Dubai Medical Journal
Volume 8, Issue no. 2, DOI 10.18502/dmj.v8i2.19001
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Research Article

Evaluation of Interactions Between Transient Receptor Potential Vanilloid 1 and Active Constituents Using Molecular Docking

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Abstract

Introduction: Pain, whether acute or chronic, imposes huge economic burdens in the societies due to complexities in the signaling pathways that lead to pain. There are many nociceptive receptors and channels that play key roles in the processing and maintenance of pain. Transient receptor potential vanilloid 1 (TRPV1) is a nonselective cation channel that is considered a crucial target in pain. Drugs that are available in the market have side effects, highlighting the need to find new analgesics that have no side effects.

Methods: In this study, we assessed the interactions between active constituents and TRPV1 receptor using molecular docking in comparison to capsazepine and the analgesic drug SB-3567791; 3-(4-chlorophenyl)-N-(3-methoxyphenyl)-2-propenamide. Molecular docking is a computational technique that provides calculation for the non-covalent binding of protein or receptor and ligand molecule(s) and aids in investigating the binding interaction between ligands and receptors. These selected active constituents belong to different classes of secondary metabolites including alkaloids, terpenoids, and flavonoids.

Results: The results indicate promising binding affinities between and TRPV1 receptor and speciofoline, mitragynine, 7-hydroxymitragynine, salvinorin, mescaline, acacetin, ladanein, vulgarin, marrubiin, geranial, neral, epi-α-cadinol (t-cadinol), myrtenol, δ-cadinene, and α-terpineol. The compound α-calacorene showed no affinity toward TRPV1 receptor at all.

Conclusion: Many active compounds are ligands for TRPV1 channel and can be assessed to determine their effects on inhibiting TRPV1 as analgesics. These results allow us to assess the effects of many of these compounds on TRPV1 channel in vivo and in vitro.

Keywords: TRPV1, active constituents, ligands, docking, receptor

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Received: October 4, 2024
Accepted: December 7, 2024
Published: June 30, 2025

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How to cite this article: Jaffal S, Khan S, Khan MI. Evaluation of Interactions Between Transient Receptor Potential Vanilloid 1 and Active Constituents Using Molecular Docking. *Dubai Medical Journal*. 2025;8(2):140–152. DOI 10.18502/dmj.v8i2.19001

1. Introduction

Pain is an important signal that alerts the body to noxious stimuli and decreases the risk of tissue damage [1]. However, chronic pain has negative consequences and represents a vital socioeconomical burden [2]. Several reports identified headache, chronic back pain, and road traffic accidents among the top 10 causes of disabilities and showed that patients with chronic pain suffer from anxiety and depression [1, 2]. At the same time, pain is considered a heterogeneous disorder that has no single cause. Thus, a large body of research has been conducted to understand the pathways responsible for pain processing and perception and to find approaches for pain management. For instance, many studies were conducted to find inhibitors for the availability and/or functionality of transient receptor potential vanilloid 1 (TRPV1), which is a thermosensitive, non-selective cation channel and a marker for a population of C-fibers (unmyelinated, slowly conducting neurons) that comprise 30–50% of all somatosensory neurons within ganglia [3]. Thus, TRPV1 channel is a marker for neurons that express the neuropeptides substance P, calcitonin gene-related peptide (CGRP), and neurokinin A [3]. This receptor is activated by extracellular, protons, capsaicin, heat, toxins, and inflammatory mediators [4]. The receptor can also be activated by isothiocyanates, menthol, thiosulfinates, and related pungent irritants [3, 4]. Multiple studies highlighted that active constituents are considered promising therapeutics for the modulation of TRP channels, including TRPV1 receptor [5]. There are several classes of active constituents, including alkaloids, terpenoids, and flavonoids [6]. Terpenoids (or isoprenoids) are one of the largest natural product families that have lipophilic characteristics, including sesquiterpenes, diterpenoids, monoterpenes [6]. Alkaloids are nitrogen-containing organic compounds that are divided into non-heterocyclic (also known as atypical alkaloids, protoalkaloids, or biological amines) and heterocyclic (also known typical alkaloids or true alkaloids) [6]. The aim of this paper was to analyze the interactions between selected active compounds and TRPV1 receptor in comparison to capsazepine and the analgesic drug SB-366791 using molecular docking to identify new natural sources of analgesics.

