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In-silico evaluation of Fragransol B from *Myristica dactyloides* for anti-inflammatory potential

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ABSTRACT

The objective of the present investigation was to uncover the drug-likeness and possible anti-inflammatory mechanism of Fragransol B, a lignan molecule isolated and characterized from *Myristica dactyloides* through *in-silico* analysis to assist in the future evaluation of the compound. A comprehensive analysis of the drug-like properties was carried out through physicochemical and ADME parameters using the SWISSADME tool. Targets and biological properties were predicated using SwissTargetPrediction and PASS online along with toxicity evaluated through ProTox-II for a variety of toxicity endpoints. Furthermore, the protein–ligand interaction of Fragransol B along with known standards was initially evaluated against targeted proinflammatory targets and enzymes to pinpoint its anti-inflammatory ability through *in-silico* molecular docking analysis. The results demonstrated that Fragransol B has drug-likeness and lead-likeness properties with specified ADMET parameters of an effective drug candidate with passive gastrointestinal absorption and blood–brain penetration. The maximum binding affinity exhibited by Fragransol B against all targets confirms the anti-inflammatory efficiency of the molecule and thus unveils the hidden molecular mechanism of the traditionally used medicinal plant *M. dactyloides*. The predicted targets also confirm the compound's anti-inflammatory potential and provide an insight into its multi-target potential. The study sheds light on future work focused on the experimental synthesis and evaluation of *in-silico* activity.

INTRODUCTION

To be established as a new drug candidate in the drug discovery process, a bioactive compound must have all of the desirable pharmacokinetic properties in all dimensions of the potent drug. Many plant-based bioactive compounds, despite having bioactive potential, fail to progress to the stage of becoming a potent drug in terms of its bioactivity. Drug resistance, as well as the use of multiple drugs for the treatment of single health problem, has changed researchers' interest toward multi-target drugs in the treatment and management of complex diseases over the years. Physicochemical properties along with absorption, distribution, metabolism, excretion, and toxicity (ADMET) properties of a compound define the effectiveness of a compound in relation to its solubility, permeability, and metabolic stability which mainly affect oral bioavailability, metabolism, clearance, toxicity, etc. (Bocci *et al*., 2017; Daina *et al*., 2017).

Throughout the drug development phase, small molecules with bioactive principles have to possess the ability to reach the specified target in their bioactive form with an effective concentration to attain their *in-vitro* bioactive potential which is mainly dependent on their pharmacokinetic and toxicity properties. Hence, an early evaluation of the compound's physicochemical, ADMET properties, as well as target and biological activity

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prediction through *in-silico* studies would help to eliminate or reduce unnecessary efforts of *in-vivo* screening, identifying novel therapeutic targets, and drug leads. In the case of multifactorial disease conditions, like chronic inflammation and cancer that depend on multiple mediators to advance, it will be highly advantageous if they can be managed effectively by a multitarget drug candidate (Koeberle and Werz, 2014).

Lignans and neolignans are well documented for a wide range of bioactivities such as antioxidant, antitumor, anti-inflammatory, anti-neurodegenerative, antiviral, and antimicrobial properties. The fact that in the past 7 years 564 different lignans and neolignans have been discovered from natural sources highlights their significance in drug development (Teponno *et al*., 2016; Zálešák *et al*., 2019). Our earlier study had identified Fragransol B, a lignan from bioactive methanol extracts of leaves and bark of *Myristica dactyloides* with antioxidant and anti-inflammatory properties (Marulasiddaswamy *et al*., 2021), has not been evaluated individually for any bioactive potential to date; it has only been identified from *Myristica fragrans* extracts evaluated for antimicrobial activity (Hada *et al*., 1988; Hattori *et al*., 1988). Although Fragransol B has been earlier characterized and identified mainly from *M. fragrans*, a well-established plant species in the Myristicaceae family with a wide variety of activities attributed to it, (Abourashed and El-Alfy, 2016; Asgarpanah, 2012; Kuete, 2017), limited attention has been received from the scientific community to validate its pharmaceutical potential. Hence, there is a need to validate its pharmaceutical potential and develop it as a drug candidate for the management of pathophysiology of inflammatory conditions. Fragransol B (2,3-dihydro-5-(2″-hydroxyethyl)-2-(4′-hydroxy-3′-methoxyphenyl)-7-methoxy-3-methylbenzofuran) a phenylpropanoid containing the dihydrobenzofuran moiety, consist of 2-aryl-3-methyl-2,3-dihydrobenzofurans with one benzylic methine, one hydroxymethyl, two methoxyl, and five aromatic protons (Hada *et al*., 1988).

