

PHYTOPHARMACOLOGICAL EVALUATION OF CALOTROPIS LEAF EXTRACT FOR CYSTIC FIBROSIS: A BIOACTIVE SCREENING AND MOLECULAR DOCKING APPROACH

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ABSTRACT

Cystic fibrosis (CF) is a life-threatening autosomal recessive disorder caused by mutations in the CFTR gene, leading to defective chloride ion transport and thick mucus accumulation, primarily affecting the lungs and digestive system. Current treatment options focus on managing symptoms, yet there remains a strong demand for novel, cost-effective therapeutics with anti-inflammatory and antioxidant properties. This study explores the phytotherapeutic potential of *Calotropis gigantea* and *Calotropis procera*, two traditional medicinal plants known for their bioactive compounds. Methanolic leaf extracts were subjected to phytochemical screening, Thin Layer Chromatography (TLC), and column chromatography. Fractionated samples were analyzed using Gas Chromatography-Mass Spectrometry (GC-MS), which identified key compounds such as pinoresinol and neophytadiene. Molecular docking studies were performed to assess their interactions with CFTR-related protein domains. Pinoresinol exhibited strong binding affinity, suggesting its potential to modulate CFTR function and reduce inflammation. These findings highlight the potential of *Calotropis*-derived compounds as a promising source for CF therapy. Further in vitro and in vivo investigations are recommended to validate their therapeutic mechanisms and efficacy. This study contributes to the growing interest in plant-based drug discovery for genetic and inflammatory disorders.

Keywords: Cystic Fibrosis, CFTR Modulation, *Calotropis gigantea*, Pinoresinol, Molecular Docking

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INTRODUCTION

Medicinal plants have long been a rich source of therapeutic agents due to their production of bioactive compounds such as tannins, terpenoids, alkaloids, and flavonoids, which exhibit antioxidant, antimicrobial, and antipyretic properties. These compounds help protect plants from herbivores, fungi, and insects, highlighting the need to explore traditional medicinal plants for novel antimicrobial agents (Waseem *et al.*, 2020). *Calotropis gigantea* and *Calotropis procera*, two Arka species, hold significant value in Ayurveda, Unani, and Siddha systems (Shripad M. Bairagi *et al.*, 2018). *Calotropis* species are native to China, Malaysia, and India, and widely found across South India, Assam, Punjab, lower Bengal, and the Himalayas up to 1000 meters. They thrive in waste lands, roadsides, and railway tracks. These *Apocynaceae* family members are perennial shrubs with latex-bearing tissues (Subhajit Mandal *et al.*, 2022). They grow 2.4–3 meters tall, with thick, pale green leaves and purple or pale greenish-yellow flowers (Shripad M. Bairagi *et al.*, 2018).

Cystic Fibrosis (CF) is a genetic, autosomal recessive disorder primarily affecting the lungs, pancreas, liver, intestines, and reproductive organs. It results from mutations in the CFTR gene, particularly $\Delta F508$, leading to thick, sticky mucus that blocks airways and ducts, causing chronic infections, malabsorption, and infertility, especially in males. CF is most prevalent among individuals of European descent, 1 in 2,500 births in Northern/Western Europe and Australia, and rarer in Africa and

Asia. Despite equal inheritance in both sexes, males are often sterile due to congenital bilateral absence of the vas deferens (CBAVD).

CFTR functions as a chloride and bicarbonate ion channel on epithelial cells. Mutations impair ion transport, leading to dehydration of mucosal surfaces and thick mucus accumulation. This disrupts mucociliary clearance, promotes infections, and leads to lung and pancreatic damage, malabsorption, CFRD, intestinal obstruction, liver issues, and infertility. Elevated salt in sweat serves as a diagnostic marker. The 1,480-amino acid CFTR protein comprises two membrane-spanning domains (MSDs), two nucleotide-binding domains (NBDs), and a regulatory (R) domain. It regulates Cl^- and HCO_3^- transport and inhibits ENaC, maintaining hydration across mucosal surfaces for functions like mucociliary clearance and enzyme secretion.

