

Analgesic Profiling of Capsicum Extracts Using the Hot Plate Test in Mice: An Integrative Analysis of Bioactive Compounds Based on FT-IR, GC-MS, and Molecular Docking Studies

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Received: 27 November 2025, Revised: 29 January 2026, Accepted: 15 February 2026, Published: 5 July 2026

Abstract

This study aims to investigate the analgesic potential of extracts from 3 *Capsicum* species, namely *Capsicum chinense* (CC), *Capsicum frutescens* (CF), and *Capsicum annuum* (CA), using a hot plate test in mice. In addition, the study identified the key bioactive compounds in the extracts through a combined analysis of Fourier Transform Infrared Spectroscopy (FTIR), Gas Chromatography-Mass Spectrometry (GC-MS), and molecular docking studies. During the experimental procedures, extracts from the stem, fruit, and seed of 3 *Capsicum* species were prepared using methanol, hexane, and distilled water as solvents. The chemical composition of these extracts was profiled and classified through Fourier-transform infrared (FTIR) spectroscopy combined with cluster analysis. Subsequently, the analgesic activity of the samples was assessed *in vivo* using the hot plate test in mice. Gas chromatography-mass spectrometry (GC-MS) was used to identify the bioactive compounds present in both the most potent and least potent extracts. Molecular docking studies were then conducted to predict the interactions between these compounds and specific analgesic protein targets, namely 5IKQ, 4TLM, and 4EJ4. The results showed that the aquadest fruit extract of CC had the highest analgesic activity, while extracts from CF and CA had minimal effects. GC-MS analysis showed that the extract with minimal effects contained terpenoids, sterols, vitamins, hydrocarbons, and fatty acids. However, CC was rich in alkaloids capsaicin (4.38%) and dihydrocapsaicin, O-acetyl- (2.26%), terpenoids (nerolidyl acetate) (11.32%), phenolics (2-methoxy-phenol) (1.37%), and fatty acid derivatives. Molecular docking studies confirmed that capsaicin and dihydrocapsaicin, O-acetyl- exhibited strong binding affinities with analgesic protein targets 5IKQ (−8.0 kcal/mol), 4TLM (−5.3 and −4.9 kcal/mol), and 4EJ4 (−7.3 and −6.5 kcal/mol) outperforming the reference drug sodium diclofenac (−8.3, −5.9, and −6.6 kcal/mol). This study described the significant analgesic potential of CC extracts, particularly the aquadest fruit extract, as a promising candidate for developing new pain management therapies.

Keywords: *Capsicum* extracts, Analgesic, FTIR, GC-MS, Molecular docking.

Introduction

Pain is a subjective sensation triggered by the stimulation of the nervous system, which serves as a protective mechanism against tissue injury or damage [1]. Consequently, effective management is a significant focus in the medical field due to its profound impact on patients' quality of life [2]. Chronic pain has been reported to affect approximately 20% of the global population. This condition is often associated with reduced physical activity, sleep disturbances, and an increased risk of psychological issues, such as depression and anxiety [3]. In patients with cancer, pain diminishes the quality of life as well as worsens the prognosis by impairing the immune response [4]. A previous study showed that pain caused by diabetic neuropathy was often poorly managed, leading to a higher risk of further complications [5]. Another study reported that musculoskeletal diseases, including osteoarthritis and lower back pain, were a leading cause of disability worldwide, reducing patients' ability to work and perform daily activities [6]. Acute pain resulting from trauma or surgery can also lead to hemodynamic disturbances, increase the risk of complications, such as thrombosis, and prolong the recovery time [7].

To overcome this prevalence, conventional analgesics, including opioids and nonsteroidal anti-inflammatory drugs (NSAIDs), have long been used, but their prolonged usage is often associated with several side effects [8]. Opioids, such as morphine and oxycodone, can lead to physical and psychological dependence, with increasing tolerance requiring higher doses for the same effect [9]. Other side effects include chronic constipation, respiratory depression, nausea, vomiting, and cognitive disturbances [10]. Long-term usage also causes paradoxical hyperalgesia, where pain intensifies with continued use [11].

NSAIDs, including ibuprofen and diclofenac, which are commonly used to reduce pain and inflammation, can cause gastrointestinal disturbances, such as gastritis, ulcers, and bleeding, specifically with prolonged use or in patients with a history of gastric issues [12]. These drugs also carry cardiovascular risks, namely hypertension, myocardial infarction, and congestive heart failure, particularly in vulnerable populations [13].

In addition, long-term use can impact kidney function, leading to analgesic nephropathy, fluid retention, and acute kidney failure in severe cases [14]. Given these diverse risks, there is an increased need for the development of safer and more effective alternative analgesics from natural sources [15].

Capsicum, commonly known as chili pepper, is one such natural source with significant analgesic potential [16]. *Capsicum* genus comprises several species rich in bioactive compounds, such as capsaicin, dihydrocapsaicin, and flavonoids, which have analgesic activity [17]. The genus comprises approximately 30 species that are widely used and commercially produced [18]. The most studied species are *Capsicum annuum* (CA), *Capsicum frutescens* (CF), and *Capsicum chinense* (CC). CA, known as bell pepper, contains abundant flavonoids and vitamins, making it a potential source of antioxidants [19]. CF, or cayenne pepper, has a higher capsaicin content, supporting its traditional use in pain and inflammation reduction [20]. Meanwhile, CC, or Katokkon chili, contains the highest levels of capsaicin, along with other compounds, such as dihydrocapsaicin and capsiate, which contribute to its analgesic and anti-inflammatory effects [21]. Despite this potential, there are still several study gaps in developing *Capsicum* as analgesic agent [22].

Several studies have focused on extraction using specific solvents but have not compared the efficacy of different solvents, such as water, methanol, or ethanol, which can affect the composition of the bioactive compounds obtained [23]. In addition, the exploration of different plant parts, including fruit, seeds, and stems, remains limited, but each part contains bioactive compounds with distinct pharmacological activities [24,25]. Previous studies have shown that there is also a lack of multidisciplinary approaches integrating *in vivo* testing, chemical identification, and molecular simulations, which offers a more comprehensive understanding of *Capsicum* analgesic potential [26]. Although there is existing literature on capsaicin as the main compound, the potential of secondary metabolites and their synergistic effects remain underexplored [27].

To address these challenges, there is an urgent need to develop a comprehensive analytical approach. Techniques, such as Fourier Transform Infrared Spectroscopy (FTIR), Gas Chromatography-Mass

Spectrometry (GC-MS), and molecular docking studies provide deeper insights into the chemical composition and mechanisms of action of compounds [25]. FTIR can identify the functional groups in the bioactive compounds, while GC-MS allows for the precise identification of the compounds present [28]. Molecular docking helps to evaluate how these compounds interact with target proteins, including the vanilloid receptor 1 (TRPV1) [29], cyclooxygenase (COX), which plays a significant role in pain modulation [30], and N-methyl-D-aspartate (NMDA). Therefore, this study aims to explore analgesic potential of *Capsicum* through an integrated approach. *In vivo* biological testing, chemical analysis, and molecular simulations were carried out to provide comprehensive scientific evidence on the effectiveness and mechanisms of action of *Capsicum* as analgesic.

Materials and methods

Plant material

Fresh samples of the stems, fruits, and seeds of CA and CF were obtained from Rosoan Village, Enrekang District, Enrekang Regency, South Sulawesi, Indonesia (coordinates: 3°28'47.53"S, 119°49'21.47"E). Meanwhile, CC samples were sourced from Pangala Village, Rindingallo District, North Toraja Regency, South Sulawesi, Indonesia (coordinates: 2°53'48.73"S, 119°48'40.21"E). Botanical authentication of the collected plant materials was carefully performed at the Botany Laboratory, Department of Biology, Mathematics, and Natural Sciences, Universitas Negeri Makassar, Indonesia. This process ensured the accurate identification and verification of the plant species used in this study [25].

Extraction methods

Plant samples were dried in an oven (IL80EN) at a stable temperature of 40 to 50 °C for 3 days to remove the moisture content. Once dried, the plant materials were ground by applying a high-speed blender (IC-10B) at 25,000 rpm and then sieved through a 40/60 mesh to obtain a fine powder. The resulting powder was stored in airtight containers to prevent contamination until further analysis. Subsequently, the bioactive compounds were extracted using an ultrasonic-assisted method (BRANSON 1800). A total of 20 g of powdered plant material was extracted with 200 mL of distilled water,

methanol (p.a., Merck), and n-hexane (p.a., Merck) in an ultrasonic bath for 30 min. The solvent-to-material ratio was maintained at 1:10 in the process to ensure extraction efficiency. After this procedure, the resulting mixture was filtered, and the solvent was evaporated using a rotary evaporator (BUCHI) or freeze-dryer (BUCHI) to obtain concentrated or dry extracts. The extraction yield was calculated using Formula (1), as described by Yasir *et al.* [24,25].

$$\% \text{ Yield extract} = \left(\frac{\text{Dry extract weight}}{\text{Weight of dried materials}} \right) \times 100\% \quad (1)$$

FTIR analysis

FTIR spectroscopy was used to analyze 27 variations of *Capsicum* extracts. A total of 5 mg of each extract was mixed with 50 mg of potassium bromide (KBr) and 5 mL of analytical-grade methanol to prepare the samples. The mixture was homogenized and dried to form pellets, which were then analyzed using a Perkin Elmer Spectrum GX FTIR spectrometer (8400S, Waltham, MA, USA). Spectral data were obtained in the wavelength range of 400 to 4,000 cm⁻¹ with 16 scans per sample. To determine the similarities and differences between the extracts, chemometric analysis using cluster analysis (CA) was performed using Minitab 18 software. Grouping was conducted with the complete linkage method, distance was measured using absolute correlation, and the number of clusters was set to 6. CA was applied to group the 27 extracts based on their spectral profiles. Extracts with similarity greater than 80% were considered to have similar chemical properties [25,31]. The analysis revealed 6 identified clusters, which were selected for *in vivo* analgesic activity testing.

Analgesic activity

Animals and ethical approval

Analgesic activity test of chili extracts (*Capsicum* spp.) was conducted on 24 male mice (*Mus musculus*) aged 35 to 60 days, weighing 20 to 35 g, healthy, and not stressed (normal activity). The animals were randomly allocated into eight experimental groups, each consisting of three mice: CC aquadest stem, CA methanol seed, CC aquadest fruit, CC methanol seed, CF hexane fruit, CF methanol fruit, NaCMC 1%, and Voltaren Emulgel®. Randomization was applied to minimize selection bias and to ensure comparable

baseline characteristics among the groups. Before the treatment, the animals were acclimatized in their cages for 7 days [32] and kept under standard conditions, fasted from food for 24 h before the experiment, and given access to water only [33]. All experimental processes were approved by Sekolah Tinggi Ilmu Farmasi Makassar ethical committee through letter number: 222/EC.1.1.B/IX/KEPK/2023.

