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LETTERS

Nanoemulsion of *Mentha piperita* essential oil active against Mycobacterium strains

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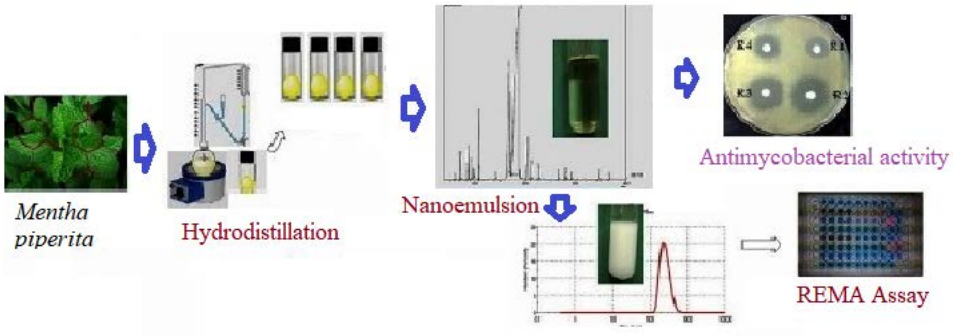
Article

ABSTRACT Present investigation aimed to study the antimycobacterial potential of *Mentha piperita* essential oil fractions, identify its active constituents by GC-MS and preparation of nanoemulsion from biologically active fraction. Four oil fractions (R1, R2, R3, R4) were collected during hydrodistillation of *Mentha piperiata* leaves and tested in the two of mycobacterial strains by conventional disc diffusion method. Oil fractions R2 and R3 demonstrated maximum zone of inhibition of 39 mm and 36 mm in *Mycobacterium smegmatis*, 33 mm and 31 mm in *Mycobacterium bovis* BCG respectively at a dilution of 75% in DMSO compared to standard drug isoniazid (23 mm in 4 µg/ml). GC-MS analysis of the most active fraction R2 reveals the presence of menthol (70.69%), isomenthone (14.63%) and neomenthol (6.82%) as major constituents. To enhance bioavailability of oil fraction, the nanoemulsions were prepared from R2 by sonication method. Nanoemulsions, N1 and N2 prepared by varying surfactant concentrations were tested in *Mycobacterium bovis* BCG using quantitative and colorimetric resazurin microtiter assay (REMA). Nanoemulsions, N1 and N2 have shown 97-100 % bacterial growth inhibition at 3.125 % concentration in the culture medium compared with the culture control.



Keywords: *Menthol, Isomenthone, Neomenthol, Antimycobacterial, Nanoemulsion.*

INTRODUCTION

Tuberculosis (TB) is fast emerging as a threat and a cause of global concern that needs both surveillance and control. An estimated 10 million people fell ill with TB in 2021 with ~1.6 million deaths being recorded among HIV-negative people.[1] After epidemic of covid-19 WHO reported tuberculosis as second leading cause of death due to acute respiratory syndrome coronavirus-2 (SARS-CoV-2).[2–4] Drug therapy is another cornerstone of TB management. Prolonged duration of the current therapy, the non-compliance of patients, the occurrence of multidrug-resistant (MDR; resistant to isoniazid and rifampicin) and extensively drug-resistant (XDR; resistant to isoniazid, rifampicin, quinolones and any one of kanamycin, capreomycin or amikacin) strains along with the increased co-incidence of HIV cases, high relapse rates and latent infection have all made the effective control and management of the disease difficult.[2] It is reported that over use of antibiotics, incomplete treatment and horizontal transfer of resistant genes among bacterial species reduce the effectiveness of antibiotics. Studies also state that administration of antibiotics may cause disturbance in gut microbiota.[3–6] The current status of the problem clearly manifests the need to develop new potent inhibitory molecules that could help not only shorten the duration of the current therapy but also provide effective treatment of MDR, XDR and latent TB.[7–11]

