ISSN: 0975-8585

Research Journal of Pharmaceutical, Biological and Chemical Sciences

Monoterpene Synthase from *Lavandula angustifolia* and Solid-Phase Microextraction- Gas Chromatography-Mass Spectroscopy Analysis of its Aroma Profile

Soodabeh Saeidnia [1]*, Gholamreza Amin [1, 2], Pantea Sarkheil [3] and Abbass Hadjiakhoondi [1, 2]

[1]Medicinal Plants Research Center, Faculty of Pharmacy, Tehran University of Medical Sciences, P. O. Box 14155-6451, Tehran, Iran.
[2] Department of Pharmacognosy, Faculty of Pharmacy, Tehran University of Medical Sciences, Tehran, Iran.
[3] Pharmaceutical Research Center, Tehran University of Medical Sciences, Tehran, Iran

ABSTRACT

Lavandula angustifolia Mill. (Labiatae) is commercially used as a fragrance and flavor in cosmetics, food and pharmaceutical industries. This aromatic species is widely grown and cultivated in various temperate regions of Persia. There are diverse reports around composition of the oil of this plant in Iran representing linalool derivatives or 1,8-cineol and camphor as the major compounds. In addition, some mono-and sesquiterpene synthases have been cloned and identified from L. angustifolia. In the present study, the aroma profile of one cultivated plant in Tehran has been extracted and analyzed via Headspace Solid-Phase Microextraction technique coupled with gas chromatography- mass spectroscopy. In order to determine the sequence of the active terpene synthase in this plant, mRNA was prepared, 3'and 5'-RACEs-PCR Method employed, cDNA sequenced and finally aligned with other recognized terpene synthases. The results showed that the cultivated plant leaves mainly comprised linalool (31.0%), linalyl acetate (18.2%) and lavandulyl acetate (10.7%) apposite of the essential oil of plant reported from dry and temperate areas. Sequencing the cDNA cloned from this plant revealed the presence of a monoterpene synthase absolutely similar to limonene synthase, responsible in formation of limonene, terpinolene, camphene and some other cyclic monoterpenes in L. angustifolia young leaves.

Keywords: Monoterpene synthase, *Lavandula angustifolia*, Labiatae, Headspace Solid phase Microextraction, Essential oil.

Corresponding author

ISSN: 0975-8585

INTRODUCTION

Terpenes and terpenoids are classified among the important bioactive natural compounds. Most of them exhibited biologically activity such as sterols, saponins and volatile oils. Recent genetic approaches are capable of isolation and characterization of the biosynthetic enzymes especially by development of molecular biological techniques. For example, sequence comparison of mono-, sesqui- and diterpene synthases revealed that they have a similar structure [1, 2]. Purification and identification of a number of monoterpene synthases indicated that they all have similar properties (like molecular mass, a divalent metal ion and neutral pH optimum requirements). The interesting point is that a terpene synthase is able to form multiple products [1, 3]. For example the pinene synthases (from sage and grand fir) can catalyze the production of both α- and β-pinenes [4].

Geranyl diphosphate (GDP) is well known as a natural substrate for monoterpene synthases. It is found that all terpene synthases employ GPD without the formation of free intermediates [5]. Since GDP could not be directly cyclized, because of the C_2-C_3 (*trans*) double bond, both isomerization and cyclization have to be created in biosynthesis [1, 4]. For this reason, monoterpene cyclases are able to catalyze both the isomerization and cyclization reactions. There have been a few monoterpene synthases involving in production of acyclic products such as myrcene and linalool [4, 6].

Most of the Lamiaceae (Labiatae) plant species produce considerable amounts of essential oils. Essential oils contain a complex mixture of aromatic and volatile compounds and are widely used in cosmetics as fragrance, in the food industry as flavoring and in the household products as scenting agents [7]. The genus *Lavandula* comprises 30 known species, three of them are economically important including *Lavandula angustifolia, Lavandula latifolia* and the hybrid *L. angustifolia* × *L. latifolia* [8]. The constituents of the essential oil of lavender have been frequently reported and linalool and linalyl acetate are mainly described as the most abundant compounds. Other characteristic components that have been identified are limonene, 1, 8-cineol, camphor, lavandulol, lavandulyl acetate and α-terpineol [9].

Literature review show that two monoterpene synthases (LaLIMS and LaLINS) and one sesquiterpene synthase (LaBERS) were cloned from lavender. LaLIMS catalyze the formation of (R)-(+)-limonene, terpinolene, (1R, 5S)-(+)-camphene, (1R, 5R)-(+)-a-pinene, β-myrcene and traces of α-phellandrene. The second enzyme LaLINS produce exclusively (R)-(-)-linalool, the main component of lavender essential oil [8]. In the present study, we aimed to analyze the aroma profile of a cultivated *L. angustifolia* in Tehran, *via* Headspace Solid-Phase Microextraction technique coupled with gas chromatography- mass spectroscopy (SPME-GC-MS), in order to determine the main components compared to other reports from diverse arid and temperate areas. Furthermore, the cloning of a monoterpene synthase related to the flavor of *L. angustifolia* essential oil has been described for the first time from Iranian cultivated plant leaves.