Hot plate test

In this study, each mouse was administered a dose of 10 mg per paw [34]. Samples including CA aquadest stem, CA methanol fruit, CC aquadest fruit, CC methanol seed, CF hexane fruit, CF methanol fruit, NaCMC 1%, and Voltaren emulgel® were applied to the soles of the test animal's paws. After a 1-minute exposure, the mouse was placed on a hotplate at 50 °C for a maximum of 15 seconds. When the mice showed signs of pain, such as licking their paw, jumping, or shaking, before the 15 seconds were up, the observation was stopped [35]. This response to the heat stimulus was observed for 1 h and 30 min at intervals of 15, 30, 45, 60, 75, and 90 min [36,37]. The percentage protection against the thermal pain stimulus was calculated according to the following Formula (2), as described by Hijazi *et al.* [38]. Percentage protection data from three replicates were analyzed using one-way ANOVA, complemented by main effects plots, following verification of normality and homogeneity assumptions. Statistical significance was determined through inferential analysis at a significance level of $\alpha < 0.05$, and all statistical procedures were conducted using Minitab® Statistical Software version 22.

$$\% \text{ Protection} = \frac{\text{Test Mean (Ta)} - \text{Control Mean (Tb)}}{\text{Control Mean (Tb)}} \times 100\% \quad (2)$$

GC-MS analysis

GC-MS analysis was carried out at the Chemical Engineering Laboratory, Politeknik Negeri Ujung Pandang (PNUP), Makassar, Indonesia. The samples exhibiting the highest analgesic activity, such as CC extract with aquadest solvent, and a representative extract with no significant effect, namely CC extract with hexane solvent (both 1 g), were dissolved in 5 mL of 96% ethanol (p.a.). The dissolution process was conducted using an ultrasonic device at 55 °C for 30 min.

Subsequently, the solution was filtered using Whatman No. 42 filter paper, and the resulting filtrate was injected into GC-MS Ultra QP 2010 instrument (Shimadzu, Japan). The chromatographic conditions included an injector temperature of 250 °C in the splitless mode, a pressure of 76.9 kPa, a carrier gas flow rate of 14 mL/min, and a split ratio of 1:10. In this study, the ion source and interface temperatures were set to 200 °C and 280 °C, respectively, with a solvent cut-off time of 3 min and a mass range of 10 to 890 m/z. The column used was an SH-Rxi-5Sil MS with a length of 30 m and an inner diameter of 0.25 mm. Furthermore, the column temperature program started at 70 °C with a hold time of 2 min, followed by a temperature increase of 10 °C/min until reaching 200 °C. This temperature was then increased further at 5 °C/min to 280 °C with a hold time of 9 min, resulting in a total analysis time of 36 min. The obtained data were analyzed using the NIST 17 and Wiley 9 libraries to identify the major compounds in the extracts with an area percentage greater than 1% [25].

Molecular docking studies

Sample preparation (Virtual screening)

The major compounds identified were then further analyzed using molecular studies to comprehend their mechanism of action and interaction with target proteins as analgesics. These compounds were identified through GC-MS analysis of CC fruit extracts with aquadest as well as hexane solvents and were subjected to molecular docking. The chemical structures of the identified compounds were retrieved from PubChem (accessed on 9 July 2024, link: <https://pubchem.ncbi.nlm.nih.gov>) and downloaded in SDF format, then converted and energy-minimized to obtain 3D structures suitable for molecular docking. The target proteins were obtained from the Protein Data Bank (accessed on 9 July 2024, link: <https://www.rcsb.org>) in PDB format [25]. The analgesic-related targets used were PDB IDs 4EJ4 (opioid receptor) [39], PDB IDs 5IKQ (COX-2) [40], and PDB IDs 4TLM (NMDA receptor) [41], which were prepared for docking by removing co-crystallized ligands and water molecules and by adding polar hydrogens.

Molecular docking

Ligand was optimized using Chimera 1.17.3 (UCSF Chimera; accessed on 13 July 2024, link:

<https://www.cgl.ucsf.edu/chimera/>). PyMOL version 2.5 (accessed on 13 July 2024, link: <https://pymol.org/2>) was used to refine protein structures, remove ligands, and eliminate water molecules. The docking process was carried out by applying PyRx AutoDock Vina (accessed on 13 July 2024, link: <https://pyrx.sourceforge.io/>), which was used to calculate the binding affinity, RMSD, amino acid residues, and bond types between the optimized ligands and receptors. Furthermore, the interaction results between the ligand and receptor were then visualized using the Biovia Discovery Studio Visualizer (accessed on 13 July 2024, link: <https://www.3dsbiovia.com>). The ligand with the lowest binding energy or the lowest docking score, along with the hydrogen-bonding interactions, was selected as the best candidate [25].

Results and discussion

Extraction Optimization

The extraction data for 3 chili species, namely CA, CF, and CC using hexane, methanol, and distilled water (aquadest) as solvents revealed significant trends regarding solvent efficiency, variation among plant parts, and interspecies differences. Furthermore, the results indicated that aquadest consistently achieved the highest extraction yields compared to methanol and hexane. This showed that aquadest, as a polar solvent, was particularly effective at extracting polar compounds such as flavonoids, alkaloids, and phenolics, which were abundant in chili plants. Meanwhile, hexane, a nonpolar solvent, produced the lowest yields, reflecting the relatively low content of nonpolar compounds like oils and lipophilic components in the samples [25].

Among the plant parts, fruits consistently demonstrated the highest extraction yields across all species, particularly when extracted with aquadest (Table 1). For instance, CF fruits yielded up to 23.5%,

while stems generally exhibited the lowest yields, due to their lower concentrations of bioactive metabolites compared to fruits and seeds. In CA, the highest yield was obtained from fruits using aquadest (18%), with methanol and aquadest depicting similar trends in extracting bioactive compounds from various plant parts. CF demonstrated the highest overall yields, with aquadest extracts yielding 23.5% from fruits and 14.5% from stems. Meanwhile, CC achieved maximum yields of 12.5% and 7% in fruits and stems, respectively, when extracted with aquadest, and methanol provided relatively balanced yields across all parts of CC.

The results were consistent with previous studies that identified distilled water-based solvents as optimal for recovering polar metabolites such as capsaicin and phenolic acids [42]. Methanol, with its dual polarity, was effective at extracting both polar and nonpolar compounds. However, the low yields from hexane were consistent with the limited presence of nonpolar metabolites such as oils and lipids in the chili samples [43]. Chili fruits were known to contain the highest concentrations of secondary metabolites like capsaicinoids, carotenoids, and flavonoids [44], while stems, serving primarily structural roles, typically contained lower metabolite levels.

CF emerged as the standout species, displaying the highest metabolite content, particularly in its fruits extracted with aquadest. Notable yields included CF fruits and stems (23.5% and 14.5%, respectively), CA fruits (18%), CC fruits (12.5%), and CF seeds extracted with methanol (15%). These results emphasized the pharmacological potential of CF as a rich source of bioactive metabolites. For further studies, the extracts were grouped based on the functional groups identified through cluster analysis. Their representative with the highest potential proceeded to *in vivo* testing using Hot Plate method on mice to assess their analgesic activity.

Table 1 Percentage yield of extracts from different species, solvents and parts of *Capsicum* plants.

Code	Samples	Plant Parts	Solvent	Extract (g)	Yield (%)
CA	<i>Capsicum annum</i>	Stem	Hexane	0.1	0.5
			Methanol	1.1	5.5
			Aquadest	2.4	12
		Seed	Hexane	2	10
			Methanol	1	5
			Aquadest	0.8	4

Code	Samples	Plant Parts	Solvent	Extract (g)	Yield (%)
CF	<i>Capsicum frutescens</i>	Fruit	Hexane	1	5
			Methanol	2	10
			Aquadest	3.6	18
		Stem	Hexane	0.2	1
			Methanol	2	10
			Aquadest	2.9	14.5
		Seed	Hexane	1	5
			Methanol	2	10
			Aquadest	0.7	3.5
		Fruit	Hexane	1	5
			Methanol	3	15
			Aquadest	4.7	23.5
CC	<i>Capsicum chinense</i>	Stem	Hexane	0.1	0.5
			Methanol	1.7	8.5
			Aquadest	1.4	7
		Seed	Hexane	1	5
			Methanol	1	5
			Aquadest	0.8	4
		Fruit	Hexane	1	5
			Methanol	2	10
			Aquadest	2.5	12.5
CA	<i>Capsicum annuum</i>	Stem	Hexane	0.1	0.5
			Methanol	1.1	5.5
			Aquadest	2.4	12
		Seed	Hexane	2	10
			Methanol	1	5
			Aquadest	0.8	4
		Fruit	Hexane	1	5
			Methanol	2	10
			Aquadest	3.6	18
CF	<i>Capsicum frutescens</i>	Stem	Hexane	0.2	1
			Methanol	2	10
			Aquadest	2.9	14.5
		Seed	Hexane	1	5
			Methanol	2	10
			Aquadest	0.7	3.5
		Fruit	Hexane	1	5
			Methanol	3	15
			Aquadest	4.7	23.5
CC	<i>Capsicum chinense</i>	Stem	Hexane	0.1	0.5
			Methanol	1.7	8.5
			Aquadest	1.4	7
		Seed	Hexane	1	5
			Methanol	1	5
			Aquadest	0.8	4
		Fruit	Hexane	1	5
			Methanol	2	10
			Aquadest	2.5	12.5

FTIR profiling analysis

FTIR referred to a technique used to identify functional groups in chemical compounds by measuring their vibrational modes, which absorbed specific wavelengths of IR light. These bands observed in FTIR spectra depended on the functional groups present in the compounds [25].

FTIR absorption regions for the compounds identified in the hexane fruit extract of CF indicated distinct functional group characteristics (**Figure 1**). Oleic acid exhibited absorption at 1,700 to 1,725 cm^{-1} (C=O) [45], a broad peak at 2,500 to 3,300 cm^{-1} (O-H), and 2,800 to 3,000 cm^{-1} (C-H). Esters such as methyl palmitate, methyl linoleate, methyl oleate, and 15-octadecenoic acid methyl ester showed peaks at 1,735 to 1,750 cm^{-1} (C=O), 2,800 to 3,000 cm^{-1} (C-H, aliphatic), and 1,620 to 1,680 cm^{-1} (C=C, alkene, in unsaturated compounds) [46]. N-hexadecanoic acid demonstrated absorption at 1,700 to 1,725 cm^{-1} (C=O, carboxylic acid), 2,500 to 3,300 cm^{-1} (O-H), and 2,800 to 3,000 cm^{-1} (C-H, aliphatic). Similarly, linoleic acid exhibited peaks at 1,700 to 1,725 cm^{-1} (C=O, carboxylic acid), 2,500 to 3,300 cm^{-1} (O-H), and 1,620 to 1,680 cm^{-1} (C=C, alkene). N-pentadecylacetamide was characterized by absorption at 1,640 to 1,690 cm^{-1} (C=O, amide), 3,200 to 3,500 cm^{-1} (N-H, amide), and 2,800 to 3,000 cm^{-1} (C-H). Nerolidyl is identified by peaks at 3,200 to 3,600 cm^{-1} (O-H, alcohol), 1,620 to 1,680 cm^{-1} (C=C, alkene), and 2,800 to 3,000 cm^{-1} (C-H). Long-chain alkanes such as heneicosane, pentacosane, hexacosane, and nonacosane exhibited a characteristic alkane signature at 2,800 to 3,000 cm^{-1} (C-H). Isofucosterol displayed absorption at 3,200 to 3,600 cm^{-1} (O-H, hydroxyl group) and 1,600 to 1,700 cm^{-1} (C=C, steroid double bond). Squalene showed peaks at 2,800 to 3,000 cm^{-1} (C-H) and 1,620 to 1,680 cm^{-1} (C=C, alkene), while dl- α -tocopherol was marked by a broad peak at 3,200 to 3,600 cm^{-1} (O-H, phenol) [47], 2,800 to 3,000 cm^{-1} (C-H), and 1,450 to 1,600 cm^{-1} (C=C, aromatic).

