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...
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; Zálešík 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; Asgarpah, 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-dihydrobenzofuran 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 ADMET 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 Kp) 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:1INQ), 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:2AZS). 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 grid 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; Lagumín 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 physicochemical 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 LD50 value indicates that Fragransol B belongs to Class IV with an LD50 ~1,743 mg/kg and has shown only immunotoxicty.

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 557, 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 Lipoxigenase-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-Lipoxigenase, 15-Lipoxigenase, ...

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**Table 3.** Physicochemical properties, drug and lead-likeness parameters of the Fragransol B, Diclofenac, and Celecoxib.

| Sl. No. | Name          | Molecular formula | Molecular weight g/mol | ClogP | No. of H bond donors | No. of H bond acceptors | RB | MR | TIPS | Log S(E) Expressions | Lipophilic availability | Bioactivity Score | Rule of three (ROS) Violation | Drug likens | Lead likens |
|-------|---------------|-------------------|------------------------|-------|---------------------|-------------------------|----|----|------|---------------------|------------------------|-------------------|---------------------------|-------------|------------|
| 1     | Fragransol B  | C_{17}H_{26}O_{3}S | 343.37                 | 0.27  | 5                   | 2                       | 2  | 91.02| 68.15| −7.79              | 0                     | 0.55              | 0             | 0             | 0             | 0             |
| 2     | Diclofenac    | C_{14}H_{18}ClNO_{2} | 296.15                 | 0.07  | 4                   | 2                       | 2  | 77.55| 49.33| −1.95              | 0                     | 0.85              | 0             | 0             | 0             | 0             |
| 3     | Celecoxib     | C_{16}H_{17}FNO_{3}S | 381.37                 | 0.12  | 4                   | 7                       | 1  | 89.96| 86.36| −4.57              | 0                     | 0.55              | 0             | 0             | 0             | 0             |

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

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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 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               | Yes              | Yes              | Yes              | −6.22                         |
| 2.      | Diclofenac  | High                | Yes          | No             | Yes              | Yes              | Yes              | No               | 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.
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     | Fragransol B   | Lipoxygenase-31JNQ | −8.155 | −8.155 | −20.374 | 37.144 | HIS, ILE, PHE | 576 |
|       | Diclofenac     |                | −9.604 | −9.606 | −27.993 | −43.355 | HIS, FE | 2 858 |
|       | Celecoxib      |                | −5.527 | −5.527 | −36.542 | −52.633 | –               | –               |
| 2     | Fragransol B   | sPLA2 1POE     | −7.697 | −7.698 | −43.872 | −59.157 | PHE, ALA | 18 |
|       | Diclofenac     |                | −11.206 | −11.208 | −42.159 | −56.403 | GLY, HIP, CA | 802 |
|       | Celecoxib      |                | −12.98 | −12.98 | −60.957 | −91.118 | ARG, GLN, ARG | 499, PHE | 504 |
| 3     | Fragransol B   | COX-2 3LN1     | −8.841 | −8.842 | −32.743 | −2.201 | TYR, ARG | 341, GLU | 510 |
|       | Diclofenac     |                | −8.256 | −8.257 | −29.076 | −36.613 | TYR, TRP | 371, TRP | 373 |
|       | Celecoxib      |                | −12.928 | −12.928 | −60.957 | −91.118 | ARG, GLN, ARG | 355, PHE | 410 |
| 4     | Fragransol B   | LOX-5 3V99     | −5.893 | −5.893 | −40.305 | −49.374 | GLN, GLN | 413, H$_2$O |
|       | Diclofenac     |                | −4.455 | −4.456 | −28.253 | −36.33 | PHE, ILE | 406, H$_2$O |
|       | Celecoxib      |                | −3.915 | −3.916 | −33.928 | −49.203 | ARG | 410 |
| 5     | Fragransol B   | Cyclooxygenase-1 3KK6 | −9.717 | −9.717 | −33.484 | −23.709 | MET, BOG | 751 |
|       | Diclofenac     |                | −8.253 | −8.254 | −29.704 | −39.545 | ARG, TYR | 385, ILE | 523, SER | 530 |
|       | Celecoxib      |                | −11.733 | −11.734 | −55.728 | −70.713 | ARG, LEU | 352, TYR | 355, SER | 516 |
| 6     | Fragransol B   | NOS—5UO1       | −8.642 | −8.642 | −58.528 | −78.324 | TRP, PHE | 589, SER | 590, HEM | 801 |
|       | Diclofenac     |                | −9.973 | −9.975 | −37.551 | −29.849 | TRP, HEM | 801 |
|       | Celecoxib      |                | −5.418 | −5.419 | −30.37 | 7.568 | ARG, VAL | 421, TRP | 683, H$_2$O |
| 7     | Fragransol B   | Cyclooxygenase-2 5F1A | −6.029 | −6.03 | −44.541 | −49.057 | HIS, THR | 212, ASN | 382, COH | 602 |
|       | Diclofenac     |                | −4.238 | −4.24 | −37.644 | −40.24 | ALA, HIS | 207, COH | 602 |
|       | Celecoxib      |                | −5.888 | −5.889 | −30.909 | −46.154 | ALA, TYR | 446, COH | 602, H$_2$O |
| 8     | Fragransol B   | TNF-α 2AZ5     | −6.347 | −6.347 | −35.862 | −41.595 | GLY | 121, H$_2$O |
|       | Diclofenac     |                | −4.797 | −4.798 | −28.671 | −37.357 | –               | –               |
|       | Celecoxib      |                | −6.69 | −6.69 | −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) 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.
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 SS; and supervision and final approval—KRK.