Since ancient times, nature played a major role as the main source of medicinal extracts used, as well as the source of several natural products that led to the development of various drugs currently used for the treatment of a wide spectrum of diseases. Based on empirical knowledge, natural product extracts, mainly plant-derived, have formed the basis of traditional medicine systems. However, it was only in the 20th century that research focused on the identification and characterization of the

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compounds responsible for the medicinal properties. Herbal medicines have always been a center of attraction for drug discovery, and many plant derived compounds have shown promising activity against multidrug resistant (MDR) pathogens. The urgent need of new therapeutic alternatives, mainly for the treatment of cancer[12–14] and infectious diseases including tuberculosis, as well as new chemical entities decline in drug development pipelines led to the rekindling of interest in "rediscovering natural products."

Peppermint (*Mentha piperita* L) is an herbaceous perennial plant of the family Lamiaceae with a tremendous medicinal use,[15] due to its soothing effect on the stomach and colon. It is also a refreshing flavoring agent for food, candy, gum and dental products.[16–18] The plant has also been used in pharmaceutical, cosmetic, insecticide[19,20] and perfumery products due to the presence of the essential oil,[21] which contains menthol as its major component.[22] Several biological activities, including antimicrobial and antioxidant are usually assigned to this oil or to some of its constituents.[23] Herbalists consider peppermint as an astringent, antiseptic, anti-inflammatory[24] antipruritic, antispasmodic, antiemetic, carminative, diaphoretic, mild bitter, analgesic, anticatarrhal, antimicrobial, rubefacient, stimulant, and emmenagogue.[25–28] Different genus of mentha plant is also used as biopesticides.[29] Peppermint oil vapor was used as an inhalant for respiratory congestion. Peppermint oil infused tea was a remedy used to treat coughs, bronchitis, and inflammation of the oral mucosa and throat.[30]

Nanotechnology is an ever-expanding field whose tentacles are gripping almost every field of science. It is a revolutionary technology that has been used in the medical industry for years and has revolutionized how doctors diagnose and treat disease.[31,32] In addition to this, nanoscale nanoemulsion have gained considerable attention due to its high kinetic stability, higher performance and explored in several fields including drug delivery and pharmaceutical system. Oral administration of several drugs and natural supplements are often results in poor compliances due to its toxicity, poor solubility and bioavailability, which intend give rise for the finding of alternative aspect of drug administration.[33,34] Nanoemulsion applications in drug delivery is found to be one of the best alternatives, by asset of their kinetic stability, high surface area per unit volume, tuneable rheology and many more positive attributes. As nano carriers, they improve therapeutic efficacy, reduce adverse toxic effects and are biocompatible.[35–42]

METHODS AND MATERIALS

MICROORGANISM

Mycobacterium species i.e., *M. smegmatis* and *M. bovis* used in this study were obtained from collaborator Professor Jaya S. Tyagi laboratory at Department of Biotechnology AIIMS, New Delhi. Strains were characterized by acid fast staining.

MENTHA PIPERITA ESSENTIAL OIL FRACTIONS AND CHEMICALS

Mentha piperita were purchased from local market of Greater Noida, leaves were separated, cleaned properly and dried in shade properly before extraction. Essential oil was extracted by hydrodistillation using Clevenger type apparatus. 500 gm of powdered leaves of mentha were distilled for 3 hours, fractions were collected at every 30 minutes interval. Fraction R1 (at 60 min), R_2 (90 min), R_3 (120 min) and R_4 (180 min) and was stored at 4˚C until used.[43,44] Standard menthol, isomenthone and neo-menthol were purchased from Sigma Aldrich, India. Solvents (Acetonitrile and DMSO) used in the study were of analytical reagent grade. Sterile discs were purchased from HiMedia. Culture medium 7H9 broth and 7H11 agar was purchased from Difco (BD and company sparks, USA), medium chain triglyceride [smilymct oil health supplement manufactured by FDC Limited], gum arabic powder, (purchased from the local market).

