In-silico Analysis of Terpenes From *Mentha rotundifolia* (L) As Human Angiotensin Converting Enzyme-Related Carboxypeptidase (ACE2) Inhibitors

Geeta Mounika\(^1\), Netala Silvia\(^1\), Gondu Eswara Rao\(^1\), Kotte Raju\(^1\), Siva Kumar B\(^2\), Ravi Chandra Sharabu\(^3\), Radha Madhavi B\(^4\), Sujit Kumar Mohanty\(^*1\)

\(^1\)Department of Pharmaceutical Chemistry, Shri Vishnu College of Pharmacy, Bhimavaram, Andhra Pradesh – 534202, India
\(^2\)Department of Pharmaceutical Chemistry, SRM College of Pharmacy, Kattankulathur – 603203, Tamil Nadu, India
\(^3\)Department of Pharmaceutical Chemistry, Hindu College of Pharmacy, Guntur, Andhra Pradesh, India
\(^4\)Department of Pharmaceutics, Shri Vishnu College of Pharmacy, Bhimavaram, Andhra Pradesh – 534202, India

**ABSTRACT**

The *Mentha* genus includes several species such as *Mentha rotundifolia* L., which is widely distributed around the Mediterranean basin, America and in western Asia. The plant is recommended in folk medicine for the treatment of various diseases. It has also been used to discover biomolecules that have significant beneficial effects with fewer side effects. *Mentha rotundifolia* (L) leaves are potential as an antihypertensive cause of terpenes which contain in them. 36 different terpenes and terpenoids have been identified and selected from this plant. This study evaluated the mechanism of phytoconstituents from the above plant in the inhibition of angiotensin-converting enzyme-related carboxypeptidase (ACE2) with molecular docking. Selected ligands were docked on the receptor (PDB ID: 1R4L) using Auto Dock Vina and analysed by PyMol. 2D and 3D structures of compounds were drawn by the Chem Draw program. The standard drug that has been taken for the study, lisinopril, has shown a binding affinity of \(-7.8\) Kcal/mol. Calacorene, one of the terpenes present in the plant, has interacted with Phe274, Asp367, Glu406, Thr445, Thr371 residues of protein and produced a docking score similar to that of the standard drug Lisinopril. In the light of the results obtained, the plant studied is promising as a source of natural hypotensive agent that can be further developed as a lead molecule.

**INTRODUCTION**

The angiotensin-converting enzyme (ACE) linked carboxy peptidase, ACE2 is a specific type I integral membrane protein having 805 amino acids, which contains 1 HEXXH + E zinc-binding consensus sequence. ACE2 has been involved in the maintenance of heart function and also as an active receptor for the corona virus that produces severe acute respiratory syndrome (SARS). The active site of ACE2 is 42% equal to that of its adjacent homolog, i.e. somatic angiotensin-converting enzyme (SACE;
Table 1: The Binding Affinity of Ligand, Standard and Compounds towards human angiotensin-converting enzyme-related carboxypeptidase (ACE2) (1R4L)