2. Materials and Methods

In the current study, molecular docking of various phytochemical compounds was performed against TRPV1 receptor. AutoDockVina software (version 4.2.6) was used to perform molecular docking interaction. AutodockTools (version 1.5.7) which is part of Molecular Graphics Laboratory (MGL) Tools suit that was used to provide the graphical interface to AutoDock software and also used to visualize and analyze molecular structures. This software was developed at the Scripps Research Institute, Molecular Graphics Laboratory, Department of Molecular Biology, La Jolla California, USA. In the present study, TRPV1 protein (pdb id: 8gfa) was downloaded from RCSB PDB database (https://www.rcsb.org) and saved in pdb format. The protein receptor was prepared using AutodockTools-1.5.7 [7]. Similarly, all ligands (speciofoline, mitragynine, 7-hydroxymitragynine, salvinorin, mescaline, acacetin, ladanein, vulgarin, marrubiin, geranial, neral, epi-α-cadinol (t-cadinol), myrtenol, δ-cadinene, and α-terpineol) were downloaded from PubChem compound database and saved in sdf format. The selected ligands were prepared using PyMOL (version; 2.5.4, developed by Schrödinger, New York, USA) and AutodockTools-1.5.7. Molecular docking was performed using AutoDockVina-4.2.6 to investigate the binding affinities of selected ligands against

TRPV1 receptor. The amino acids of binding sites were determined and a grid box was created having dimensions of center_x=102.052, center_x=86.589, center_x=89.908 with grid spacing of size_x=24, size_y=24, size_z=24, and exhaustiveness=8. The compounds docked with the lowest binding energy and best pose were chosen and saved. The results were visualized using Protein-Ligand Interaction Profiler (PLIP) tool (developed at Heidelberg University in Germany by Structural Bioinformatics Groups), Proteins Plus (developed at University of Hamburg in Germany by Center for Bioinformatics) and Discovery Studio 2021 (developed by BIOVIA). Moreover, the hydrogen bond and hydrophobic interactions were evaluated with PLIP [7, 8].

3. Results

In the present study, the binding affinities of various compounds were investigated using AutoDockVina-4.2.6. The AutoDock Tools-1.5.7 graphical user interface was used to add polar hydrogen and to create Kollman charges for the TRPV1 receptor. The active sites of TRPV1 binding domain consist of PHE-543, ALA:546, LEU:547, PHE-591, LEU-663, ALA-666, LEU:670, LEU-553 THR:550, and TYR:511 [9, 10]. The binding affinities of each ligand were evaluated by enclosing the TRPV1 binding domain in the grid box. The results indicate promising binding affinity toward TRPV1 receptor except for α-calacorene, which shows no affinity at all. The binding energy, hydrogen bond, and hydrophobic interaction are listed in Table **1**. Figures **1** and **2** show the 3-dimensional (3D) and 2-dimensional (2D) images of respective ligands and their interactions with TRPV1 receptor. Moreover, the heat map indicates the binding affinity as shown in Figure **3**. In comparison, capsazepine and the analgesic drug SB-366791 were also docked against TRPV1 receptor, showing binding affinities of -8.7 and -9.3 kcal/mol, respectively (Figure **4**). All the selected compounds were set in the same binding pocket as compared to capsazepine and SB-366791.

Table 1: Molecular docking of selected compounds against TRPV1.