ANTIMYCOBACTERIAL SCREENING BY DISC DIFFUSION ASSAY

Initial antimycobacterial screening of neat and diluted fractions (R_1, R_2, R_3, R_4) of mentha essential oils were carried out in two of mycobacterial strains viz *M. smegmatis* and *M. bovis* BCG. Neat oil (100%) and diluted (in DMSO) oil fractions were tested. Two dilutions; 50% (prepared by mixing equal amount of oil and DMSO) and 25% (by mixing 250 µL of oil in 750 µL of DMSO) similarly 250 µg of standard menthol in 1 ml of DMSO. Samples were sterilized by passing through 0.45µ syringe filters. Isoniazid (10µg/ml) was used as positive control.

Briefly, Stock culture of *M. smegmatis* and *M. bovis* BCG strain were inoculated into Middlebrook 7H9 medium (containing 10% ADC [Albumin dextrose complex], 0.2% glycerol and 0.1% Tween-80) for 2-3 days in *M. Smegmatis* and 4-5 days in *M. bovis* respectively until final OD_{595} of the culture reached 0.40. These primary cultures were sub cultured in the same media to prepare secondary culture of 0.40 OD. Secondary cultures were serially diluted into plane media (no ADC) to get final OD of 0.0004 for *M. smegmatis* and 0.004 for *M. bovis* BCG strain. 500 µl (2×10^4 cells of *smegmatis*, 2×10^5 cells of *bovis*) of this culture was used to prepare lawn. Bacterial lawns were prepared in 7H11 agar containing 10% ADC and 0.5% glycerol. The prepared bacterial lawn was dried for 30 minutes. Each plate was divided into 4 quartets, and each quartet were loaded with 10 µl of either mentha essential oil fraction or dilutions of mentha oil or standard drug isoniazid or DMSO as negative control. Plates were closed and incubated at 37^0C for 48-72 hrs (*M. smegmatis*) and 3-4 weeks (*M. bovis* BCG strain). Zone of inhibitions (ZOI) was located on the surface of medium, the diameter (in millimeter) of non-growth were determined and compared with DMSO/vector controls. All work was carried out in biosafety cabinet (-ve pressure/BSL-2 level).

CHARACTERIZATION OF *MENTHA PIPERITA* ESSENTIAL OIL FRACTION BY GC-MS ANALYSIS

GC analysis was carried out on Netel Micro 9100 GC/MS system with 5973 mass selective detector equipped with a DB 624 MS capillary column (30 m × 0.25 mm, 0.25 µm film thickness), using helium as carrier gas at a flow rate of 1.3 mL/min. Column temperature was programmed from 80 to 210 °C at a rate of 15 °C/min, 210 °C to 250 °C at a rate of 5 °C/min, 250 °C to 280 °C at a rate of 15 °C/min and for 9 min at 280 °C. Components of the fractions were identified by comparison with

standard compound data. Test solutions were prepared by addition of acetonitrile to 1% concentration. The GC/MS analysis was carried out using Shimadzu GC-MS, Model QP-2010 Ultra, plus with thermal desorption System TD 20. Detector: Column: Rtx-5 MS 30 m length, 0.25 mm ID, 0.25 mm film thickness (Cross bond 5% diphenyl/95% dimethyl polysiloxane). Mass spectroscopy, carrier gas: helium, oven temperature program: (start temperature = 50 ˚C with a hold time 2 min, while the final temperature 280 ˚C with a hold time 4 min at Rate of 5 ˚C/minute). GC-MS program was as follow: Ion Source temperature 230 ˚C, interface temperature 270 ˚C, solvent cut time 3.5 min, detector gain mode is relative, detector gain 0.00 kv, run time 40 min, ACQ mode is Scan, event time 0.20 sec, scan speed 3.333, start m/z 40.00 and end m/z is 650.0.