With this background, the current research was focused on the comprehensive *in-silico* evaluation of Fragransol B for the physicochemical and the ADMET properties of this compound were also assessed to ensure its candidature as an effective drug candidate. Furthermore, its anti-inflammatory efficiency was evaluated by employing protein–ligand docking studies against a set of proinflammatory targets. In addition, efforts were also made to predict the target as well as the prediction of biological activity of this compound.

MATERIALS AND METHOD

Chemical structure preparation for *in-silico* studies

The structure files for Fragransol B (PubChem ID: 14015413), a bioactive molecule identified from the methanol extracts of leaves and bark of *M. dactyloides* (Marulasiddaswamy *et al*., 2021), along with standard anti-inflammatory drugs Diclofenac (PubChem ID: 3033) and Celecoxib (PubChem ID: 2662), were retrieved in ".sdf" and ".mol" file formats from the public chemical database—PubChem (https://pubchem.ncbi.nlm.nih.gov/).

Physicochemical properties and ADME parameters

Physicochemical Properties, lipophilicity, water solubility, pharmacokinetics, drug-likeness, and medicinal chemistry parameters are well-established parameters of the drug discovery process to characterize an effective drug candidate. These parameters were examined using SwissADME, an online web-based method (http://www.swissadme.ch/index.php) (Daina *et al*., 2017). The algorithm computes properties such as molecular weight, fraction Csp3, RB, no. of H bond acceptors, no. of H bond donors, MR, TPSA, water solubility Log Se [Estimated SOLubility (ESOL)], lipophilicity Qlog Po/w, and drug likeness—Lipinski (RO5) violation, bio-availability score along with lead likeness—rule of three (RO3) violation. Pharmacokinetic parameters like human gastrointestinal absorption [GI (HIA)], blood–brain barrier permeation (BBB), permeability glycoprotein (P-gp), cytochrome P450 inhibitor (CYP), and skin permeability coefficient (Log *K*p) are also evaluated.

Assessment of toxicity of Fragransol B

Early evaluation of a compound's toxicity for harmful effects on humans, animals, plants, and the environment is very essential for the development of a new drug candidate and significantly reduces the necessity of animal models for the evaluation along with cost reduction. The algorithm computes the toxicity for various toxicity endpoints, such as acute toxicity, hepatotoxicity, cytotoxicity, carcinogenicity, mutagenicity, immunotoxicity, adverse outcomes pathways (Tox21), and toxicity targets, based on the molecular similarity, pharmacophores, different fragments in the molecular structure of the compounds. Fragransol B's toxicity was assessed using the ProTox-II web server for a variety of toxicity endpoints (hepatotoxicity, immunotoxicity, genetic toxicity endpoints, especially cytotoxicity, mutagenicity, and carcinogenicity) (Banerjee *et al*., 2018).

Biological activity prediction of Fragransol B

The PASS online, an *in-silico* server for the prediction of biological properties and possible targets, was used to investigate Fragransol B's biological activity spectrum. PASS algorithm with a training set of over 260,000 drug-like biologically active compounds (drugs, drug candidates, lead compounds, and toxic compounds) simultaneously predicts 3,678 kinds of activity (95% mean accuracy) based on multilevel neighbors of atoms descriptors of the molecular structures of active compounds in comparison with the training set. The ratios of "probability to be active (Pa)" and "probability to be inactive (Pi)" were used to predict and rank biological properties. A higher "Pa" indicates higher probability of a compound to be bioactive (Lagunin *et al*., 2000).

Ligand-based target prediction of Fragransol B

Understanding the likely targets of an active compound early during the drug discovery process would aid in the repurposing of the compound for various bioactive potentials. The SwissTargetPrediction tool, which primarily predicts targets based on ligand-based screening in comparison with known compiled in curated, cleansed collections of known actives, was used to investigate possible protein targets of the chosen phytochemical through ligand-based screening (Daina *et al*., 2019). The query molecule can be uploaded as SMILES or through drawing in MarvinJS molecular editor, and after selecting a species from *Homo sapiens*, *Mus musculus*, and *Rattus norvegicus*, the targets can be predicted.

Anti-inflammatory molecular docking studies to predict the best fit

Inflammation is a complex condition that needs a multitarget drug to control since different proinflammatory mediators participate in the disease's progression to chronic conditions. The Protein Data Bank (PDB; https://www.rcsb.org) was used to retrieve the X-ray crystallography structures of proinflammatory targets such as Lipoxygenase-3 (Soybean) complex with epigallocatechin (PDB ID:1JNQ), human secretory phospholipase A2 (sPLA2) (PDB ID:1POE), structure of celecoxib bound COX-2 (PDB ID:3LN1), Stable-5-LOX in complex with arachidonic acid (PDB ID:3V99), cyclooxygenase-1 in complex with celecoxib (PDB ID:3KK6), nitric oxide synthase (NOS) (PDB ID:5UO1), Salicylate bound to human cyclooxygenase-2 (PDB ID:5F1A), and tumour necrosis factor alpha (TNF-α) (PDB ID:2AZ5). The anti-inflammatory mode of action of Fragransol B along with the standard anti-inflammatory drugs Diclofenac, and Celecoxib was determined using Schrodinger's Maestro platform (Version 11.2).