ACKNOWLEDGMENTS

The authors were financially supported through the UGC: National Fellowship for Higher Education (NFHE) for this study. We thank Department of Studies in Computer Science, University of Mysore for providing Schrodinger’s Maestro software (Version 11.2). The authors are thankful to the Sophisticated Analytical Instrument Facility (SAIF), IIT Bombay instrumentation facility. The authors are also grateful to the Institution of Excellence (IOE), University of Mysore, for the instrumentation facility.

ETHICAL APPROVAL

Not applicable.

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

| Sl No. | PASS-predicted bioactivity spectra | SwissTarget-predicted targets |
|--------|-----------------------------------|-------------------------------|
| 01     | Pa 0.828  Pi 0.010 HIF1A expression inhibitor | P-glycoprotein 1 |
| 02     | Pa 0.804  Pi 0.005 Caspase three stimulant | Dopamine D2 receptor |
| 03     | Pa 0.740  Pi 0.003 Free radical scavenger | Tyrosine-protein kinase ITK/TSK |
| 04     | Pa 0.777  Pi 0.041 Membrane integrity agonist | Kinesin-like protein 1 |
| 05     | Pa 0.507  Pi 0.144 Membrane permeability inhibitor | Cyclooxygenase-1 |
| 06     | Pa 0.656  Pi 0.009 Hepatoprotectant | Serotonin 1a (5-HT1a) receptor |
| 07     | Pa 0.599  Pi 0.022 Vasodilator, peripheral | Interleukin-1 receptor-associated kinase 4 |
| 08     | Pa 0.596  Pi 0.032 Cytoprotectant | Cyclooxygenase-2 |
| 09     | Pa 0.577  Pi 0.023 HMOX1 expression enhancer | Serine/threonine-protein kinase MRCK-A |
| 10     | Pa 0.556  Pi 0.012 Antimutagenic | Serine/threonine-protein kinase receptor R3 |
| 11     | Pa 0.537  Pi 0.009 Myc inhibitor | Aldose reductase |
| 12     | Pa 0.564  Pi 0.039 JAK2 expression inhibitor | PI3-kinase p110-alpha/p85-alpha |
| 13     | Pa 0.542  Pi 0.018 Antinociceptive | Beta-secretase 1 |
| 14     | Pa 0.551  Pi 0.028 Vasoprotector | Sorbitol dehydrogenase |
| 15     | Pa 0.521  Pi 0.007 NOS2 expression inhibitor | Serine/threonine-protein kinase Chk1 |
| 16     | Pa 0.522  Pi 0.013 Chemopreventive | Aldo-keto reductase family 1 member B10 |
| 17     | Pa 0.588  Pi 0.079 Fibrinolytic | Tyrosine-protein kinase receptor TYRO3 |
| 18     | Pa 0.513  Pi 0.026 Spasmyolytic | Proto-oncogene tyrosine-protein kinase MER |
| 19     | Pa 0.549  Pi 0.068 TP53 expression enhancer | ATP-sensitive inward rectifier potassium channel 1 |
| 20     | Pa 0.491  Pi 0.018 Lipid peroxidase inhibitor | Serine/threonine-protein kinase WEE1 |
| 21     | Pa 0.484  Pi 0.021 Caspase eight stimulant | Serine/threonine-protein kinase mTOR |
| 22     | Pa 0.457  Pi 0.008 Antioxidant | Rho-associated protein kinase 2 |
| 23     | Pa 0.378  Pi 0.008 Transcription factor NF kappa B inhibitor | PI3-kinase p110-alpha subunit |
| 24     | Pa 0.411  Pi 0.044 Antiasthmatic | Estrogen receptor alpha |
| 25     | Pa 0.433  Pi 0.079 Anti-inflammatory | Estrogen receptor beta |
| 26     | Pa 0.354  Pi 0.073 TNF expression inhibitor | PI3-kinase p110-delta subunit |
| 27     | Pa 0.320  Pi 0.053 Anti-inflammatory, ophthalmic | PI3-kinase p110-beta subunit |
| 28     | Pa 0.261  Pi 0.064 Transcription factor NF kappa A inhibitor | PI3-kinase p110-gamma subunit |
| 29     | Pa 0.127  Pi 0.020 12-Lipoxygenase inhibitor | Mitogen-activated protein kinase kinases 8 |
| 30     | Pa 0.107  Pi 0.019 15-Lipoxygenase inhibitor | Mu opioid receptor |
CONFLICT OF INTEREST

The authors have declared no conflict of interest.

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How to cite this article:
Marulasiddaswamy KM, Nuthan BR, Channarayapatna-Ramesh S, Bajpe SN, Sekhar S, Kini KR. In-silico evaluation of Fragransol B from Myristica dactyloides for anti-inflammatory potential. J Appl Pharm Sci, 2021; 11(11):112–120.