In the aquadest fruit extract of CC, as well as propanoic acid, 2-methyl- was characterized by peaks at 1,700 to 1,725 cm^{-1} (C=O, carboxylic acid) and 2,500 to 3,300 cm^{-1} (O-H, carboxylic acid). Furthermore, 1-Propanol, 2,2-dimethyl-, and acetate displayed absorption at 1,735 to 1,750 cm^{-1} (C=O, ester), 2,800 to 3,000 cm^{-1} (C-H, aliphatic), and 3,200 to 3,600 cm^{-1} (O-H, alcohol, when free alcohol was present). Phenol, 2-methoxy- exhibited peaks at 3,200 to 3,600 cm^{-1} (O-H, phenol), 1,450 to 1,600 cm^{-1} (C=C, aromatic), and 1,000 to 1,300 cm^{-1} (C-O, ether). Palmitoleic acid shows absorption at 1,700 - 1,725 cm^{-1} (C=O, carboxylic acid), 2,500 - 3,300 cm^{-1} (O-H), and 1,620-indicated cm^{-1} (C=C, alkene). Capsaicin was identified by its characteristic peaks at 3,200 to 3,600 cm^{-1} (O-H, phenol) [48], 1,650 to 1,700 cm^{-1} (C=O, amide), 2800–3000 cm^{-1} (C-H), and 1,450 to 1,600 cm^{-1} (C=C, aromatic) [49].

Lastly, dihydrocapsaicin, O-acetyl- showed peaks at 1,735 to 1,750 cm^{-1} (C=O, ester) and 2,800 to 3,000 cm^{-1} (C-H, aliphatic) [50]. These data were integrated with GC-MS and *in silico* results to validate the presence of these compounds in the extracts and correlate their functional groups with biological activities. The presence of characteristic FTIR peaks could confirm the structural features predicted by GC-MS, enhancing the reliability of compound identification [25]. FTIR profile provided valuable insights into the functional groups and primary components of chili species extracts, including CA, CF, and CC, with variations in the solvents and plant parts, which were essential for chemical characterization and phytochemical studies.

The hierarchical cluster analysis based on functional groups detailed the process (**Figure 2**), where observations were progressively merged into larger clusters based on similarity and distance levels [31]. Initially, the analysis started with 27 distinct clusters, each representing individual observations or small groups, and ultimately resulted in 6 clusters with high similarity levels (> 80%) [31, 25].

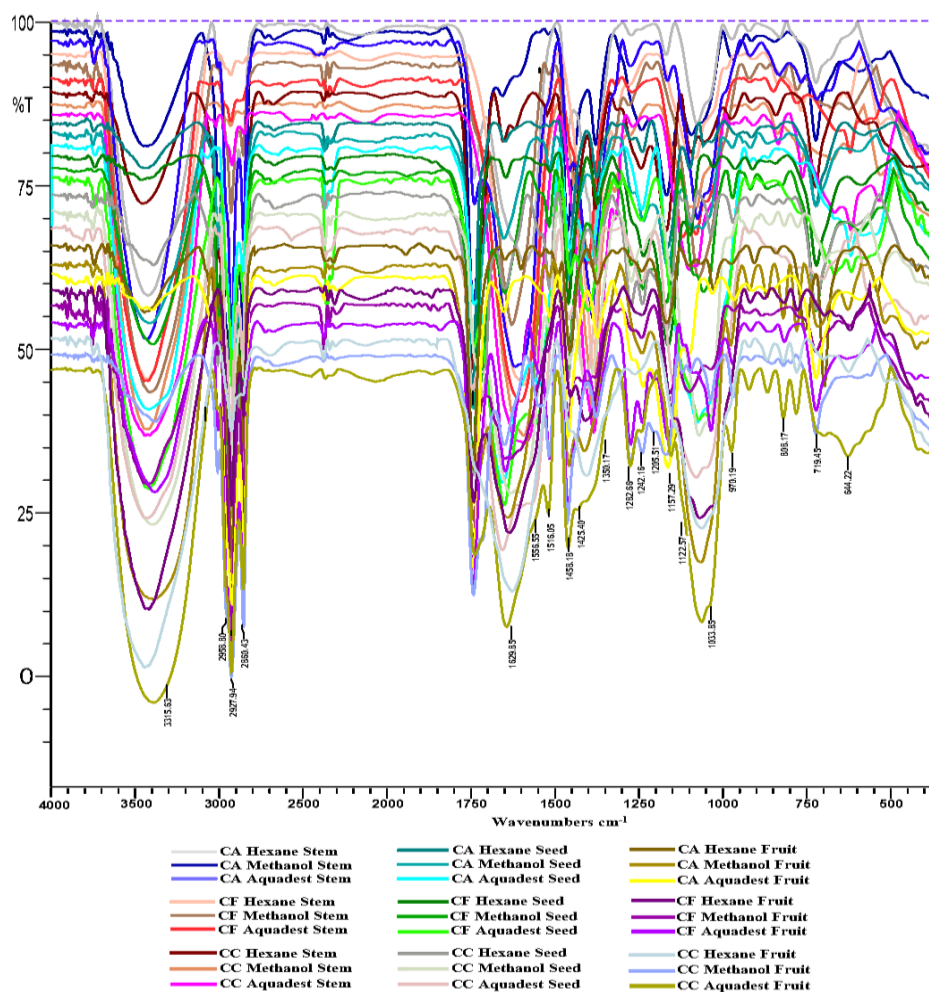


Figure 1 FTIR spectra of *Capsicum* extracts (CA, *Capsicum annuum*; CF, *Capsicum frutescens*; CC, *Capsicum chinense*) extracted using aquadest, methanol, and hexane from stem, seed, and fruit parts.

Cluster 1 consisted of extracts from CA hexane stem, CF aquadest fruit, CA methanol stem, CC methanol stem, CF aquadest seed, CA aquadest seed, CA hexane seed, CF aquadest stem, CA methanol seed, and CF hexane fruit, with a similarity of 83.62%. The second one included extracts from CF hexane stem, CF methanol seed, CA methanol fruit, CC methanol fruit, and CF hexane seed, with a similarity of 90.67%. Cluster 3 was composed of extracts from CA aquadest stem, CF methanol stem, and CC aquadest stem, with a similarity of 90.26%. Meanwhile, cluster 4 contained only CC aquadest fruit extract with a similarity of 77.56%, while 5 included extracts from CC hexane stem, CC aquadest seed, CC hexane seed, CC methanol seed, and CA aquadest fruit, with a similarity of 87.58%. Cluster 6 contained extracts from CA hexane fruit, CF methanol fruit, and CC hexane fruit, with a similarity of

99.82%. The final grouping reflected the hierarchical relationships among the observations, capturing similarities and distances throughout the clustering process [51]. Representatives from each cluster were selected for further analgesic activity testing because clusters with similar functional groups based on FTIR data were likely to exhibit similar pharmacological effects [25]. This novel approach in natural product studies simplified the screening process by focusing on extracts with similar functional group compounds, facilitating the identification of potential candidates without the need to test each extract individually [25]. The extracts selected for analgesic testing were CA methanol seed extract from Cluster 1, CA methanol fruit extract from 2, CA aquadest stem extract from 3, CC aquadest fruit extract from 4, CC methanol seed extract from 5, and CF methanol fruit extract from cluster 6.

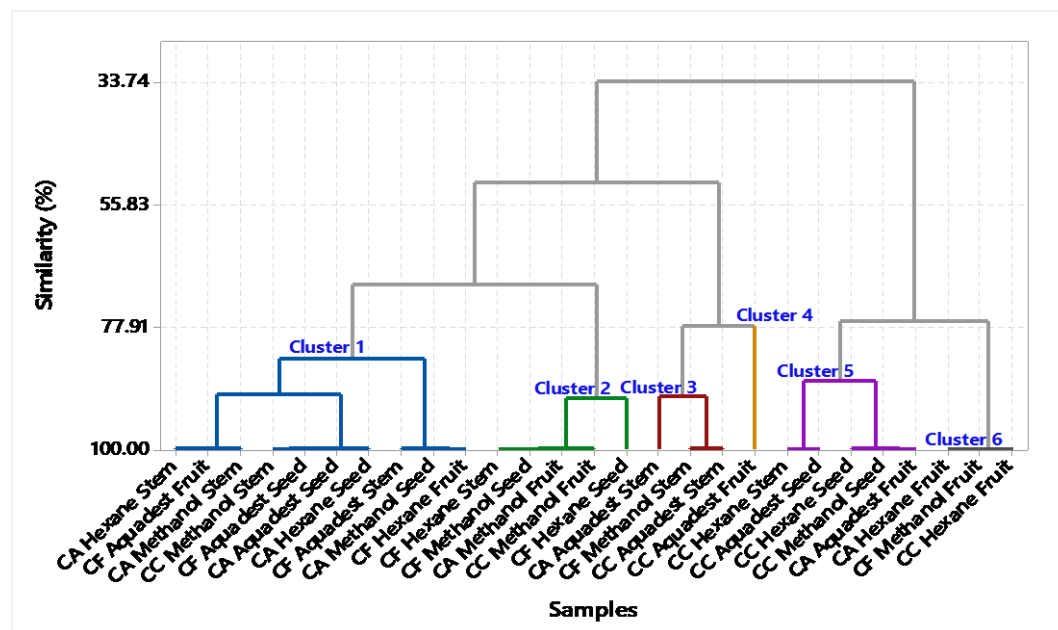


Figure 2 The dendrogram illustrating the classification of samples through cluster analysis (CA), with samples in clusters 1 to 6 represented by distinct colored lines for each respective cluster.

Analgesic activity

The results of analgesic activity test using Hot Plate method revealed significant differences in pain-relieving activity between the positive control (Voltaren Emulgel®), the negative control (NaCMC 1%), and various 10 mg extracts from CF, CC, and CA. Based on the results of the one-way ANOVA supported by the main effects plot for analgesic activity (%), the ranking of analgesic efficacy from highest to lowest is as follows: Voltaren Emulgel® > CC aquadest fruit extract > CF methanol fruit extract > CF hexane fruit extract > CA aquadest stem extract > CA methanol fruit extract > CC methanol seed extract > NaCMC 1%.

Voltaren emulgel® demonstrated high analgesic efficacy, reaching 100% at the 45-minute mark and maintaining this level through to the 90-minute mark, which indicated strong pain-relieving potential. However, 1% NaCMC did not exhibit significant analgesic activity in the test [52]. Extracts from *Capsicum* plants, although showing increased analgesic activity over time, still had lower efficacy than the

positive control. The highest activity reached 75% for CC aquadest fruit extract at the 90-minute mark. Other extracts, such as CF methanol fruit, CA methanol fruit, and CA aquadest stem, demonstrated relatively lower activities, with 36%, 14%, and 21%, respectively, at the 90-minute mark.

