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Original Article

# PREDICTION OF ACTIVE COMPOUNDS OF *MUNTINGIA CALABURA* AS POTENTIAL TREATMENT FOR CHRONIC OBSTRUCTIVE PULMONARY DISEASES BY NETWORK PHARMACOLOGY INTEGRATED WITH MOLECULAR DOCKING

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**ABSTRACT**

**Objective:** Electronic cigarettes (E-Cigarettes) are often advertised as a safe alternative to smoke cessation. The number of E-Cigarettes users (vapers) has increased in many countries. The health impact of E-Cigarettes research topics still counting constitutes initiating Chronic Obstructive Pulmonary Disease (COPD). This research aimed to analyze the interaction between genes from E-Cigarettes causing COPD with *Muntingia Calabura* leaves, which has umpteen pharmacological effects through Bioinformatics.

**Methods:** The related genes in E-Cigarettes compounds underlying COPD conditions were screened and intersected towards *M. Calabura*'s genes target. The constructed networks were analyzed for their protein-protein interaction and pathway possibilities. The gene with the best betweenness centrality, closeness centrality, and degree value was validated using molecular docking methods for its interaction with *M. Calabura* leaves.

**Results:** 12 target genes of *M. Calabura* and COPD were ALB, MMP-9, ICAM-1, GADPH, VEGFA, MPO, AKT1, ELANE, CXCR2, CFRTR, HSPA1A, and ADRB2. MMP-9 had the best value and then became the gene docked with *M. Calabura* compounds. The signaling propensity probably was PI3K/AKT pathway. *M. Calabura* has potentiated as a neutrophil inhibitor to balance protease/anti-protease. From molecular docking analyses, we found that 5,7-Dihydroxy-6-methoxyflavone gave the best conformation with MMP-9 with a binding affinity value of-10 kcal/mol.

**Conclusion:** *M. Calabura* can be considered a natural source of candidates for COPD treatment.

**Keywords:** COPD, e-cigarettes, Muntingia Calabura, Cytoscape, Molecular docking, Protease inhibitor


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## INTRODUCTION

With the advancement of technology, hundreds of devices were made to support a better human life. For example, Electronic cigarettes or E-Cigarettes (E-Cig) are often advertised as an alternative way to help someone quit smoking. E-Cigarettes are an example of electronic nicotine delivery systems (ENDS) devices consisting of heating elements that deliver liquid substances through an aerosol system to be inhaled [1]. In 2020, in the United States, 3.6 million youth (middle and high school students) used an e-cigarette, 1 in 5 high schools, and 1 in 10 middle school students [2]. There was an outbreak in the same year; 2,807 hospitalized e-cigarette product use-associated lung injury (EVALI) cases or deaths have been reported [3]. In Indonesia, from RISKESDAS data in 2018, 10.9% of electronic users came from students aged 10-18 [4]. Research conducted in Indonesia shows that 10.68% of 920 respondents (16-24 y old) were E-Cigarettes users [5]. 53% of users in the US were curious to try this device [6]. Moreover, this kind of cigarette is smokeless, so people believe that this device is safer [1]. In contrast, the research conducted these past years refute this notion. Besides Nicotine, other compounds are vegetable glycerol as a humectant, flavoring agent, carbonyl, and metals [1]. Garcia-Arcos *et al.* conducted mice exposed to E-Cigarettes aerosol with nicotine liquid could lead to Chronic Obstructive Pulmonary Disease (COPD) characterized by airways enlargement, mucus hypertrophy, the release of inflammation mediators, and changes in cilia motion [7]. The toxicity effect from E-Cigarettes was comparable with conventional cigarettes, including DNA damage, cytotoxicity, and stimulating inflammation [8]. E-Cigarettes elevate IL-6, and MUC5AC protein production [9], encouraging TLR9 expression [10] and IL-8 [11]. Also, in e-cigarette users, some biomarkers, such as IL1-β, IL-6, and IL-8, were significantly higher than in nonsmokers [12]. At short-term exposure, E-Cigarettes increase the density of mice's platelet and their α-granule secretion [13]. COPD is a curable disease interpreted with airflow limitation manifested from alveolar abnormalities acquired by harmful particle exposure [14]. Males who use e-cigarettes and conventional cigarettes were 3.46 times more likely to get COPD [15]. Many mechanisms are responsible for the progression of COPD, such as necroptosis [16], apoptotic, DNA damage, reactive oxygen species, and inflammation that plays supremely, followed by bronchial remodeling [17] related to regulation like protease/anti-protease imbalance, which has a strong correlation with MMP's family expression (MMP2, MMP9, and MMP12) [18] and adhesion factors ICAM1 to lead mucus hypersecretion [19]. The research on this topic still counts on finding Therapy to alleviate the patient's condition.