ISSN: 0975-8585

MATERIALS AND METHODS

General Procedure

Chemical reagents and solvents were purchased from Merck Co. (Germany). Gel and plasmid extraction kits were from Invitrogen Co. (UK). RNeasy Plant Mini Kit was prepared from Qiagen (USA), and vectors and E. coli competent cells were from Invitrogen. Polymerase chain reactions were performed on a Primus 25 (Peqlab, Germany) thermal cycler.

Plant Materials

The plant used in this study was grown in the Herboratum of Faculty of Pharmacy, Tehran University of Medical Sciences. The plant identified by Dr. Gholamreza Amin, Department of Pharmacognosy (the same institution) as *Lavandula angustifolia*. Lavender young leaves were harvested from one *L. angustifolia* plant grown outside in a mini-garden under natural conditions.

cDNA Preparation

Total RNA was extracted from the early growing stage of the *L. angustifolia* leaves using RNeasy Plant Mini Kit and reverse transcribed with oligo (dT) primer [ad: 5'-GCT GTC AAC GAT ACG CTA CGT AAC GGC ATG ACA GTG TTT TTT TTT TTT TTT TTT-3'] designed to have an adaptor sequence at the 5'-end to obtain the cDNA for the 3'-RACE method. The resulting cDNA of the leaves was used as a template in subsequent PCR with Taq or KOD Dash DNA polymerases with various combinations of sense and antisense degenerate primers. The temperature program was designed on thermal cycler and started at 94˚C (3 min), followed by 33 cycles (94˚C for 30 s, 46˚C for 30 s and 72˚C for 1min), then 72 ˚C for 2 min. Elongating times were different (30-60 s) based on the expected length of the amplified fragment. The size of monoterpene synthase sequences (partial not complete) was estimated by gel electrophoresis. PCRs were repeated using the same primers to obtain more amounts of DNA, for cloning into the vector. The similarity of cloned sequences to known sequences was checked with NCBI pBLAST.

3'and 5'-RACEs-PCR Method

The 3'-RACE method was used to amplify the 3'-end of the monoterpene synthase. First, the polymerase chain reaction for 3'-RACE was carried out with degenerate primer [5'- TAG ATG ATG TTT ACG AT-3'] and an adaptor primer [amm: 5'- GGC CAC GCG TCG ACT AC-3']. The PCR was performed by KOD Dash DNA polymerase (0.2 µL), amm primer (0.3 µL), degenerate primer (0.6 µL), dNTP (0.1mM, 2 µL), DNA template (1 µL) and appropriate amounts of recommended buffer, DMSO and water by the temperature program (94°C for 20 s, 40°C for 15 s and 72°C 30 s, 33 cycles). The subsequent PCR gave a 900 bp product, which was electrophoresed on 0.8% agarose gel and purified using the Gel-M™ Gel Extraction System (Viogene). The resulting DNA fragment was cloned into the plasmid vector pCR 2.1 using the

ISSN: 0975-8585

TOPO TA cloning kit (Invitrogen). Specific antisense primer [LAV-I: 5'-ACC CCA TTC GTA GTT GTC GCA GAA CG-3'] designed on the basis of the sequence obtained from 3'-RACE.

A poly C tail was appended to the cDNA for 5'-RACE by terminal dideoxynucleotidyl transferase, and the cDNA was purified on a PCR-M column (Viogene).To clone the 5'-end of the transcript (5'-RACE), cDNA was synthesized from mRNA with Reverse Transcriptase and gene specific reverse primers based on the known sequence parts (LAV-I). The purified product (64 µL) was amplified with terminal deoxynucleotidyl transferase (1 µL) , dCTP (10 mM, 5 µL) and 16 µL of TdT buffer in PCR at 37˚C for 90 min, 70˚C for 10 min and 4˚C for 10 min to synthesize an oligo (dC) -tail. The cDNA (1 µL) was used as a template in PCR (temperature program: 94°C for 30 s, 52°C for 20 s, 74°C 50 s, 35 cycles) with dNTPs (10 mM, 2µL), oligo (dT) anchor primer as for 3'-RACE (0.3 µL), gene specific primer (10 µM, 0.3 µL), KOD Dash DNA polymerase (0.8 µL) and appropriate amounts of buffer and water. Furthermore, a touchdown protocol similar to that of used for 3'-RACE was applied.

SPME- GC-MS Analysis

Head space Solid-phase microextraction (SPME) coupled to gas chromatography and mass spectrometry has been applied for analyzing the essential oil directly evaporated from *L. angustifolia* young leaves. GC-MS was performed on a cross-linked 5% methyl phenyl siloxane (HP-5, 30 m × 0.25 mm i.d., 0.25 µm film thickness), carrier gas, He; split ratio, 1:15; quadruple mass spectrometer Hewlett-Packard 6890) operating at 70 eV ionization energy. In order to obtain the retention index for each compound, normal alkanes (C8-C25) were injected at the same temperature and condition. The components were identified by comparison of their retention indices (RI, DB-5) and mass fragmentation with those reported in the literature [10]. Percentage of each component was calculated on the basis of the peak area.