Preparation of proteins targets

Selected X-ray crystallography structures of different proinflammatory targets for this study were refined using the Protein Preparation Wizard tool. Initially, proteins were pre-processed by assigning bond orders, followed by the addition of hydrogens, creating the disulfide bonds and modifications. Furthermore, protein structures were refined by optimizing hydrogen bond assignment using PROPKA for which pH was adjusted to 7 ± 2. Restrained minimization was used to minimize non-hydrogen atoms by default root-mean-square deviation to 0.3Å with the OPLS3 force field (Madhavi Sastry *et al*., 2013). Finally, the refined structures were used for the ligand-target GLIDE docking process.

Preparation of ligands

In the docking analysis, *in-silico* molecular interactions of Fragransol B and the positive controls Diclofenac, and Celecoxib with various inflammatory targets were studied. The ligands were prepared beforehand using the LigPrep tool to produce all possible tautomers and stereoisomers, as well as three-dimensional (3D) coordinates. The OPLS3 force field was used to minimize energy and the possible states were generated using Epik at pH 7 ± 2. All the generated stereoisomers were used for the GLIDE docking process (Balakumar *et al*., 2010; Madhavi Sastry *et al*., 2013).

GLIDE docking

The GLIDE docking module was used to assess the interaction between selected ligands and proinflammatory targets. All refined proteins were given a receptor gird box based on the ligand and ligands were docked using the GLIDE docking module's extra-precision (XP) mode. The binding affinity of the ligand expressed as XP score (kcal/mol) was used to determine the anti-inflammatory potential of the phytochemicals (Friesner *et al*., 2004; Joshi *et al*., 2016).

RESULTS AND DISCUSSION

Small molecules derived from various natural sources showing tremendous bioactive potential during *in-vitro* evaluation often fail to pass the drug development process since they struggle to maintain the same effect when it comes to *in-vivo* conditions. This is due to their physicochemical and ADMET properties that play a significant role in determining the efficacy of the drug candidate (Banerjee *et al*., 2018; Daina and Zoete, 2016; Daina *et al*., 2017). The prime aim of this investigation was to validate the anti-inflammatory potential of Fragransol B using protein–ligand docking experiments against a wide range of proinflammatory targets and to evaluate its physicochemical and ADMET properties. In addition, efforts were also made to find its possible targets and to predict its biological activity(ies) (Daina *et al*., 2019; Lagunin *et al*., 2000).

Fragransol B belongs to lignans and neolignans class of compounds known for various bioactive properties highlighting their importance in the field of drug development (Teponno *et al*., 2016; Zálešák *et al*., 2019). With this in mind, the research was planned to evaluate this compound through in silico tools for further development as a drug candidate. As per our knowledge, there have been no studies on the bioactive potential of Fragransol B.

The physiochemical properties, drug and lead-likeness parameters, and pharmacokinetic parameters of Fragransol B evaluated through SwissAMDE are summarised in Tables 1 and 2. Fragransol B is falling within Lipinski's rule and bioavailability radar (Fig. 1). The bioavailability of the compound under the biological system plays an important role in its effectiveness with respect to its target, which is primarily based on oral bioavailability. This is predominantly addressed by six major physicochemical properties of the molecule: lipophilicity, size, polarity, solubility, flexibility, and saturation. Score of TPSA analysis highlighted the effective oral absorption efficiency of Fragransol B and proves the passive absorption of the molecule by GI tract. In addition, BOILED EGG model analysis indicates the ability of Fragransol B to penetrate the blood–brain barrier through the central nervous system by P-glycoprotein (PGP+) (Fig. 2) (Daina and Zoete, 2016). It is interesting to note that these properties of Fragransol B are similar to the standards Diclofenac and Celecoxib used in the study. The drug's effectiveness is governed by its ability to reach its target at the right dosage, which is determined primarily by lipophilicity, size, polarity, solubility, flexibility, and saturation. As a result, these parameters play an important role in the drug's binding to its target.

Assessing the toxicity of a compound for different toxicity endpoints is very critical for the drug development process since it will minimize the risks during animal studies and clinical evaluation. The toxicity of the compound and the LD_{50} value indicates that Fragransol B belongs to Class IV with an LD_{50} −1,743 mg/kg and has shown only immunotoxicity.