No.	Ligand-protein interaction	Binding energy (Kcal/mol)	Hydrophobic interaction	Hydrogen bond
1	Speciofoline-TRPV1	-6.7	PHE A:543, ILE A:573, LEU A:577, ALA B:666, ILE B:669, LEU B:670	THR A:550
2	Mitragynine-TRPV1	-7.5	LEU A:515, LEU A:547, LEU A:553, ILE A:569, ILE A:573, LEU B:670, LEU B:670	TYR A:511, TYR A:511
3	7-Hydroxymitragynine-TRPV1	-6.6	TYR A:511, LEU A:515, LEU A:515, LEU A:553, ILE A:569, GLU A:570, ILE A:573, LEU B:670	TYR A:511, THR A:550, THR A:550
4	Salvinorin-TRPV1	-7.1	PHE A:507, TYR A:511, LEU:547, THR A:550, PHE B:587, ALA B:666, LEU B:670	THR A:550
5	Mescaline-TRPV1	-4.7	ALA A:646, LEU A:647, PHE B:591, ALA B:666, LEU B:670	TYR A:511, LEU A:547
6	Acacetin-TRPV1	-8.4	LEU:515, LEU:547, THR:550, LEU:553, ILE:573, PHE B:587, LEU B:670, TYR A:511	THR A:550, ARG A:557, ALA A:566, GLU A:570
7	Ladanein-TRPV1	-7.9	LEU A:515, ILE A:573, ALA B:666, LEU B:670, LEU B:670, TYR A:511	TYR A:511, THR A:550, ASN A:551
8	Vulgarin-TRPV1	-8.4	LEU A:515, THR A:550, LEU A:553, LEU A:553, ALA A:566, GLU A:570, PHE B:587, LEU B:670	TYR A:554, ALA A:566

Table 1: Continued.

No.	Ligand-protein interaction	Binding energy (Kcal/mol)	Hydrophobic interaction	Hydrogen bond
9	Marrubiin-TRPV1	-7.5	TYR A:511, LEU A:515, PHE A:543, ALA:546, ILE A:573, PHE A:591, PHE B:591, ALA B:666, LEU B:670	
10	Geranial-TRPV1	-6.0	TYR A:511, LEU A:515, LEU A:553, LEU A:553, ALA A:566, ILE:569, PHE B:587	ARG A:557, GLN A:701
11	Neral-TRPV1	-5.9	TYR A:511, LEU A:553, GLU A:570, ILE A:573, PHE B:587, LEU B:670	SER A:512, ARG A:557
12	α-Calacorene-TRPV1	—	—	—
13	t-Cadinol-TRPV1	-7.0	PHE A:543, ALA A:546, LEU A:547, LEU A:547, PHE B:591, PHE B:591, LEU B:663, ALA B:666, LEU B:670, LEU B:670	THR A:550, THR A:550
14	Myrtenol-TRPV1	-5.7	PHE A:543, ALA A:546, LEU A:547, PHE B:591, PHE B:591, LEU B:663, LEU B:670	
15	δ-Cadinene-TRPV1	-7.5	PHE A:543, LEU A:547, LEU A:547, PHE B:591, PHE B:591, LEU B:663, PHE B:666, LEU B:670, LEU B:670	
16	α-Terpineol-TRPV1	-6.6	TYR A:511, LEU A:553, ALA A:566, ILE A:569, ILE A:573, PHE B:587, LEU B:670	