| Sl.No | Chemical constituent               | Highest Dock score | Type of bonding (Hydrophobic bonding-HPB, Hydrogen bonding-HB) |
|-------|-----------------------------------|--------------------|-----------------------------------------------------------------|
| 1     | (-)Germacrene D                   | -6.8               | Thr371, Asp367, Phe274                                          |
| 2     | (-)T-muurolol                     | -6.4               | Asp269, Phe274, Trp271                                          |
| 3     | (+)Germacrene D                   | -6.7               | Thr371, Leu370, Asp367, Thr445, Phe274                          |
| 4     | (+)T-muurolol                     | -6.5               | Asp967, Phe274                                                  |
| 5     | 1,2-epoxymenthyl acetate          | -5.8               | Phe274, Leu370, Thr371, His374                                  |
| 6     | 1,6-germacradien-5-ol             | -6.8               | Phe274, Asp367, Asp269, Trp271                                  |
| 7     | Allo-cimene                        | -6.1               | Phe274                                                          |
| 8     | α- caryophyllene alcohol          | -7.3               | HPB: Phe274, Thr445, Asp357, Glu406, Leu370, His374, Thr371, HB: Thr371 |
| 9     | α-pinene                          | -5.5               | Thr445, Phe274                                                  |
| 10    | Aromadendrene                     | -7.2               | Asp367, Phe274, Leu370                                          |
| 11    | β-pinene                          | -5.5               | Thr445, Phe274                                                  |
| 12    | Bicyclogermacrene                 | -6.9               | Arg273, Phe274, Trp271, Arg273                                  |
| 13    | Borneol                           | -5.8               | Phe274                                                          |
| 14    | Bornyl acetate                    | -5.9               | Phe274, Leu370, Thr445, Thr371                                  |
| 15    | Cadina-1,4-diene                  | -7.1               | Thr371, His374, Asp367, Phe274                                  |
| 16    | Calacorene                        | -7.8               | HPB: Phe274, Asp367, Glu406, Thr445, Thr371                    |
| 17    | Camphene                          | -5.4               | Leu370, Phe274, Thr446                                          |
| 18    | Carvotanacetone                   | -5.4               | Leu370, Phe274                                                  |
| 19    | Caryophyllene oxide               | -7                 | Phe274, Thr371, Thr276                                          |
| 20    | Cis-calamenene                    | -7.2               | Phe274, Arg273, Thr276, Thr371                                  |
| 21    | Cis-piperitoneoxide               | -5.7               | Phe274, Leu370, His374, thr371                                  |
| 22    | Epi-bicyclosesquiphellandrene     | -7.1               | His374, Glu406, Asp367, Phe274                                  |
| 23    | Hydroxypiperitone                 | -5.6               | Phe274, Asp367                                                  |
| 24    | Jasmone                           | -5.6               | Thr445, Phe274                                                  |
| 25    | Limonene                          | -5.8               | Phe274                                                          |
| 26    | Myrcene                           | -5.7               | Leu144, Glu145, Cys344, Ser128, His345                           |
| 27    | Neophytadiene                     | -5.6               | Phe274, Asp367, His374, Thr371                                  |
| 28    | P-cymene                          | -6.1               | Phe274, Thr445                                                  |
| 29    | P-cymenene                        | -6.3               | Phe274, Thr276                                                  |
| 30    | Piperitenone oxide                | -5.7               | Phe274, Tyr515                                                  |
| 31    | Piperitenone                      | -6                 | Phe274, Thr371, His374                                          |
| 32    | Sabinene                          | -5.4               | Thr445, Phe274, Asp367, Thr371                                  |
| 33    | Sabinene hydrate                  | -5.7               | Phe274, Thr371                                                  |
| 34    | Terpinen-4-ol                     | -6.1               | Phe274, Asp367                                                  |
| 35    | Terpinolene                       | -5.8               | Phe274, Asp367                                                  |
| 36    | Viridiϐlorol                      | -7.2               | Thr276, Thr371, Thr445, Phe274, His374, Asp367, HB: Thr445      |
| 37    | XXH(co-crystallized ligand of 1R4L)| -7.4               | Phe274, Thr276, HB: Thr371                                      |
| 38    | Lisinopril                        | -7.8               | Phe274, Trp271                                                  |
EC 3.4.15.1), a peptidyl dipeptidase which plays a meaningful role in the renin-angiotensin system for maintaining BP homeostasis (Towler et al., 2004).

ACE inhibitors are prescribed for regulating chronic and acute elevated blood pressure, left ventricular dysfunction and failure of heart, prevention of strokes and kidney ailments in individual suffering from high BP or diabetes. But there are various issues related to these drugs toleration and safety. The USFDA is conducting global investigations on the limits of impurities, particularly nitrosamine, in these particular drugs. These drugs are generally contraindicated in Pregnancy or breastfeeding cases. These drugs, if prescribed with COX inhibitors, results in the decrease of hypotensive effects of ACE Inhibitors (Sidorenkov and Navis, 2014).

*Mentha* plant is one of the privileged medicinal plants in ancient medicine. This particular plant is mainly a leafy plant grouped in the Lamiaceae family. Starting from ancient times, various parts of this plant have been prescribed for various treatments. The plant possess cardiovascular (Awang, 1999; Hawthorn et al., 1988), pulmonary (Balakrishnan, 2015), gastrointestinal (Mikaili et al., 2013), neuro-psychiatric, endocrine, immune-modulatory, antimicrobial, Hypotensive and antispasmodic activities (Shahbazi, 2015; Singh et al., 2015; Pattnaik et al., 1997).

The active phytochemicals isolated in the diethyl ether extract of *M. rotundifolia* are Sabinene, Terpinen-4-ol, Piperitenone, Piperitoneoxide, 1,2-epoxymethyl acetate, Germacrene D, Jmuurolol, α-pinene, α-caryophyllene alcohol, Aromadendrene, β-pinene, Borneol, Cadina-1,4-diene, Calorecne, Camphene, α-carvotanacetone, cis-calamenene, Caryophyllene oxide, epi-bicyclosesquiphellandrene, Jasmine, Limonene, Lisinopril, Myrcene, Neophytadiene, p-Cymene, p-Cymenene (Brada et al., 2006).

Terpenes and terpenoids are a broad group of natural compounds possessing important biological activities and are prescribed for human diseases. Taxol (anticancer drug) and Artimesin (antimalarial drug) are the most well-known terpene derivatives. In the last few decades, many new terpenoids from the marine environment has been identified, with new structures and promising bioactivities, with more to be discovered in the future. Semisynthetic derivatives of terpenes also play a major role in the development of terpenoid-derived molecules. Recent technologies like environmental genomics and other “-omics” will surely help in the isolation and development of new terpenoids from nature (Wang et al., 2005).