RESULTS AND DISCUSSION

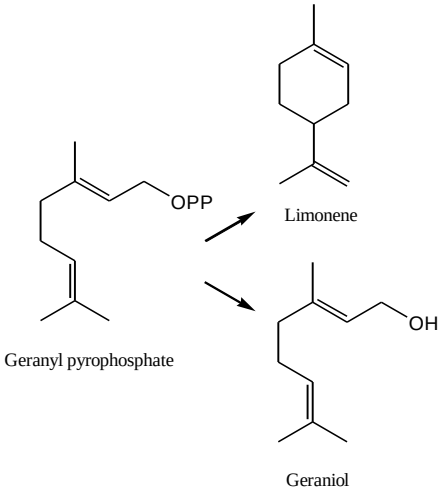

Fig 1: Cyclic and linear monoterpenes biosynthetic pathway.

ISSN: 0975-8585

In the present study, PCR process were performed with degenerate primers designed regarding to the conserved amino acid sequence in various plant terpene synthases. Sequence analysis was carried out using the cDNA prepared from the young leaves of *L. angustifolia* as a template. The results of partial cloning revealed the core sequence containing the 3'-flanking region of limonene synthase, on the other hand the partial sequence was similar to monoterpene synthase genes reported in GenBank in advance, so that the 5'- and 3'-ends were cloned by RACE-PCR to get the full-length sequences. In order to clone the full length of limonene synthase cDNA, the 5'-RACE technique was utilize to complete the remained 5'-region (Figs 1, 2). The transcript from leaves' mRNA was identical and previously designed as LaLIMS [8] .

The open reading frame of the above mentioned limonene synthase consists of 1809 bp, coding for protein with 602 amino acids and the predicted molecular mass of 70.3 kDa. Literature review shows two characteristic motives for monoterpene synthases as DDxxD and (N,D)D(L,I,V)x(S,T)xxxE, which are reported to completely conserved in limonene synthase sequences [8]. These motives are essential for substrate (*e.g.* Mn^{2+}, Mg^{2+}) binding and ionization [11]. A distinguished part of the active site of such enzymes, which are frequently found in monoterpene synthases, is LQLYEASFLL and well conserved in LaLIMS [12].

```
M.longifolia            --------GAAAAACA-TAGAAAGAGAGCGGAAGG---AAAGATTAATCATGGCTCTCAA 48
P.citriodora            AATCCAAGAAAAAATAACAGATAAAAAAAGGGAAATCTAAAGATTAATC---GATCTCAA 57
L.angustifolia_lin_     ----------------------------------------ATGTCGATCA------ATA- 13
L.angustifolia_lim_     ----------------------------------------ATGTCTATCA------TTAG 14
                                                                *  *  ***        *
M.longifolia            AGTGTTTAGTGTTGCAACTCAAATGGCGATTCCTAGCAAGCTAACGAGATGTCTTC--AA 106
P.citriodora            AATGTATACCGGTGTGATGAATATGGCGTTTCCTATGAAGCCAGCTAATTATCTTCATAA 117
L.angustifolia_lin_     -----------------TCAACATG------CCTGC--AGCC-----GCCGTCCTC---- 39
L.angustifolia_lim_     CATGCATGTGGGAATCCTTAATAGG------CCTGC--AGCTTA-TAACCATCTTCGCAA 65
                                  * * *       ***    ***          ** **
M.longifolia            CCCTCACACTTGAAATCCTCTCCAAAATTGT----TAT--------CTAGCACTA--ACA 152
P.citriodora            CTCCGGCAGTAGCAA--CTCTTCAAAATTGTGCGGTGTCTCTTCTACTAGTACTAGAGCA 175
L.angustifolia_lin_     ----------------------------CGCC------------CT----TTT---C- 49