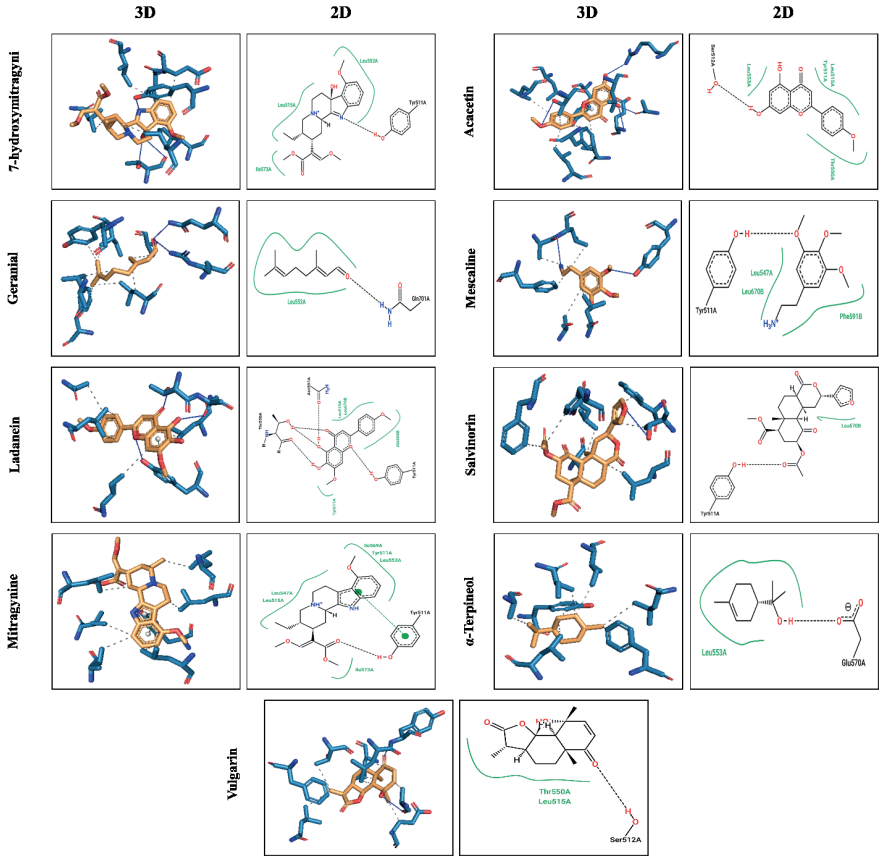

Figure 1: Molecular docking evaluation of selected ligands against TRPV1 receptors. The figure demonstrates binding interactions of ligands with TRPV1 receptors and their 3D and 2D visualization.

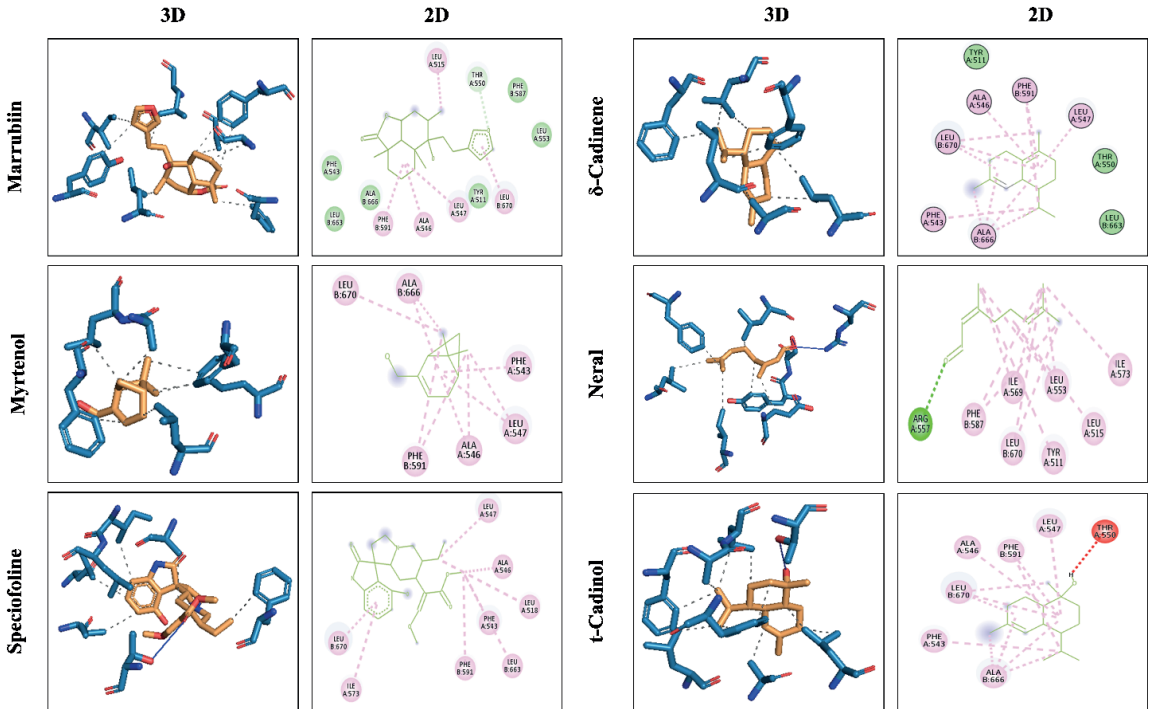

Figure 2: Molecular docking evaluation of selected ligands against TRPV1 receptors. The figure demonstrates binding interactions of ligands with TRPV1 receptors and their 3D and 2D visualization.

