A SYSTEMATIC ANALYSIS OF COMPOUNDS PRESENT IN *OCIMUM TENUIFLORUM* (TULSI) REGARDING ITS ANTI-INFLAMMATORY PROPERTIES USING *IN-SILICO* TECHNIQUES

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ABSTRACT

The objective of this study was to gather data, create a database of the compounds present in *Ocimum tenuiflorum* (*O. tenuiflorum*), and gather related literature on the compounds found. A thorough literature search was performed to gather information on compounds present in *O. tenuiflorum*, including chemical structures, relative abundance, presence in different plant parts, and availability from chemical supply vendors. The compounds' chemical structures were refined using Discovery Studio Visualizer and Chimera software for future *in-silico* docking studies. The structures with cleaned structural geometry were obtained through D.S. Visualizer for docking in the future. From the literature search of previously presented articles, it was found that methyl eugenol had the greatest percent composition in *O. tenuiflorum*. After searching the Protein Data Bank, COX-1, COX-2, and NF Kappa B were found to be the main protein targets of *O. tenuiflorum* compounds in the arachidonic acid inflame matory pathway. Thus, the anti-inflammatory properties of *O. tenuiflorum* have been analyzed in this article for future *in silico* docking.

1 INTRODUCTION

Tulsi is a plant of the species *Ocimum*, scientifically known as *Ocimum tenuiflorum L.* and more commonly known as "the holy basil."[5] It is native to India and parts of North and Eastern Africa, China, Hainan Island, and Taiwan, where it is also titled as "the elixir of life" or "the queen of herbs."[5] Numerous parts of *O. tenuiflorum,* such as the leaves, stems, and flower spikes, are traditionally used in Ayurveda and Siddha medicine for treating conditions such as coughs, bronchitis, fever, and bile disturbances.[5] Ayurveda and Siddha medicines are two of the most ancient medicinal branches of India based on herbal and mineral compounds. *O. tenuiflorum* has also been vividly known for its anti-inflammatory, antiseptic, and other numerous organ protective properties.[5] Based on its prominent medicinal uses, the *Ocimum tenuiflorum* was chosen for further investigation. In this study, the focus was on inflammation because it is a well-known symptom of numerous infectious and non-infectious diseases. Various in vitro and in vivo studies have documented the anti-inflammatory effects of *O. tenuiflorum*, and it has been suggested that *O. tenuiflorum* has many bioactive secondary metabolites that help inhibit certain inflammatory pathways synergistically or alone.[6]

Arachidonic acid is the major polyunsaturated fatty acid present in mammalian systems, which is oxygenated by three important pathways—the cyclooxygenase (COX), the lipoxygenase (LOX), and the epoxygenase pathways.[10] The inhibition of COX and LOX pathways in arachidonic acid metabolism in conjunction with major *O. tenuiflorum* compounds like eugenol and linoleic acid contributes to the anti-inflammatory action of *O. tenuiflorum*, observed in both acute and chronic inflammatory models in animals.[4] This observation suggests that certain compounds present within *O. tenuiflorum are* responsible for anti-inflammatory responses in ani-

mal models. This implication can be further explored to determine which compounds in *O. tenuiflorum* may induce anti-inflammatory activity in humans. As a result, *O. tenuiflorum* has demonstrated anti-inflammatory effects normally observed in nonsteroidal anti-inflammatory drugs (NSAID) such as phenylbutazone, ibuprofen, naproxen, aspirin, and indomethacin.[4] The inhibition of the cyclooxygenase enzymes COX-1 and COX-2 is what drives the NSAID mechanism.[1] Although NSAIDS are effective, they have a few common side effects, such as nausea, dyspepsia, vomiting, and skin reactions. On the contrary, herbal medications are devoid of such side effects, which illustrates the importance of conducting a study of the compounds found in O. tenuiflorum: it may open the path for herbal NSAIDs without these side effects.[1]