Indonesia has diverse potential natural medicine sources, one of them is *M. Calabura* which has the immense pharmacological ability, including anti-nociceptive [20], anti-proliferative [21], antimicrobial, cytotoxicity [22], anti-oxidant, anti-inflammatory both fruit [24], also stem bark parts [25] and hepatoprotective [26] from the leaves. Flavonoid is *M. Calabura*'s primary bioactive compound responsible for pharmacological effects in *M. Calabura* [27]. Flavonoid content in leaves was almost twice greater than in stem bark [28]. This plant grows and is fast-paced to be cultivated in Southeast Asia, the US, India, and Brazil [29]. Therefore, this plant could be considered a tremendous therapy for diseases involving inflammation pathways, such as COPD.

We can use Network Pharmacology integrated with molecular docking to bridge this covered potential of *M. Calabura* with COPD. These systematic approaches combine some web-based databases and applications to reduce the time and give us a strong background before *in vivo* research execution [30]. The gene-target relationship can be predicted as well as the pathways perspective. In this study, we conducted the intersection of protein-protein interaction from COPD, E-Cigarette impacted genes, and genes target of *M. Calabura* isolates using Cytoscape [23]. After clustering the nodes, some genes with high degree, betweenness, and closeness centrality values were analyzed to give pathways and mechanisms liability. Finally, the

bonding strength between the gene and the isolates was done using PyRx, and AutoDock Vina, then visualized by Discovery Studio.

## MATERIALS AND METHODS

### Genes target collection of COPD and *M. Calabura*

The chemical substances per puff of electronic cigarettes were revealed by Cunnigham *et al.* [31]. Each chemical substance information, structure, genes targ*et al.* so SMILES canonical form was collected from website databases such as PubChem (https://pubchem.ncbi.nlm.nih.gov/), Swiss Prediction (http://www.swissadme.ch/), PharmMapper (https://www.bio.tools/pharmmapper-drug#!), GeneCards V4.12 (https://www.genecards.org/), OMIM (https://omim.org/), Pharmgkb (https://www.pharmgkb.org/) and TTD (http://db.idrblab.net/ttd/). The compound of *M. Calabura* was retrieved from PubChem, while the gene target was collected with the same method.

### Network construction

The network from the gene collection was constructed using Cytoscape 4.2.8 [23]. Cytoscape 4.2.8, installed with STRING disease databases, can generate a COPD network. After that, we can construct an E-cigarette network and an *M. Calabura* network. Merge the intersection of the E-cigarette network with the COPD network to obtain the possibility gene that can lead to COPD from the E-cigarette compound. This network then intersected with the *M. Calabura* network.

### Protein-protein interaction and pathways analysis

To make the analysis more manageable, we can cluster the network to create a pathway hypothesis. Installed CytoCluster [32] can be used for this. After choosing the best cluster with the smallest p-value, the network can be analyzed to observe degree, closeness, and betweenness centrality values. This study use STRING https://string-db.org/ [30], STITCH http://stitch.embl.de/ and The Human References Interactome http://www.interactome-atlas.org/ databases to complete and compare the involved genes as well as interaction analysis. By the same website database like STRING and STITCH, we can see the possible pathways related to COPD and *M. Calabura's* intervention. STRING can be directed to some pathways websites such as Celular Network Biology Jensenlab https://jensenlab.org/ and KEGG [33] Pathway database https://-www.genome.jp/.

### Virtual Screening and molecular docking

The gene target structure was collected from the website https://www.rcsb.org/ [34]. The protein target was complex primarily with its ligand, small molecules which can be metabolites, drugs, or cofactors. After the 3D structure was downloaded, the preparation protein included water and unnecessary ligand deletion, the addition of hydrogen atoms, and optimizing missing atoms; the last step was to save this prepared protein into pdbqt format. This file would be a macromolecule for molecular docking [33]. The 14 ligands were also prepared using PyRx [35] to minimize the energy via open babel and save them as pdbqt.

