Utilization of the Japanese Peppermint Herbal Water Byproduct of Steam Distillation as an Antimicrobial Agent

Naofumi Ohtsu¹*, Yoshihito Kohari¹, Masataka Gotoh¹, Reiji Yamada¹, Yuichi Nagata², and Miki Murata¹

¹ School of Earth, Energy and Environmental Engineering, Kitami Institute of Technology, 165 Koen-cho, Kitami, Hokkaido 090-8507, JAPAN
² Kitami Hakka Tsusho Co. Ltd., Oroshi-machi 1-5-2, Kitami, Hokkaido 090-0056, JAPAN

Abstract: The present study provides valuable data that the herbal water byproduct of Japanese peppermint, produced during the steam distillation extraction of an essential oil, can be utilized as an antibacterial agent. The major ingredient in the herbal water from Japanese peppermint ‘Hokuto’ was menthol, with a concentration close to its water solubility. The herbal water produced showed excellent antibacterial efficacy against typical gram-negative and gram-positive bacterial strains of Escherichia coli and Staphylococcus aureus, respectively, and the antibacterial efficacy was maintained even when the herbal water was diluted up to an appropriate concentration of 50%. The efficacy of the herbal water against E. coli was higher than that against S. aureus, which is likely because of the difference in the efficacy of menthol against these two different bacterial strains. The excellent antibacterial efficacy of the herbal water is mainly attributed to the function of menthol, while other trace ingredients also contributed to the antibacterial efficacy. The Japanese peppermint herbal water byproduct, generally treated as industrial waste and disposed, can be easily commercialized as an antibacterial agent if efforts are made to maintain a constant menthol concentration throughout the steam distillation essential oil extraction process.

Key words: Japanese peppermint, herbal water, antibacterial agent, hito

1 INTRODUCTION

Mint is an important source of natural monoterpenes such as menthol and menthone, and various species have been widely planted globally¹, ². The essential oil obtained from mint is a major industrial product, and has been utilized as an additive in pharmacy, food, and cosmetics³, ⁴. Even now, extraction of the essential oil from natural plants is conducted by steam distillation, a process that has been used since ancient times⁵, ⁶. Steam distillation is a simple process; dried plants are vaporized together with the steam, and thereafter, the essential oil and an aqueous solution are generated simultaneously when the steam is condensed. The essential oil is marketed as an industrial product; on the other hand, the remaining aqueous solution, deemed "herbal water," is generally abandoned as industrial waste. However, the herbal water is considered to include a part of the essential oil, because the essential oil and herbal water are in contact with each other throughout the extraction process⁷-¹⁰. Finding a beneficial use for this wasted herbal water would enhance the value of mint, and would be profitable for agricultural producers.

It is well known that menthol has antioxidant and antibacterial functions¹¹-¹⁵; therefore, the herbal water produced from mints may also be utilized as an antibacterial agent if it includes a sufficient menthol concentration. Verma et al.¹⁶ demonstrated that the isolated oil produced by collecting the herbal-water’s organic ingredient using hexane had sufficient antibacterial efficacies against both gram-positive and gram-negative strains. Using the oil isolated from herbal water as an antibacterial agent would be a promising method of utilizing the herbal water, although it would be more beneficial to use the herbal water itself as a functional product without further chemical treatment. Nevertheless, reports investigating any functions of the herbal water itself could not be found, to the best of our knowledge.

Among the mint species, Japanese peppermint (Mentha avensis L.) is a commercially viable species that has com-
Comparatively high amounts of menthol, and has been widely planted in India, North America, Europe, and Japan as a natural source of menthol. The essential oil produced from Japanese mint contains a comparatively high amount of menthol\(^{15}\); accordingly, it is conjectured that a high amount of menthol is also present in the herbal water. Menthol is a simple monoterpene known to possess remarkable biological functions, such as antifungal, antibacterial, and antipruritic activities. In the present study, therefore, we investigated the chemical composition and antibacterial efficacies of the herbal water produced through steam distillation of Japanese peppermint. Moreover, the relationship between the activity and the chemical composition was also evaluated. Together with these results, we discussed the possibility of utilizing the Japanese peppermint herbal water as an antibacterial agent in industry.