L.angustifolia_lim_     CTTGGACAGGAGAG-----CTTCAAAGCCGCGCCATGT--------CT----CTT---CT 105
                                                                **     *    *
M.longifolia            GTAGTAG-TCGGTCTCGCCTCCGTGTGTATTGCTCCTCCTCGCAACT------------C 199
P.citriodora            GCTACAGCTCGCCTCCGCCTCCGGCTGCGTTGCTCGTT---GCAACT------------C 220
L.angustifolia_lin_     GCTGCT-------------------------------C---ACAACTACA---------T 66
L.angustifolia_lim_     ACTGCCGCCGCCACTCGCCTCCGGGTTTCTTGCGCCAC---ACAACTAGAAATTAAGTCC 162
                                                          *****
M.longifolia            ACTACTGAG---AGACGATCCGGAAACTACAACCCTTCTCGTTGGGATGTCGAATTCATC 256
P.citriodora            AGTGATCAA---CGACGATCTGGAAACTACAGTCCTTCCTTTTGGAATACCGATTATATT 277
L.angustifolia_lin_     GTCGATGAAACCCGACGCTCCGGAAACTACCGCCCCTCGGCTTGGGATTCCAACTACATC 126
L.angustifolia_lim_     GTCGATGAAACCCGACGCTCCGGAAACTACAACCCTACCGCTTGGGATTTCAACTACATC 222
                          * *     **** ** *********   **  *   **** **  * * *  **
M.longifolia            CAATCCCTCCACAGTGATTATGAGGAGGACAAACACGCGATTAGGGCTTCT---GAGCTG 313
P.citriodora            CTATCTCTCAACTGTGACTATGAGGACGAGAGACGCATGAGAGGGGCTGCTGGTGAGCTG 337
L.angustifolia_lin_     CAATCTCTCAATTCTCAGTATAAGGAAAAGAAGTGCTTGACAAGGCTAGAA---GGGCTG 183
L.angustifolia_lim_     CAATCCCTCGACAATCAGTATAAGAAAGAGAGGTACTCGACAAGACACGCT---GAGCTG 279
                        * *** *** *   * * *** ** *  * *     *  **   *        * ****
M.longifolia            GTCACTTTGGTGAAGATGGAATTGGAGAAAGAAACGGATCATATTCGACAACTTGAGTTG 373
P.citriodora            GTTGAGCAAGTGAAGATGCTGATGGAGAAAGAAACAGATCCTATTGTACAGCTTGAGTTG 397
L.angustifolia_lin_     ATTGAGCAAGTGAAGGAACTGAAGGGGACAAAAATGGAGGCTGTTCAACAATTGGAGTTG 243
L.angustifolia_lim_     ACTGTGCAAGTGAAGAAGCTGCTGGAGGAAGAAATGGAAGCGGTTCAAAAGTTGGAATTG 339
                          ******      ** *  * ***  **     **  * *  * ** ***
M.longifolia            ATCGATGACTTGCAGAGGATGGGGCTGTCCGATCATTTCCAGAATGAGTTCAAAGAAATC 433
P.citriodora            ATTGATGACCTTCAAAAGCTGGCTCTCTCTCACTATTTCGAGAAAGAAATCAAGGAAATA 457
L.angustifolia_lin_     ATTGATGACTCGCAGAATCTGGGATTATCATATTATTTTCAAGATAAAATTAAACATATC 303
L.angustifolia_lim_     ATTGAGGATTTGAAGAACCTGGGAATATCTTACCCATTTAAGGACAATATCCAACAGATT 399
                        ** ** **     * *   ***   * **  *    **  *  *  *  *  *  * **
M.longifolia            TTGTCCTCTATATATCTCGACCAT---CACTATTACAAGAACCCTTTTCCAAAAGAAGAA 490
P.citriodora            TTATTCA----ACATCAGTACTATA--TATGATGACAAGAAC----------AGGGAG 499
L.angustifolia_lin_     TTGAATTTGATATATAATGATCACAAATATTTTTACGATAGTGA------AGCTGAAGGA 357
L.angustifolia_lim_     TTAAATCAAATATATAATGAGCACAAATGTTGCCACAACAGTGA------AGTGGAAGAA 453
                        **         * **    *  *           ** * *                *
```