The relationship between analgesic activity results and the previously conducted FTIR data showed that extracts with similar functional groups [53], such as those found in CC aquadest Fruit and CF methanol fruit, exhibited similarities in pharmacological effects, including analgesic activity. In this study, FTIR-based cluster analysis, grouping the extracts according to functional group similarities, indicated that extracts containing similar chemical components were likely to exhibit comparable biological effects [54]. Therefore, the integrative approach using FTIR and cluster analysis facilitated the selection of extracts with better potential, which could be further investigated, although these extracts had not reached the same efficacy levels as the positive control [25].

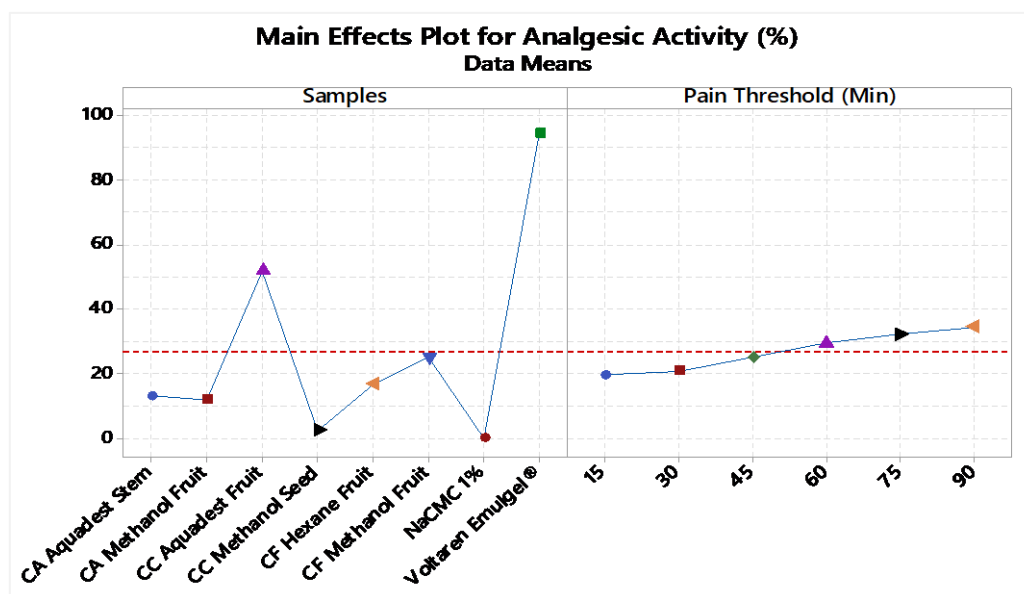


Figure 3 Main effects plot analysis of the effects of different capsicum extract variations on analgesic activity.

As the extract with the highest analgesic activity, CC aquadest fruit proceeded to GC-MS testing for a more detailed identification of its chemical components. Furthermore, *in silico* testing was conducted to analyze the potential bioactive interactions of this extract with 3 key protein targets involved in pain modulation. This included the vanilloid receptor 1 (TRPV1) using structure 4EJ4 [39,55], the delta opioid receptor, and COX using structure 5IKQ (Human Cyclooxygenase-2) [40], as well as N-methyl-D-aspartate (NMDA) receptor using structure 4TLM (GluN1/GluN2B NMDA receptor, structure 2) [41]. Meanwhile, CF hexane fruit, which demonstrated low analgesic activity, was selected as a

representative of extracts with weaker effects for comparison of its chemical content and efficacy. This approach was expected to provide a clearer understanding of the relationship between the chemical composition of the extracts and their analgesic activity and to identify potential candidates for the development of natural pain relief therapies.

GC-MS metabolite characterization

The chemical profiling of CF hexane fruit extract and CC aquadest fruit extract, based on GC-MS analysis (Table 2), showed significant differences in their chemical compositions and potential bioactivities [25].

Table 2 GC-MS analysis of compounds in hexane extract of *Capsicum frutescens* fruit and aquadest extract of *Capsicum chinense* fruit.

Compounds Detected	Molecular formula	MW (g/mol)	Pubchem (CID)	RT (M in)	SI (%)	Area (%)
<i>Capsicum frutescens</i> (CF) hexane fruit extract						
Oleic acid	C ₁₈ H ₃₄ O ₂	282	445639	19.025	88	1.06
Methyl palmitate	C ₁₇ H ₃₄ O ₂	270	8181	21.127	97	3.90
N-hexadecanoic acid	C ₁₆ H ₃₂ O ₂	256	985	22.129	95	6.74
Methyl linoleate	C ₁₉ H ₃₄ O ₂	294	5284421	24.938	96	6.66
Methyl oleate	C ₁₉ H ₃₆ O ₂	296	5364509	25.084	94	5.37
15-octadecenoic acid, methyl ester	C ₁₉ H ₃₆ O ₂	296	5364490	25.217	85	2.26
Linoleic acid	C ₁₈ H ₃₂ O ₂	280	5280450	26.271	93	6.88
N-pentadecylacetamide	C ₁₇ H ₃₅ N ₂ O	269	12009452	26.659	85	6.59
Nerolidol	C ₁₅ H ₂₆ O	222	8888	29.086	86	22.64
Heneicosane	C ₂₁ H ₄₄	296	12403	29.417	94	3.10

Compounds Detected	Molecular formula	MW (g/mol)	Pubchem (CID)	RT (M in)	SI (%)	Area (%)
Isofucosterol	C ₂₉ H ₄₈ O	412	5281326	29.892	89	1.23
Pentacosane	C ₂₅ H ₅₂	352	12406	33.233	97	1.06
Hexacosane	C ₂₆ H ₅₄	366	12407	36.600	96	1.08
Squalene	C ₃₀ H ₅₀	410	638072	38.342	98	3.43
Nonacosane	C ₂₉ H ₆₀	408	12409	39.667	97	1.42
Dl- α -tocopherol	C ₂₉ H ₅₀ O ₂	430	2116	44.167	96	1.64
Total						75.06
<i>Capsicum chinense</i> (CC) aquadest fruit extract						
Propanoic acid, 2-methyl-	C ₄ H ₈ O ₂	88	6590	4.700	87	3.50
1-Propanol, 2,2-dimethyl-, acetate	C ₇ H ₁₄ O ₂	130	13552	4.906	87	7.73
Phenol, 2-methoxy-	C ₇ H ₈ O ₂	124	460	8.894	92	1.37
(6E)-8-methyl- 6-nonenoic acid	C ₁₀ H ₁₈ O ₂	170	5365959	13.225	94	1.16
Hexadecanoic acid, methyl ester	C ₁₇ H ₃₄ O ₂	270	8181	21.044	96	1.89
Palmitoleic acid	C ₁₆ H ₃₀ O ₂	254	445638	21.668	97	1.31
N-hexadecanoic acid	C ₁₆ H ₃₂ O ₂	256	985	22.076	95	12.58
9,12-Octadecadienoic acid (Z,Z)-	C ₁₉ H ₃₄ O ₂	294	5284421	26.080	96	1.68
Oleic acid	C ₁₈ H ₃₄ O ₂	282	445639	26.167	85	1.59
7-tetradecenal, (Z)-	C ₁₄ H ₂₆ O	210	5364468	26.270	87	2.19
Linoleic acid ethyl ester	C ₂₀ H ₃₆ O ₂	308	5282184	26.372	94	2.37
Octadecanoic acid	: C ₁₈ H ₃₆ O ₂	284	5281	26.717	93	2.91
Nerolidyl acetate	C ₁₇ H ₂₈ O ₂	264	5363426	28.996	86	11.32
Capsaicin	C ₁₈ H ₂₇ NO ₃	305	1548943	34.746	97	4.38
Dihydrocapsaicin, O-acetyl-	C ₂₀ H ₃₁ NO ₄	349	91715808	35.182	89	2.26
Total						58.24

Note: MW (molecular weight); PubChem (PubChem compound identifier); RT (retention time); SI (similarity index).

Furthermore, CF hexane fruit extract contained 16 identified compounds with a total area percentage of 75.06%. Notable constituents included nerolidyl (22.64%), a sesquiterpene alcohol with reported anti-inflammatory and analgesic properties [56]. Fatty acids such as n-hexadecanoic acid (6.74%) and linoleic acid (6.88%) contributed to its pharmacological profile, supported by evidence of their anti-inflammatory effects [57,58]. Furthermore, methyl esters such as methyl linoleate (6.66%) and methyl oleate (5.37%) could enhance the membrane permeability and exhibit bioactivity. Squalene (3.43%), a triterpene, provided antioxidant benefits, while dl- α -tocopherol (1.64%), a form of vitamin E, added further antioxidant capacity [59].

In this study, CC aquadest fruit extract contained 15 compounds with a total area percentage of 58.24%. Hydrophilic and semihydrophilic compounds dominate,

including n-hexadecanoic acid (12.58%) and nerolidyl acetate (11.32%), both of which exhibited notable anti-inflammatory potential. Linoleic acid ethyl ester (2.37%) and other fatty acid derivatives further enhanced the extract's anti-inflammatory profile. Capsaicinoids, such as capsaicin (4.38%) and dihydrocapsaicin, O-acetyl (2.26%), were particularly predominant. These compounds were directly associated with analgesic effects through TRPV1 receptor activation [60]. In comparison, CF hexane fruit extract was rich in lipophilic compounds, including fatty acids, esters, and terpenes, which enhanced membrane permeability and exhibited synergistic pharmacological effects [61].

CC aquadest extract emphasized capsaicinoids, which were primarily responsible for its analgesic activity. The presence of fatty acids and terpenes in both extracts suggested overlapping anti-inflammatory mechanisms. However, the distinct hydrophilic and

lipophilic compound profiles indicated different potential applications. Meanwhile, CF hexane fruit extract could be more suitable for lipid-based formulations targeting anti-inflammatory effects, CC aquadest extract showed potential for analgesic applications through capsaicin-mediated pathways. These results were consistent with previous studies on chili extracts, describing their potential to manage inflammation and pain [62]. Therefore, the superior analgesic effect of the distilled water extract is likely due to the synergistic action of polar bioactive constituents, such as flavonoids, polyphenols, tannins, saponins, and other hydrophilic metabolites, and warrants further molecular docking and *in vitro* studies to clarify their mechanisms of action.

Molecular docking analysis

Table 3 showed the binding free energy values and amino acid residues interacting with the pain target proteins 5IKQ, 4TLM, and 4EJ4 for the several tested compounds. These data provided insights into molecular interactions between these compounds and pain target proteins, as well as how the results correlated with

analgesic activity assays and the chemical profiles of *Capsicum* extracts identified through GC-MS.