2 EXPERIMENTAL PROCEDURES

2.1 Plant material and steam distillation

The plant materials of Japanese peppermint (\textit{Mentha aquensis} L. cv. ‘Hokuto’) were collected from an experimental field at Kitami Hakka Tsusho Co. Ltd. (43.54° N and 143.54° E) in September, 2016. The climate of the field is semi-humid and the average temperature during the cultivation was about 17°C.

The collected aerial parts were shade-dried at room temperature and divided into leaves and stems, after which they were stored at -80°C. The essential oils were extracted only from the dried leaves through the steam distillation method with apparatus purchased from Tokyo Sei-sakusyo Co. Ltd. Briefly, a body for the distillation was made of heat-resistant glass, including a plate of stainless steel, and the steam passing through the plants was water-cooled, thereby producing the essential oil and herbal water simultaneously. In total, 50 g of dried leaves were used as a starting material, and the distillation was stopped when 200 mL of herbal water, as well as ca. 3.0 mL of essential oil, was collected.

2.2 Chemical composition analysis using gas chromatography

Ingredients in the essential oil and herbal water were identified using a gas chromatography-mass spectrometer (GC-MS; QP-2010 Ultra, Shimadzu Ltd.) equipped with an InertCap Pure-WAX (polyethylene glycol)-fused silica capillary column (60 m × 0.25 mm; 0.25 μm film thickness). The injector and interface temperature were maintained at 250°C, and the flow rate of helium as a carrier gas was 1 mL·min\(^{-1}\). The essential oil was added with tridecane as an internal standard, and was diluted with n-hexane, after which 0.1 μL of the sample liquid was injected. In the case of the herbal water, its ingredients were extracted three times using chloroform. The chloroform, including the ingredients, was added with tridecane, after which 1.0 μL of sample liquid was injected. The column temperature was programmed to increase from 50 to 180°C at a rate of 5°C·min\(^{-1}\) and to maintain the temperature for 10 min. Here, the split ratio was 1:20 for the essential oil analysis and 1:2 for the herbal water extract analysis. The mass spectrum was scanned from \textit{m/z} 30-1000 amu. Peak identification was then carried out using the NIST14 GC-MS database\(^{20}\). The retention indices (RI) for the essential oil and herbal water components were calculated using alkane standard solution C8-C20.

After the identification of the ingredients, their chemical compositions were determined using a gas chromatograph (GC-17A, Shimadzu Ltd.) equipped with a flame ionization detector (GC-FID). Chromatographic conditions, such as the capillary column, flow rate of helium gas carrier, split ratio, and oven parameters were the same as the above-mentioned for the GC-MS analysis. The detector temperature was maintained at 250°C. The relative and absolute concentrations of l-menthol were calculated using an internal standard method based on the GC-FID peak areas, and the other chemical components were calculated based on the GC-FID peak areas using the FID response factor\(^{35}\).

2.3 Antibacterial test

The antibacterial efficacy of the herbal water was examined using the \textit{Escherichia coli} (\textit{E. coli}) strain ATCC 25922 and \textit{Staphylococcus aureus} (\textit{S. aureus}) strain ATCC 6538P. Prior to the antibacterial test, \textit{E. coli} and \textit{S. aureus} were cultured on nutrient agar plates at 37°C. A bacterial suspension of 2 × 10^8 CFU mL\(^{-1}\) was then prepared using a 500-fold diluted nutrient broth (1/500NB). The herbal water was diluted into various concentrations by the 1/500NB, which was used as a testing solution. Then, 30 μL of the bacterial suspension was added to 3 mL of each testing solution, and was incubated for 4 h at 37°C with 65 rpm shaking. After the incubation, the colony forming unit (CFU) in the bacteria-containing testing solution was determined by the Miles and Misra method\(^{36}\) using a nutrient agar plate. In the present study, the antibacterial efficacies of the herbal-water-containing solution were expressed by the following equation:

\[
\text{CFU ratio} = \frac{\text{CFU in a herbal water containing solution}}{\text{CFU in a solution without herbal water}}
\]