The effect of Fragransol B on different proinflammatory targets was evaluated through *in-silico* molecular interaction studies. Table 3 summarizes the interaction of Fragransol B with the various proinflammatory targets indicating the XP score (kcal/mol) and H-bond interacting residues. The binding affinity of Fragransol B was favored by hydrogen-bond interaction with the inflammatory marker enzyme Lipoxygenase-3 residues HIS 518, ILE 557, and PHE 576 with XP score (kcal/mol) −8.155. This offers a strong case for future investigation to unveil its efficacy in treating asthma, inflammation, arthritis, and psoriasis (Kühn *et al*., 2005). It is well known that phospholipase A2 plays a significant

role in systemic and acute inflammatory conditions (Balsinde *et al*., 1999). However, there is a dearth of natural specific PLA2 inhibitors, due to which there is a continuous interest in finding new pharmacologic inhibitors to treat various inflammatory disorders. In this context, the interaction of Fragransol B with Human sPLA2 (−7.697 kcal/mol) by hydrogen bond formation with residues PHE 5 and ALA 18 demonstrated in the present study appears to be particularly potent in inhibition of PLA2. Fragransol B also exhibited notable binding affinity with COX-2 when assessed with celecoxib bound COX-2 3LN1 (−8.841 kcal/mol), by hydrogen bond formation with amino acid residues TYR 341, ARG 499, and GLU 510 which act as an entry point for the COX-2 active site. These results add TYR 341, ARG 499, and GLU 510 to the list of previously cataloged COX-2 active site amino acid residues (Llorens *et al*., 1999).

The interaction between Fragransol B and LOX-5 through hydrogen interactions with residues—GLN 363 and GLN 413—has a strong binding affinity with this enzyme (XP score = −5.893 kcal/mol). It was observed that the positive standards Diclofenac and Celecoxib were comparatively less competitive in establishing strong affinity with the active site of LOX-5 compared to Fragransol B highlighting its higher efficiency in inhibiting this enzyme (Table 3 and Fig. 6). Among all the test targets, the binding affinity of Fragransol B with the binding pocket of cyclooxygenase-1 was found to be very high (−9.717 kcal/mol) favored by hydrogen-bond interactions with residues MET 522 and BOG 751. NOS is another important target enzyme with which Fragransol B showed significant interaction in the present study by binding to the active site TRP 414, and other residues PHE 589, SER 590, and HEM 801 through hydrogen bonding (XP score = −8.642 kcal/mol).

Finding new inhibitor molecules for TNF-α has considerable therapeutic potential in treating various diseases including cancer (Zia *et al*., 2020). Our analysis recorded an XP score of −6.347 kcal/mol for the interaction of Fragransol B with this target protein. Several earlier studies have set an XP value greater than −6.5 kcal/mol as a cut-off score for further experimental consideration of the molecule as a drug candidate (Table 3 and Fig. 6) (Zia *et al*., 2020). Thus, our results highlight the possible role of Fragransol B in treating diseases influenced by the dysregulation of TNF-α.

Among the tested proinflammatory targets used in the present investigation, the maximum inhibitory interaction by Fragransol B was observed with COX-1, followed by COX-2, LOX-3, Nos, and PLA-2 enzymes. Minimum inhibition was observed with TNF-α and LOX-5. Binding modes and molecular interactions of Fragransol B, Diclofenac, and Celecoxib with Lipoxygenase-3, Secretory Phospholipase, and cyclooxygenase-2 are shown in Figures 4–6.

The anti-inflammatory potential conferred by docking studies is well supported through the *in-silico* bioactivity spectra prediction and target prediction mainly based on the structure similarity screening. Table 4 and Figure 3 show the different probable targets and bioactive potential with emphasis given to the anti-inflammatory potential of the compound. The compound shows probable inhibitory effects on NOS 2 expression, Transcription factor NF kappa B, TNF expression, transcription factor NF kappa A, 12-Lipoxygenase, 15-Lipoxygenase,

Table 1. Physiochemical properties, drug and lead-likeness parameters of the Fragransol B, Diclofenac, and Celecoxib.

Sl. no.	Name	Physiochemical properties								Drug likeness			Lead likeness
		Molecular formula	Molecular weight g/mol	Fraction Csp3	RB	No. of H bond acceptors	No. of H bond donors	MR	TPSA	Water solubility Log S^{e} (ESOL)	Lipophilicity Qlog $_{Po/W}$	Lipinski (RO5) violation	Bio-availability score	Rule of three (RO3) violation
1.	Fragransol B	$C_{19}H_{22}O_5$	330.37	0.37	5	5	2	91.02	68.15	−3.79	2.89	0	0.55	0
2.	Diclofenac	$C_{14}H_{11}Cl_2NO_2$	296.15	0.07	4	2	2	77.55	49.33	−4.65	3.66	0	0.85	1
3.	Celecoxib	$C_{17}H_{14}F_3N_3O_2S$	381.37	0.12	4	7	1	89.96	86.36	−4.57	3.4	0	0.55	1

RB = No. of rotatable bonds; MR = Molar refractivity; TSPA = Topological polar surface area.