ISSN: 0975-8585

```
M.longifolia            AGGGATCTCTACTCCACATCTCTTGCATTTAGGCTCCTCAGAGAACATG-TTTTCAAGTC 549
P.citriodora            AGGGATTTGTACTCGACAACTCTTGCATTCAGACTTCTTAGACAACACGGTTATCAAGTT 559
L.angustifolia_lin_     ATGGATTTGTATTTTACAGCTCTTGGATTTAGACTCTTTAGACAACATGGTTTTAAAGTC 417
L.angustifolia_lim_     AAGGATTTGTATTTCACGGCTCTTCGATTCCGACTCCTTAGACAACAGGGTTTTGAAGTC 513
                        * **** * ** *  **  *****  ***  * **  * *** **** * ** * ****

M.longifolia            GCACAAGAGGTATTCGACAGTTTCAAGAACGAGGAGGGTG---AGTTCAAAGAAAGCCTT 606
P.citriodora            CCTCAAGAGTTGTTCGAATGTTTCAAGAACGACAAGGGTG---AGTTCAAAGAAAGCCTT 616
L.angustifolia_lin_     TCCCAAGAAGTATTTGATCGTTTCAAGAACGAGAATGGTACGTATTTCAAGC---AC--- 471
L.angustifolia_lim_     TCTCAAGAAGTATTTGATCATTTCAAGAACGAGAAGGGTACAGATTTCAAGCCAAACCTT 573
                         * *****  * ** **   ************  * ***    * *****      *

M.longifolia            AGCGACGACACCAGAGGATTGTTGCAACTGTATGAAGCTTCCTTTCTGTTGACGGAAGGC 666
P.citriodora            AGCAATGACACCAAAGGATTGTTGCAACTGTACGAAGCTTCATTCCTATTGACAGAAGGC 676
L.angustifolia_lin_     ---GACGATACAAAGGGATTGTTGCAGCTCTACGAAGCATCATTCCTAGTGCGAGAAGGC 528
L.angustifolia_lim_     GCTGACGATACTAAAGGACTATTGCAACTTTACGAAGCATCTTTCCTATTAAGAGAAGCT 633
                            * ** ** *  *** * ***** ** ** ***** ** ** **  *    ****

M.longifolia            GAAACCACGCTCGAGTCAGCGAGGGAATTCGCCACCAAATTTTTGGAGGAAAGAGTGAA- 725
P.citriodora            GAAACAACACTCGAGTTAGCAAGAGAATTTGCCACCAAATTTCTGCAGGAAAAAGAAAAA 736
L.angustifolia_lin_     GAAGAGACACTCGAACAAGCACGAGAATTTGCCACCAAATCCCTACAAAGAAAACTTGA- 587
L.angustifolia_lim_     GAAGATACACTTGAGTTAGCTCGACAATTTTCAACCAAATTACTGCAGAAAAAAGTCGA- 692
                        ***   ** ** **   ***  *  ****  * *******   *  *   ** *    *

M.longifolia            --CGAGGGTGGTGTTGAT--GGC-GAC------CTTTTAACAAGAAT-CGCATATTCTTT 773
P.citriodora            CACAATATTGATGATGAT--GAT-GACACTAATCTTATATCGTGTGTGCGC-CACTCTTT 792
L.angustifolia_lin_     --TGAGGATGGTGATGG---AATTGACGCCAATATCGAATCATGGATCCGC-CACTCTCT 641
L.angustifolia_lim_     --TGAGAATGGTGATGATAAAATAGAGGATAATCTATTATTATGGATTCGC-CGTTCTTT 749
                            *   ** ** **        **        *   *    *  * ***    *** *

M.longifolia            GGACATCCCACTTCATTGGAGGATTAAAAGGCCAAATGCACCTGCGTGGATCGAATGGTA 833
P.citriodora            GGACATGCCAATTTATTGGAGGATTCAAAGGC-AAATGCAAGGTGGTGGATTCATGCCTA 851
L.angustifolia_lin_     GGAGATCCCACTTCATTGGAGGGCTCAGAGGCTAGAGGCGAGATGGTTCCTAGATGCTTA 701
L.angustifolia_lim_     GGAGCTCCCGCTTCATTGGAGGGTGCAAAGGCTAGAAGCAAGAGGGTTCTTGGATGCTTA 809
                        ***  * **  ** ********    * **** * * **      **   *  *    **

M.longifolia            TAGGAAGAGGCCCGACATGAATCCAGTAGTGTTGGAGCTTGCCATACTCGACTTAAATAT 893
P.citriodora            TAATAGGAGAACTCACATTAATCCACTTGTGTTGGAGCTTTCCAAACTTGACTTCAATAT 911
L.angustifolia_lin_     TGCGAGAAGGCCCGACATGAACCCCGTTATCTTCGAGCTTGCTAAACTCAACTTCAATAT 761
L.angustifolia_lim_     CGTTAGAAGGCCCGACATGAATCCAATTGTTTTTGAGCTCGCCAAACTCGACTTCAATAT 869
                            *  **  *  **** ** **  *  * ** *****  * * ***  **** *****

M.longifolia            TGTTCAAGCACAGTTTCAAGAAGAGCTTAAAGAATCCTTCAGGTGGTGGAGAAATACTGG 953
P.citriodora            TATTCAAGCACAATATCAGCAAGAACTTAAACAAGACTTAAGGTGGTGGAGAAATACATG 971
L.angustifolia_lin_     TGTCCAAGCAACACAACAAGAAGAATTGAAAGCTCTCTCGAGGTGGTGGAGTAGTTTAGG 821
L.angustifolia_lim_     TACCCAAGCAACACAACAAGAAGAACTGAAAGATCTCTCGAGGTGGTGGAATAGTACAGG 929
                        *   ******      **  ****  * ***     **  **********  * *    *


M.longifolia            GTTTGTTGAGAAGCTGCCCTTCGCAAGGGATAGACTGGTGGAATGCTACTTTTGGAATAC 1013
P.citriodora            CATTGCTGAGAAGCTTCCCTTTGCAAGGGATAGGCTCGTGGAATCCTACTTTTGGAGTAC 1031
L.angustifolia_lin_     CCTAGCTGAAAAACTCCCATTTGTGAGGGATAGGCTTGTGGAAAGCTACTTTTGGGCTAT 881
L.angustifolia_lim_     CCTTGCCGAAAAACTCCCATTCGCGAGGGATCGGGTTGTTGAGTCCTACTTCTGGGCAAT 989
                          * *  ** ** ** ** ** *  ****** *  * ** **   ****** ***   *
```