Preparation of *Mentha piperita* Nanoemulsion

The nanoemulsion was prepared by mentha oil fraction R_2 (showed maximum inhibition zone in primary screening) following the reported method of Liang *et.al.*, 2012[45], with slight modification. Aqueous phase was prepared by dispersing 12% (w/w) dried gum arabic powders in deionized water with continuous stirring at 2000 rpm for 12 hours at room temperature (maintained at 25 ºC). Oil phase was prepared by mixing oil fraction R_2 and MCT at a ratio of 4:1 (v/v). Nanoemulsion was prepared by mixing three different ratios of oil and aqueous phases vis O/W ratio; 1:1, 2:3 and 3:2. The oil and water phases were mixed and sonicated at an ultrasonic frequency of 27375 Hz for 20 Minutes, the temperature of the vessel was maintained at 4-8 ºC.

Droplet size determination

The dispersed droplet size distribution in the nanoemulsions were carried out by photon correlation spectroscopy (PCS) instrument (Zetasizer Nano ZS, Malvern Zen 3600, UK). Prior to measurements, nanoemulsions were diluted in double distilled water (40 times) to avoid particle interactions and additional scattering during measurement. The dispersant viscosity was set at 0.8872cP at 25 ºC.

Freeze/thaw cycle

Stability of nanoemulsion was studied by freeze/thaw cycle. Nanoemulsions were frozen in a -20 ºC freezer for overnight (12 ± 2 hrs), then thawed at 40ºC for 1 hour and stored at 25ºC. Freeze/thaw process was carried out with 5, 10 and 20 times diluted sample (in distilled water) of the nanoemulsions.

Sedimentation

Nanoemulsion samples were subjected to centrifugation at 10,000 rpm at 4ºC for 20 minutes using REMI, C-24+ refrigerated centrifuge machine. The centrifugation process was repeated with 5, 10 and 40 times diluted samples also.

UV-vis spectroscopic study of nanoemulsion

The UV-vis spectra of nanoemulsions were recorded in UV 3092 UV-vis spectrophotometer (Labindia Analytical, India). The solutions of nanoemulsions were prepared by the addition of 2µl of corresponding nanoemulsion in 5 ml (2500 times) of distilled water and sample was shacked briskly before transferring into cuvette (3 ml). Samples were subjected to UV-VIS spectrums in the scanning mode (190-900 nm).

Stability of Nanoemulsion

The stability of nanoemulsions (Phase separation) were monitored by long term storage of nanoemulsion at room temperature (between 25±5 ºC) and stability were monitored by measuring the change in color, thickness, turbidity, heterogeneity in the texture (formation of two or more layers) and droplet size during one month of storage.

Antimycobacterial activity of nanoemulsion using Resazurin microplate assay (REMA)

Drug susceptibility testing (DST) of nanoemulsions using resazurin reduction assay (REMA) were carried out under aerobic condition as described by Taneja NK and Tyagi, 2007 with minor modifications[46]. The assay was performed in black, clear-bottomed, 96-well microplates (Becton Dickinson, USA). Nanoemulsions (N1 and N2) were filtered sterilized using Millex GV filters (0.45 µm pore size, Millipore). 100µL of each nanoemulsion (in triplicate) was diluted in 100 µL of 7H9 broth medium supplemented with 0.05% glycerol (without Tween 80) in the microplates and further diluted in subsequent wells to prepare to 2-fold serial dilutions of the nanoemulsions. 100µl of *M. bovis* BCG cultures (4×10^7 Cells) was added to each well after dilution. Control wells contained medium only (M), bacteria only (CC) and nanoemulsion only (to detect auto fluorescence of nanoemulsions). The plates were incubated at 37°C for 5-7 days. Subsequently, freshly prepared and filter sterilized 0.02% resazurin (30µl) and 20% Tween 80 (12.5µl) were filter sterilized and added in each well. The plates were further incubated at 37°C for 24 to 48 h and observed for a color change from blue to pink. Fluorescence was measured by excitation at 530 nm and emission at 590 nm using Gemini XS spectroflourimeter (SpectraMAX) in bottom reading mode. Visual MIC was defined as the lowest concentration of nanoemulsion that prevented a colour change. For flourometric MIC, background fluorescence from medium (M) and nanoemulsions were subtracted. Percentage inhibition of viability was defined as