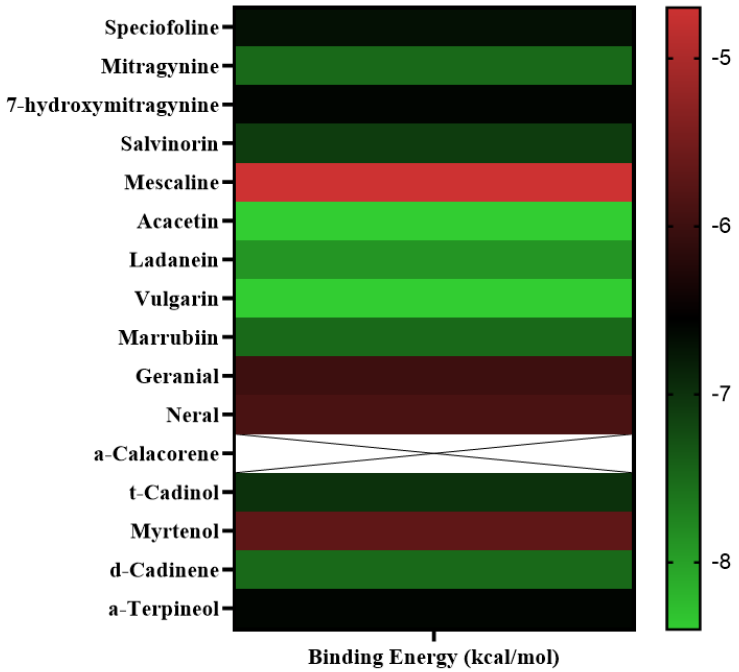

Figure 3: Heat map showing binding energies of all selected ligands against TRPV1 receptor.

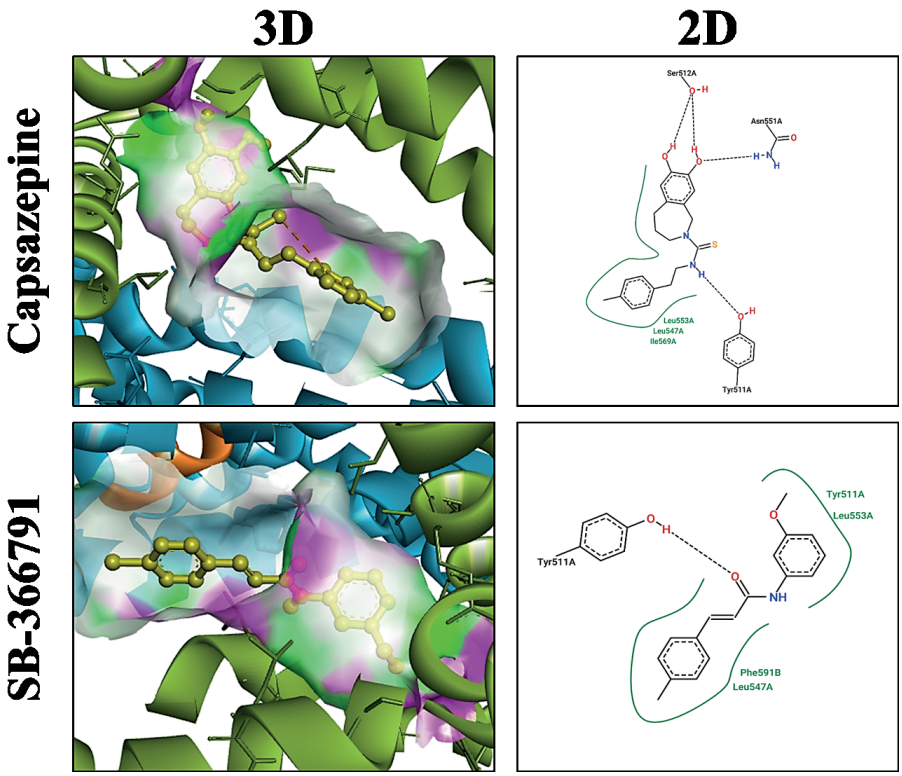

Figure 4: Molecular docking evaluation of capsazepine and anagesic drug SB-3567791 against TRPV1 receptor. The figure demonstrates binding interactions of ligands with TRPV1 receptor and their 3D and 2D visualization.