Virtual Screening was done using PyRx and AutoDock Vina [36]. The virtual Screening can be obtained after the prepared ligand and macromolecule are set. Select the AutoDock bar and click Vina Wizard. Make sure that each of the Ligands and Macromolecules was in their place.

On the Run Vina below Vina Wizard, the Grid Box must be decided; ensure that the box is within the protein's active site. After all, set, run the Vina, and wait until binding affinity data and RMSD (Root Mean Square Deviation) data can be downloaded. The result shows that the best isolate with the lowest (most negative binding affinity, kcal/mol) and RMSD value closest to 0 was chosen.

Discovery Studio Visualizer v21.1.0.20298 is a valuable tool for analyzing and visualizing the protein residue's interaction with ligand compounds. This result is then compared with the native ligand of the protein [37]. This study was conducted by Laptop *HP*IntelCorei5 8$^{th}$ Gen Radeon Graphics and the Software Application Cytoscape 4.2.8, AutoDock Tools 1.5.6, and Discovery Studios Visualizer v21.1.0.20298.

## RESULTS

We found 25 detected compounds from E-Cigarettes based on the research article by Cunningham *et al.* [31]. They were Nicotine, propylene glycol, glycerol, Polycyclic Aromatic Hydrocarbons (Naphthalene, 1-Methylnaphtalene, 2-Methylnapthalene, Fluorene, Phananthrene, Anthracene, Flouranthene, Pyrene), Carbonyls (Formaldehyde, Acetaldehyde, Acetone, Propionaldehyde, Acrolein, Isobutyraldehyde, Glycolaldehyde, Glyoxal, Methylglyoxal) and metals (iron, aluminum, chromium, molybdenum, dan zinc).

The genes target of each compound were generated from different databases such as PubChem, Swiss Adme and Swiss Prediction (http://www.swissadme.ch/), Pharm Mapper (https://www.bio.tools/pharmmapper#), GeneCardsV4.12 (https://www.genecards.org/), OMIM(https://omim.org/); Pharmgkb (https://www.pharmgkb.org/) and TTD (http://db.idrblab.net/ttd/) [38]. We searched multiple databases because not all the chemical compound targets can be found in one website database. For example, SwissADME could not find any targets for small molecules (atom C<5), while the volatile heated compound in E-Cigarettes consists of many small molecules. From the genes target inputted into Cytoscape, there were 7371 nodes with 215247 edges.

While COPD Network, the Cytoscape is integrated with some databases, such as PubMed, STRING, and STITCH. There were contains 100 nodes with 1409 edges constructed.

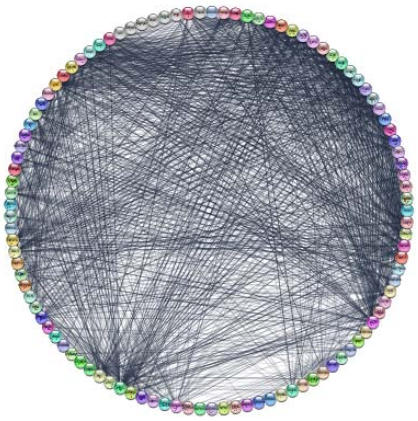


**Fig. 1: Chronic obstructive pulmonary disease (COPD) network**

Both networks then merged and intersected to find the same genes that indicate the possibility of the underlying pathogenesis of COPD due to E-Cigarettes disclosure. The highest 20 genes with closeness centrality value of nearly 1.0from 75 genes and 1114 nodes were TNF, ALB, IL-6, GAPDH, AKT-1, ACTB, IL-1b, CXCL8, FN-1, INS, IL-10, VEGFA, TLR-4, CD-4, MMP-9, MPO, CCL2, CRP, ICAM-1 and IL-4. The intersected network is shown in fig. 2.

The next step was to retrieve the network from *M. Calabura* leaves isolates. As mentioned in the introduction above, the leaves had two times greater flavonoid content [28]. From PubChem, we found 48 articles related to *M. Calabura*. Five of them were the isolation of its leave available. The isolate had potent pharmacological activity such as antiplatelet [39], cytotoxicity [40], and inflammation mechanism [41-43].