Table 2. Pharmacokinetic parameters of the Fragransol B, Diclofenac, and Celecoxib.

Sl. No.	Name	GI tract absorption	BBB permeant	P-gp substrate	CYP1A2 inhibitor	CYP2C19 inhibitor	CYP2C9 inhibitor	CYP2D6 inhibitor	CYP3A4 inhibitor	Log K_p (skin permeation; cm/s)
1.	Fragransol B	High	Yes	Yes	No	No	No	Yes	Yes	−6.22
2.	Diclofenac	High	Yes	No	Yes	Yes	Yes	Yes	No	−4.98
3.	Celecoxib	High	No	No	Yes	No	Yes	No	No	−6.21

GI (HIA) = Human gastrointestinal absorption; BBB = Blood–brain barrier permeation; P-gp = Permeability glycoprotein; CYP = Cytochrome P450 inhibitor; Log *K*p = Skin permeability coefficient.

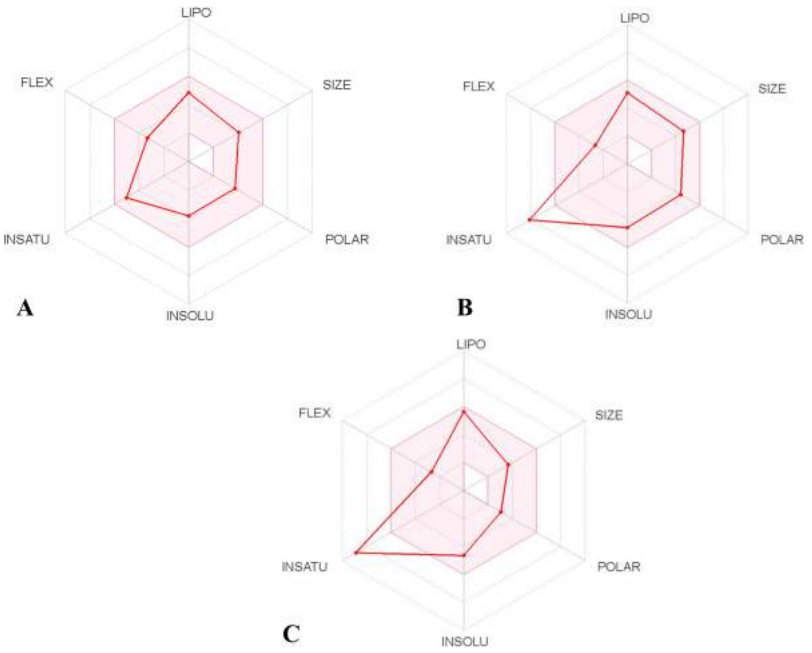

Figure 1. Bioavailability radar of (A) Fragransol B, (B) Celecoxib, and (C) Diclofenac with a pink area representing the optimal range for each property (lipophilicity: XLOGP3 between −0.7 and + 5.0; size: MW between 150 and 500 g/mol; polarity: TPSA between 20 and 130 Å2; solubility: log S not higher than 6; saturation: fraction of carbons in the sp3 hybridization not less than 0.25; and flexibility: no more than nine rotatable bonds).

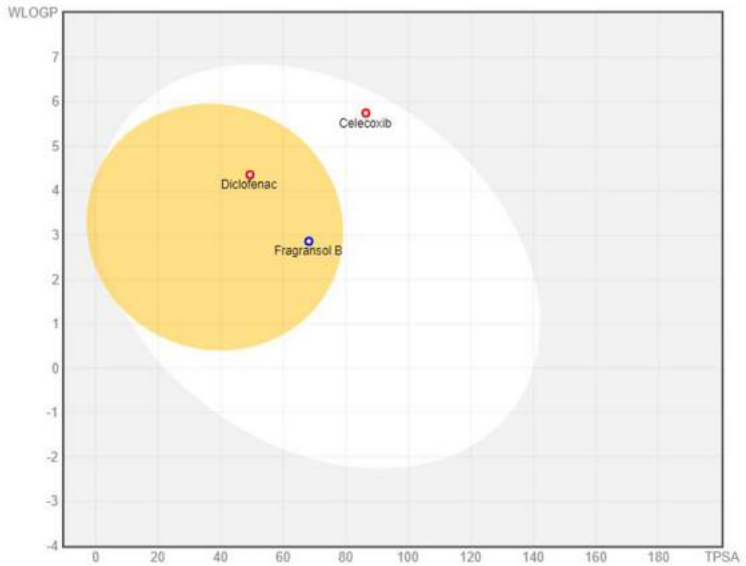

Figure 2. BOILED-egg plot representing the passive gastrointestinal absorption (HIA) and BBB of the compound.