4. Discussion

Pain is one of the main health problems that urge us to look for natural therapies with fewer side effects. TRPV1 receptor is a highly validated pain target associated with pain and nociception [11]. In this research, we evaluated the possible interaction between selected active constituents and TRPV1 receptor in comparison to capsazepine and the analgesic drug SB-366791 using molecular docking, which is a computational technique that provides calculation for the non-covalent binding of protein or receptor and ligand molecule(s) and aids in investigating the binding interaction between ligands and receptors [12]. The current results revealed significant binding affinities and non-covalent binding such as hydrophobic interactions and/or hydrogen bonding of selected ligands towards the binding sites of TRPV1 receptor. In more detail, promising binding affinities were identified between TRPV1 receptor and speciofoline, mitragynine, 7-hydroxymitragynine, salvinorin, mescaline, acacetin, ladanein, vulgarin, marrubiin, geranial, neral, epi-α-cadinol (t-cadinol), myrtenol, δ-cadinene, and α-terpineol. The compound α-calacorene showed no affinity towards TRPV1 receptor at all. The tested compounds belong to different classes of active constituents including alkaloids, flavonoids, and terpenoids. These compounds were identified in multiple medicinal plants that have key therapeutic values such as anti-nociceptive, antioxidant, anti-microbial, and anti-inflammatory effects. In more detail, González et al. reported that δ-cadinene, epi-α-cadinol (t-cadinol), and α-cadinol represent 16.5%, 12%, and 8.8% respectively of the

constituents in the volatile oil and methanolic extracts of *Xenophyllum poposum* that is used to alleviate intestinal inflammation, rheumatism, and abdominal pain in folk medicine [13]. Marrubiin is a diterpenoid lactone which exists in many medicinal plants of the genus *Marrubium* (*Lamiaceae*) [14]. Particularly, marrubiin isolated from *Marrubium vulgare* demonstrated anti-nociceptive properties [15]. Additionally, in research conducted to reduce the lactone ring of marrubiin to produce marrubiinic acid, it was shown that it has more potent analgesic activity than maarubiin itself [16]. Also, ladanein is a flavonoid that was isolated from different *Ballota* species and other medicinal plants that showed anti-cancerous activity such as *Marrubium vulgari* [17, 18].

Vulgarin is another compound that was identified as a ligand for TRPV1 receptor in this study. Vulgarin is an eudesmanolide sesquiterpene that was isolated from *Artemisia judaica* and was refluxed with iodine to produce two derivatives that were purified and spectroscopically identified as naproxen methyl ester analogs. Speciofoline, mitragynine, and 7-hydroxymitragynine are alkaloids available in many medicinal plants such as kratom (*Mitragyna speciosa*). Importantly, Todd et al. reported that mitragynine and 7-hydroxymitragynine are partial agonists for μ opioid receptor, while speciofoline did not have affinity for μ-, δ- or ƙ opioid receptors [19]. In our study, we identified that the three alkaloids tested in the study of Todd et al. are ligands for TRPV1 receptor and exhibit similarity to capsazepine and SB-366791 [19]. Salvinorin A is another tested compound that we used in this study. It is an agonist for kappa opioid, cannabinoid, TRPA1 and TRPV1 receptors and exhibits anti-nociceptive activity in thermal and chemical assays [20]. Mescaline (3,4,5-trimethoxyphenethylamine) is a naturally occurring alkaloid that has psychedelic properties and is present in many medicinal plants [21]. Mescaline showed similar neurological and biochemical patterns to morphine with respect to anti-nociceptive effects in rabbits [22]. According to Carballo-Villalobos et al., acacetin is a bioflavonoid that exhibit anti-nociceptive/anti-inflammatory effects [23]. In another study, the antipyretic, anti-nociceptive, and spasmolytic response effects of acacetin were reported [13, 24]. Additionally, geranial and neral are monoterpene aldehydes that exist in many medicinal plants that have anti-nociceptive properties such as *Pimenta pseudocaryophyllus* and *Cymbopogon citratu* [25, 26]. In a study conducted on citral, 3,7-dimethyl-2,6-octadienal (an isomer of the two aldehydes: neral cis isomer and geranial trans isomer), citral was effective in reducing orofacial pain (acute and chronic) in a mechanism that involves TRPV1, TRPM3, and TRPM8 receptors [25, 27]. The anti-nociceptive effect of citral was also reported by Nishijima et al. [26]. Previous studies did not determine whether the anti-nociceptive effect of citral is related to renal or geraniol or both compounds that join as the isomer citral. α-Calacorene is another active constituent that was examined in this paper. It is identified in many medicinal plants that exhibited valuable therapeutic effects such as the Brazilian medicinal plant *Xylopia laevigata*, whereby its leaves have antioxidant, anti-inflammatory, anti-cancerous, and anti-nociceptive effects by acting on the descending pathway of the central nervous system [28]. Additionally, α-calacorene is one of the volatile constituents that were identified in the aqueous extract and essential oil of *Baccharis heterophylla* and was effective in decreasing pain [29]. In a study conducted by Zhang et al., δ-cadinene and α-terpineol were determined as constituents in essential oil recipe prepared from multiple Chinese herbs such as long pepper, saffron, myrrh, cinnamon, and white pepper, which were used in traditional