The native ligand (meclofenamic acid) for 5IKQ target protein (**Figure 4**) had the strongest binding affinity [63], with a bond-free energy of -9.0 kcal/mol, and formed multiple hydrogen bonds with amino acids such as Tyr 385, and Ser 530. This suggested that it strongly inhibited COX-2, potentially acting as an anti-inflammatory or analgesic agent. Diclofenac sodium with a bond-free energy of -8.3 kcal/mol bound to Leu 352, Tyr 358, Ser 530. Although not as strong as meclofenamic acid, but its strong binding suggested that it could act as an effective COX-2 inhibitor, consistent with its clinical use as an anti-inflammatory drug. Capsaicin and dihydrocapsaicin, which are associated with analgesic activity, exhibited strong binding affinity toward COX-2, with a binding energy of -8.0 kcal/mol. Furthermore, it interacted with Ser 353, and Ser 530, which were key residues included in the enzyme's activity. This reinforced the role of capsaicin and dihydrocapsaicin in pain relief through COX-2 inhibition, supporting its known analgesic properties.

Table 3 Bond-free energy values and amino acid residues binding to analgesic protein targets (5IKQ, 4TLM, and 4EJ4).

Compounds	Protein Target	Bond-free energy (kcal/mol)	H-bond Interaction
Meclofenamic acid	5IKQ	-9.0	Tyr 385, Ser 530
Diclofenac sodium		-8.3	Leu 352, Tyr 358, Ser 530
Oleic acid		-6.7	NI
Methyl palmitate		-6.6	Arg 120, Tyr 355
N-hexadecanoic acid		-6.5	Arg 120, Tyr 355
Methyl linoleate		-6.9	Arg 120, Tyr 355
Methyl oleate		-6.7	Arg 120
15-octadecenoic acid, methyl ester		-6.9	Arg 120
Linoleic acid		-6.9	Arg 120
N-pentadecylacetamide		-6.7	Arg 120
Nerolidol		-6.9	Arg 120
Heneicosane		-6.7	NI
Isofucosterol		-4.0	NI
Pentacosane		-7.2	NI
Hexacosane		-7.1	NI
Squalene		-8.5	NI
Nonacosane		-7.5	NI
DL- α -tocopherol		-8.8	NI
Propanoic acid, 2-methyl-		-4.3	ALA 151, ARG 496
1-Propanol, 2,2-dimethyl-, acetate		-4.9	ALA 527
Phenol, 2-methoxy-		-5.3	MET 522, VAL 523, ALA 527
(6E)-8-methyl- 6-nonenic acid		-6.3	TYR 385, SER 530

Compounds	Protein Target	Bond-free energy (kcal/mol)	H-bond Interaction
Hexadecanoic acid, methyl ester		−6.5	ARG 120, TYR 355
Palmitoleic acid		−6.7	ARG 120, TYR 355
9,12-octadecadienoic acid (Z,Z)-		−6.9	ARG 120, TYR 355
7-tetradecenal, (Z)-		−6.2	ARG 120, TYR 355
Linoleic acid ethyl ester		−7.3	TYR 385, SER 530
Octadecanoic acid		−6.8	ARG 120
Nerolidyl acetate		−7.4	TYR 385, SER 530
Capsaicin		−8.0	SER 353, SER 530
Dihydrocapsaicin, O-acetyl-		−8.0	SER 353, SER 530
4-[(1R,2S)-3-(4-benzylpiperidin-1-yl)-1-hydroxy-2-methylpropyl]phenol	4TLM	−6.0	Gly 112
Diclofenac sodium		−5.9	Ile 133, Ser 108, Tyr 109, Thr 110
Oleic acid		−4.3	Ser 132
Methyl palmitate		−4.2	Thr 110
N-hexadecanoic acid		−3.7	Gly 112, Leu 135
Methyl linoleate		−4.3	Thr 110
Methyl oleate		−3.9	NI
15-octadecenoic acid, methyl ester		−4.0	NI
Linoleic acid		−4.2	NI
N-pentadecylacetamide		−4.5	Ser 132, Ile 133
Nerolidol		−4.9	NI
Heneicosane		−3.9	NI
Isofucosterol		−6.0	NI
Pentacosane		−3.7	NI
Hexacosane		−3.4	NI
Squalene		−4.5	NI
Nonacosane		−3.6	NI
DI-α-tocopherol		−5.2	Thr 110
Propanoic acid, 2-methyl-		−3.5	Ser 108, His 134, Leu 135, Ser 136
1-Propanol, 2,2-dimethyl-, acetate		−3.8	Tyr 109, Gly 112, Leu 135, Ser 136
Phenol, 2-methoxy-		−4.3	His 134
(6E)-8-methyl- 6-nonenic acid		−3.4	Gly 112
Hexadecanoic acid, methyl ester		−3.7	Gly 112, Leu 135
Palmitoleic acid		−4.2	NI
9,12-octadecadienoic acid (Z,Z)-		−4.2	NI
7-tetradecenal, (Z)-		−4.0	NI
Linoleic acid ethyl ester		−4.2	Arg 115
Octadecanoic acid		−3.8	Ile 133
Nerolidyl acetate		−4.9	Arg 115
Capsaicin		−5.3	Tyr 109
Dihydrocapsaicin, O-acetyl-		−4.9	Tyr 109, Arg 115
(4bS,8R,8aS,14bR)-7-(cyclopropylmethyl)-5,6,7,8,14,14b-hexahydro-4,8-methano[1]benzofuro[2,3-a]pyrido[4,3-b]carbazole-1,8a(9H)-diol	4EJ4	−10.9	Asp 128, Tyr 129
Diclofenac sodium		−6.6	Asp 128
Oleic acid		−5.6	Gln 105, Lys 108, Tyr 109
Methyl palmitate		−4.8	NI
N-hexadecanoic Acid		−5.3	Gly 307, Tyr 308

Compounds	Protein Target	Bond-free energy (kcal/mol)	H-bond Interaction
Methyl linoleate		−6.0	Met 199, Leu 200
Methyl oleate		−5.7	NI
15-octadecenoic acid, methyl ester		−5.3	His 278
Linoleic acid		−5.4	Phe 218, His 278
N-pentadecylacetamide		−5.2	Asp 128, Ile 304
Nerolidol		−6.5	NI
Heneicosane		−5.2	NI
Isofucosterol		−7.7	NI
Pentacosane		−5.2	NI
Hexacosane		−5.0	NI
Squalene		−6.8	NI
Nonacosane		−5.3	NI
Dl-α-tocopherol		−6.4	Gln 105
Propanoic acid, 2-methyl-		−4.1	Asp 128, Tyr 308
1-Propanol, 2,2-dimethyl-, acetate		−4.6	NI
(6E)-8-methyl- 6-nonenoic acid		−5.0	NI
Hexadecanoic acid, methyl ester		−5.3	Gly 307, Tyr 308
Palmitoleic acid		−5.6	Phe 218, His 278
9,12-octadecadienoic acid (Z,Z)-		−5.5	Lys 214, His 278
7-tetradecenal, (Z)-		−5.4	NI
Linoleic acid ethyl ester		−5.8	NI
Octadecanoic acid		−5.7	Gln 105, Tyr 308
Nerolidyl acetate		−6.3	His 278, Phe 218
Capsaicin		−7.3	Tyr 219, His 278
Dihydrocapsaicin, O-acetyl-		−6.5	Asp 128, Lys 214, Leu 200

Note: “NI” indicates that the amino acid residues did not interact with the analgesic protein target (5IKQ, 4TLM, and 4EJ4) via H-bond interactions and were not found in the native ligand interactions.

Linoleic acid ethyl ester and nerolidyl acetate, exhibited moderate binding energies (−7.3 to −7.4 kcal/mol) and interacted with residues like Tyr 385, and Ser 530. These interactions contributed to the anti-inflammatory and analgesic effects, potentially modulating COX-2. Oleic acid, heneicosane, isofucosterol, pentacosane, hexacosane, squalene, nonacosane, and dl- α -tocopherol showed weaker binding energies −4.0 to −8.5 kcal/mol), and did not form hydrogen bonds (NI). Despite this potential, the compounds could still have biological activity through other mechanisms, such as influencing membrane fluidity or other enzyme pathways. Other fatty acids and esters such as n-hexadecanoic acid, methyl palmitate, methyl linoleate, methyl oleate, and 15-octadecenoic acid, methyl ester, with bond-free energies around −6.2 to −6.9 kcal/mol, formed hydrogen bonds with various residues like Arg 120, and Tyr 385. This suggested their interaction with the target protein, although their

binding affinity was lower. Therefore, this analysis suggested that many compounds, specifically those with strong to strong binding affinities (meclofenamic acid, capsaicin, and dihydrocapsaicin), had significant anti-inflammatory and analgesic potential through their interaction with COX-2 protein target.

In **Figure 5**, the native ligand (4-[(1R,2S)-3-(4-benzylpiperidin-1-yl)-1-hydroxy-2-methylpropyl]phenol) for 4TLM target protein [64] had binding free energy of −6.0 kcal/mol and interacted with the amino acid residue Gly 112 through hydrogen bonds. This suggested its potential for therapeutic applications in pain management or other medicinal uses. Diclofenac sodium had a binding free energy of −5.9 kcal/mol, and it interacted with Ile 133, Ser 108, Tyr 109, and Thr 110. This indicated a moderate interaction, which was consistent with the known mechanism of NSAIDs, binding to COX enzymes to inhibit prostaglandin synthesis, thereby reducing inflammation and pain.

Capsaicin and dihydrocapsaicin (with -5.3 and 4.9 kcal/mol) were indicated for their specific interaction with Tyr 109 and Arg 115, which suggested their effective interaction with the protein target and exerted analgesic effects, possibly through the modulation of inflammatory pathways [65].

Oleic acid depicted moderate to strong binding with specific amino acids (Ser 132), which explained their anti-inflammatory or analgesic potential. Fatty acids like oleic acid, methyl palmitate, and methyl linoleate had moderate binding energies (ranging from -4.0 to -4.3 kcal/mol) and were involved in interactions with key amino acids like Ser 132 and Thr 110, which

contributed to their potential effects on the protein target. Nerolidyl acetate had slightly stronger interactions with Arg 115, suggesting their potential candidates for the modulation of the target protein. These results suggested that compounds like capsaicin, dihydrocapsaicin, and 4-[(1R,2S)-3-(4-benzylpiperidin-1-yl)-1-hydroxy-2-methylpropyl]phenol could be developed as leads for analgesic drug design, supported by their ability to interact with key residues involved in pain and inflammation. Further studies, including *in vitro* and *in vivo* assays, were warranted to validate these molecular interactions and their therapeutic potential.