$$\% \text{ inhibition of viability} = 1 - \left(\frac{Test\ well\ flourescence}{mean\ fluorescence\ of\ triplicate\ CC\ well}\right) \times 100$$

The lowest dilution of nanoemulsion effecting an inhibition of 90% was considered as the MIC.

Result and Discussion

Chemical composition of essential oil fractions

The composition of mentha essential oil fractions were analyzed by GC and component were identified by comparing the relative retention times with those of authentic samples and literature reported.[41] While analyzing the GC-data of tested essential oil fractions it was noted that the fraction R1, R2 R3 and R4 contains 89%, 70.69%, 68.4% and 58% of menthol as a major constituent respectively (supplementary data S1-S7). We noticed that two minor constituents (with effective percentage

>5.0%) are present in all the tested fractions, as shown in Figure 1b. These constituents were identified by comparing their retention time with reported literature. All constituents present in most active R2 fraction were further analysized by GC-MS and result shown in Table-1. It contains menthol (70.69%), isomenthone (14.89%) and neomenthol (6.85%) as major constituents.

Table 1. Chemical Composition of fraction R2 of *M. piperita* essential oil

Peaks	Component	Retention Time	Area	Area %
1.	Tetrahydrofuran <2,5-diethyl->	5.961	1502191	0.04
2.	Pinene <alpha->	7.084	38493744	0.98
3.	Sabinene	8.556	14666342	0.37
4.	Pinene <beta->	8.714	38234110	0.98
5.	Myrcene	9.245	23302553	0.60
6.	Hexanol <ethyl->	9.699	11736593	0.30
7.	Limonene	10.884	117259599	2.00
8.	2-Oxabicyclo[2.2.2] Octane, 1,3,3-Trimethyl-	10.950	8606633	0.22
9.	1,6-Octadien-3-ol,3,7-dimethyl-	14.313	9693763	0.25
10.	Cyclohexanone, 5-methyl-2-(1-methylethyl (Isomenthone)	16.257	572819738	14.63
11.	Neomenthol	16.850	38905677	6.82
12	Menthol	17.177	2658876556	70.7
13.	Cyclohexanol, 5-methyl-2-(1-methylethyl)	18.156	6129594	0.16
14.	Piperitone	21.228	25208445	0.64
15.	Menthyl acetate	22.717	17819633	0.46
16.	Cyclobuta[1,2:3,4]dicyclopentene, 1,2,3,3A,3B	26.549	6143519	0.16
17.	Caryophyllene <(E)->	28.049	14269445	0.36
18.	Isovalerate <octyl->	29.083	2713455	0.07
19.	Caryophyllene oxide	34.591	8615861	0.22
20.	Phenyl acetate <cis-3-hexenyl->	36.649	2300229	0.06

ANTIMYCOBACTERIAL ACTIVITY OF ESSENTIAL OILS

No bacterial growth was observed in plates containing neat and 50% diluted fractions of R1, R2, R3 and R4 (Figure 1, panel-i). Fractions R1, R2, R3 and R4 showed clear zone of inhibition against two of the tested mycobacterial strains at 75% dilution. Fractions R2, R3, R1 and R4 have shown 39 mm, 36, 19, 16 in M. smegmatis and 33 mm, 31 mm, 19 mm, 18 mm in *M. bovis* BCG respectively. Pure menthol has shown 10 mm in *M. smegmatis* and 08 mm in *M. bovis*.

