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In silico Analysis of MicroRNA of (*Mentha X Piperita*) Peppermint

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ABSTRACT

Mentha piperita, commonly known as peppermint, is a well-known medicinal plant with a range of therapeutic properties. In this study, we conducted an *in-silico* analysis of microRNAs and ESTs in *Mentha piperita* to gain insight into its genetic makeup. We obtained 29 EST sequences and 36 microRNAs from the NCBI database and analyzed them using various bioinformatics tools. Functional annotation of the microRNAs revealed that only 29% of the miRNAs met the criteria for AU content between 30% and 70%. Out of these, target genes were identified for only 9 sequences, which were annotated with basic roles in biological processes and molecular functions. Phylogenetic analysis confirmed the conservation of *Mentha piperita* with Arabidopsis thaliana. Additionally, we calculated E-values and energies for the ESTs and microRNAs, respectively. Our findings provide valuable insights into the genetic makeup of *Mentha piperita* and may aid in further understanding its therapeutic properties. Future research could involve the experimental validation of the predicted targets of the identified microRNAs and the characterization of the function of the ESTs.

Keywords: Mentha; EST sequence; Structural and functional annotation; Gene ontology; Phylogeny

INTRODUCTION

Mentha piperita L. (peppermint), belongs to the family Lamiaceae, one of the most significant industrial herbs with a variety of essentials oils. Mint is a member of the Lamiaceae family, which is the largest family of plants with 236 genera and 7000 species and genus *Mentha*, consist of 15 hybrids, cultivars, subspecies, varieties and 42 species [1]. *Mentha piperita* is generally called as peppermint is a perennial herb widely used by greater part of people globally in various forms like leaf, leaf extract oil and leaf water. Due to its rich flavor and fragrance peppermint has economic value both in the domestic and international market and an important export commodity that fetches good foreign revenue. The peppermint oil logs for various antispasmodic activities like antivomiting, carminativum, stomachic and antimicrobial properties. The main compounds such as menthol, menthone, and menthofuran which gives unique aroma [2]. *In-silico* refers to analysis which is carried out in a computer environment, rather than in the laboratory (*in vitro*). Plant

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micro RNA's are small non-coding RNA's that have a length of 21 to 24 nucleotides which have role in growth and in metabolic and defense progress. Mostly, miRNAs associate with the target mRNAs by 3' UTR to curb its expression. It also interacts with gene promoters and 5' UTR coding sequence; in addition under specific condition it activates gene expression. This miRNA can also be identified by the use of Expressed Sequence Tag (EST) and a Genome Survey Sequence (GSS). EST analysis has gained significance when compared with other approaches like conserved miRNAs and be capable of identifying without knowing the whole genome sequences. It offers straight confirmation for miRNA expression than from genomic sequence surveys without any much specified software. By this method several miRNAs are effectively recognized in diverse plant species. Many plant miRNAs are evolutionarily conserved from species to species. MiRNA precursors display high variability; their mature sequences display extensive sequence complementarity to their target mRNA sequences. MiRNAs play important roles in plant post-transcriptional gene regulation by targeting mRNAs for cleavage or repressing translation [3]. MiRNAs are involved in plant development, signal transduction, protein degradation, response to environmental stress and pathogen invasion, and regulate their own biogenesis. MiRNAs regulate the expression of many important genes; a majority of these genes are transcriptional factors. Here we use homology base method meaning searching for micro RNA sequence which are involved in anti-oxidant. The specie we selected that is mentha peppermint.

MATERIALS AND METHODS

Searching of EST's in NCBI

In this study we collect total 1316 EST sequences of (*Mentha X Piperita*) Peppermint from National Center for Biotechnology Information NCBI. Furthermore, to screen all these 1316 EST using Mirbase online search tool with the following E-value 0.01 to 0.099 [4].

Identification of Structural and Functional Annotation

Structural annotation of these microRNA to identify their structure through online server Mfold, using Mfold to determine the best possible secondary structure and energies of these microRNA which uses energy minimization method, protein coding sequences were removed prior to prediction. Functional annotation is the main step for finding the function of obtained microRNA EST sequences [5]. Functional analysis can be done by different database here we Blast2GO database because this also give the graph along with the function of EST's microRNA. In order to find function of the obtained EST's sequences first you need to target the genes for the particular microRNA, because the function of microRNA is ultimately defining by the genes. For identifying the target gene for those microRNA PsRNA target software were used.