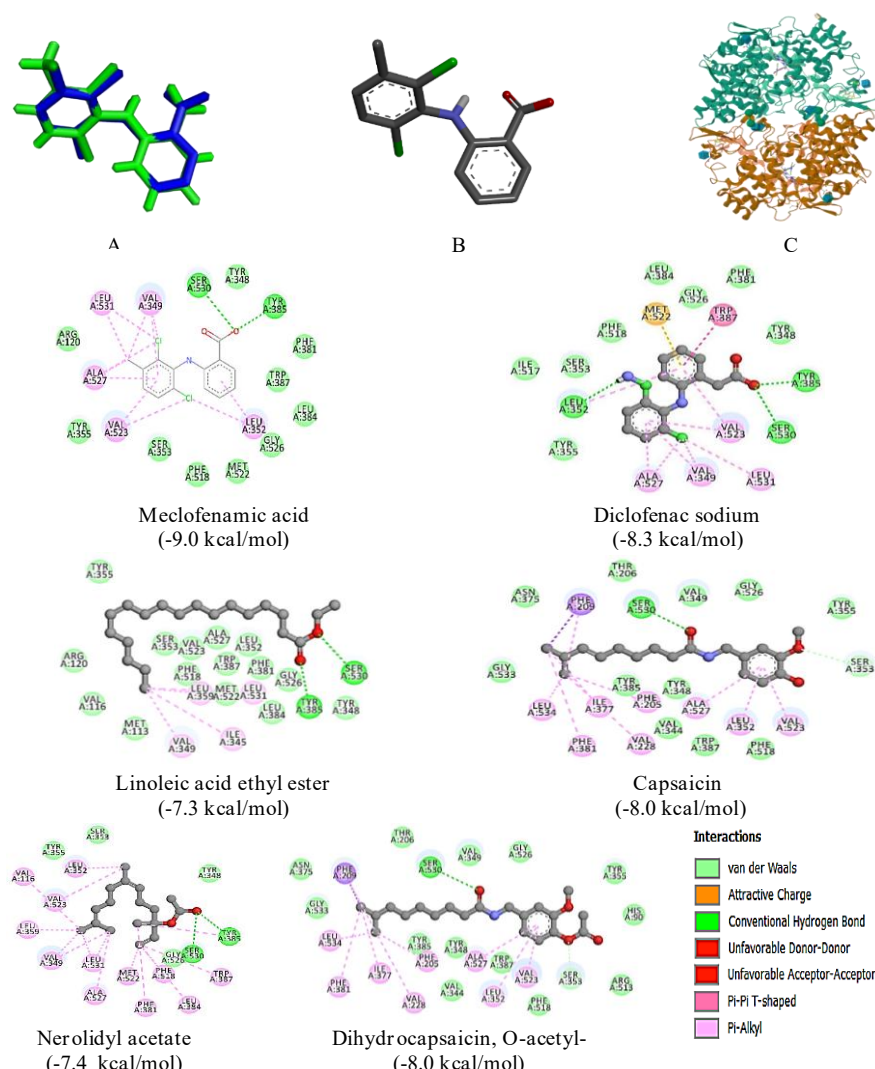


Figure 4 Structure of the analgesic protein target (5IKQ) (C) along with the native ligand (meclofenamic acid) (B). The re-docking of the native ligand (co-crystal) into the analgesic protein target pocket validates the method, resulting in a root mean square deviation (RMSD) value of $0.01 \text{ \AA}$ (A). Additionally, the interactions of the compounds in the extract, the native ligand, and the positive control with the analgesic protein target were illustrated, emphasizing their binding affinities and interactions within the target pocket.

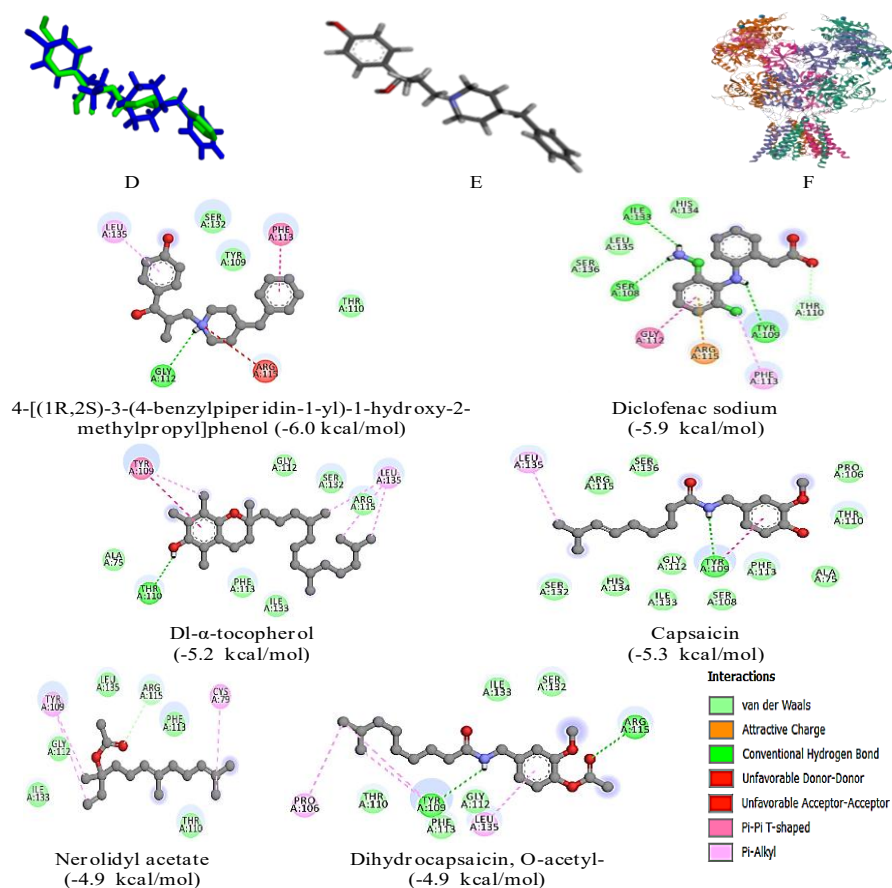


Figure 5 Structure of the analgesic protein target (4TLM) (F) along with the native ligand (4-[(1R,2S)-3-(4-benzylpiperidin-1-yl)-1-hydroxy-2-methylpropyl]phenol) (E). The re-docking of the native ligand (co-crystal) into the analgesic protein target pocket validates the method, resulting in a root mean square deviation (RMSD) value of 1.321 Å (D). Additionally, the interactions of the compounds in the extract, the native ligand, and the positive control with the analgesic protein target were illustrated, emphasizing their binding affinities and interactions within the target pocket.

The native ligand (4bS,8R,8aS,14bR)-7-(cyclopropylmethyl)-5,6,7,8,14,14b-hexahydro-4,8-methano [1]benzofuro[2,3-a]pyrido[4,3-b]carbazole-1,8a(9H)-diol for the 4EJ4 target protein [66] (**Figure 6**) had binding free energy of -10.9 kcal/mol and interacted with Asp 128 and Tyr 129. This suggested its potential as a powerful inhibitor, possibly for a receptor involved in inflammatory or neurological pathways. Diclofenac sodium exhibited a moderate binding energy of -6.6 kcal/mol, reflecting its interaction with the delta-opioid receptor through key residues such as Asp214. Capsaicin and dihydrocapsaicin showed stronger binding energies (-7.3 kcal/mol and -6.5 kcal/mol, respectively) and formed interactions with important residues, including Tyr219 and His278. These interactions likely contribute to their analgesic effects, which are associated with modulation of the TRPV1 receptor [67]. Oleic acid and other fatty acids (such as linoleic acid, and methyl oleate) exhibited moderate to

strong binding energies and interactions with key residues, supporting their potential anti-inflammatory and analgesic properties. Nerolidyl and nerolidyl acetate (with binding energies of -6.5 kcal/mol and -6.3 kcal/mol, respectively) showed significant interactions, despite lacking direct hydrogen bond interactions, suggesting that other non-covalent forces contributed to their activity. This dataset revealed several promising compounds with strong binding affinities and specific amino acid interactions, which supported their potential in treating inflammatory, analgesic, or other related conditions. (4bS,8R,8aS,14bR)-7-(cyclopropylmethyl)-5,6,7,8,14, 14b-hexahydro-4,8-methano[1] benzofuro [2,3-a] pyrido [4,3-b]carbazole-1,8a(9H)-diol was evident with the strongest binding, while compounds like capsaicin, dihydrocapsaicin and diclofenac sodium maintained strong interactions with residues known to be associated with their pharmacological effects.

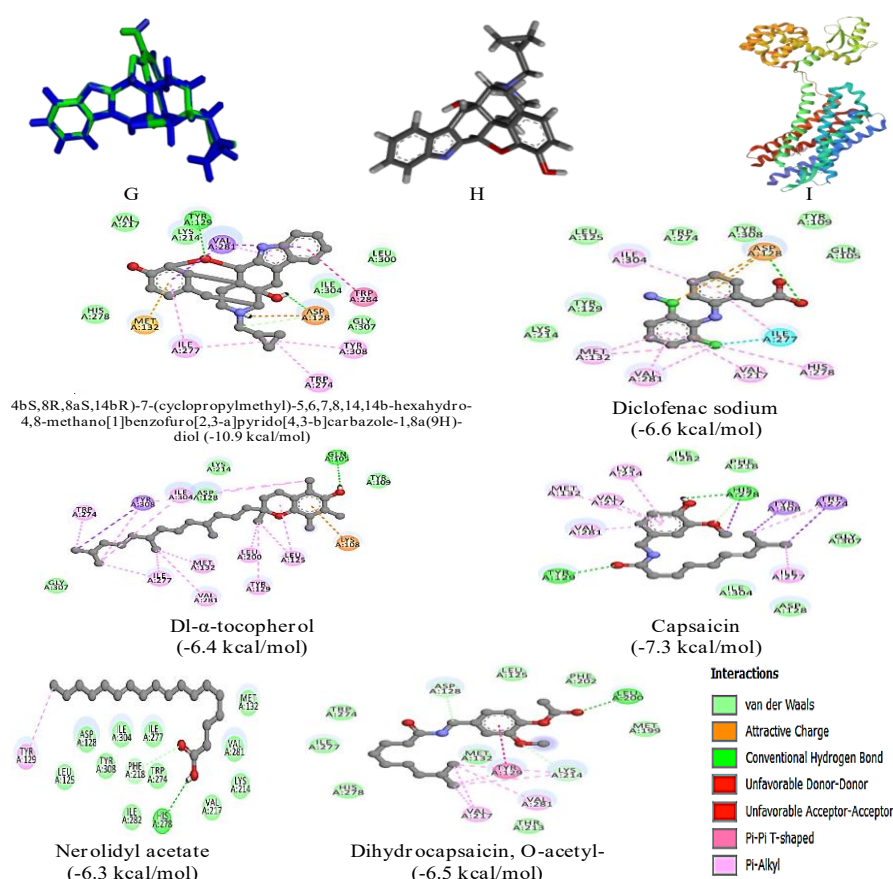


Figure 6 Structure of the analgesic protein target (4EJ4) (I) along with the native ligand (4bS,8R,8aS,14bR)-7-(cyclopropylmethyl)-5,6,7,8,14,14b-hexahydro-4,8-methano[1]benzofuro[2,3-a]pyrido[4,3-b]carbazole-1,8a(9H)-diol (H). The re-docking of the native ligand (co-crystal) into the analgesic protein target pocket validates the method, resulting in a root mean square deviation (RMSD) value of 0.01 Å (G). Additionally, the interactions of the compounds in the extract, the native ligand, and the positive control with the analgesic protein target were illustrated, emphasizing their binding affinities and interactions within the target pocket.