O. tenuiflorum has various transcription factors, one of which is the nuclear factor-Kappa B (NF Kappa B). NF Kappa B is one of the major regulators of inflammation, cellular transformation, tumor cell survival, proliferation, invasion, angiogenesis, and metastasis as compared to the other transcription factors.[11] Plants of the *Ocimum* species are known to have an abundance of terpenes.[7] Additionally, plant isolates containing terpenoids (a modified class of terpenes) have been found to suppress NF kappa B signaling, a protein complex linked to the pathogenesis of inflammatory diseases, cancer, viral infection, and autoimmune diseases.[7] Thus, the study of *O. tenuiflorum* for anti-inflammatory properties is vital. There are numerous compounds present in the *Ocimum* species amongst which methyl eugenol, β-selinene, γ-murolene, rosmarinic acid (phenolic), ursolic acid, and camphene are some major ones based on their percentage of makeup. These and other compounds from *O. tenuiflorum* have been characterized and analyzed in this study to form an extensive database. This database is used to determine which of the compounds identified would interact with desirable molecular targets and decrease inflammation in future studies.

Therefore, in this study, an *in-silico* approach was used to refine the compounds present in *O. tenuiflorum* and to develop a highly detailed database to be used for future purposes, such as investigating how an individual compound from *O. tenuiflorum* extract will interact with these molecular targets. This interaction would be detailed using the *in-silico* docking mechanism, which is a molecular modeling technique that is used to predict how a protein (enzyme) interacts with small molecules (ligands) to deduce their mechanism of action on targets found in future dry labs.

2 METHODOLOGY

First, a literature search was conducted through Pubchem to create a list of well-known compounds present in *O. tenuiflorum* and their percent composition in the part of the plant they are found in.[8] The vendors that distribute the compound in the USA were identified from PubChem as well (TABLE 1).[8] Next, the collection of the virtual structures of all the compounds was created and the files were saved in the SDS (2D) formats (FIGURE 1). Then, within the structural data, the pharmacology and biochemistry of the compounds were reviewed for each compound from PubChem in order to get an idea of the mechanism of action and the aspects of human interaction associated with the individual compounds.[8] This was an adaptation process, so new compounds were added to the list when and if they were discovered throughout the study.

Later, software such as Discovery Studio (D.S.) Visualizer (BIOVIA Dasasult Systemes, San Diego, CA, USA) and Chimera were downloaded.[9] Chimera was used to convert the mol format files into sybyl mol 2 format files for compatibility within D.S. Visualizer. The sybyl mol 2 format files were then opened in D.S. visualizer, wherein hydrogen atoms were added to the structure. The geometry of the hydrogen atoms was cleaned and 3D coordinates were assigned to them. The three-dimensional structure was then saved as sybyl mol 2 format files (FIGURE 2). The formats sybyl mol 2 and SDS were employed to have a better approximation of the structure of the compounds, and to notice the 2D and 3D differences in the molecules, which would visually assist in the molecular docking.

And finally, another literary search was conducted on the Protein Data Bank for the inflammatory molecular pathways and *O. tenuiflorum* within