Phylogenetic Analysis

Evolutionary tree is constructed to find out relation among present sequences and their root sequences [6]. Phylogenetic tree also constructed to find out the ancestral relation among *Arabidopsis thaliana* and *Mentha Peppermint* specie. Phylogenetic tree uses a character based method called maximum likelihood that show the distance among texa. The tool that are used for the construction of phylogenetic tree is RxMI and MegaXtool, that uses the MI tree construction and its probability based approach and for construction of phylogenetic tree and evolutionary model [7].

RESULTS

Total 1316 EST of *Mentha piperita* was retrieved (based on the basis of size), analyzed and annotated through the CAP3 assembly program. In the screening all the 1316 EST's sequence only 29 miRNA sequence was retrieve [8]. After that all the 29 EST's sequence only 36 miRNA were found meet the criteria of required E-value. We blast each EST's sequence individually in mirbase and search for value. The result also shows and given number of A, G, C, U/T and A-T/U count are listed in **Table 1**.

Table 1: Screening the ESTs sequence with lowest E-Value.

S. no	Accession number	Sequences	E values
1	MIMAT0027646	>hsa-miR-6873-5p MIMAT0027646 CAGAGGGAAUACAGAGGGCAAU	0.099
2	MIMAT0015455	>bmo-miR-3270 MIMAT0015455 UGUCUUUUUCCUAAAACGAUUCA	0.036
3	MIMAT0009979	>hsa-miR-2054 MIMAT0009979 CUGUAAUAUAAAUUUAAUUUAUU	0.042

4	MIMAT0030650	>prd-miR-7912-5p MIMAT0030650 CAUUGCAUUUGGUUGUCCUUGGCU	0.080
5	MIMAT0024577	>bta-miR-574 MIMAT0024577 UGAGUGUGUGUGUGUGAGUGUGUG	0.034
6	MIMAT0017325	>mmu-miR-466i-5p MIMAT0017325 UGUGUGUGUGUGUGUGUG	0.050
7	MIMAT0004795	>hsa-miR-574-5p MIMAT0004795 UGAGUGUGUGUGUGAGUGUGU	0.089
8	MIMAT0005837	>mmu-miR-1187 MIMAT0005837 UAUGUGUGUGUGUAUGUGUGUAA	0.077
9	MIMAT0005854	>mmu-miR-467g MIMAT0005854 UAUACAUACACACACAUAUAU	0.093
10	MIMAT0022724	>hsa-miR-1277-5p MIMAT0022724 AAAUAUAUAUAUAUAUGUACGUAU	0.073
11	MIMAT0021390	>rmi-miR-5318 MIMAT0021390 UUGUAUAACUGCAGAAGACUUUUCUCC	0.034
12	MIMAT0017613	>aly-miR831-5p MIMAT0017613 AGAAGAGGUACAAGGAGAUGAGA	0.080
13	MIMAT0032837	>cel-miR-8210-5p MIMAT0032837 UGCCUUCUUUCCUUGUGUCGCCGAC	0.054
14	MIMAT0032835	>cel-miR-8209-5p MIMAT0032835 AAAACGAAGAAAGAAGAAGAA	0.043
15	MIMAT0044920	>pab-miR11418 MIMAT0044920 ACCCACCGCCGCUGCCGCCGC	0.094
16	MIMAT0017325	>mmu-miR-466i-5p MIMAT0017325 UGUGUGUGUGUGUGUGUG	0.078
17	MIMAT0022724	>hsa-miR-1277-5p MIMAT0022724	0.055