Recent in silico studies explore natural compounds such as pinoresinol and neophytodine for CFTR modulation. Pinoresinol binds to NBD1, potentially enhancing CFTR stability, while neophytodine targets transmembrane domains, indicating potential correction of misfolded proteins. Commercial CFTR modulator drugs like ivacaftor, lumacaftor, and tezacaftor modulate CFTR activity, ivacaftor as a potentiator and others as correctors. These improve lung function but are expensive and mutation-specific (Emanuele Bellacchio *et al.*, 2023; Giada Righetti *et al.*, 2020). Natural compounds show promising docking scores and fewer side effects compared to commercial drugs. Pinoresinol and

neophytodine offer lower toxicity and cost benefits, warranting further in vitro and in vivo research (Areeba Akram *et al.*, 2024).

MATERIALS AND METHODS

Sample collection and preparation

Leaves of *Calotropis gigantea* and *Calotropis procera* were collected from roadside areas in the suburbs of Avadi, Chennai, India. The collected leaves were thoroughly washed under running water to remove adhering soil, dust, and other external contaminants. The washed leaves were subsequently air-dried at room temperature for three weeks. Following air-drying, the leaves were further subjected to heat treatment in a hot-air oven at 40°C for 2 h to remove residual moisture and facilitate efficient grinding. The dried leaves were then pulverized using a mechanical grinder to obtain a fine and relatively homogeneous powder. The powdered material was passed through a suitable filtering mesh to ensure uniform particle size. The resulting powdered plant material was transferred to clean cylindrical plastic containers and stored at room temperature in a dark environment until further extraction. Storage under dark conditions was employed to minimize exposure to light and thereby help preserve the phytochemical constituents of the plant material.

Soxhlet Extraction

The powdered leaves of *C. gigantea* and *C. procera* were subjected to Soxhlet extraction using methanol as the extraction solvent. For each extraction, 82.5 g of finely powdered plant material was extracted with 330 mL of methanol, corresponding to a plant material-to-solvent ratio of 1:4 (w/v), based on the proportion of 250 g plant material to 1000 mL solvent. The extraction was performed for 3 h under controlled heating conditions. The extraction temperature was maintained at approximately 30°C. Following completion of the extraction, the methanolic extract was carefully collected and transferred into a clean beaker. The solvent was subsequently allowed to evaporate gradually at room temperature. The beaker was covered with aluminum foil containing small perforations to minimize environmental contamination while permitting gradual evaporation of methanol. The resulting concentrated extract was retained for subsequent chromatographic analysis.

Thin Layer Chromatography (TLC)

Thin-layer chromatography (TLC) was performed to evaluate the phytochemical profile of the methanolic *Calotropis* extracts and to identify a suitable mobile phase for subsequent column chromatographic separation. A total of 14 solvent systems with different polarities were evaluated using combinations of chloroform, ethyl acetate, and methanol. Chloroform was used as the relatively non-polar component, ethyl acetate as an intermediate-polarity solvent, and methanol as the polar component. The solvent systems evaluated are presented in Table 1. The solvent systems were evaluated based on the degree of migration, number of visible bands, and resolution between separate components. Solvent systems producing comparatively distinct and well-resolved bands were considered suitable for subsequent column chromatography.

Column Chromatography

The methanolic *Calotropis* extract was initially mixed thoroughly with silica gel to facilitate dry loading onto the chromatographic column. Ethyl acetate was added to the extract-silica mixture to facilitate uniform dispersion and formation of a slurry. The resulting slurry was allowed to air-dry until the ethyl acetate had evaporated completely, yielding a dry powdered material in which the extract constituents were adsorbed onto the silica gel. The dried silica-bound extract was subsequently divided into three batches for loading onto the chromatographic column.

Column chromatography was performed using a glass column packed with silica gel as the stationary phase. The three batches of silica-bound extract were separately loaded onto the column and eluted using solvent systems selected based on the preliminary

TLC optimization. The solvent systems selected for the three batches were as follows: Batch 1 (Ratio 4): 95% ethyl acetate + 5% methanol, Batch 2 (Ratio 10): 65% ethyl acetate + 35% methanol and Batch 3 (Ratio 12): 55% ethyl acetate + 45% methanol. For each batch, the eluate was collected into three separate test tubes according to the observed elution pattern and timing. The collected fractions were visually examined for the presence of separate bands and differences in coloration. Where required, the fractions were further subjected to TLC analysis to assess the separation pattern and relative purity of the obtained fractions.