14 isolates structure had been collected as well as their genes target (1) (2R,3R)-3,5,7-Trihydroxyflavanone; (2) (2R,3R)-7-Methoxy-3,5,8-trihydroxyflavanone; (3) (2S)-5'-Hydroxy-7,8,3',4'-tetramethoxyflavan; (4)(2S)-5-Hydroxy-7-methoxyflavanone; (5)(2S)-7,8,3',4',5'Pentame-thoxyflavan; (6) (2S)-7-Hydroxyflavanone; (7) 2',4'-Dihydroxychalcone; (8) 2',4'-Dihydroxydihydrochal-cone; (9) 5,4-Dihydroxy-3,7-dimethoxyflavone (Kumatakenin); (10) 5,7-Dihydroxy-3-methoxyflavone; (11)5,7-Dihydroxy-6-methoxyflavone; (12)5-Hydroxy-3,8-dimethoxyflavone; (13)5-Hydroxy-7-meth-oxyflavone; and (14)Methyl gallate. Those 14 isolates had 560 nodes with 4691 edges constructed network. We excluded the structure that had no genes target.

We had intersected the *M. Calabura* network with the merged COPD-E. Cigarettes network. The genes were ALB, MMP-9, AKT-1, VEGFA, MPO, GAPDH, ICAM-1, ELANE, CXCR2, CFTR, HSPA1A, ADBR2, and MMP12, the network captured in fig. 3. The nodes' border would be thicker in line with the higher value of betweenness and closeness centrality also its degree. The degree value indicates the number of edges linked to a given node [44] Here, MMP9, AKT-1, and ALB nodes had a high degree that may equate to the hub genes related to biological functions. At the same time, betweenness centrality emulates the importance of the node entrenched in the number of shortest paths for each node. For PPI analysis's closeness, betweenness centrality, and degree value result as per table 1.

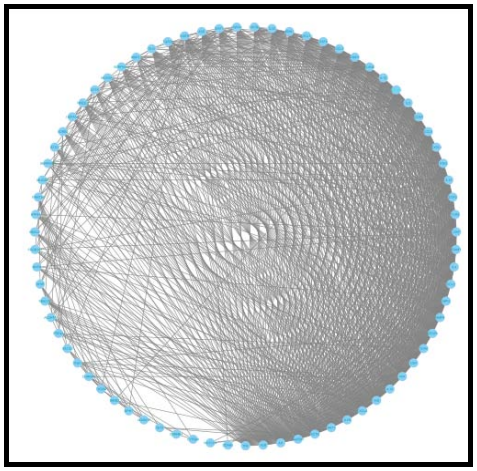


**Fig. 2: COPD and E-Cigarettes intersected network**

Using CytoCluster–ClusterONE [32] plugin in Cytoscape, we can make the network into a cluster with only minimal protein complexes at three proteins with a threshold $\partial$ value of 0.8 (by default). The only cluster in this network consists of 8 nodes. ALB, MMP9, ICAM-1, GADPH, VEGFA, MPO, AKT-1, and ELANE. All of these genes were related to COPD pathogenesis in many pathways.

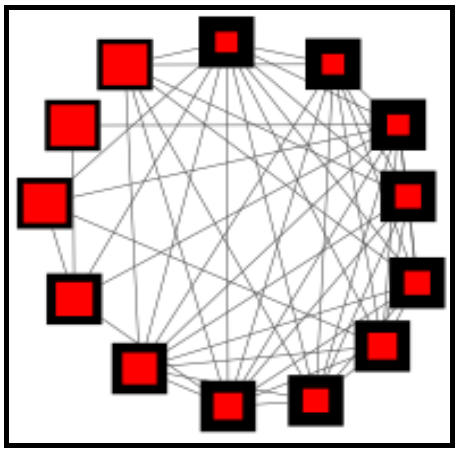


**Fig. 3: 13 Nodes of *M. Calabura* target COPD due to E-Cigarette exposure**

We used STRING, STITCH, and Interactome database services for pathway analysis. Fig. 4 is the graph generated from the STITCH database that shows the interaction mode of each node. STRING is integrated with some pathways analysis database websites. One of them is Jensenlab http://jensenlab.org/, which revealed tissue expression related to the genes. The genes inputted to STRING would generate other information such as pathways, biological processes, cellular components, UniProt, and protein domains.