	COMPOUNDS	PERCENTAGE FOUND	VENDORS	PART OF PLANT
1.	1,8-CINEOLE	TRACE(T)	ALL	LEAVES
2.	1,10 DI-EPI-CUBENOL	1.8	N/A	LEAVES
3.	3,4-DIMETHOXYCINNAMIC ACID (PHENOLIC)	N/A	ALL	N/A
4.	3.4.5-TRIMETHOXYCINNAMIC ACID	N/A	1,2,4	N/A
5.	4.4′-METHYLENE-BIS (2-METHYL ANILINE)	N/A	N/A	N/A
6.	A-ELEMENE	0.5	2	FLOWER SPIKES
7.	A-HUMULENE	0.2	4	LEAVES
8.	A-PINENE	0.2	2	LEAVES
9.	A-TERPINEOL	TRACE(T)	1,2,3	LEAVES
10.	ALLOAROMADENDRENE	1.16	N/A	N/A
11.	APIGENIN	N/A	2	LEAVES
12.	B-BOURBONENE	0.2	N/A	LEAVES
13.	B-CUBEBENE	0.1	2,4	FLOWER SPIKES
14.	B-PINENE	0.1	2,3	LEAVES
15.	**B-SELINENE**	**3.3**	**2,4**	**LEAVES**
16.	B- SESQUIPHELLANDRENE	0.2	N/A	LEAVES, FLOWER SPIKES
17.	BAICALIN (LAVONOIDS)	N/A	2,4	N/A
18.	BENZALDEHYDE	0.44	2,4	LEAVES
19.	BICYCLOGERMACRENE	TRACE(T)	N/A	LEAVES
20.	CAFFEIC ACID (PHENOLIC)	N/A	ALL	LEAVES
21.	**CAMPHENE**	**0.1**	**1,2,3**	**LEAVES**
22.	CAMPHOR	0.1	2,3	LEAVES
23.	CARNOSIC ACID	N/A	2,4	N/A
24.	CHRYSOERIOL (FLAVON)	N/A	1,2,4	N/A
25.	CI A-COPAENE	1.9	N/A	LEAVES, FLOWER SPIKES
26.	CI A-CUBEBENE	TRACE(T)	4	FLOWER SPIKES
27.	CI B-CARYOPHYLLENE	4.1	2,3,4	LEAVES
28.	CI B-GURJUNENE	TRACE(T)	N/A	LEAVES
29.	CI BORNEOL	2.4	2	LEAVES
30.	CI TERPIN-4-OL	0.1	2,3	LEAVES, STEM
31.	CUBEBOL	0.3	N/A	LEAVES
32.	DI (ETHYLHEXYL) PHTHALATE	N/A	1,2	N/A
33.	DI-N-BUTYL PHTHALATE, DIBUTYL PHTHALATE	N/A	1,2,4	LEAVES

TABLE 1: *This table shows various compounds found in specific parts of* O. tenuiflorum *such as the leaves, stem, or flower spikes. It also includes their percentage composition, the vendors who supply them in the USA, and their PubChem SID and Purchasable Chemical ID. Some of the compounds have trace(t) in their "Percentage Found" column, which implies that the compound is present in a quantity too small to be measured.* PubChem[8]

	COMPOUNDS	PERCENTAGE FOUND	VENDORS	PART OF PLANT
34.	DIOSMETIN (FLAVONE GLYCOSIDE)	N/A	1,2,4	N/A
35.	(E)-A-BERGAMOTENE	0.72	2,4	LEAVES, FLOWER SPIKES
36.	(E)-B-OCIMENE	1.9	2,4	LEAVES
37.	E-METHYL CINNAMATE	1.5	ALL	LEAVES, STEM
38.	EPI-A-CADINOL	1.03	N/A	LEAVES
39.	ESTRAGOL	N/A	1,2,4	LEAVES, FLOWER SPIKES
40.	EUGENOL	0.9	ALL	LEAVES
41.	**Γ-MUROLENE**	**5.82**	**2**	**LEAVES**
42.	GERMACRENE A	0.7	N/A	LEAVES
43.	GERMACRENE D	2.3	2,4	LEAVES
44.	GERANIOL	TRACE(T)	ALL	LEAVES
45.	GLOBULOL	1.05	2,4	N/A
46.	ISOSAKURANETIN (LAVANONE)	N/A	4	N/A
47.	KAEMPFEROL	N/A	ALL	N/A
48.	LINALOOL	0.5	3	LEAVES
49.	LIMONENE	0.2	1,2	LEAVES
50.	LUTEOLIN (FLAVONOID)	0.5	N/A	LEAVES
51.	METHYL CHAVICOL	TRACE(T)	1,2,3	LEAVES
52.	**METHYL EUGENOL**	**82.9**	**2,3,4**	**LEAVES**
53.	MYRTENAL	N/A	2	LEAVES
54.	NEVADENSIN (FLAVONES, GLYCOSIDES)	N/A	2,4	N/A
55.	P-CYMENE	TRACE(T)	1,2,3	FLOWER SPIKES
56.	PEDUNCULIN	N/A	N/A	N/A
57.	PERMETHRIN	N/A	1,2	N/A
58.	PEINIDIN (ANTHOCYANIDINS)	N/A	N/A	N/A
59.	**ROSMARINIC ACID (PHENOLIC)**	**0.27**	**2,4**	**LEAVES, STEMS**
60.	SABINENE	TRACE(T)	2	LEAVES
61.	Δ-CADINENE	1.1	2,4	LEAVES
62.	(TRANS)-B-FARNESENE	0.12	2,4	LEAVES
63.	(TRANS)-B-GUAIENE	0.29	N/A	LEAVES
64.	**URSOLIC ACID**	**2.5**	**1,2,4**	**LEAVES**
65.	XANTHOMICROL	N/A	2	STEM
66.	(Z)-3-HEXANOL	1.8	1,2	LEAVES

