed using a 0.2% agar. This solution allows a homogeneous distribution of essential oils and maximizes the germ/compound contact. In this agar solution, the following dilutions were prepared as 1/2, 1/10th, 1/25th, 1/50th, 1/100th, 1/200th, 1/300th and 1/500th. Test tubes containing each 9 ml of solution B were sterilized for 20 min at 121 °C and cooled to 50 °C, then 1 ml of each dilution was added aseptically in order to obtain a final concentration of 1/20th, 1/100th, 1/250th, 1/500th, 1/1000th, 1/2000th, 1/3000th and 1/5000th. The tubes were shacked to disperse the essential oil in the culture medium. Negative and positive controls were also prepared. Negative control contained sterile Middlebrook 7H9 broth with glycerol without adding mycobacterial suspension. Or, the positive control contained mycobacterial suspension without adding essential oil. All tubes were incubated at 36 °C during 7 days. Then, 200 μl from each tube was inoculated on Lowenstein–Jensen slants for mycobacterial culture as previously described [17]. The experiments were repeated three times for each essential oil and the average results were reported.

Statistical analyses

Statistical analyses were performed with GraphPad Prism 6.0 (GraphPad Software Inc., San Diego, CA) using the Student's t-test to compare the antimycobacterial activity between medicinal plant essential oils, and to also compare the antimycobacterial activity of *M. barbata* against *M. tuberculosis* strain (ATCC 27294) and MDR *M. tuberculosis* clinical strain. The tests were two-sided, with a type I error set at $\alpha = 0.05$.

Results

During the last decades, due to the emergence and spread of MDR *Mycobacterium* spp. strains worldwide, demand for searching novel antimycobacterial agents is being increased. The medicinal plant essential oils as natural substances could represent a potential source of new antimycobacterial agent. In this study, three essential oils extracted from Lebanese plants (*M. barbata, E. globulus* and *J. excelsa*) were studied for antimycobacterial activity. In order to evaluate their antimycobacterial activity, a new protocol was adapted and validated. Our data showed that *M. barbata, E. globulus* and *J. excelsa* essential oils were 100% active against studied strains (Table 1; Fig. 1). Even with a high dilution, the investigated essential oils showed a high antimycobacterial activity on all the tested strains. The results showed that *M. barbata*, *E. globulus* and *J. excelsa* essential oils totally inhibit the mycobacterial growth whatever

Table 1
Antibacterial effect of *M. barbata*, *E. globulus*, and *J. excelsa* essential oils on the studied mycobacterial strains, the results represent the higher essential oils dilution conferring a complete growth inhibition against the studied strains.

Mycobacterial strain	Higher essential oil dilution conferring antimycobacterial activity		
	M. barbata	*E. globulus*	*J. excels*
M. kansasii (ATCC® 12478™)	1/1000	1/100	1/500
M. gordonae (ATCC® 14470™)	1/500	1/250	1/500
M. tuberculosis (ATCC® 27294™)	1/1000	1/250	1/500
M. tuberculosis MDR (CMUL 157)	1/250	1/250	1/250

*The experiments were repeated three times for each essential oil, and the reported dilutions were the same in all series.

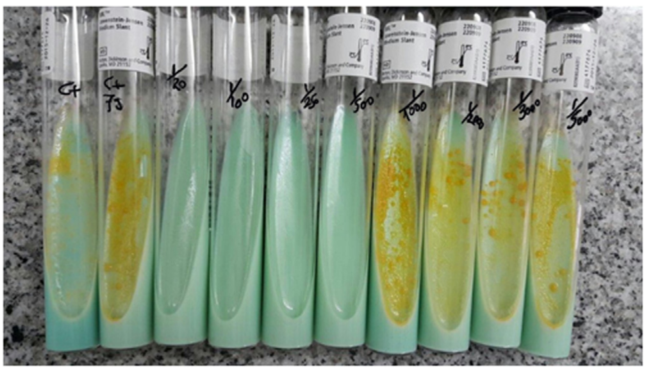

Fig. 1. Antimycobacterial activity of *M. barbata* essential oil against *M. gordonae* (ATCC® 14470™), with a complete growth inhibition (100% inhibition) obtained at a maximum dilution of 1/500.

the tested strains for the dilution 1/250, 1/100 and 1/250, respectively. The experiments were highly reproducible, delivering the same results for every repeated test. The total inhibition (100%) is defined by the absence of mycobacterial growth after 7 days of incubation. Out of studied mycobacterial strains, *M. barbata* essential oil was the most effective in comparison with *E. globulus* essential oil (p-value = 0.001), and *J. excelsa* essential oil (p-value = 0.28). In addition, *M. barbata* essential oil was significantly more effective on wild-type *M. tuberculosis* strain (ATCC 27294) than MDR *M. tuberculosis* strain (p-value = 0.005). Regarding *E. globulus*, essential oil extracted from this plant was the less active in comparison with the other investigated essential oils.