		AAAUAUAUAUAUAUGUACGUAU	
18	MIMAT0036467	>tch-miR-1277-5p MIMAT0036467 UAUAUAUAUAUAUGUACGUAU	0.081
19	MIMAT0032837	>cel-miR-8210-5p MIMAT0032837 UGCCUUCUUUCCUUGUGUCGCCGAC	0.054
20	MIMAT0004885	>mmu-miR-467c-5p MIMAT0004885 UAAGUGCGUGCAUGUAUAUGUG	0.097
21	MIMAT0009979	>hsa-miR-2054 MIMAT0009979 CUGUAAUAUAAAUUUAAUUUAUU	0.098
22	MIMAT0005837	>mmu-miR-1187 MIMAT0005837 UAUGUGUGUGUGUAUGUGUGUAA	0.097
23	MIMAT0043929	>lja-miR11146-3p MIMAT0043929 GCAACGACAUGUAUAGUUGGAGG	0.076
24	MIMAT0023986	>cgr-miR-598 MIMAT0023986 UACGUCAUCGUCGUCAUCGUUAUC	0.057
25	MIMAT0005837	>mmu-miR-1187 MIMAT0005837 UAUGUGUGUGUGUAUGUGUGUAA	0.097
26	MIMAT0022724	>hsa-miR-1277-5p MIMAT0022724 AAAUAUAUAUAUAUGUACGUAU	0.082
27	MIMAT0036057	>chi-miR-211 MIMAT0036057 UUCCCUUUGUCAUCCUUUGCCC	0.060
28	MIMAT0000668	>mmu-miR-211-5p MIMAT0000668 UUCCCUUUGUCAUCCUUUGCCU	0.072
29	MIMAT0012223	>dps-miR-980-5p MIMAT0012223 AGUCUCUCACAUGGCUGGUCUAGC	0.097
30	MIMAT0022724	>hsa-miR-1277-5p MIMAT0022724	0.070

		AAAUAUAUAUAUAUGUACGUAU	
31	MIMAT0017325	>mmu-miR-466i-5p MIMAT0017325 UGUGUGUGUGUGUGUGUG	0.019
32	MIMAT0017325	>mmu-miR-466i-5p MIMAT0017325 UGUGUGUGUGUGUGUGUG	0.050
33	MIMAT0024577	>bta-miR-574 MIMAT0024577 UGAGUGUGUGUGUGAGUGUGUG	0.074
34	MIMAT0034619	>eca-miR-9074 MIMAT0034619 UGACUAAUAGGAAAUUUUAAGUGAC	0.079
35	MIMAT0022724	>hsa-miR-1277-5p MIMAT0022724 AAAUAUAUAUAUAUGUACGUAU	0.087
36	MIMAT0001322	>ath-miR414 MIMAT0001322 UCAUCUUCAUCAUCAUCGUCA	0.029

To find a novel /unique miRNA 1316 EST sequences were screened for identifying miRNAs of lowest E values. The criterion of lowest E value was between 0.01-0.099. mirbase is an online search engine used to get the micro RNA's of our specie at the end of screening all the 1316 EST's only 36 miRNAs were found that meets the criteria of required E value. We blast each EST sequence individually in mirbase and search for values that are of our interest in the transcript Table [9-11]. After getting the required E value we open its accession number and we have its structure and also an option of get sequence we click on it and copy mature RNA sequence and paste that sequence in the word file. The table given below shows the result of screening and also number of A, G, C, U/T, and A-T/U counts are listed in **Table 2**.

Table 2: Screening of different ESTs sequence with count of A, G, C, U/T, and A-T/U.

S. no	MiRNA	A Count	G Count	C Count	U/T Count	Total	GC Count	A-T/U Count
1	>hsa-miR-6873-5p MIMAT0027646 CAGAGGGAAUACAGAGGGCAAU	9	8	3	2	22	11	11
2	>bmo-miR-3270 MIMAT0015455 UGUCUUUUUCCUAAAACGAUUCA	2	2	5	10	23	7	16

3	>hsa-miR-2054 MIMAT0009979 CUGUAAUAUAAAUUUAAUUUAUU	9	1	1	12	23	2	21
4	>prd-miR-7912-5p MIMAT0030650 CAUUGCAUUUGGUUGUCCUUGGCU	2	6	5	11	24	11	13
5	>bta-miR-574 MIMAT0024577 UGAGUGUGUGUGUGUGAGUGUGUG	2	12	0	10	24	12	12
6	>mmu-miR-466i-5p MIMAT0017325 UGUGUGUGUGUGUGUGUGUG	0	10	0	10	20	10	10
7	>hsa-miR-574-5p MIMAT0004795 UGAGUGUGUGUGUGUGAGUGUGU	2	11	0	10	23	11	12
8	>mmu-miR-1187 MIMAT0005837 UAUGUGUGUGUGUAUGUGUGUAA	4	8	0	11	23	8	15
9	>mmu-miR-467g MIMAT0005854 UAUACAUACACACACAUAUAU	10	0	5	6	21	5	16
10	>hsa-miR-1277-5p	11	2	1	10	24	3	21