VENDORS

1) **TIM TEC**
2) **MUSECHEM**
3) **ACROS ORGANICS**
4) **ZINC**

TABLE 1 CONTINUED

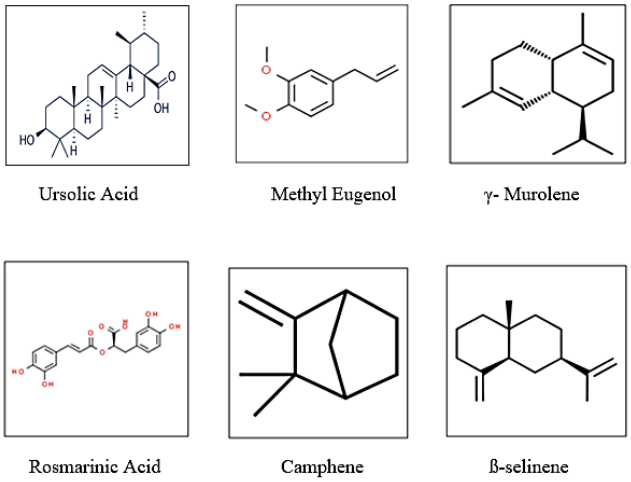

FIGURE 1: *The 2D structures of compounds present in* O. tenuiflorum *are shown here, such as ursolic acid, methyl eugenol, γ-murolene, rosmarinic acid, camphene, and β-selinene. Some of the figures that were downloaded from PubChem portray the oxygen and hydroxide atoms in red fonts.*

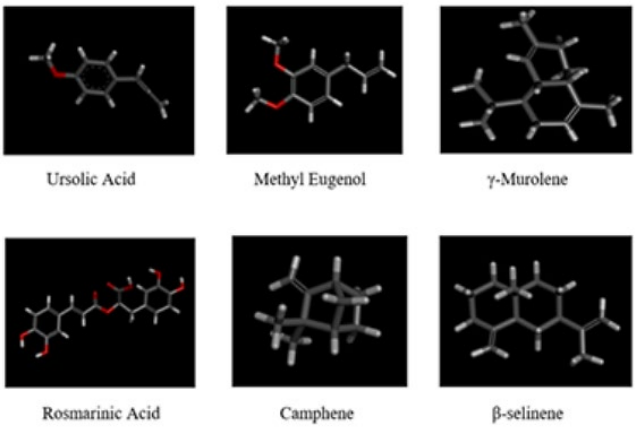

FIGURE 2: *3D structures of a few of the major compounds present in* O. tenuiflorum, *such as camphene, methyl eugenol, rosmarinic acid, β-selinene and γ-murolene with hydrogen atoms added and their geometry cleaned.*

the context of inflammation and its molecular targets concerning humans.[2] This search was conducted to determine which molecular target should be further used to analyze the docking compatibilities of the compiled *O. tenuiflorum* compounds.