Discussion

Essential oils are becoming increasingly popular to be used for a wide variety of applications, such as alternative natural medicines and aroma therapeutics. Indeed, *M. barbata*, *E. globulus* and *J. excelsa* are three medicinal plants which grow in temperate regions and are widely present in Middle Eastern region, particularly in Lebanon. However, despite that many studies worldwide reported the potential application of essential oils as promising antimicrobial agents, only very rare data are available in the literature regarding the antimicrobial activity of these essential oils. The present study represents the first report on antimycobacterial activity of Lebanese essential oils against *M. tuberculosis* and atypical mycobacteria. Moreover, the evaluation assay adopted in this study has allowed us to evaluate the antimycobacterial activity of essential oils accurately. In comparison with other methods reported in the literature, this assay is easier to implement and less complicated [12,15]. Numerous investigations conducted on the assessment of the antimicrobial activity of essential oils extracted from *Micromeria* spp. have reported a significant activity against bacteria and fungi [18,19]. Our previous studies reported that the essential oil of *M. barbata* have potentially a rich source of antimicrobial agents [19]. In addition, regarding *E. globulus* and *J. excelsa*, most studies have focused on foodborne pathogens and food spoilage bacteria. Indeed, several essential oils have been approved as food additives and fall in the category of generally recognized as safe (GRAS) by the FDA [20]. However, few data are available concerning the bactericidal activity of essential oils against *Mycobacterium* spp. including *M. tuberculosis* and non-tuberculosis mycobacteria [12,15]. Some previous studies on mycobacteria have shown promising results reporting bactericidal effects of crude plant extracts and their constituents or the root of the plant. Synergistically effects have been documented with *Melaleuca leucodendron* essential oil when combined with kanamycin, isoniazid and rifampicin against *M. tuberculosis*. For instance, these combinations increased the potency of isoniazid 4-fold against *M. tuberculosis* [21]. Another study found that essential oil isolated from the roots of *Euclea natalensis* decreased the MIC 4- to 6-fold for isoniazid and rifampicin, respectively [22]. The observed antimycobacterial activity can be attributed to the presence of some components such as carvacrol, α-terpinly acetate, cymene, thymol, pinene, linalool [19]. These natural compounds, naturally present in various essential oils, are known to have antimicrobial activity against various microorganisms.

Furthermore, an aforementioned study showed a high antimicrobial activity of the essential oil extracted from the berries of *J. excelsa*. Its main component, α-pinene, has a high microbiostatic activity against many clinically important bacteria and yeasts. The essential oil of *J. excelsa* has been analyzed by GC/MS. Forty-four components were identified, with the presence of very high amounts of α-pinene followed by α-cedrol which are already known to exhibit antimicrobial activity.

In the same context, *Eucalyptus* essential oil has antimicrobial effects against many bacteria, including *M. tuberculosis* and methicillin-resistant *Staphylococcus aureus*, viruses, and fungi. In a striking case, a TB infected patient who had refused conventional treatment, employed *E. globulus* oil inhalation during 3 weeks. After 10 days of using *Eucalyptus* oil the malaise reduced, appetite improved, cough subsided, and weight was gained [23]. Numerous studies reported that *Eucalyptus* essential oil may play an effective adjunctive role in the treatment of respiratory problems, such as tuberculosis, pneumonia, bronchitis, asthma and chronic obstructive pulmonary disease [23–26].

On the other hand, many other essential oils were also investigated and confirming their effects on mycobacterial strains. For instance, the essential oil of *Myrtus communis* was reported as effective against wild-type *M. tuberculosis* H37Rv, wild-type *M. tuberculosis* H37Ra, clinical resistant *M. tuberculosis* and *Mycobacterium avium*. Similarly, clove, cumin, and cinnamon essential oils were active against *M. tuberculosis* strains, with very low minimum inhibitory concentration values [27].

In conclusion, to our best knowledge, this study is the first investigation regarding the antimycobacterial activity of essential oils in Lebanon. Our data indicates that the three essential oils of *M. barbata, E. globulus* and *J. excelsa* have antibacterial activity against *M. tuberculosis* and atypical mycobacteria. The essential oil of *M. barbata* was significantly the more effective of the three tested. These results are encouraging to study more on these medicinal plants, particularly *M. barbata*. Nevertheless, the main limitations of this study are the small sample size of strains and the absence of cytotoxicity experiments, and thus conclusions made here will need to be confirmed in a larger ongoing study including MDR and XDR strains, and evaluating the cytotoxicity of these promising essential oils.