Antibacterial tests were performed with \(n = 3\) and repeated at least twice. All statistical analyses were performed by using ANOVA with a Student–Newman–Keuls (SNK) post-hoc test to identify levels of significance (\(p < 0.01\)).
3 RESULTS
3.1 Comparison of the chemical composition of the essential oil and the herbal water extract from Japanese peppermint
The Japanese peppermint, *Mentha arvensis* L. cv. 'Hokuto', was bred in 1987 for the large amount of essential oil present in it as well as its medium l-menthol content. In fact, in our present study, the essential oil yield from the leaf parts was approximately 6%. The chemical compositions of the essential oil and herbal water extract from *Mentha arvensis* L. 'Hokuto' are listed in Table 1. In the essential oil, 32 compounds were identified, and further-

| Compound          | RT (min) | RI | Content of Constituent in essential oil (%) | Content of Constituent in herbal water (mg/L) |
|-------------------|----------|----|---------------------------------------------|-----------------------------------------------|
| α-Pine ne         | 10.6     | 1017 | 0.3 ± 0.0                                   |                                               |
| β-Pine ne         | 14.4     | 1100 | 0.4 ± 0.2                                   |                                               |
| Sabinen           | 15.1     | 1115 | 0.5 ± 0.5                                   |                                               |
| β-Myrcene         | 17.1     | 1160 | 0.2 ± 0.0                                   |                                               |
| D-Limonene        | 18.6     | 1191 | 0.3 ± 0.0                                   |                                               |
| Eucalyptol        | 19.0     | 1200 | 0.3 ± 0.0                                   |                                               |
| (Z)-β-Ocimene     | 20.2     | 1230 | 0.1 ± 0.0                                   |                                               |
| (E)-β-Ocimene     | 20.9     | 1247 |                                            |                                               |
| 3-Octanol         | 26.0     | 1386 | 0.5 ± 0.0                                   | 3.2 ± 0.3                                     |
| l-Menthone        | 28.6     | 1468 | 16.1 ± 0.1                                  | 52.8 ± 2.9                                    |
| Isomenthone       | 29.4     | 1493 | 3.5 ± 0.0                                   | 19.2 ± 3.7                                    |
| β-Bourbonene      | 30.3     | 1525 |                                            |                                               |
| Linalool          | 30.7     | 1539 |                                            |                                               |
| Menthyl acetate   | 31.4     | 1565 | 2.0 ± 0.1                                   |                                               |
| Isopulegol        | 31.6     | 1571 | 0.8 ± 0.1                                   | 2.8 ± 0.4                                     |
| Isopulegone       | 31.7     | 1575 |                                            |                                               |
| Neomenthol        | 32.2     | 1594 | 1.4 ± 0.0                                   | 6.2 ± 0.4                                     |
| Caryophyllene     | 32.6     | 1608 | 0.1 ± 0.0                                   |                                               |
| Neoisomenthol     | 33.1     | 1627 |                                            |                                               |
| l-Menthol         | 33.7     | 1650 | 66.4 ± 0.1                                  | 371.7 ± 7.0                                   |
| l-Pinocarveol     | 33.9     | 1659 |                                            |                                               |
| (E)-β-Famesene    | 34.1     | 1667 |                                            |                                               |
| Lavandulol        | 34.3     | 1673 |                                            |                                               |
| (Z)-Verbenol      | 34.5     | 1680 |                                            |                                               |
| Humulene          | 34.7     | 1687 |                                            |                                               |
| α-Terpineol       | 34.9     | 1695 | 0.5 ± 0.2                                   |                                               |
| endo-Borneol      | 35.1     | 1703 |                                            |                                               |
| Piperitone oxide  | 35.4     | 1716 |                                            |                                               |
| Germacrene D      | 35.6     | 1722 |                                            |                                               |
| Piperitone        | 35.9     | 1736 | 1.2 ± 0.7                                   | 34.4 ± 2.9                                    |
| (Z)-Isopiperitenol| 36.4     | 1753 |                                            |                                               |
| 2,2-Dimethylcyclohexyl acetate | 43.4 | 1992 |                                             |                                               |
| Total identified  |         |     | 94.7 ± 1.6                                 |                                               |