To substantiate the difference in the activity shown by the fractions, measure of effective concentration of one of the main constituent menthol was carried out by GC-analysis. We noted that the fraction R2 showing maximum inhibitory zone (39 and 33 mm) contains 70.69% of menthol, whereas fraction R1 having 89 % of menthol showed less zone of inhibition (19 mm) compared with R2. On the other hand, fraction R4 having menthol concentration nearly 58% showed almost similar ZOI (16 and18 mm) as of fraction R1, clearly indicate that only menthol is not responsible for the biological activity, there may be cumulative effect of some other constituent in inhibiting the growth of Mycobacterium. Further pure menthol also demonstrated a lower ZOI of 8-10 mm in two of test strains. Comparative scrutiny of GC-data of all essential oil fractions revealed presence of two other minor constituents (>5.0%) in all the tested fractions, along with menthol (Figure 1b). These constituents along with menthol may be cumulatively governing the antimycobacterial activity of oil fractions. Fractions showed varied concentration of isomenthone and neomenthol along with menthol (Figure 1b). This data is further supported by lower antimycobacterial activity of pure menthol (Figure 1c).

PREPARATION AND CHARACTERIZATION OF NANOEMULSIONS

Oils are complex hydrophilic mixtures and have poor pharmacokinetics due to low water miscibility, they cannot be mixed directly to water based bacterial culture medium therefore, nanoemulsion was prepared from most active R2 fraction. Nanoemulsions were prepared by using O/W ratio of 1:1, 2:3 and 3:2. All three types have shown variations in mean droplet size; the smallest droplet size of 220 nm was obtained in the case of N1 (O/W: 1/1) while N2 and N3 have shown higher diameter of 381 nm and 775 nm respectively (Table 2, Zeta sizer data were shown in (**supplementary information S8)**. The high diameter of 775 nm was shown by N3, which may be attributed to higher oil phase proportion as excessive oil volume fraction may not be covered by the emulsifier. Only N1 and N2 were further characterized.

PHYSICAL STABILITY OF NANOEMULSIONS

The freeze-thawing of nanoemulsions N1 and N2 did not showed any phase separation or transition. The thermodynamic nanoemulsion retains their original form after 20-30 minutes, when they are kept at room temperature (25º±3º C). No precipitation was observed after centrifugation for 10,000 rpm for 20 minutes at in neat and as well as diluted nanoemulsions.[47]

UV-VIS SPECTROSCOPIC ANALYSIS OF NANOEMULSION

Nanoemulsions appear nearly transparent in the visible spectrum and exhibit very little scattering despite significant refractive index contrast. Quantitative measurement of the optical transparency of nanoemulsions in the visible and ultraviolet wavelengths were shown by UV-VIS analysis.[48] We have not observed any absorption in the nanoemulsions N1 and N2 at 600 nm indicating the transmission in the visible spectrum is nearly

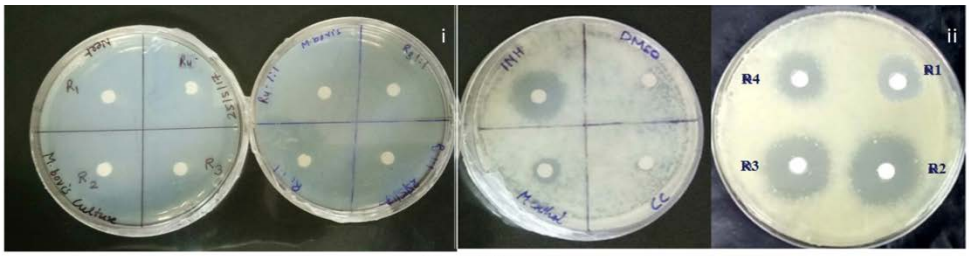

Figure 1(a)