ISSN: 0975-8585

```
M.longifolia            TGGGATCATCGAGCCACGTCAGCATGCAAGTGCAAGGATAATGATGGGCAAAGTCAACGC 1073
P.citriodora            TGGGATCATTCAGCCTCGTCAACATGAAAATGCAAGGATAATGATGGCAAAAGCTCTTGC 1091
L.angustifolia_lin_     TCCACTCTTTGAGCCTCATCAATATGGATATCAAAGAAAAGTGGCCACCAAGATCATAAC 941
L.angustifolia_lim_     GGGAACCTTTGAGCCTCATCAATATGGTTATCAGAGAGAACTTGTCGCCAAGATTATTGC 1049
                              * *  **** * ***  ***    *   **   * *       **        *

M.longifolia            TCTGATTACGGTGATCGATGATATTTATGATGTCTATGGCACCTTAGAAGAACTCGAACA 1133
P.citriodora            TCTAATAACCACGTTAGATGATGTTTACGATGTCTACGGTACCTTAGAAGAACTCGAGCT 1151
L.angustifolia_lin_     CCTAATCACATCTTTAGACGATGTTTACGATATCTATGGCACGTTAGATGAATTGCAACT 1001
L.angustifolia_lim_     TCTAGCAACAGTTGTAGATGATGTTTACGATGTATATGGTACGTTAGAGGAACTGGAACT 1109
                         **    **     * ** *** **** *** * ** ** ** ***** *** *  * *

M.longifolia            ATTCACTGACCTCATTCGAA-GATGGGATATAAACTCAATCGACCAACTTCCCGATTACA 1192
P.citriodora            GTTCACCGAGGCGATTAGAA-GATGGGAAATCAGTTCAATTGACCAACTTCCTAACTACA 1210
L.angustifolia_lin_     ATTTACGAAC-TTATTTGAAAGATGGGATAATGCATCAATCGGCCGACTTCCTGAATACT 1060
L.angustifolia_lim_     ATTTACAGATGCCATTCGGA-GATGGGATCGTGAATCAATCGACCAACTTCCTTACTACA 1168
                         ** **  *    *** * * *******       ***** * ** ******  * ***

M.longifolia            TGCAACTGTGCTTTCTTGCACTCAACAACTTCGTCGATGATACATCGTACGATGTTATGA 1252
P.citriodora            TGCAACTCTGTTTTCTTACAATCAACAACTTTGTCGACGATACTGCCTACGATGTCATGA 1270
L.angustifolia_lin_     TGCAATTGTTCTATTTCGCAATCCACAACTTTGTTTCCGAGGTGGCTTACGACATTCTCA 1120
L.angustifolia_lim_     TGCAGCTATGCTTTTTGACTGTCAACAACTTTGTTTTCGAGCTTGCTCATGATGTTCTTA 1228
                        ****  * *  * * *  *  ** ******* **    **     *  * **  *  * *

M.longifolia            AGGAGAAAGGCGTCAACGTTATACCCTACCTGCGGCAATCGTGGGTGGATTTGGCGGATA 1312
P.citriodora            AAGAGAAAGATATCAACATCATCCCGTATCTACGAAAATCGTGGGTGGATTTGGCTGAGG 1330
L.angustifolia_lin_     AAGAAAAGGGTTTCACTAGTATTGTATATTTACAGAGATC-TGGGTGGATTTGCTAAAAG 1179
L.angustifolia_lim_     AGGATAAGAGTTTCAACTGCTTACCACATTTACAGAGATCGTGGCTAGACTTGGCTGAAG 1288
                        * ** **     ***      *     *  * *    *** *** * ** ***    *

M.longifolia            AGTATATGGTAGAGGCACGGTGGTTCTACGGCGGACACAAACCAAGTTTGGAAGAGTATT 1372
P.citriodora            CATATCTGGTAGAGGCGAAATGGTTCTATGGCGGATATAAACCAAATTTG---------- 1380
L.angustifolia_lin_     GATACCTAAAAGAGGCAAAG-GGTACAATAGTGGATACACGCCAAGCCTCGAGGAATATT 1238
L.angustifolia_lim_     CATATCTTGTCGAGGCTAAGTGGTACCACAGTAGATATACACCGAGCCTCGAGGAATATC 1348
                          **  *    *****     *** * *  *  ** * *  ** *   *

M.longifolia            TGGAGAACTCATGGCAGTCGATAAGTGGGCCCTGTA-TGTTAACGCACATATTCTTCCGA 1431
P.citriodora            ------------------------------------------------------------
L.angustifolia_lin_     TCGACAACGCATTCATGACAAT-AGGGGCCCCTCCGGTACTATCGCAAGCTTATTTCACA 1297
L.angustifolia_lim_     TCAATATTGCAAGAGTTTCAGTTACGTGTCCCACTA-TAGTTTCACAAATGTACTTTGCA 1407

M.longifolia            GTAACAGATTCGTTCACAAAGGAGACCGTCGACAGTTTGTACAAATACCACGATTTAGTT 1491
P.citriodora            ------------------------------------------------------------
L.angustifolia_lin_     TTA-------------GGAAGCTCATCATCGAGAGCATGTACGAATATGACAACATACTT 1344
L.angustifolia_lim_     TTACCAATTCCGATAGAGAAACCGGTCATCGAGATCATGTACAAATACCACGACATACTT 1467


M.longifolia            CGTTGGTCATCCTTCGTTCTGCGGCTTGCTGATGATCTGGGAACCTCGGTGGAAGAGGTG 1551
P.citriodora            ------------------------------------------------------------
L.angustifolia_lin_     CGCGTTTCGGGAATGCTCGTGAGGCTTCCCGATGACCTAGGAACATCATCGTTCGAGATG 1404
L.angustifolia_lim_     TACCTCTCAGGAATGCTTCTAAGGCTTCCTGATGATCTAGGAACAGCATCGTTTGAGTTG 1527
```