	MIMAT0022724 AAAUAUAUAUAUAUAUGUACGUAU							
11	>rmi-miR-5318 MIMAT0021390 UUGUAUAACUGCAGAAGACUUUUCUCC	7	4	6	10	27	10	17
12	>aly-miR831-5p MIMAT0017613 AGAAGAGGUACAAGGAGAUGAGA	11	9	1	2	23	10	13
13	>cel-miR-8210-5p MIMAT0032837 UGCCUUCUUUCCUUGUGUCGCCGAC	1	5	9	10	25	14	11
14	>cel-miR-8209-5p MIMAT0032835 AAAACGAAGAAAGAAGAAGAA	15	5	1	0	21	6	15
15	>pab-miR11418 MIMAT0044920 ACCCACCGCCGCUGCCGCCGC	2	5	13	1	21	1	3
16	>mmu-miR-466i-5p MIMAT0017325 UGUGUGUGUGUGUGUGUGUG	0	10	0	10	20	10	10
17	>hsa-miR-1277-5p MIMAT0022724	11	2	1	10	24	3	21

	AAAUAUAUAUAUAUAUGUACGUAU							
18	>tch-miR-1277-5p MIMAT0036467 UAUAUAUAUAUAUGUACGUAU	8	2	1	10	21	3	18
19	>cel-miR-8210-5p MIMAT0032837 UGCCUUCUUUCCUUGUGUCGCCGAC	1	5	9	10	25	14	11
20	>mmu-miR-467c-5p MIMAT0004885 UAAGUGCGUGCAUGUAUAUGUG	5	7	2	8	22	9	13
21	>hsa-miR-2054 MIMAT0009979 CUGUAAUAUAAAUUUAAUUUAUU	9	1	1	12	23	2	21
22	>mmu-miR-1187 MIMAT0005837 UAUGUGUGUGUGUAUGUGUGUAA	4	8	0	11	23	8	15
23	>lja-miR11146-3p MIMAT0043929 GCAACGACAUGUAUAGUUGGAGG	7	8	3	5	23	11	12
24	>cgr-miR-598 MIMAT0023986	4	4	7	9	24	11	13

	UACGUCAUCGUCGUCAUCGUUAUC							
25	>mmu-miR-1187 MIMAT0005837 UAUGUGUGUGUGUAUGUGUGUAA	4	8	0	11	23	8	15
26	>hsa-miR-1277-5p MIMAT0022724 AAAUAUAUAUAUAUAUGUACGUAU	11	2	1	10	24	3	21
27	>chi-miR-211 MIMAT0036057 UUCCCUUUGUCAUCCUUUGCCC	1	2	9	10	22	11	11
28	>mmu-miR-211-5p MIMAT0000668 UUCCCUUUGUCAUCCUUUGCCU	1	2	8	11	22	10	12
29	>dps-miR-980-5p MIMAT0012223 AGUCUCUCACAUGGCUGGUCUAGC	4	6	7	7	24	13	11
30	>hsa-miR-1277-5p MIMAT0022724 AAAUAUAUAUAUAUAUGUACGUAU	11	2	1	10	24	3	21
31	>mmu-miR-466i-5p MIMAT0017325	0	10	0	10	20	10	10

	UGUGUGU GUGUGUG UGUGUG							
32	>mmu-miR-466i-5p MIMAT0017325 UGUGUGU GUGUGUG UGUGUG	0	10	0	10	20	10	10
33	>bta-miR-574 MIMAT0024577 UGAGUGU GUGUGUG UGAGUGU GUG	2	12	0	10	24	12	12
34	>eca-miR-9074 MIMAT0034619 UGACUAA UAGGAAA UUUUAAG UGAC	10	5	2	8	25	7	18
35	>hsa-miR-1277-5p MIMAT0022724 AAAUAUAU AUAUAUAU GUACGUA U	11	2	1	10	24	3	21
36	>ath-miR414 MIMAT0001322 UCAUCUU CAUCAUC AUCGUCA	5	1	7	8	21	8	13

The structural annotation of these microRNAs to identify their structures through online software called Mfold that uses these sequences to determine the best possible secondary structures and energies of these micro RNA's. The miRNA structure that is low energy to our required parameters. These 36 miRNA structures are chosen because lowest energy structures are more stable in nature to our required E-value as shown in Supplementary Figure [12].