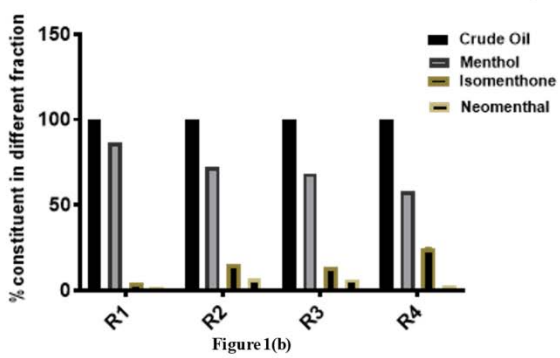

Figure 1(b)

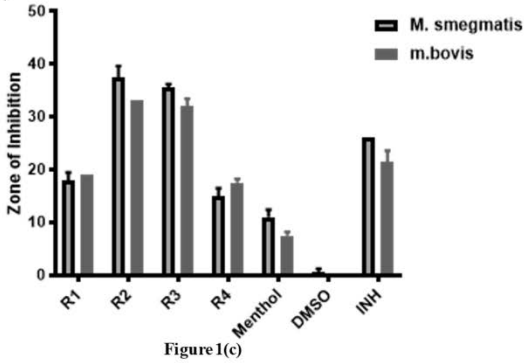

Figure 1(c)

Figure 1: (a) Antimycobacterial activity of essential oil fractions in *M. bovis* BCG strain by disc diffusion assay. (b) Bardiagram representing effective concentrations of menthol, isomenthone and neo-menthol based on GC-analysis R1, R2, R3 and R4 fraction of essential oil. (c) Zone of inhibitions shown by essential oil fractions tested against two bacterial strain in disc diffusion assay (INH 4 µg/ml).

Table 2: Mean droplet size and polydispersity index of nanoemulsions

Nanoemulsion	Oil phase: aqueous phase (v/v)	Mean droplet size (nm)	Polydispersity index
N1	1:1	220	0.062
N2	2:3	381	0.195
N3	3:2	775	0.732

100%, especially toward visible wavelengths, indicating a high degree of transparency. Spectrum of both nanoemulsions N1 and N2 showed characteristic peak of menthol at 198 (Abs 3.171) and 197 nm (Abs 3.012) nm respectively (**Supplementary Information S9**).

Storage Stability of Nanoemulsions

To check the stability extended storage test were performed by measuring the mean diameter of nanoemulsions during the storage time of 1, 15, and 30 days at room temperature (25°±5° C). At day one, the mean diameter of nanoemulsion N1 (1:1), N2 (2:3) was found to be 220.2 and 381.0 respectively which increases by 30-50 nm during 30 days interval. We have noted increased polydispersity index (PDI) which indicates that some aggregation occurred during the storage.[42] It was observed that the rate of increase in mean diameter is more during first 15 days, afterwards minute changes in the diameter were observed (**Figure 2**).

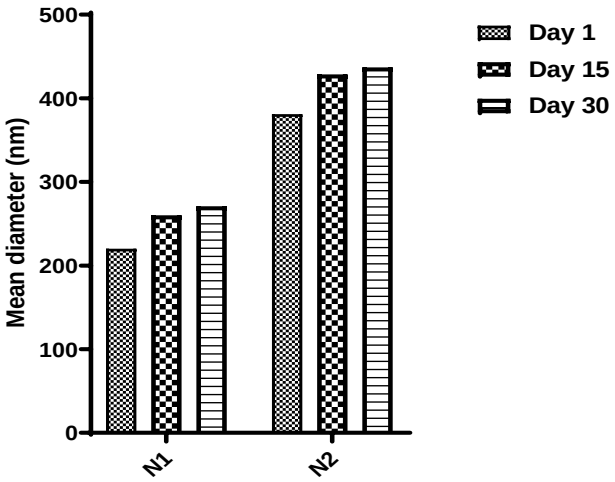

Figure 2: Variation in mean diameter of nanoemulsion N1 and N2 over the storage time

During the storage no phase separation or creaming of nanoemulsions were observed. The consistency of mean diameter of emulsions over storage times indicated a better stability of O/W nanoemulsions. Before subjected to biological activity nanoemulsion was passed through 0.45 µ filter. Passage of nanoemulsion through 0.45 µ filter further confirmed that the droplet size were smaller than 450 nm.