ISSN: 0975-8585

```
M.longifolia            AGCAGAGGCGATGTGCCGAAATCACTTCAGTGCTACATGAGTGACTACAATGCATCGGAG 1611
P.citriodora            ------------------------------------------------------------
L.angustifolia_lin_     GAGAGAGGCGACGTGCCGAAATCGGTCCAGCTATACATGAAGGAAACAAATGCTACGGAG 1464
L.angustifolia_lim_     AAGAGAGGTGATGTGCAAAAAGCAGTCCAGTGTTATATGAAGGAAAGAAATGTTCCTGAA 1587

M.longifolia            GCGGAGGCGCGGAAGCACGTGAAATGGCTGATAGCGGAGGTGTGGAAGAAGATGAATGCG 1671
P.citriodora            ------------------------------------------------------------
L.angustifolia_lin_     GAGGAGGCGGTGGAGCACGTGAGGTTTTTGAATCGGGAGGCGTGGAAGAAGATGAACACG 1524
L.angustifolia_lim_     AATGAGGCACGAGAACATGTGAAGTTTCTGATTCGGGAGGCGTCGAAGCAGATAAACACC 1647

M.longifolia            GAGAGGGTGTCGAAGGATTCTCCATTCGGCAAA-GATTTTATAGGATGTGCAGCTGATTT 1730
P.citriodora            ------------------------------------------------------------
L.angustifolia_lin_     GCGGAGGCGGCCGGTGATTCTCCGTT-AGTGAGTGACGTGGTGGCGGTGGCGGCGAATCT 1583
L.angustifolia_lim_     GCGATGGCGACCG---ATTGTCCATTTACTGAA-GATTTTGCTGTGGCTGCAGCGAATCT 1703

M.longifolia            AGGAAGGA-GGCGCAGTTGATGTACCATAATGGAGATGGGCACGGCACACAACATCCTAT 1789
P.citriodora            ------------------------------------------------------------
L.angustifolia_lin_     TGGAAGGGCGGCGCAGTTTATGTATTTCGACGGAGATGGTA---------ACCAGTCTAG 1634
L.angustifolia_lim_     TGGAAGAGTGGCGAATTTTGTGTACGTCGACGGAGATGGTTTTGGCGTGCAACACTCAAA 1763

M.longifolia            AATACATCAACAAATGACCAGAACCTTATTCGAGCCCTTTGCATGAGAGGTGATGATGAT 1849
P.citriodora            ------------------------------------------------------------
L.angustifolia_lin_     TTTGCAGCAGTGGATTGTGAGCATGCTGTTCGAGCCGTACGCATGA-------------- 1680
L.angustifolia_lim_     AATATATGAACAGATTGGAACCCTGATGTTCGAGCCATATCCCTAA-------------- 1809

M.longifolia            GAGCCATCGTTTACTTACTTAAATTCTACCAAAGTTTTTCGAAGGCATAGTTTGTAATTC 1909
P.citriodora            ------------------------------------------------------------
L.angustifolia_lin_     ------------------------------------------------------------
L.angustifolia_lim_     ------------------------------------------------------------

M.longifolia            TTCAAGCACCAAATGGAATAAGGAGAATCGGCTCAAACAACGTGGCATTTGCCACCACGT 1969
P.citriodora            ------------------------------------------------------------
L.angustifolia_lin_     ------------------------------------------------------------
L.angustifolia_lim_     ------------------------------------------------------------

M.longifolia            GAGCACAAGGAGAGTCTGTCGTCGTTTATGGATGAACTATTCAATTTTATGCATGTAATA 2029
P.citriodora            ------------------------------------------------------------
L.angustifolia_lin_     ------------------------------------------------------------
L.angustifolia_lim_     ------------------------------------------------------------


M.longifolia            ATTAAGTTCAAGTTCAAGAGCCTTCTGCATATTTAACTATGTACTTG 2076
P.citriodora            -----------------------------------------------
L.angustifolia_lin_     -----------------------------------------------
L.angustifolia_lim_     -----------------------------------------------
```