Conclusions

In conclusion, this study demonstrates that *Capsicum* extracts exhibit significant analgesic activity, as evidenced by a time-dependent effects reduction in pain responses *in vivo* and supported by complementary spectroscopic and computational analyses. The findings indicate that the analgesic effects are mediated by bioactive constituents capable of modulating pain-related pathways, as reflected by characteristic functional groups and strong binding affinities toward relevant analgesic protein targets. The concordance between *in vivo* efficacy and *in silico* interaction profiles provides mechanistic insight into the analgesic potential of *Capsicum* extracts and highlights their promise as natural sources of analgesic agents for future pain management applications.

Acknowledgements

The authors gratefully acknowledge Almarisah Madani University for providing essential laboratory facilities and thank all collaborators and institutions for their invaluable contributions and technical assistance, which were instrumental to the completion of this study. The authors also acknowledge the Directorate General of Research and Development, Ministry of Higher Education, Science, and Technology, Republic of Indonesia, for publication funding in 2025 (Grant No. 0488/C/DT.06.01/2025), as well as the Rector of Almarisah Madani University, Makassar 90245, Indonesia for the issuance of the official Rector's Decree (No. 014/R/UNIVERSAL/XII/2025).

Declaration of Generative AI in Scientific Writing

The authors acknowledge the use of generative AI tools (e.g., ChatGPT Go, Elicit Basic, Gemini version 2.5, and Grammarly Edu) solely for language editing and grammatical refinement during the preparation of this manuscript. No content generation, data analysis, or interpretation was performed by AI. The authors assume full responsibility for the content, analyses, and conclusions presented in this work.

CRedit author statement

Suwahyuni Mus: Conceptualization; Methodology; Supervision. **Akbar Awaluddin:** Data curation; Investigation; Software. **Nursamsiar:** Investigation; Data curation. **Hesty Setiawati:** Visualization; Writing – Reviewing and Editing. **Deniyati:** Formal analysis; Software. **Widya Ariati:** Writing – Reviewing and Editing; Visualization. **Fitriani Fajri Ahmad:** Data curation; Investigation. **Arifuddin Yunus:** Software; Formal analysis. **Rezky Yanuarty:** Data curation; Investigation. **Risna:** Investigation; Data curation. **Lukman Hardia:** Writing – Reviewing and Editing. **Hapidah:** Methodology; Validation. **Muzayyidah:** Formal analysis; Software. **Gemini Alam:** Writing – Reviewing and Editing; Visualization. **Budiman Yasir:** Writing – Original Draft Preparation; Validation; Writing – Reviewing and Editing; Supervision.

References

- [1] A Peghetti, R Seri, E Cavalli and V Martin. *Pain Management*. Cham: Springer, 2024, p. 537-570.
- [2] FF Pandelani, SLN Nyalunga, MM Mogotsi and VB Mkhathswa. Chronic pain: Its impact on the quality of life and gender. *Frontiers in Pain Research* 2023; **4**, 1253460.
- [3] T Tabernacki, D Gilbert, S Rhodes, K Scarberry, R Pope, M McNamara, S Gupta, S Banik and K Mishra. The burden of chronic pain in transgender and gender diverse populations: Evidence from a large US clinical database. *European Journal of Pain* 2025; **29(2)**, e4725.
- [4] P Tiberio, M Balordi, M Castaldo, A Viganò, F Jacobs, C Benvenuti, R Torrisi, A Zambelli, A Santoro and RD Sanctis. Empowerment, pain control, and quality of life improvement in early triple-negative breast cancer patients through pain neuroscience education: A prospective cohort pilot study protocol (EMPOWER Trial). *Journal of Personalized Medicine* 2024; **14(7)**, 711.
- [5] D Blaibel, CJ Fernandez and JM Pappachan. Acute worsening of microvascular complications of diabetes mellitus during rapid glycemic control: The pathobiology and therapeutic implications. *World Journal of Diabetes* 2024; **15(3)**, 311-317.
- [6] S Tushingham, J Cottle, M Adesokan, OO Ogwumike, A Ojagbemi, B Stubbs, F Fatoye and OO Babatunde. The prevalence and pattern of comorbid long-term conditions with low back pain and osteoarthritis in low- and middle-income countries: A systematic review and meta-analysis. *International Journal of Health Promotion and Education* 2024.
- [7] C Zanza, T Romenskaya, F Racca, E Rocca, F Piccolella, A Piccioni, A Saviano, G Formenti-Ujlaki, G Savioli, F Franceschi and Y Longhitano. Severe trauma-induced coagulopathy: Molecular mechanisms underlying critical illness. *International Journal of Molecular Sciences* 2023; **24(8)**, 7118.
- [8] A Tsoupras, DA Gkika, I Siadimas, I Christodouloupoulos, P Efthymiopoulos and GZ Kyzas. The multifaceted effects of non-steroidal and non-opioid anti-inflammatory and analgesic drugs on platelets. *Pharmaceuticals* 2024; **17(5)**, 627.
- [9] I Badshah, M Anwar, B Murtaza and MI Khan. Molecular mechanisms of morphine tolerance and dependence: Novel insights and future perspectives. *Molecular and Cellular Biochemistry* 2024; **479**, 1457-1485.
- [10] A Zimmerman and A Laitman. Safe management of adverse effects associated with prescription opioids in the palliative care population: A narrative review. *Journal of Clinical Medicine* 2024; **13(10)**, 2746.
- [11] A Manhapra, RR MacLean, R Rosenheck and WC Becker. Are opioids effective analgesics and is physiological opioid dependence benign? Revising current assumptions to effectively manage long-term opioid therapy and its deprescribing. *British Journal of Clinical Pharmacology* 2024; **90(12)**, 2962-2976.

- [12] N Alhammadi, AH Asiri, FM Alshahrani, AY Alqahtani, MMA Qout, RA Alnami, AS Alasiri and AS Al-Zomia. Gastrointestinal complications associated with non-steroidal anti-inflammatory drug use among adults: A retrospective, single-center study. *Cureus* 2022; **14(6)**, 26154.
- [13] S Liang, X Wang and X Zhu. Insights from pharmacovigilance and pharmacodynamics on cardiovascular safety signals of NSAIDs. *Frontiers in Pharmacology* 2024; **15**, 1455212.
- [14] JM LaForge, K Urso, JM Day, CW Bourgeois, MM Ross, S Ahmadzadeh, S Shekoohi, EM Cornett, AM Kaye and AD Kaye. Non-steroidal anti-inflammatory drugs: Clinical implications, renal impairment risks, and AKI. *Advances in Therapy* 2023; **40**, 2082-2096.
- [15] TT Bakare, HO Uzoeto, LN Gonlepa, S Cosmas, JN Ajima, AV Arazu, SP Ezechukwu, CM Didiugwu, GO Ibiang, AG Osotuyi and OA Durojaye. Evolution and challenges of opioids in pain management: Understanding mechanisms and exploring strategies for safer analgesics. *Medicinal Chemistry Research* 2024; **33**, 563-579.
- [16] H Duranova, V Valkova and L Gabriny. Chili peppers (*Capsicum* spp.): An update on current knowledge. *Phytochemistry Reviews* 2022; **21**, 1379-1413.
- [17] K Patel and KD Patel. Biological importance, pharmacological activities, and nutraceutical potential of capsanthin: A review of *Capsicum* plant capsaicinoids. *Current Drug Research Reviews* 2024; **16(1)**, 18-31.
- [18] GNC Ferreira, GV Ferraz, LR da Silva, AC Oliveira, LR de Almeida, MF Costa, RNO Silva, VB da Silva, ÂC de Almeida Lopes and RLF Gomes. Assessment of phenotypic divergence and hybrid development in ornamental peppers (*Capsicum* spp.). *Genetic Resources and Crop Evolution* 2025; **72**, 5499-5513.
- [19] Z Kebu, A Gure and GJ Molole. Total phenolic and flavonoid content, lipophilic components, and antioxidant activities of *Capsicum annum* varieties grown in Omo Nada, Jimma, Ethiopia. *Natural Product Communications* 2024; **19(12)**, 1-11.
- [20] AT Jolayemi and JAO Ojewole. Comparative anti-inflammatory properties of capsaicin and ethyl-acetate extract of *Capsicum frutescens* Linn. [Solanaceae] in rats. *African Health Sciences* 2013; **13(2)**, 357-361.
- [21] V Rastogi, M Porwal, MS Sikarwar, B Singh, P Choudhary and BC Mohanta. A review on phytochemical and pharmacological potential of Bhut Jolokia (a cultivar of *Capsicum chinense* Jacq.). *Journal of Applied Pharmaceutical Science* 2024; **14(5)**, 79-90.
- [22] H Duranova, V Valkova and L Gabriny. Chili peppers (*Capsicum* spp.): The spice not only for cuisine purposes: An update on current knowledge. *Phytochemistry Reviews* 2022; **21**, 1379-1413.
- [23] T Lefebvre, E Destandau and E Lesellier. Selective extraction of bioactive compounds from plants using recent extraction techniques: A review. *Journal of Chromatography A* 2021; **1635**, 461770.
- [24] B Yasir, AD Astuti, M Raihan, R Natzir, Subehan, A Rohman and G Alam. Optimization of Pagoda (*Clerodendrum paniculatum* L.) extraction based by analytical factorial design approach, its phytochemical compound, and cytotoxicity activity. *Egyptian Journal of Chemistry* 2022; **65(9)**, 421-430.
- [25] B Yasir, S Mus, S Rahimah, RM Tandiongan, KP Klara, N Afrida, NRK Nisaa, R Risna, AW Jur, G Alam and A Rohman. Antimicrobial profiling of *Piper betle* L. and *Piper nigrum* L. against methicillin-resistant *Staphylococcus aureus* (MRSA): Integrative analysis of bioactive compounds based on FT-IR, GC-MS, and molecular docking studies. *Separations* 2024; **11(11)**, 322.
- [26] A Maharjan, BMK Vasamsetti and JH Park. A comprehensive review of capsaicin: Biosynthesis, industrial productions, processing to applications, and clinical uses. *Heliyon* 2024; **10(21)**, e39721.
- [27] F Wang, Y Xue, L Fu, Y Wang, M He, L Zhao and X Liao. Extraction, purification, bioactivity and pharmacological effects of capsaicin: A review. *Critical Reviews in Food Science and Nutrition* 2022; **62(19)**, 5322-5348.
- [28] MDO Al-Bedairy, ZSH Al-Saadi, ZRK Al-Majidi, MSK Al-Humairi, FAAR Al-Groushi, MBBM Al-Waeli, MAJ Al-Abode and AHN Al-Aazawei. Anticoagulant, antioxidant, and antimicrobial activity: Biological activity and investigation of