a) RI: Retention Index
more, a total of 17 components could be quantified (94.7 ± 1.6%). It is well known that the l-menthol content in essential oil obtained from *Mentha arvensis* is higher than that obtained from other *Mentha* species. For instance, Hussain et al.\(^\text{21}\) reported that the l-menthol content in *Mentha arvensis* was 78.9%, whereas that in *Mentha piperita* was only 4.83%. Similar to these results, the menthol content in the essential oil, *Mentha* species. Other main components of the essential oil, l-menthone (16.1 ± 0.1%), isomenthone (3.5 ± 0.0%), menthy acetate (2.0 ± 0.1%), neomenthol (1.4 ± 0.0%), and piperitone (1.2 ± 0.7%) were confirmed. Furthermore, it was revealed that α-pinene (0.3 ± 0.0%), β-pinene (0.4 ± 0.2%), sabinene (0.5 ± 0.5%), D-limonene (0.3% ± 0.0%), and eucalyptol (0.3 ± 0.0%) were also present in the essential oil, although in trace amounts. On the other hand, in the herbal water extract, only seven quantifiable components were found. This smaller number of components is probably due to the solubility of the oil components in water. The first and second major components were l-menthol (71.7 ± 0.0 mg/L) and l-menthone (52.8 ± 2.9 mg/L). The other five components quantified were piperitone (34.4 ± 2.9 mg/L), isomenthone (19.2 ± 3.7 mg/L), neomenthol (6.2 ± 0.4 mg/L), 3-octanol (3.2 ± 0.3 mg/L), and isopulegol (2.8 ± 0.4 mg/L).

### 3.2 Antibacterial efficacy of the herbal water produced from Japanese peppermint

The antibacterial efficacy of herbal water solutions with various concentrations are shown in Fig. 1. Here, *E. coli* and *S. aureus* were used as typical gram-positive and gram-negative strains, respectively. The horizontal axis denotes the concentration of herbal water included in the solution, wherein the 0% solution corresponds to the 1/500NB solution without herbal water. Regarding *E. coli* (Fig. 1(a)), the CFU ratio of the solution containing over 50% herbal water was below 0.002, indicating that the CFU in the 50% solution was below 1/500 compared to the 0% solution. The ratio became zero in the solution containing herbal water beyond a 75% concentration, meaning that the bacteria added into the testing solution were killed completely. Statistical analysis also demonstrated the significant difference between the 0% solution and the other herbal water solution, whereas the difference depending on the concentration was not found. Obviously, the herbal water produced from the Japanese peppermint has sufficient antibacterial efficacy against *E. coli*, and, in addition, it can be said that sufficient antibacterial efficacy against *E. coli* is expected, even after a dilution of up to 50%.

Likewise, antibacterial efficacy was also observed against *S. aureus* (Fig. 1(b)). The CFU ratio of the 50% solution was 0.5, which was statistically lower than that of the 0% solution. The CFU ratio decreased sequentially to 0.3 and 0.05 in the 60% and the 75% solutions, respectively, and
these ratios were statistically lower than that of the 50% ratio. A ratio of zero was confirmed when using the 100% solution, which corresponds to herbal water without dilution. It should be noted that the antibacterial efficacy against \textit{S. aureus} was lower than that against \textit{E. coli}. However, we here emphasize that cultivation in the testing solution was only for 4 h. The antibacterial efficacy is considered to be enhanced by prolonging the cultivating time, and hence, the antibacterial efficacy of the diluted herbal water was considered to be sufficient against \textit{S. aureus}. In conclusion, we can say that the herbal water produced from Japanese herbal water can be used as an antibacterial agent against typical gram-positive and gram-negative bacteria, even if the solution is diluted to an appropriate concentration.

3.3 Predominant ingredient responsible for antibacterial efficacy

To reveal the predominant ingredient responsible for the antibacterial property, we evaluated the antibacterial efficacies of individual chemical components of the herbal water. Based on the analytical result in Table 1, we focused on menthol and menthone, which were the first and second major components of the herbal water. A single-component solution, composed of a prescribed concentration of menthol or menthone, was prepared by dissolving commercially purchased menthol and menthone reagents into the 1/500NB solution. Here, the reagents used were \textit{l}-menthol reagent of over 99% purification, purchased from Wako Pure Chemical Ltd., and \textit{l}-menthone reagent of about 90% purification, from Sigma-Aldrich Ltd. The concentration ranges of the single-component solutions were determined based on the results of the chemical composition analysis (Table 1).

The antibacterial efficacies of the menthol solution against \textit{E. coli} and \textit{S. aureus} are shown in Fig. 2(a). Here, the value within the bracket at the horizontal axis indicates the concentration of the herbal water, including the equivalent menthol concentration. In the case of \textit{E. coli}, the CFU ratio at a concentration of 150 mg·L$^{-1}$ (equivalent to 40% herbal water) was about 0.5, and the ratio was lowered below 0.05 by increasing the concentration to 250 mg·L$^{-1}$ (equivalent to 66.8% herbal water). A CFU ratio of zero was found when using a menthol solution with a concentration of over 350 mg·L$^{-1}$. On the other hand, in the case of \textit{S. aureus}, a decrease in the ratio was not found in the 150 mg·L$^{-1}$ menthol solution, but the ratio decreased to 0.8 in the 250 mg·L$^{-1}$ solution, and then, was lowered up to 0.005 in the 450 mg·L$^{-1}$ solution.