3 RESULTS

A detailed database was created in TABLE 1 to compile an extensive list of 66 compounds in *O. tenuiflorum*. TABLE 1 also displays information about the percentage of a particular compound present in certain parts of *O. tenuiflorum*, such as the leaves, stem, flower spikes, etc. In TABLE 1, it is evident that most of the compounds were available from the list of US-based vendors like Tim Tec, Musechem, Acros organics, and ZINC. The PubChem SID and Purchasable Chemical ID were also available for all of the compounds. The highest percentage of composition for a compound in *O. tenuiflorum* was for methyl eugenol, about 82.9% within the leaves, which was available from Musechem, Acros organics, and ZINC (TABLE 1). Overall, a total of 66 compounds were identified to be present in *O. tenuiflorum*. Out of those compounds, many were in trace amounts and some did not have any data on the percentage of their presence (TABLE 1).

Next, 2D structures in SDS format files were obtained from PubChem in the form of 2D ChemDraw files (FIGURE 1). FIGURE 1 shows the chemical structure of a few major compounds found in *O. tenuiflorum*. The compounds seen were not ready for docking since they had not been refined yet to allow for further *in-silico* processes due to their structural ambiguity. It would be difficult to dock them; therefore, further work on each compound was needed, such as adding hydrogens and removing unwanted ions (FIGURE 1). Next, the refined structures were obtained from D.S. Visualizer in order to transfer the coordinates to proceed to the *in-silico* docking step (FIGURE 2). The refined structure of several *O. tenuiflorum* compounds are portrayed in FIGURE 2.

Finally, the literature search on the Protein Data Bank yielded that COX-1, COX-2, and NF Kappa B were the anti-inflammatory target proteins that interact with certain compounds found in *O. tenuiflorum*. However, within the Protein Data Bank the COX-2 protein was the most prevalent target found in humans and COX-1 had the least human entries. These targets will be explored in future studies with the collected *O. tenuiflorum* database compounds.

4 DISCUSSION & CONCLUSION

For this study, a thorough database was created for various compounds present in different parts of *O. tenuiflorum*. This database is being stored on the computers in the Arora lab to be used for future studies. During the literary search of these structures, it was found that methyl eugenol was the most abundant compound and **γ**-Murolene was the second most abundant compound. Both compounds are present in the leaves of *O. tenuiflorum*. These compounds are significant because they are present in such high abundance, which suggests that they might have an important role in the anti-inflammatory effect of *O. tenuiflorum*. To test this statement, these structures will have to be run through *in-silico* docking modules to determine whether the abundance has any correlation with the anti-inflammatory effect.

Moreover, the purpose of attaining the 2D files was to make the structures available before refinement so that they could be reviewed when required. The refined structures with cleaned geometry were obtained so that their docking sites were clear of any hindrance and to ensure that docking could be done properly in the future. Also, the target proteins were identified from the Protein Data Bank so that they could be used along with their ligands to dock. Thus, the future goal is to use *in-silico* approaches to discover how compounds will interact with various molecular targets and inflammatory pathways, specifically COX-2, as it is the most prevalent target yielded in most searches related to human query within the Protein Data Bank. The database gathered in this study will be crucial for future studies to identify which compounds present in *O. tenuiflorum are* attributed to its anti-inflammatory properties. This information could in turn allow for the development of herbal anti-inflammatory medication with minimal side effects■

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Jinay Patel is Rutgers University graduate, having B.S degree in Biotechnology and Plant Science from School of Environmental and Biological Science. His research was on *O. tenuiflorum* to gather the data on the compounds present within it and study its anti-inflammatory properties using In-Silico techniques. He is currently working as a QC analyst at Roche Molecular Systems within enzymatic department and in the future, he would like to attend graduate school and study drug development. Education in drug development for him would entail discovering new medicines and treating diseases to support better patient care. The following research on *O. tenuiflorum* is one of the crucial steps for him in attaining this goal.