The functional annotationselect those microRNA whose AU content between 30% and 70%. Only 29% miRNA filled with these criteria in which we found target genes for only 10 sequences. The complete 9 sequences annotated which have basic role in biological process and molecular level is listed in **Table 3** [13].

Table 3: Functional annotation of *Mentha piperita* with respect 9 different sequence of biological and molecular level.

Peppermint MiRNA	Function	GO Term Annotations (biological process)	GO term annotations (molecular functions)
>mmu-miR-466i-5p MIMAT0017325	Protein phosphorylation electron transport chain	Cellular process. GO :0009987	Electron transfer activity GO: 0009055

UGUGUGUGUGUGUGUGUGU G	Cell redox homeostasis Activation of GTPase activity		Phosphotransferase activity GO: 0016773 GTPase activator activity GO: 0005096
>bmo-miR-3270 MIMAT0015455 UGUCUUUUUCCUAAAACGAU UCA	Cellular response to nitrogen starvation Cellular response to fatty acid Glucosinolate catabolism	Cellular metabolic process GO: 0044237	ATP binding GO: 0005524 Ascorbic acid binding GO: 0031418
>hsa-miR-6873-5p MIMAT0027646 CAGAGGGAAUACAGAGGGCAAU	Somatic cell DNA recombination	Regulation of biological process GO: 0065007	Sequence specific DNA binding GO: 0043565
>prd-miR-7912-5p MIMAT0030650 CAUUGCAUUUGGUUGUCCUUGGCU	Defense process Response to herbivore Response to fungus	Response to stimulus GO: 0050896	Not known
>bta-miR-574 MIMAT0024577 UGAGUGUGUGUGUGUGAGU GUGUG	Nucleosome assembly	Cellular component organization GO: 0071840	Histone binding GO: 0042393
>hsa-miR-8485 MIMAT0033692 CACACACACACACACACGUA U	Intra cellular signal transduction Transmembrane Protein serine/ threonine kinase signaling pathway	Signaling GO: 0023052	Transmembrane signaling receptor serine threonine kinase activity GO: 0004674
>mmu-miR-467c-5p MIMAT0004885 UAAGUGCGUGCAUGUAUAUGUG	Regulation of vesicle fusion	Localization GO: 0051179	Protein dimerization activity GO: 0046983
>rmi-miR-5318 MIMAT0021390 UUGUAUAACUGCAGAAGACU UUUCUCC	Cell proliferation Cell differentiation	Cell proliferation GO: 0008283	2 iron, 2 sulpher cluster binding GO : 0051537
>aly-miR831-5p MIMAT0017613 AGAAGAGGUACAAGGAGAUG AGA	Double DNA break repair via homologous	Reproductive process GO: 0022414	Core promoter sequence specific activity GO : 0001046 mRNA binding GO: 0003739

InterPro is a database of protein families, domains and functional sites in which identifiable features found in known proteins can be applied to new protein sequences in order to functionally characterize them. Next step to annotate these sequences which can be done by merging the GOs (obtained from inter pro database) into annotation domain of Blast2Go as shown in **Figure 1** [14]. Blast2go helps us to know about our data distribution that how much sequences were annotated with Gene Ontology (GOs). The functional annotation refers to the analysis of the biological processes associated with these components. Those 9 sequences have roles in biological process and at molecular level as well as shown in **Figure 2**.

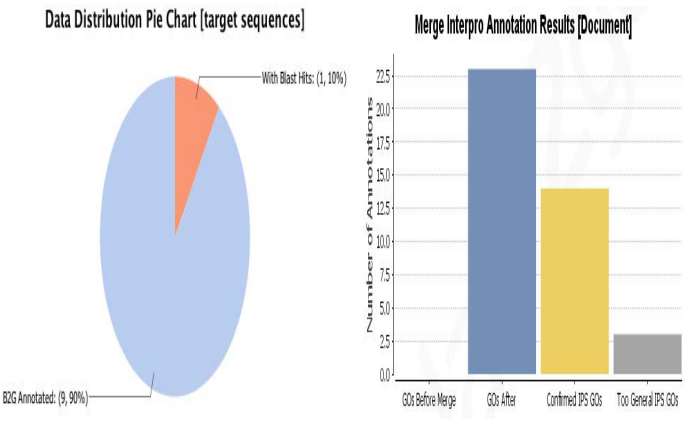

Figure 1: Functional annotation of merge interpro domain reference with gene ontology.