In contrast to the observations for menthol, a decrease in the CFU ratio with an increasing concentration of menthone was hardly observed (Fig. 2(b)). Surprisingly, the ratio against \textit{E. coli} was rather increased with an increased concentration of menthone, and the ratio in the 50 mg·L$^{-1}$ menthone solution was almost double to that observed in the 0% solution. The CFU ratio against \textit{S. aureus} was

![Fig. 2](image_url)  
**Fig. 2** Variation of the colony forming units (CFU) ratio in the single-component solution depending on the concentration of (a) menthol and (b) menthone. Here, the CFU ratio was calculated using the following equation: \((\text{CFU ratio}) = (\text{CFU in a herbal water containing solution}) / (\text{CFU in a solution without herbal water})\). Error bars show the standard deviation of the testing result \((n = 3)\).
hardly changed in the menthol solution ranging below a concentration of 50 mg·L\(^{-1}\).

It was obvious that the predominant ingredient contributing to antibacterial efficacy in the herbal water was menthol, notwithstanding the bacterial strains. In addition, the low antibacterial efficacy against \textit{S. aureus} compared to that against \textit{E. coli} is likely to have originated from the difference in the efficacy of menthol against the two strains. Yet, the antibacterial efficacy does not seem to be explained by the effect of menthol only. To clarify, variations in the bacterial ratio of \textit{S. aureus} against the menthol concentration were plotted with respect to both the menthol solution and herbal water (Fig. 3). Here, the concentration of menthol in the herbal water was calculated based on the quantitative analytical result (Table 1). The CFU ratio decreases gradually with an increase in concentration, but the ratio in the herbal water was significantly lower than that in the menthol solution throughout the whole concentration range. This plot clearly indicates that the efficacy cannot be explained only based on the effect of menthol. Ingredients with trace concentrations, as well as the existence of other ingredients with antibacterial effects undetected by gas chromatography, are considered as possible candidates contributing to the difference in efficacy. Nevertheless, further experimental investigations using other analytical approaches are important for elucidating the reason for these differences.

4 DISCUSSION

There is no doubt that it is possible to utilize the herbal water byproduct from Japanese peppermint 'Hokuto' as an antibacterial agent due to the function of menthol. Thus, the concentration of menthol in the herbal water produced is considered to be a key factor determining the quality of the agent. Unevenness in performance is never permitted in a commercialized product; nevertheless, its value may be easily affected by the conditions of the steam distillation process, such as the apparatus, amounts of dried plant and water, and surrounding temperature. To investigate this, we repeated the production of herbal water via steam distillation many times, and confirmed the variation in menthol content in the water samples produced. As a result, we could confirm that there was negligible variation in the concentration of menthol in herbal water extracted using the apparatus under the same distillation conditions. This result indicated that maintaining the concentration of menthol could be accomplished by fixing the process condition, if using the same materials. From this, we deduce that the commercialization of Japanese peppermint herbal water as an antibacterial agent can be easily realized.

5 CONCLUSION

The herbal water of Japanese peppermint, obtained as a byproduct of the steam distillation process for extracting an essential oil, was mainly composed of menthol and menthone. The herbal water produced showed excellent antibacterial efficacy against \textit{E. coli} and \textit{S. aureus} of typical
The Japanese Peppermint Herbal Water as an Antimicrobial Agent

ACKNOWLEDGMENT

The authors gratefully acknowledge Prof. Hasumi from the Kitami Institute of Technology for his advice in the collaborated research project. The authors also thank Kitami Hakka Tsusho Co. Ltd. for providing the peppermint plants used in our experiment. This research was conducted as a part of the research project, “Agricultural Engineering Unit of Research on Okhotsuk Region Advanced Agriculture (AURORA)”, at the Kitami Institute of Technology.