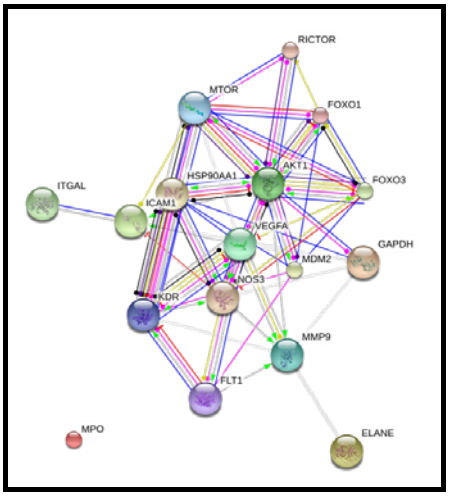



**Fig. 4: STITCH analysis of *M. Calabura* genes target towards COPD due to E-Cigarettes network**

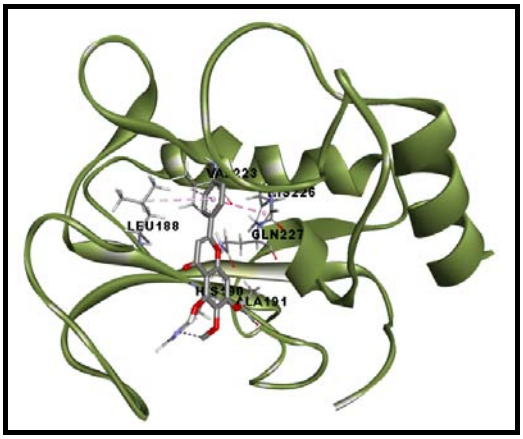
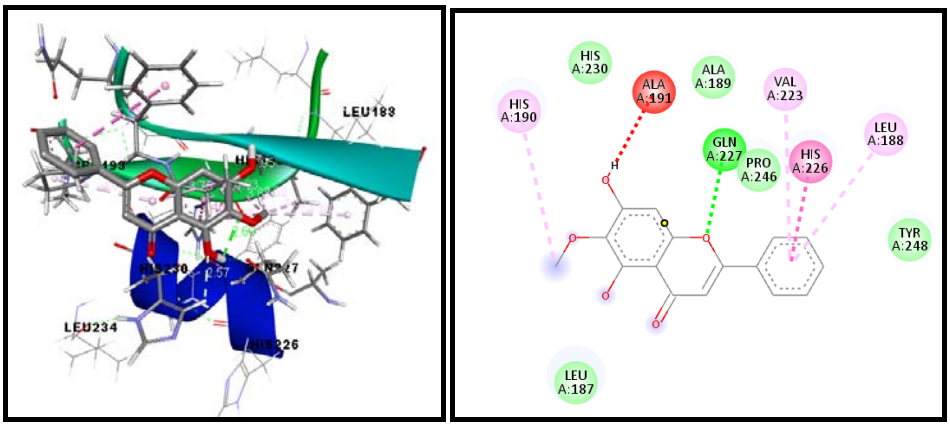



**Fig. 5: The docking result between 5,7-Dihydroxy-6-methoxyflavone and MMP-9**

We wanted to know the expression in the tissue. As directed to Jensen's lab, those genes appeared as human genes that correlate with neutrophil and protease inhibitor complexes. Another platform we used to find protein-protein interaction was The Human Reference Interactome http://www.interactome-atlas.org/. This website has a filter feature to see the gene expressed in a specific tissue, such as the lung, with low-medium and high-confidence value options. ICAM-1 was revealed as the highest specificity gene expressed in the lung.

## DISCUSSION

The anti-protease imbalance was one of the COPD pathogenesis [45] that affected tissue remodeling and inflammation, which led to the degradation of the extracellular matrix unit. MMP-9 [46], MMP-12 [18] and neutrophil regulation [47] have important roles in COPD. The research about how E-Cigarettes cause COPD was limited; the first research successfully developed a mice COPD model conducted by Garcia *et al.* [7] that indicated the elevation of MMP9, MMP 12, IL-6, and IL-8. MMPs in the airways are produced normally in the human body, but the triggering exogen factor, such as cigarette smoke exposure, can elevate these MMPs production. Moreover, MMP-9 related to alveolar bronchodilation facilitates the migration of bronchiolar cells into regions of injury, while the decreasing MMP-9 indicates repairing progress due to cigarette smoke-induced airway epithelial injury [48]. The pathways from this analysis were pointed to PI3K/AKT pathway. Veiling PI3K (phosphatidylinositol 3-kinase) can inhibit downstream signaling pathways such as AKT (Protein kinase B) to regulate autophagy and induce alveolar epithelial cells' apoptosis in COPD [49].