REMA ASSAY RESULT OF NANOEMULSION

Nanoemulsions, N1 and N2 were tested in *Mycobacterium bovis* BCG strain using quantitative and colorimetric resazurin microtiter assay (REMA). Both Nanoemulsions N1 and N2 have shown 97-100% bacterial growth inhibition at lowest concentration of 3.125% in the culture medium compared to culture control. Culture and gum arabic controls did not showed any bacterial growth inhibition, as expected. INH had shown 80% growth inhibition at 4 µg/ml concentration while media control did not show any bacterial growth (**Figure 3**).

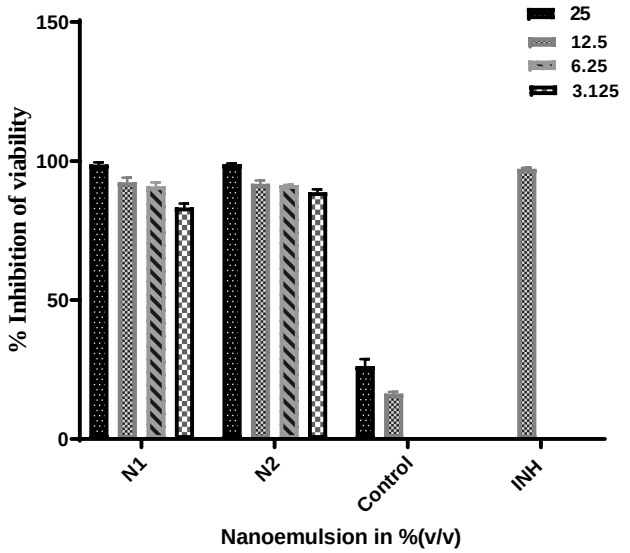

Figure 3: The percentage inhibition of *M. bovis* BCG viability in REMA assay by N1 and N2 nanoemulsions.

CONCLUSION

It is well studied that active principle of the peppermint oil L-menthol is responsible for various pharmacological activities.[23,49–52,48,53,54] We explored the essential oil from particular plant *M. piperita* leaves for growth inhibitory effect against two of *Mycobacterial* sp. Initial screening of essential oil fractions were carried out in fast growing *Mycobacterium tuberculosis* commensal strain i.e., *Mycobacterium smegmatis.* Fractions showed promising activity were further tested in *Mycobacterium bovis* BCG (a surrogate *M. tb*. strain). We have noted in our study that the antimycobacterial activity of mentha oil is not only governed by menthol but also other oil constituents. It was noted that oil fractions containing higher concentration of menthol showed lower antimycobacterial activity then oil fraction containing lower concentration of menthol along with other constituents have shown better activity. Further characterization of biologically active mentha oil fraction by GC-MS analysis indicates the presence of two other constituents like isomenthone and neomenthol present in substantial amount along with menthol. The % of these constituents alters the biological activity, which confer that these constituents may be synergistically enhancing the antimycobacterial potential of mentha oil. Oils shows poor pharmacokinetics through oral route of administration. They cannot be tested directly in broth medium therefore we have prepared and characterized nanoemulsion from biologically active mentha oil fraction. Nanoemulsion were diluted in bacterial medium and tested for antimycobacterial activity in calorimetric REMA assay. Nanoemusion N1 prepared 1:1 oil/Water ratio have shown maximum bacterial growth inhibition of 98% against *M. bovis* BCG strain. Further investigations are needed to establish clear synergetic mechanism of menthol and other constituents and studies of nanoemulsions in clinical & MDR mycobacterial strains.

CONFLICT OF INTEREST

There are no conflicts to declare.

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