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Competing interests

None declared.

Ethical approval

Not required.

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References

[1] Daniel TM. The history of tuberculosis. Respir Med 2006;100(11):1862–70, http://dx.doi.org/10.1016/j.rmed.2006.08.006. PubMed PMID: 16949809.
[2] WHO. Global tuberculosis report; 2018.
[3] Herbert N, Sharma V, Masham BS, Sheehan BS, Hauser J, Zumla A. Concrete action now: UN High-Level Meeting on Tuberculosis. Lancet Infect Dis 2018;18(7):709–10, http://dx.doi.org/10.1016/S1473-3099(18)30171-3. PubMed PMID: 29580817.
[4] Floyd K, Glaziou P, Zumla A, Raviglione M. The global tuberculosis epidemic and progress in care, prevention, and research: an overview in year 3 of the End TB era. Lancet Respir Med 2018;6(4):299–314, http://dx.doi.org/10.1016/S2213-2600(18)30057-2. PubMed PMID: 29595511.
[5] Dheda K, Gumbo T, Maartens G, Dooley KE, McNerney R, Murray M, et al. The epidemiology, pathogenesis, transmission, diagnosis, and management of multidrug-resistant, extensively drug-resistant, and incurable tuberculosis. Lancet Respir Med 2017;5(4):291–360, http://dx.doi.org/10.1016/S2213-2600(17)30079-6. PubMed PMID: 28344011.
[6] Hoffmann H, Kohl TA, Hofmann-Thiel S, Merker M, Beckert P, Jaton K, et al. Delamanid and bedaquiline resistance in *Mycobacterium tuberculosis* ancestral Beijing genotype causing extensively drug-resistant tuberculosis in a Tibetan refugee. Am J Respir Crit Care 2016;193(3):337–40, http://dx.doi.org/10.1164/rccm.201502-0372LE. PubMed PMID: 26829425.
[7] Veziris N, Bernard C, Guglielmetti L, Le Du D, Marigot-Outtandy D, Jaspard M, et al. Rapid emergence of *Mycobacterium tuberculosis* bedaquiline resistance: lessons to avoid repeating past errors. Eur Respir J 2017;49(3), http://dx.doi.org/10.1183/13993003.01719-2016. PubMed PMID: 28182568.
[8] Xu J, Wang B, Hu M, Huo F, Guo S, Jing W, et al. Primary clofazimine and bedaquiline resistance among isolates from patients with multidrug-resistant tuberculosis. Antimicrob Agents Chemother 2017;61(6), http://dx.doi.org/10.1128/AAC.00239-17. PubMed PMID: 28320727; PubMed Central PMCID: PMC5444180.
[9] Demirci B, Yusufoglu HS, Tabanca N, Temel HE, Bernier UR, Agramonte NM, et al. *Rhanterium epapposum* Oliv. essential oil: chemical composition and antimicrobial, insect-repellent and anticholinesterase activities. Saudi Pharm J 2017;25(5):703–8, http://dx.doi.org/10.1016/j.jsps.2016.10.009. PubMed PMID: 28725142; PubMed Central PMCID: PMC5506638.
[10] Chouhan S, Sharma K, Guleria S. Antimicrobial activity of some essential oils-present status and future perspectives. Medicines 2017;4(3), http://dx.doi.org/10.3390/medicines4030058. PubMed PMID: 28930272; PubMed Central PMCID: PMC5622393.
[11] Orchard A, Viljoen A, van Vuuren S. Antimicrobial essential oil combinations to combat foot odour. Planta Med 2018;84(9-10):662–73, http://dx.doi.org/10.1055/a-0592-8022. PubMed PMID: 29579744.
[12] Chraibi M, Farah A, Lebrazi S, El Amine O, Iraqui Houssaini M, Fikri-Benbrahim K. Antimycobacterial natural products from Moroccan medicinal plants: Chemical composition, bacteriostatic and bactericidal profile of *Thymus satureioides* and *Mentha pulegium* essential oils. Asian Pac J Trop Biomed 2016;6(10):836–40, http://dx.doi.org/10.1016/j.apjtb.2016.08.002.
[13] Nguta JM, Appiah-Opong R, Nyarko AK, Yeboah-Manu D, Addo PGA. Medicinal plants used to treat TB in Ghana. Int J Mycobacteriol 2015;4(2):116–23, http://dx.doi.org/10.1016/j.ijmyco.2015.02.003.
[14] Nguta JM, Appiah-Opong R, Nyarko AK, Yeboah-Manu D, Addo PG, Otchere I, et al. Antimycobacterial and cytotoxic activity of selected medicinal plant extracts. J Ethnopharmacol 2016;182:10–5, http://dx.doi.org/10.1016/j.jep.2016.02.010. PubMed PMID: 26875647; PubMed Central PMCID: PMC4801013.