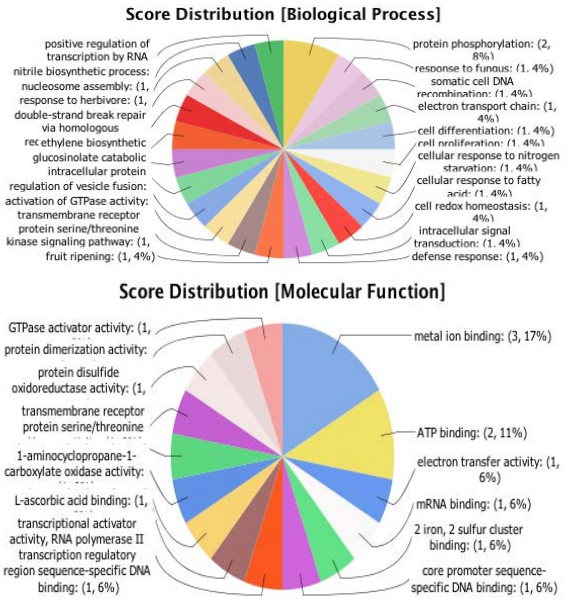

Figure 2: Functional annotation of *Mentha piperita* with reference to gene ontology biological function, molecular function.

The phylogeny analysis of *Mentha* has been blasted with Arabidopsis thaliana to confirm the conserved domain by using the online package NCBI database [15]. Based on blast results, the phylogenetic tree was constructed to confirm the conservation of *Mentha piperita* with *Arabidopsis thaliana*. The transition and transversion of nucleotide are under observation to have tree and cladogram. The branch length show among the relationship between species as shown in **Figure 3**.

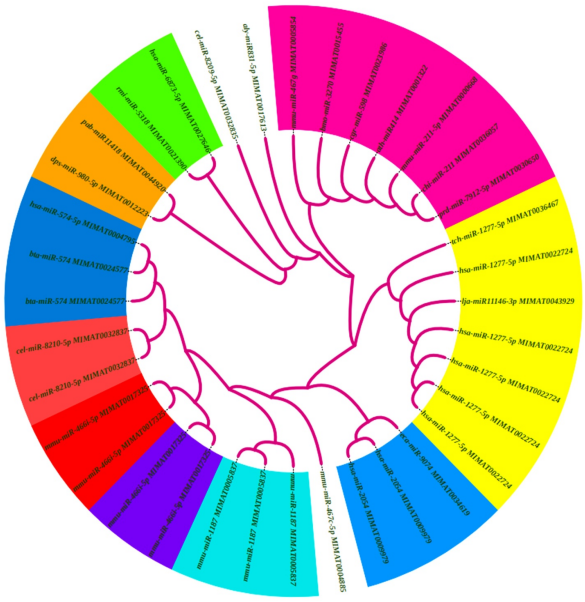

Figure 3: Phylogenetic tree analysis using the neighbor-joining method by MEGA X.