- bioactive natural compounds using FTIR and GC-MS techniques. *Journal of Current Medical Research and Opinion* 2024; **7(4)**, 2236-2249.
- [29] CA Amaya-Rodriguez, K Carvajal-Zamorano, D Bustos, M Alegría-Arcos and K Castillo. A journey from molecule to physiology and *in silico* tools for drug discovery targeting the transient receptor potential vanilloid type 1 (TRPV1) channel. *Frontiers in Pharmacology* 2024; **14**, 1251061.
- [30] M Rudrapal, WA Eltayeb, G Rakshit, AA El-Arabey, J Khan, SM Aldosari, B Alshehri and M Abdalla. Dual synergistic inhibition of COX and LOX by potential chemicals from Indian daily spices investigated through detailed computational studies. *Scientific Reports* 2023; **13**, 8656.
- [31] B Yasir and G Alam. Chemometrics-assisted fingerprinting profiling of extract variation from Pagoda (*Clerodendrum paniculatum* L.) using TLC-densitometric method. *Egyptian Journal of Chemistry* 2023; **66(13)**, 1589-1596.
- [32] MF Khan, G Khodve, S Yadav, K Mallick and S Banerjee. Probiotic treatment improves post-traumatic stress disorder outcomes in mice. *Behavioural Brain Research* 2025; **476**, 115246.
- [33] P Villiger, C Calvet, EM Pastor-Arroyo, CA Wagner and P Seebeck. Thermoneutral environment improves mouse welfare and reduces stress in metabolic cages. *Lab Animal* 2025; **54(11)**, 303-312.
- [34] J Deshpande, S Jeyakodi and V Juturu. Tolerability of capsaicinoids from *Capsicum* extract in a beadlet form: A pilot study. *Journal of Toxicology* 2016; **2016(1)**, 6584649.
- [35] M Gautam, A Yamada, AI Yamada, Q Wu, K Kridsada, J Ling, H Yu, P Dong, M Ma, J Gu and W Luo. Distinct local and global functions of mouse A β low-threshold mechanoreceptors in mechanical nociception. *Nature Communications* 2024; **15**, 2911.
- [36] FA Khan, G Ali, K Rahman, Y Khan, M Ayaz, OF Mosa, A Nawaz, SS ul Hassan and S Bungau. Efficacy of 2-hydroxyflavanone in rodent models of pain and inflammation: Involvement of opioidergic and GABAergic anti-nociceptive mechanisms. *Molecules* 2022; **27(17)**, 5431.
- [37] M Gupta, UK Mazumder, RS Kumar, P Gomathi, Y Rajeshwar, BB Kakoti and VT Selven. Anti-inflammatory, analgesic and antipyretic effects of methanol extract from *Bauhinia racemosa* stem bark in animal models. *Journal of Ethnopharmacology* 2005; **98(3)**, 267-273.
- [38] MA Hijazi, A El-Mallah, M Aboul-Ela and A Ellakany. Evaluation of analgesic activity of *Papaver libanoticum* extract in mice: Involvement of opioid receptors. *Evidence-Based Complementary and Alternative Medicine* 2017; **2017(1)**, 8935085.
- [39] Z Fakhar, A Hosseinpouran, OQ Munro, S Sarmadi and S Gharaghani. Revealing key structural features for developing new agonists targeting δ opioid receptor: Combined machine learning and molecular modeling perspective. *Medicine in Drug Discovery* 2024; **21**, 100176.
- [40] NH Zilani, A Islam, P Biswas, Anisuzzman, H Hossain, JA Shilpi, N Hasan and G Hossain. Metabolite profiling, anti-inflammatory and analgesic potentials of edible herb *Colocasia gigantea* and molecular docking study against COX-II enzyme. *Journal of Ethnopharmacology* 2021; **281**, 114577.
- [41] W Liu, X Jiang, Y Zu, Y Yang, Y Liu, X Sun, Z Xu, H Ding and Q Zhao. A comprehensive description of GluN2B-selective N-methyl-D-aspartate (NMDA) receptor antagonists. *European Journal of Medicinal Chemistry* 2020; **200**, 112447.
- [42] JA Olguín-Rojas, LA Vázquez-León, M Palma, MT Fernández-Ponce, L Casas, G Fernández Barbero and G del Carmen Rodríguez-Jimenes. Re-valorization of red Habanero chili pepper (*Capsicum chinense* Jacq.) waste by recovery of bioactive compounds: Effects of different extraction processes. *Agronomy* 2024; **14(4)**, 660.
- [43] MF Fabela-Morón, JC Cuevas-Bernardino, T Ayora-Talavera and N Pacheco. Trends in capsaicinoids extraction from habanero chili pepper (*Capsicum chinense* Jacq.): Recent advanced techniques. *Food Reviews International* 2020; **36(2)**, 105-134.
- [44] K Islam, A Rawoof, A Kumar, J Momo, I Ahmed, M Dubey and N Ramchiary. Genetic regulation, environmental cues, and extraction methods for

- higher yield of secondary metabolites in *Capsicum*. *Journal of Agricultural and Food Chemistry* 2023; **71(24)**, 9213-9242.
- [45] LT Wang, Q Chen, RY Hong and MR Kumar. Preparation of oleic acid modified multi-walled carbon nanotubes for polystyrene matrix and enhanced properties by solution blending. *Journal of Materials Science: Materials in Electronics* 2015; **26**, 8667-8675.
- [46] VK Ahluwalia. *Infrared spectroscopy*. Cham: Springer Nature, Switzerland, 2023, p. 179-231.
- [47] X Zheng, Y Hao, M Zhao, Z Ye, X Zhang, K Zhang, Y Lin and S Liang. Efficient enzymatic synthesis of D- α -tocopherol acetate by *Carica papaya* lipase-catalyzed acetylation of D- α -tocopherol in a solvent-free system. *LWT* 2024; **202**, 116289.
- [48] JP Colli-Pacheco, CR Rios-Soberanis, VM Moo-Huchin and E Perez-Pacheco. Study of the incorporation of oleoresin *Capsicum* as an interfacial agent in starch–poly(lactic acid) bilayer films. *Polymer Bulletin* 2023; **80**, 9077-9095.
- [49] A Ahmad and H Ayub. *Fourier transform infrared spectroscopy (FTIR) technique for food analysis and authentication*. Singapore: Springer Nature, Singapore, 2022, p. 103-142.
- [50] M Baranska and H Schulz. Determination of alkaloids through infrared and Raman spectroscopy. *The Alkaloids: Chemistry and Biology* 2009; **67**, 217-255.
- [51] A Bouguettaya, Q Yu, X Liu, X Zhou and A Song. Efficient agglomerative hierarchical clustering. *Expert Systems with Applications* 2015; **42(5)**, 2785-2797.
- [52] S Cheon, JS Kim, MR Woo, SH Ji, S Park, F ud Din, JO Kim, YS Youn, KT Oh, SJ Lim, SG Jin, JE Chung and HG Choi. Establishment of nanoparticle screening technique: A pivotal role of sodium carboxymethylcellulose in enhancing oral bioavailability of poorly water-soluble aceclofenac. *International Journal of Biological Macromolecules* 2024; **277(2)**, 134246.
- [53] NK Azam, P Biswas, A Khandker, MI Tareq, SJ Tauhida, TA Shishir, S Bibi, A Alam, NH Zilani, NA Albekairi, A Alshammari, S Rahman and N Hasan. Profiling of antioxidant properties and identification of potential analgesic inhibitory activities of *Allophylus villosus* and *Mycetia sinensis* employing *in vivo*, *in vitro*, and computational techniques. *Journal of Ethnopharmacology* 2025; **336**, 118695.
- [54] PN Sampaio and CC Calado. Enhancing bioactive compound classification through the synergy of Fourier-transform infrared spectroscopy and advanced machine learning methods. *Antibiotics* 2024; **13(5)**, 428.
- [55] YM Bian, XB He, YK Jing, LR Wang, JM Wang and XQ Xie. Computational systems pharmacology analysis of cannabidiol: A combination of chemogenomics-knowledgebase network analysis and integrated *in silico* modeling and simulation. *Acta Pharmacologica Sinica* 2019; **40**, 374-386.
- [56] WK Chan, LTH Tan, KG Chan, LH Lee and BH Goh. Nerolidol: A sesquiterpene alcohol with multi-faceted pharmacological and biological activities. *Molecules* 2016; **21(5)**, 529.
- [57] J Alves, H Gaspar, J Silva, C Alves, A Martins, F Teodoro, P Susano, S Pinteus and R Pedrosa. Unravelling the anti-inflammatory and antioxidant potential of the marine sponge *Cliona celata* from the Portuguese coastline. *Marine Drugs* 2021; **19(11)**, 632.
- [58] SA Segovia, MH Vickers, XD Zhang, C Gray and CM Reynolds. Maternal supplementation with conjugated linoleic acid in the setting of diet-induced obesity normalises the inflammatory phenotype in mothers and reverses metabolic dysfunction and impaired insulin sensitivity in offspring. *The Journal of Nutritional Biochemistry* 2015; **26(12)**, 1448-1457.
- [59] J Yang, K Zhang, S Bai, Q Zeng, J Wang, H Peng, Y Xuan, Z Su and X Ding. Effects of maternal and progeny dietary vitamin E on growth performance and antioxidant status of progeny chicks before and after egg storage. *Animals* 2021; **11(4)**, 998.
- [60] W Zhang, Y Zhang, J Fan, Z Feng and X Song. Pharmacological activity of capsaicin: Mechanisms and controversies. *Molecular Medicine Reports* 2024; **29(3)**, 38.
- [61] G El-Saber Batiha, A Alqahtani, OA Ojo, HM Shaheen, L Wasef, M Elzeiny, M Ismail, M Shalaby, T Murata, A Zaragoza-Bastida, N Rivero-Perez, AM Beshbishy, KI Kasozi, P Jeandet and

- HF Hetta. Biological properties, bioactive constituents, and pharmacokinetics of some *Capsicum* spp. and capsaicinoids. *International Journal of Molecular Sciences* 2020; **21(15)**, 5179.
- [62] A Almotayri, M Jois, J Radcliffe, M Munasinghe and J Thomas. The effects of red chilli, black pepper, turmeric, and ginger on body weight: A systematic review. *Human Nutrition & Metabolism* 2020; **19**, 200111.
- [63] BJ Orlando and MG Malkowski. Substrate-selective inhibition of cyclooxygenase-2 by fenamic acid derivatives is dependent on peroxide tone. *Journal of Biological Chemistry* 2016; **291(29)**, 15069-15081.
- [64] CH Lee, W Lü, JC Michel, A Goehring, J Du, X Song and E Gouaux. NMDA receptor structures reveal subunit arrangement and pore architecture. *Nature* 2014; **511**, 191-197.
- [65] T Laorob, J Ngoenkam, A Nuiyen, P Thitiwuthikiat, D Pejchang, W Thongsuk, U Wichai, S Pongcharoen and P Paensuwan. Comparative effectiveness of nitro dihydrocapsaicin, new synthetic derivative capsaicinoid, and capsaicin in alleviating oxidative stress and inflammation on lipopolysaccharide-stimulated corneal epithelial cells. *Experimental Eye Research* 2024; **244**, 109950.
- [66] S Granier, A Manglik, AC Kruse, TS Kobilka, FS Thian, WI Weis and BK Kobilka. Structure of the δ -opioid receptor bound to naltrindole. *Nature* 2012; **485**, 400-404.
- [67] JD Smith, VT Stillerová, M Dračinský, M Popr, HLA Gaustad, Q Lorenzi, H Smrčková, JK Reinhardt, MA Liénard, L Bednárová, P Šácha and T Pluskal. Discovery and isolation of novel capsaicinoids and their TRPV1-related activity. *European Journal of Pharmacology* 2025; **999**, 177700.