Computational techniques are generally used to predict the activity of various compounds. In silico studies add axiomatically to starting pharmaceutical developments and are crucial in the identification of specific targets. The aim of this study is to analyse the activity of *M. roduntifolia* as human angiotensin-converting enzyme-related carboxypeptidase (ACE2) inhibitors by the help of computational techniques.

**Figure 1:** (1a) Calacorene position in the protein 1R4L and (1b) interactions of calacorene with Phe274, Asp367, Thr445, Thr371, Glu406.

### Experimental

**Material and Methods**

A desktop operated by Windows 10pro, Intel® CoreTM i3-8100 (3.6 GHz), 64-bit, hard disc drive 1TB and RAM memory 4 GB were used to run the molecular docking process. Softwares used for In silico docking were Autodock 1.5.6, Autodock Vina 1.1.2 program and visualized by PyMol 1.3. The 3D structure of the enzyme used in this research (human angiotensin-converting enzyme-related carboxypeptidase (ACE2) was obtained from Protein Data Bank (PDB code: 1R4L) through the website http://www.rcsb.org/pdb. 2D and 3D structures of compounds were generated by using ChemDraw.

### RESULTS AND DISCUSSION

**In-silico Analysis**

Various terpenes obtained from *Mentha rotundifolia* leaves were docked into the protein (1R4L) binding pocket, and the dock scores were shown in Table 1. A docking score equivalent to that of the standard ligand was observed, with calcorene having a value of -7.8. The binding pocket and interactions of Calacorene were shown in Figure 1.

### CONCLUSIONS

From the result, it can be concluded that Calacore has the highest activity in inhibiting the
human angiotensin-converting enzyme-related carboxypeptidase (ACE2). Moreover, the binding scores also revealed that calacorene has a similar affinity towards the receptor as the standard drug Lisinopril. There is no ambiguity that more terpenes and its synthetic derivatives will become available and will have a more significant role in the treatment of various human ailments in future.

Acknowledgement
The authors are thankful to Shri Vishnu College of Pharmacy and its Management for the research facilities.

Funding Support
The authors declare that there is no funding support for this research work.

Conflict of Interest
The authors declare that there is no conflict of interest.

REFERENCES
Awang, D. V. 1999. Tyler’s herbs of choice: The Therapeutic Use of Phytomedicinals. CRC Press, third edition.

Balakrishnan, A. 2015. Therapeutic Uses of Peppermint-A Review. Journal of Pharmaceutical Science and Research, 7(7):474–476.

Brada, M., Bezina, M., Marlier, M., Lognay, G. C. 2006. Chemical Composition of the Leaf Oil of Mentha rotundifolia(L.) from Algeria. Journal of Essential Oil Research, 18(6):663–665.

Hawthorn, M., Ferrante, J., Luchowski, E., Rutledge, A., Wei, X. Y., Triggle, D. J. 1988. The actions of peppermint oil and menthol on calcium channel-dependent processes in intestinal, neuronal and cardiac preparations. Alimentary Pharmacology and Therapeutics, 2(2):101–118.

Mikaili, P., Mojaverrostami, S., Moloudizargari, M., Aghajanshakeri, S. 2013. Pharmacological and therapeutic effects of Mentha Longifolia L. and its main constituent, menthol. Ancient Science of Life, 33(2):131–138.

Pattnaik, S., Subramanyam, V. R., Bapaji, M., Kole, C. R. 1997. Antibacterial and antifungal activity of aromatic constituents of essential oils. Microbios, 89(358):39–46.

Shahbazi, Y. 2015. Chemical Composition and In VitroAntibacterial Activity ofMentha spicataEssential Oil against Common Food-Borne Pathogenic Bacteria. Journal of Pathogens, 2015:1–5.

Sidorenkov, G., Navg, G. 2014. Safety of ACE inhibitor therapies in patients with chronic kidney disease. Expert Opinion on Drug Safety, 13(10):1383–1395.

Singh, R., Shushni, M. A., Belkheir, A. 2015. Antibacterial and antioxidant activities of Mentha piperita L. Arabian Journal of Chemistry, 8(3):322–328.

Towler, P., Staker, B., Prasad, S. G., Menon, S., Tang, J., Parsons, T. 2004. ACE2 X-Ray Structures Reveal a Large Hinge-bending Motion Important for Inhibitor Binding and Catalysis. Journal of Biological Chemistry, 279(17):17996–18007.

Wang, G., Tang, W., Bidigare, R. R. 2005. Terpenoids as Therapeutic Drugs and Pharmaceutical Agents. Natural Products, Humana Press Inc, Totowa, New Jersey.