**6A** **6B**

**Fig. 6: A(3D Visualization); B (2D Visualization) the binding site and ligand interaction of 5,7-Dihydroxy-6-methoxyflavone to MMP-9**

**Table 1: Genes target list of *M. Calabura* towards COPD due to E-Cigarettes exposure**

| Genes name | Human genes for neutrophil Z-score | Human genes for protease inhibitor complex Z-score | Closeness centrality/betweenness centrality/ Degree |
|---|---|---|---|
| ALB | 6.3 | 7 | 0.76/0.014/9 |
| MMP-9 | 5.9 | 5.3 | 0.76/0.058/9 |
| ICAM-1 | 6.2 | 4.6 | 0.72/0.014/8 |
| GADPH | 5.2 | 7.4 | 0.68/0.039/7 |
| VEGFA | 5.6 | 5.1 | 0.72/0.05/8 |
| MPO | 7 | 3.8 | 0.72/0.014/8 |
| AKT1 | - | 6.3 | 0.76/0.163/9 |
| ELANE | 6.6 | 5 | 0.68/0.077/7 |
| CXCR2 | 6.1 | - | 0.619/0.004/6 |
| CFRTR | - | - | 0.59/0.06/4 |
| HSPA1A | - | 3.6 | 0.52/0.01/3 |
| ADRB2 | - | - | 0.5/0.004/2 |
| MMP-12 | 4.7 | 3.4 | 0.5/0.002/3 |

**Table 2: Virtual screening result of *M. Calabura* isolates and MMP9**

| Isolate name | Binding Affinity kcal/mol | RMSD ub (A) | RMSD lb (A) |
|---|---|---|---|
| 5,7-Dihydroxy-6-methoxyflavone | -10.0 | 0.0 | 0.0 |
| (2S)-5-Hydroxy-7-methoxyflavanone | -9.9 | 0.0 | 0.0 |
| 5-Hydroxy-7-methoxyflavone | -9.6 | 0.0 | 0.0 |
| (2R,3R)-3,5,7-Trihydroxyflavanone | -9.5 | 0.0 | 0.0 |
| (2R,3R)-7-Methoxy-3,5,8-trihydroxyflavanone | -9.5 | 0.0 | 0.0 |
| (2S)-7-Hydroxyflavanone | -9.4 | 0.0 | 0.0 |
| 2',4'-Dihydroxychalcone | -9.4 | 0.0 | 0.0 |
| 2',4'-Dihydroxydihydrochalcone | -9.4 | 0.0 | 0.0 |
| Control Positive: SB-3CT | -8.4 | 0.00 | 0.00 |

Since the highest gene related to COPD from this network analysis was MMP-9, we conducted the virtual Screening and then molecular docking with 14 isolates to get information on which isolate can bind into the site active of MMP-9. The best-fitted isolate, from the binding affinity-10 kcal/mol and RMSD value 0.00, was 5,7-Dihydroxy-6-methoxyflavone which is to be dated known as oroxylin A.

This substance perfectly binds onto MMP-9 residues with better binding affinity values than SB-3CT [48] 2-[(4-Phenoxyphenyl) sulfonylmethyl-thiirane, indicated for MMP9 inhibitor. The detailed result is as per table 2. Lower binding affinity implies that this compound and the macromolecules need low activation energy with the possibility of spontaneous reaction [49]. Moreover, there were four interaction types: van der Waals interaction (Leu187, Tyr248, Pro246, Ala189, His230), hydrogen bond interaction (Gln227), Pi-Pi Stacked interaction, and Pi-Alkyl interaction which gain effectivity and could be a potential therapy for drug development.

## CONCLUSION

*M. Calabura* can give intervention to ameliorate COPD condition as a neutrophil inhibitor by blocking MMP9 via PI3K-Akt signaling pathways. 5,7-Dihydroxy-6-methoxyflavone has a good RMSD score (0) with-10 kcal/mol. Further *in vivo* study is required to get comprehensive molecular mechanisms from the bioinformatics approach.

## FUNDING

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## AUTHORS CONTRIBUTIONS

AB devised the project and the main conceptual ideas. and designed the experiment. FF designed the experiment. NN worked out almost all of the technical details and performed the experiments. NN proposed the experiment in discussions with FF and AB NN, and AB wrote the manuscript.

**CONFLICT OF INTERESTS**

We assure you that there is no conflict of interest related to this study.

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