Hui medicine to prepare aromatic drugs to relief pain and inflammation [30]. Myrtenol is a plant-derived monoterpene alcohol that causes the inhibition of cell migration and the release of inflammatory mediators and is involved in the signaling pathways of certain nociceptive receptors [31]. This constituent decreased carrageenan-induced interleukin 1β and myeloperoxidase activity [31]. Finally, we recommend examining the effects of the previously mentioned compounds using in vitro and in vivo assays to ascertain whether they act as agonists or antagonists for TRPV1. Finding novel antagonists for TRPV1 receptor can open a new gate for pain relief.

By looking at the published research regarding the pharmacokinetics and bioavailability of these compounds, it was found that these compounds have variable bioavailability. Speciofoline, mitragynine, and 7-hydroxymitragynine alkaloids primarily undergo hepatic metabolism. mitragynine shows moderate bioavailability but has potential for addiction and adverse effects like hepatotoxicity and respiratory depression, especially at higher doses. Detailed research, specifically on speciofoline's pharmacokinetics, is limited [32]. Salvinorin A is known for rapid brain uptake and a short duration of action. It is primarily metabolized in the liver with low bioavailability. While it shows low toxicity at moderate doses, high doses can result in intense psychological effects [33, 34]. Mescaline's toxicity is generally low, but high doses may induce cardiovascular and psychological side effects [35]. In contrast, Acacetin has moderate bioavailability and low toxicity in animal models, and Ladanein has limited detailed pharmacokinetic data [36]. Vulgarin was reported to have moderate oral absorption and liver metabolism, though more data on its toxicity is needed [37]. Marrubiin has high bioavailability, and animal studies indicate a favorable toxicity profile supporting its therapeutic potential [38]. Regarding geranial, neral, epi-α-cadinol (t-cadinol), myrtenol, δ-cadinene, and α-terpineol: these monoterpenes and sesquiterpenes are known for their moderate bioavailability and metabolism through cytochrome P450 enzymes. Generally low in toxicity, certain compounds like δ-cadinene and α-terpineol may cause mild irritation at high concentrations [37].

5. Conclusion

Many active constituents were identified as promising anti-nociceptive compounds that can be devoid of side effects in comparison to the available drugs in the market. There is need to identify novel active constituents that can be effective in decreasing the functionality and/or signaling of main pain targets such as TRPV1 receptor and to conduct in vivo and in vitro studies that can confirm the effects of these active constituents. This area of research can open a gate towards finding new therapies for pain.

Acknowledgments

This article was published with the support of the Deanship of Scientific Research and Graduate Studies at Amman Arab University, Amman, Jordan.

Statement of Ethics

The researchers adhered to the RCSB PDB database usage and policies.

Ethical Approval

The research was based exclusively on data available publicly from PubChem compound database and RCSB PDB database.

Informed Consent Statement

Not applicable.

Conflict of Interest Statement

Authors have no financial disclosure, non-financial relationship and activities, and conflict of interest. Purposely failing to report any conflict of interest might be considered a form of misconduct.

Funding/Support Sources

None.

Author Contributions

Sahar Jaffal provided the idea and wrote and edited the manuscript. Salman Khan and Muhammad Khan performed the molecular docking. Salman Khan revised the manuscript.

Data Availability Statement

Research data are available in the manuscript.

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