Table 3. Binding mode and molecular interaction of Fragransol B, Diclofenac, and Celecoxib with proinflammatory targets.

Sl. No.	Test compounds	Target protein	PDB Id	Docking score	XP Score (kcal/mol)	Glide energy	Glide E_{model}	H-bond interacting residues
1					Lipoxygenase-31JNQ			
	Fragransol B			−8.155	−8.155	−20.374	37.144	HIS 518, ILE557, PHE 576
	Diclofenac			−9.604	−9.606	−27.993	−43.335	HIS 518, FE2 858
	Celecoxib			–	–	–	–	–
2					sPLA2 1POE			
	Fragransol B			−7.697	−7.698	−43.872	−59.157	PHE 5, ALA 18
	Diclofenac			−11.206	−11.208	−42.159	−56.403	GLY 27, HIP 47, CA 802
	Celecoxib			−5.527	−5.527	−36.542	−52.633	–
3					COX-2 3LN1			
	Fragransol B			−8.841	−8.842	−32.743	−2.201	TYR 341, ARG 499, GLU 510
	Diclofenac			−8.256	−8.257	−29.076	−36.613	TYR 371, TRP 373
	Celecoxib			−12.298	−12.298	−60.957	−91.118	ARG 106, GLN 178 ARG 499, PHE 504
4					LOX-5 3V99			
	Fragransol B			−5.893	−5.893	−40.305	−49.374	GLN 363, GLN 413, H_2O
	Diclofenac			−4.455	−4.456	−28.253	−36.33	PHE 177, ILE 406, H_2O
	Celecoxib			−3.915	−3.916	−33.928	−49.203	ALA 410
5					Cyclooxygenase-1 3KK6			
	Fragransol B			−9.717	−9.717	−33.484	−23.709	MET 522, BOG 751
	Diclofenac			−8.253	−8.254	−29.704	−39.545	ARG 120, TYR 385, ILE 523, SER 530
	Celecoxib			−11.733	−11.734	−55.728	−70.713	ARG 120, LEU 352, TYR 355, SER 516
6					NOS—5UO1			
	Fragransol B			−8.642	−8.642	−58.528	−78.324	TRP 414, PHE 589, SER 590, HEM 801
	Diclofenac			−9.973	−9.975	−37.551	−29.849	TRP 414, HEM 801
	Celecoxib			−5.418	−5.419	−30.37	7.568	ARG 419, VAL 421, TRP 683, H_2O
7					Cyclooxygenase-2 5F1A			
	Fragransol B			−6.029	−6.03	−44.541	−49.057	HIS 207, THR 212, ASN 382, COH 602
	Diclofenac			−4.238	−4.24	−37.644	−40.24	ALA 202, HIS 207, COH 602
	Celecoxib			−5.888	−5.889	−30.909	−46.154	ALA 443, TYS 446, COH 602, H_2O
8					TNF-α 2AZ5			
	Fragransol B			−6.347	−6.347	−35.862	−41.595	GLY 121, H_2O
	Diclofenac			−4.797	−4.798	−28.671	−37.357	–
	Celecoxib			−6.69	−6.691	−35.953	−49.766	TYR 151

Cyclooxygenase-1, and Cyclooxygenase-2, demonstrating its potential as an anti-inflammatory drug candidate.

Overall, the *in-silico* examination of Fragransol B has demonstrated its anti-inflammatory potential in this study. In addition, molecular interaction studies, target and bioactivity predictions made using the available online software tools, resulted in substantiating the multiple pharmacological effects and biochemical mechanisms of Fragransol B (Table 4). The results indicate that Fragransol B can participate in the processes for the development of a new drug for treating various anti-inflammatory disorders. Moreover, the data on ADMET properties also justify the use of Fragransol B as a promising drug candidate since it meets the required cut-off points for a compound to be considered as a potential drug.

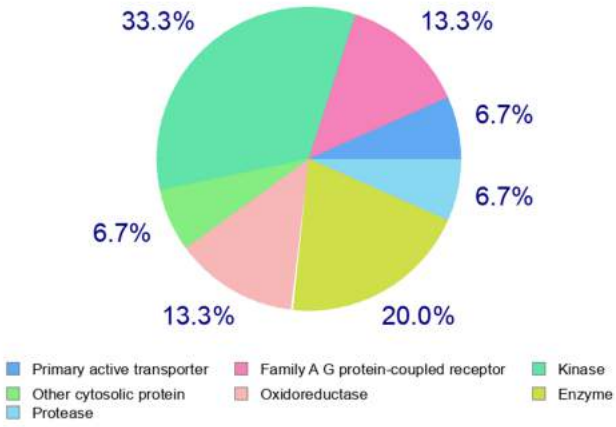

Figure 3. Different classes of predicted targets of the Fragransol B.