[15] Li H, Doucet B, Flewelling AJ, Jean S, Webster D, Robichaud GA, et al. Antimycobacterial natural products from endophytes of the medicinal plant *Aralia nudicaulis*. Nat Prod Commun 2015;10(10):1641–2. PubMed PMID: 26669092.
[16] Rusch-Gerdes S, Domehl C, Nardi G, Gismondo MR, Welscher HM, Pfyffer GE. Multicenter evaluation of the mycobacteria growth indicator tube for testing susceptibility of *Mycobacterium tuberculosis* to first-line drugs. J Clin Microbiol 1999;37(1):45–8. PubMed PMID: 9854062; PubMed Central PMCID: PMC84164.
[17] Banu S, Mahmud AM, Rahman MT, Hossain A, Uddin MK, Ahmed T, et al. Multidrug-resistant tuberculosis in admitted patients at a tertiary referral hospital of Bangladesh. PLoS One 2012;7(7):e40545, http://dx.doi.org/10.1371/journal.pone.0040545. PubMed PMID: 22808189; PubMed Central PMCID: PMC3394739.
[18] Herken EN, Celik A, Aslan M, Aydinlik N. The constituents of essential oil: antimicrobial and antioxidant activity of *Micromeria congesta* Boiss. & Hausskn. ex Boiss. from East Anatolia. J Med Food 2012;15(9):835–9, http://dx.doi.org/10.1089/jmf.2011.0315. Epub 2012/08/09. PubMed PMID: 22871061.
[19] El Omari K, Hamze M, Alwan S, Jama C, Chihib NE. Antifungal activity of the essential oil of *Micromeria barbata* an endemic Lebanese *Micromeria* species collected at North Lebanon. J Mater Environ Sci 2016;7(11):4158–67.
[20] Ribeiro-Santos R, Andrade M, Melo NRd, Sanches-Silva A. Use of essential oils in active food packaging: recent advances and future trends. Trends Food Sci Technol 2017;61(Complete):132–40, http://dx.doi.org/10.1016/j.tifs.2016.11.021.
[21] Mossa JS, El-Feraly FS, Muhammad I. Antimycobacterial constituents from *Juniperus procera*, *Ferula communis* and *Plumbago zeylanica* and their in vitro synergistic activity with isonicotinic acid hydrazide. Phytother Res 2004;18(11):934–7, http://dx.doi.org/10.1002/ptr.1420. PubMed PMID: 15597311.
[22] Bapela NB, Lall N, Fourie PB, Franzblau SG, Van Rensburg CE. Activity of 7-methyljuglone in combination with antituberculous drugs against *Mycobacterium tuberculosis*. Phytomedicine 2006;13(9–10):630–5, http://dx.doi.org/10.1016/j.phymed.2006.08.001. PubMed PMID: 16987644.
[23] Sadlon AE, Lamson DW. Immune-modifying and antimicrobial effects of *Eucalyptus* oil and simple inhalation devices. Altern Med Rev 2010;15(1):33–47. PubMed PMID: 20359267.
[24] Ben-Arye E, Dudai N, Eini A, Torem M, Schiff E, Rakover Y. Treatment of upper respiratory tract infections in primary care: a randomized study using aromatic herbs. Evid Based Complement Alternat Med 2011;2011:690346, http://dx.doi.org/10.1155/2011/690346. PubMed PMID: 21052500; PubMed Central PMCID: PMC2967840.
[25] Yadav N, Chandra H. Suppression of inflammatory and infection responses in lung macrophages by *Eucalyptus* oil and its constituent 1, 8-cineole: role of pattern recognition receptors TREM-1 and NLRP3, the MAP kinase regulator MKP-1, and NFkappaB. PLoS One 2017;12(11):e0188232, http://dx.doi.org/10.1371/journal.pone.0188232. PubMed PMID: 29141025; PubMed Central PMCID: PMC5687727.
[26] Ali B, Al-Wabel NA, Shams S, Ahamad A, Khan SA, Anwar F. Essential oils used in aromatherapy: a systemic review. Asian Pac J Trop Biomed 2015;5(8):601–11, http://dx.doi.org/10.1016/j.apjtb.2015.05.007.
[27] Sergio A, Fabiola C, Guadalupe N, Blanca R, León H. Evaluation of antimycobacterium activity of the essential oils of cumin (*Cuminum cyminum*), clove (*Eugenia caryophyllata*), cinnamon (*Cinnamomum verum*), laurel (*Laurus nobilis*) and anis (*Pimpinella anisum*) against *Mycobacterium tuberculosis*. Adv Biol Chem 2013;3:480–4, http://dx.doi.org/10.4236/abc.2013.35052.