DISCUSSION

Peppermint, commonly known as *Mentha piperita*, is a perennial herb from the Lamiaceae family [16]. For its medical benefits, including its antispasmodic actions, anti-inflammatory and antibacterial peppermint has been used for centuries. Additionally, it is employed in the food and beverage company as a flavoring ingredient. In order to comprehend the molecular processes underlying peppermint's different pharmacological characteristics, recent research has concentrated on locating novel genes and miRNAs in this plant. In this study, we used EST analysis to identify 29 novel genes from peppermint [17]. The functional annotation of these genes revealed their potential roles in various biological processes, including cellular redox homeostasis, electron transport chain, and protein phosphorylation. Furthermore, we identified 36 miRNAs in peppermint, and their functional annotation suggested their involvement in cellular response to nitrogen starvation, glucosinolate catabolism, and somatic cell DNA recombination. The phylogenetic analysis confirmed the conservation of *Mentha piperita* with *Arabidopsis thaliana*. In addition, we also calculated the E-values and energies for the identified ESTs and miRNAs, providing further insights into their potential functions. Our findings provide a valuable resource for further studies on the molecular mechanisms underlying the medicinal properties of peppermint. Previous studies have also reported the medicinal properties of peppermint, including its antimicrobial, anti-inflammatory, and antispasmodic effects. Moreover, the identification of novel genes and miRNAs in peppermint has been previously reported, providing insights into the molecular mechanisms underlying its pharmacological properties [18]. Our study builds on these findings, providing novel insights into the potential functions of newly identified genes and miRNAs in peppermint. In this study, we performed transcriptome analysis of *Mentha piperita* and identified 29 Expressed Sequence Tags (ESTs) using the NCBI database with E-values ranging from 2e-20 to 8e-04. These ESTs were found to be involved in various biological processes including protein phosphorylation, electron transport chain, cell redox homeostasis, activation of GTPase activity, cellular response to nitrogen starvation, cellular response to fatty acid, glucosinolate catabolism, somatic cell DNA recombination, defense process, response to herbivore, response to fungus, nucleosome assembly, cellular component organization, intracellular signal transduction, transmembrane protein serine/threonine kinase signaling pathway, regulation of vesicle fusion, cell proliferation, cell differentiation, double DNA break repair via homologous, and reproductive process. Furthermore, we also identified 36 microRNAs (miRNAs) in *Mentha piperita* with AU content ranging between 30% and 70%. The energies of these miRNAs were calculated using the online tool RNAfold, with values ranging from -32.1 to -25.6 kcal/mol. The miRNAs were found to have basic roles in various biological processes and molecular functions such as protein phosphorylation, electron transport chain, cellular metabolic process, somatic cell DNA recombination, and regulation of biological process, cellular component

organization, signaling, localization, and cell proliferation [19]. To confirm the conservation of *Mentha piperita* with Arabidopsis thaliana, we performed phylogenetic analysis using the online package NCBI database. The results showed that the transition and transversion of nucleotides were under observation to have tree and cladogram, which confirmed the conservation of *Mentha piperita* with Arabidopsis thaliana. Overall, our study provides valuable insights into the transcriptome and miRNA profile of *Mentha piperita* and sheds light on the conservation of this plant with Arabidopsis thaliana. Further investigation into the medicinal properties and prospective uses of plant can be enhanced by the discovery of ESTs and miRNAs involved in various biological processes and molecular functions [20].

CONCLUSION

In conclusion, this study provides valuable insights into the identification and functional annotation of ESTs and miRNAs in Peppermint (*Mentha piperita*). The ESTs obtained were identified to be associated with different biological processes, such as stress responses, indicating their potential roles in the growth, metabolism, and development of Peppermint and signal transduction. The miRNAs identified in this study were discovered to have diverse functions regulation of gene expression, modulation of cellular signaling pathways and cellular responses to various stresses. Additionally, the phylogenetic analysis revealed the conservation of Peppermint with *Arabidopsis thaliana*. The functional annotation of miRNAs revealed that only 29% of the identified miRNAs fulfilled the AU content criteria between 30% and 70%. However, among these miRNAs, 9 sequences were found to have a basic role in molecular functions and biological processes.

FUTURE RECOMMENDATION

In future studies, it would be interesting to perform functional validation experiments for the identified ESTs and miRNAs to confirm their roles in peppermint growth and development. Additionally, further investigation of the miRNAs that did not meet the AU content criteria may provide insights into their potential roles in peppermint. The results of this study may provide a foundation for future research aimed at improving the yield and quality of peppermint, which could have potential benefits for the pharmaceutical and food industries.

DATA AVAILABILITY STATEMENT

The data that support the findings were derived from the following resources available in the public domain (GenBank) at https://www.ncbi.nlm.nih.gov/genbank/

CONFLICTS OF INTEREST

The authors declare that there are no conflicts of interest

AUTHOR CONTRIBUTIONS

S.I (Saqib Ishaq), J.K. and R.T designed the study. S.I (Saqib Ishaq), J.K, R.T and O.H performed various in silico analyses. R.T. and S.I wrote the initial MS. A.A, M.I.H, K.G and Y.A, R.N revised the MS. All authors have read and agreed to the published version of the manuscript.

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INSTITUTIONAL REVIEW BOARD STATEMENT

Not applicable.

INFORMED CONSENT STATEMENT

Not applicable.

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