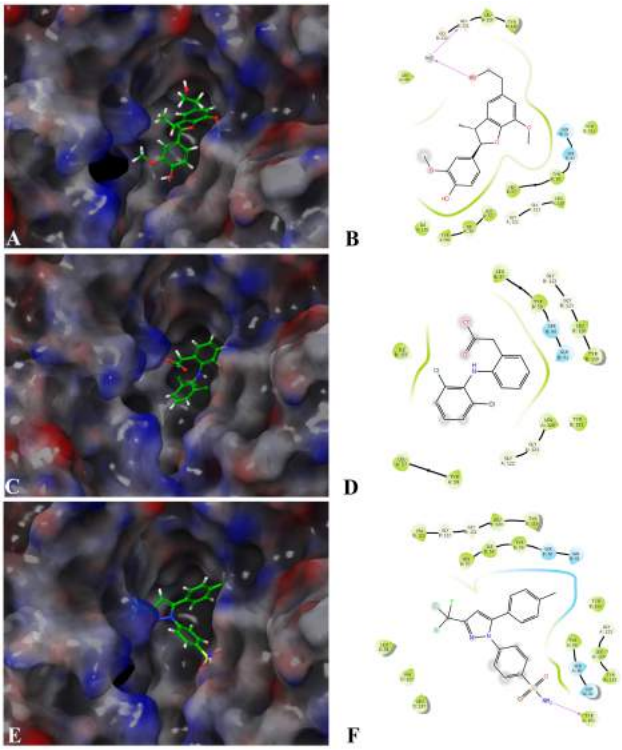

Figure 4. Binding mode and molecular interaction of selected compounds with TNF-α (2AZ5). (A and B) Fragransol B, (C and D) Diclofenac, and (E and F) Celecoxib.

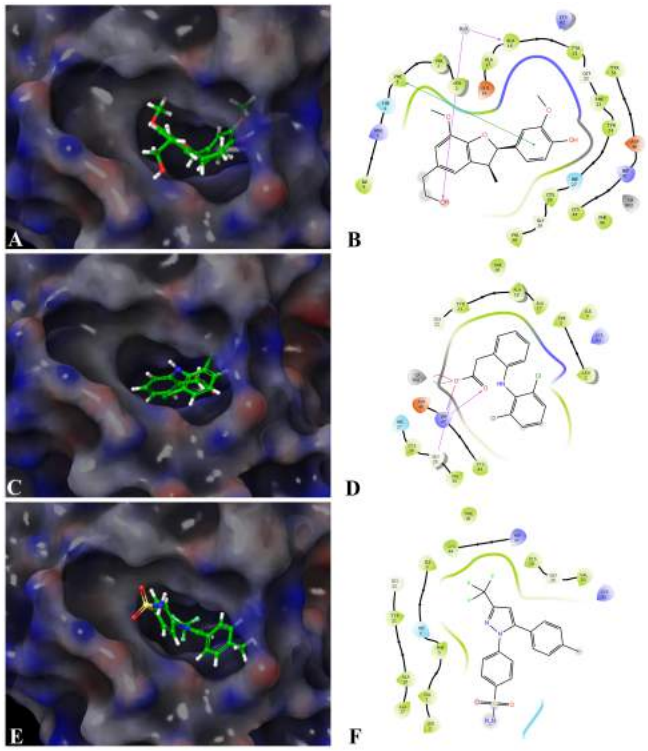

Figure 5. Binding mode and molecular interaction of selected compounds with Human sPLA2 (PDB ID:1POE). (A and B) Fragransol B, (C and D) Diclofenac, and (E and F) Celecoxib.

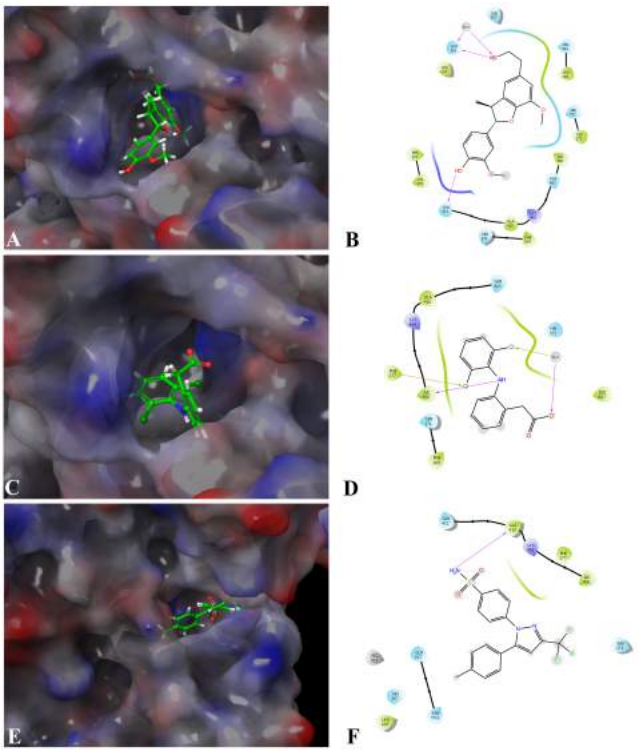

Figure 6. Binding mode and molecular interaction of selected compounds with LOX-5 (3V99). (A and B) Fragransol B, (C and D) Diclofenac, and (E and F) Celecoxib.