References

1) Lawrence, B.M. The composition of commercially important mints. in Mint the Genus Mentha (Lawrence, B.M. ed.). CRC Press, Boca Raton (2006).
2) Patel, T.; Ishiiji, Y.; Yosipovitch, G. Menthol: A refreshing look at this ancient compound. J. Am. Acad. Dermatol. 57, 873-878 (2007).
3) Kolassa, N. Menthol differs from other terpenic essential oil constituents. Regul. Toxicol. Pharm. 65, 115-118 (2013).
4) Etzold, B.; Jess, A.; Nobis, M. Epimerisation of menthol stereoisomers: Kinetic studies of the heterogeneously catalysed menthol production. Catal. Today 140, 30-36 (2009).
5) Cerpa, M.G.; Mato, R.B.; Cocero, M.J. Modeling steam distillation of essential oils: Application to lavandin super oil. AIChE J. 54, 909-917 (2008).
6) Cassel, E.; Vargasa, R.M.F.; Martinez, N. et al. Steam distillation modeling for essential oil extraction process. Ind. Crops Prod. 29, 171-176 (2009).
7) Eikani, M.H.; Golmohammud, F.; Rowshanzamir, S.; Mirza, M. Recovery of water-soluble constituents of rose oil using simultaneous distillation-extraction. Flavour Fragr. J. 20, 555-558 (2005).
8) Machale, K.W.; Niranjan, K.; Pangarkar, V.G. Recovery of dissolved essential oils from condensate waters of basil and Mentha arvensis distillation. J. Chem. Technol. Biotechnol. 69, 362-366 (1997).
9) Plotto, A.; Roberts, D.; McDaniel, M. Aroma quality of lavender water: a comparative study. Perf. Flav. 26, 44-64 (2001).
10) Rao, B.R.; Kaul, P.N.; Syamasundar, K.V.; Ramesh, S.R. Water soluble fractions of rose-scented geranium (Pelargonium species) essential oil. Bioresour. Technol. 84, 243-246 (2002).
11) Osaka, K.; Saeki, T.; Yasuda, H. et al. The antibacterial activities of peppermint oil and green tea polyphenols, alone and in combination, against enterohemorrhagic Escherichia coli. Biocontrol Sci. 4, 1-7 (1999).
12) Kotan, R.; Kordali, S.; Cakir, A. Screening of antibacterial activities of twenty-one oxygenated monoterpenes. Z. Naturforsch., C: Biosci. 62, 507-513 (2007).
13) Işcan, G.; Kirimen, N.; Kurkcioglu, M. et al. Antimicrobial screening of Mentha piperita essential oils. J. Agric. Food Chem. 50, 3943-3946 (2002).
14) Inouye, S.; Takizawa, T.; Yamaguchi, H. Antibacterial activity of essential oils and their major constituents against respiratory tract pathogens by gaseous contact. J. Antimicrob. Chemother. 47, 565-573 (2001).
15) Hajlaoui, H.; Shoussii, M.; Ben Jannet, H. et al. Comparison of chemical composition and antimicrobial activities of Mentha longifolia L. ssp. longifolia essential oil from two Tunisian localities (Gabes and Sidi Bouzid). Ann. Microbiol. 58, 513-520 (2008).
16) Verma, R.S.; Pandey, V.; Padalia, R.C. et al. Chemical composition and antimicrobial potential of aqueous distillate volatiles of Indian peppermint (Mentha piperita) and spearmint (Mentha spicata). J. Herbs Spices Med. Plants 17, 258-267 (2011).
17) Vimolmangkang, S.; Sithithaworn, W.; Vannavanich, D. et al. Productivity and quality of volatile oil extracted from Mentha spicata and M. arvensis var. piperascens grown by a hydroponic system using the deep flow technique. J. Nat. Med. 64, 31-35 (2010).
18) NIST 14: Mass Spectral Library & Search Software (2014).
19) Scanlon, J.T.; Willis, D.E. Calculation of flame ionization detector relative response factors using the effective carbon number concept. J. Chromatogr. Sci. 23, 333-340 (1985).
20) Miles, A.A.; Misra, S.S.; Irwin, J.O. The estimation of the bactericidal power of the blood. J. Hyg. 38, 732-749 (1938).
21) Hussain, A.I.; Anwar, F.; Nigam, P.S. et al. Seasonal variation in content, chemical composition and antimicrobial and cytotoxic activities of essential oils from four Mentha species. J. Sci. Food Agric. 90, 1827-1836 (2010).