Fig 2: Alignment of nucleotide sequences (cDNA) of the isolated monoterpene synthase (*L. angustifolia_lim*) with other limonene synthases (*M. longifolia* and *P. citriodora*) and linalool synthase of lavender (*L. angustifolia*_lin). Alignment was carried out using free ClustalW software (http://www.ch.embnet.org/index.html).

ISSN: 0975-8585

Fig 3: Tree view for cloned cDNA from *Lavandula angustifolia* in comparison to other similar monoterpene synthase mRNA (using free software of BLAST, NCBI).

Limonene is one of the cyclic monoterpenes, found in plant kingdom, as the simple example for cyclization of terpenoids. Its biosynthesis is started from geranyl pyrophosphate (GPP), which is a common precursor for all monoterpenes. Limonene synthases promote the cyclization of GPP into limonene and have been identified and reported from *Perilla, Mentha* and Abies [4, 13, 14]. Terpene synthases (TPS) have been cloned from several plant species and their phylogenetic relationships well found. These enzymes have been classified into six sections TPSa-f. Although there have been some differences among the various groups, there are several conserved motifs in all TPSs such as mTPSs signature arginine-rich N-terminal RR(x8)W motif [15, 16].

Table 1: Chemical composition of the essential oil of *Lavandula angustifolia* young leaves obtained by SPME

Compound Names	Retention Indices DB-5	Percentage (%)
myrcene	987	3.0
limonene	1030	1.3
1,8-cineole	1034	0.9
trans-ocimene	1057	2.2
linalool	1099	31.0
camphor	1140	0.9
borneol	1165	1.2
terpinen-4-ol	1180	2.9
α-terpineol	1190	6.3

linalyl acetate	1260	18.2
lavandulyl acetate	1289	10.7
Caryophyllen+	1404	5.2
cyclic monoterpenes	-	13.5
linear monoterpenes	-	65.1
sesquiterpenes	-	5.2
total	-	83.8

The results of head space SPME-GC-MS analysis of the aroma profile of *L. angustifolia* showed that linalool (31.0%), linalyl acetate (18.2%), lavandulyl acetate (10.7%) and alpha-terpineol (6.3%) were found as the major compounds (Table 1). Although the profile of the main components is in agreement with those reported in the literature [7, 17, 18], there is a wide variation in the quantitative composition of lavender oil. Genotype, altitude, microclimate and cultivation condition can affect the composition of the oil [19, 20]. For instance, 1, 8-cineole, camphor and borneol were found as the major compounds from *L. angustifolia* which had been collected from Yazd, an arid and temperate area located at the central part of Iran [21].

ACKNOWLEDGEMENT

This research has been supported by Tehran University of Medical Sciences & health Services grant No. 13306.

REFERENCES

[1] Bohlmann J, Meyer-Guene G, Croeau R. Proc Natl Acad Sci USA 1998; 95: 4126-4133.
[2] Maruyama T, Ito M, Kiuchi F, Honda G. Biol Pharm Bull 2001; 24: 373-377.
[3] Rajaonarivony JIM, Gershenzon J, Croteau R. Arch Biochem Biophys 1992; 296: 49-57.
[4] Bohlmann J, Steele CL, Croteau R. J Biol Chem 1997; 272: 21784-21792.
[5] Croteau R. Chem Rev 1987; 87: 929-954.
[6] Pichersky E, Lewinsohn E, Croteau R. Arch Biochem Biophys 1995; 316: 803-807.
[7] Evandri MG, Battinelli L, Daniele C, Mastrangelo S, Bolle P, Mazzanti G. Food Chem Toxicol 2005; 43: 1381-1387.
[8] Landmann C, Fink B, Festner M, Dregus M, Engel KH, Schwa W. Arch Biochem Biophys 2007; 465: 417- 429.
[9] Flores G, Blanch GP, Ruiz del Castillo ML, Herraiz M. J Sep Sci 2005; 28: 2333-2338.
[10] Adams RP. Identification of essential oil components by gas chromatography/ Quadruple Mass Spectroscopy. Allured: Carol Stream, IL, 2001
[11] Whittington DA, Wise ML, Urbansky M, Coates RM, Croteau RB, Christianson DW. Natl Acad Sci USA 2002; 99: 15375-15380.
[12] Wise ML, Savage TJ, Katahira E, Croteau R. J Biol Chem 1998; 273: 14891-14899.
[13] Yuba A, Yazaki K, Tabata M, Honda G, Croteau R. Arch Biochem Biophys 1996; 332; 280-287.

[14] Colby SM, Alonso WR, Katahir EJ, McGarvey DJ, Croteau R. J Biol Chem 1993; 268: 23016-23024.
[15] Demissie ZA, Sarker LS, Mahmoud SS. Planta 2011; 233: 685-696.
[16] Williams DC, McGarvey DJ, Katahira EJ, Croteau R. Biochemistry 1998; 37:12213-12220.
[17] Fakhari AR, Salehi P, Heydari R, Ebrahimi SN, Haddad PR. J Chromatogr A 2005; 1098: 14-18.
[18] Verma RS, Rahman LU, Chanotiya CS, Verma RK, Chauhan A, Yadav A, Singh A, Yadav AK. J Serb Chem Soc 2010; 75: 343-348.
[19] Lawrence BM. Essential Oils, 1991–1994. Allured Publishing Corp, Wheaton, 1994.
[20] Renaud ENC, Charles DJ, Simon JE. J Essent Oil Res 2001; 13: 269-273.
[21] Abroomand Azar P, Torabbeigi M, Sharifan A, Tehrani MS. Food Biosci Technol 2011; 1: 19-24.