Table 4. Predicted bioactivity spectra of Fragransol B depicting different bioactivities and probable targets.

Sl No.	PASS-predicted bioactivity spectra			SwissTarget-predicted targets
	Pa	Pi	Activity	
01	0.828	0.010	HIF1A expression inhibitor	P-glycoprotein 1
02	0.804	0.005	Caspase three stimulant	Dopamine D2 receptor
03	0.740	0.003	Free radical scavenger	Tyrosine-protein kinase ITK/TSK
04	0.777	0.041	Membrane integrity agonist	Kinesin-like protein 1
05	0.507	0.144	Membrane permeability inhibitor	Cyclooxygenase-1
06	0.656	0.009	Hepatoprotectant	Serotonin 1a (5-HT1a) receptor
07	0.599	0.022	Vasodilator, peripheral	Interleukin-1 receptor-associated kinase 4
08	0.596	0.032	Cytoprotectant	Cyclooxygenase-2
09	0.577	0.023	HMOX1 expression enhancer	Serine/threonine-protein kinase MRCK-A
10	0.556	0.012	Antimutagenic	Serine/threonine-protein kinase receptor R3
11	0.537	0.009	Myc inhibitor	Aldose reductase
12	0.564	0.039	JAK2 expression inhibitor	PI3-kinase p110-alpha/p85-alpha
13	0.542	0.018	Antinociceptive	Beta-secretase 1
14	0.551	0.028	Vasoprotector	Sorbitol dehydrogenase
15	0.521	0.007	NOS2 expression inhibitor	Serine/threonine-protein kinase Chk1
16	0.522	0.013	Chemopreventive	Aldo-keto reductase family 1 member B10
17	0.588	0.079	Fibrinolytic	Tyrosine-protein kinase receptor TYRO3
18	0.513	0.026	Spasmolytic	Proto-oncogene tyrosine-protein kinase MER
19	0.549	0.068	TP53 expression enhancer	ATP-sensitive inward rectifier potassium channel 1
20	0.491	0.018	Lipid peroxidase inhibitor	Serine/threonine-protein kinase WEE1
21	0.484	0.021	Caspase eight stimulant	Serine/threonine-protein kinase mTOR
22	0.457	0.008	Antioxidant	Rho-associated protein kinase 2
23	0.378	0.008	Transcription factor NF kappa B inhibitor	PI3-kinase p110-alpha subunit
24	0.411	0.044	Antiasthmatic	Estrogen receptor alpha
25	0.434	0.079	Anti-inflammatory	Estrogen receptor beta
26	0.354	0.073	TNF expression inhibitor	PI3-kinase p110-delta subunit
27	0.320	0.053	Anti-inflammatory, ophthalmic	PI3-kinase p110-beta subunit
28	0.261	0.064	Transcription factor NF kappa A inhibitor	PI3-kinase p110-gamma subunit
29	0.127	0.020	12-Lipoxygenase inhibitor	Mitogen-activated protein kinase kinasekinase 8
30	0.107	0.019	15-Lipoxygenase inhibitor	Mu opioid receptor

CONCLUSION

In-silico study carried out with Fragransol B isolated and characterized from *M. dactyloides* against proinflammatory targets demonstrated its capability to participate in the drug development process for treating anti-inflammatory disorders. In addition, the results of the present investigation uncovered for the first time the molecular mechanisms of Fragransol B in significantly inhibiting proinflammatory cytokines and marker enzymes involved in the inflammatory pathways. Furthermore, our investigation indicated the workable or reliable targets which will simplify the experimental design to prove the effectiveness of the lead compound through a realistic approach. This also provides scientific evidence for the use of *M. dactyloides* for the treatment of various anti-inflammatory disorders.

AUTHORS' CONTRIBUTIONS STATEMENT

All the authors have made substantive intellectual contributions to the content of this manuscript in the following areas: concept and design—KMM and KRK; data acquisition and analysis—KMM; drafting manuscript—KMM, SCR, BRN, and KRK; critical revision of manuscript SNB, BRN, and SS; and supervision and final approval—KRK.

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ETHICAL APPROVAL

Not applicable.

CONFLICT OF INTEREST

The authors have declared no conflict of interest.

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