TLC Analysis of Column Fractions

A second round of TLC analysis was performed using the fractions obtained from column chromatography. Fractions from Batch 1 (Ratio 4), Batch 2 (Ratio 10), and Batch 3 (Ratio 12) were evaluated to compare the separation profiles obtained using the different mobile-phase compositions. Among the tested batches, Batch 3, corresponding to the solvent system containing 55% ethyl acetate and 45% methanol, exhibited comparatively distinct and well-resolved bands. The improved resolution observed with this solvent system indicated more effective chromatographic separation of the extract constituents. Based on the TLC profile, the Batch 3 fraction was selected for subsequent GC-MS analysis.

Gas Chromatography–Mass Spectrometry Analysis

The fraction exhibiting the most distinct TLC separation was subjected to gas chromatography–mass spectrometry (GC-MS) analysis for characterization of its volatile and semi-volatile chemical constituents. GC-MS combines chromatographic separation by gas chromatography with mass spectrometric detection, enabling the separation and tentative identification of individual chemical constituents present in a complex extract.

During gas chromatographic analysis, the components of the extract are separated according to their physicochemical properties and interaction with the chromatographic column. The separated constituents generate individual chromatographic peaks, which can subsequently be characterized based on their mass spectra. In the mass spectrometric component, compounds are ionized and detected according to their mass-to-charge ratio (m/z), generating characteristic mass spectral profiles.

The chemical constituents were tentatively identified by comparing their obtained mass spectra and chromatographic characteristics with those available in an appropriate reference spectral library. The resulting chromatogram was used for qualitative assessment of the chemical composition, while the relative abundance of detected constituents was evaluated from their corresponding peak areas where applicable.

Molecular Docking Analysis

Molecular docking analysis was performed using AutoDock version 4.2.6 to investigate the potential binding interactions between selected compounds identified from the GC-MS analysis and selected protein targets. Molecular docking is a computational approach used to predict the preferred orientation and interaction of a ligand within the binding site of a target macromolecule.

AutoDock 4.2.6 employs the Lamarckian Genetic Algorithm (LGA) to explore the conformational and orientational space of ligand-protein complexes. The docking procedure combines a genetic algorithm with local search optimization to identify energetically favorable ligand conformations within the defined binding region.

The selected protein structures and ligand structures were prepared prior to docking. Docking calculations were performed within the predefined binding region of the target protein, and the resulting poses were evaluated according to their predicted binding energies and interaction profiles. The docking results were used to identify compounds exhibiting favorable predicted interactions with the selected protein targets.

The predicted ligand-protein complexes obtained from molecular docking were subsequently analyzed using BIOVIA Discovery

Studio Visualizer (version 21). The software was used to visualize the three-dimensional orientation of docked ligands within the protein binding sites and to characterize ligand–residue interactions. The amino acid residues involved in the predicted ligand-binding regions were examined, and the interactions between the ligands and surrounding residues were visualized. Two-dimensional interaction diagrams were generated where appropriate to identify potential hydrogen bonds, hydrophobic interactions, and other non-covalent interactions contributing to ligand stabilization within the binding site. The docking poses and corresponding interaction profiles were used to facilitate comparative assessment of the binding behavior of the selected phytochemical constituents against the respective protein targets.

RESULTS

Thin Layer Chromatography

Thin-layer chromatography (TLC) was performed using 14 different solvent systems with varying proportions of chloroform, ethyl acetate, and methanol to optimize the separation of phytochemical constituents present in the methanolic *Calotropis* extract. Among the 14 solvent systems evaluated, solvent systems 4, 10, and 12 produced comparatively distinct and well-resolved bands, indicating effective separation of the extract constituents.

Solvent system 4, consisting of 95% ethyl acetate and 5% methanol, produced sharp and clearly distinguishable bands with moderate migration. The separation profile indicated that this solvent composition was suitable for the migration and resolution of relatively moderately polar to semi-polar constituents.

Solvent system 10, comprising 65% ethyl acetate and 35% methanol, resulted in increased migration of the extract constituents compared with solvent system 4. The bands obtained were comparatively broader, suggesting enhanced mobility of more polar constituents in the presence of increased methanol concentration.

Solvent system 12, consisting of 55% ethyl acetate and 45% methanol, exhibited further enhancement in the migration of polar constituents. The solvent system produced a distinct separation pattern and facilitated the differentiation of compounds with higher polarity.

Overall, the TLC profiles demonstrated that changes in mobile-phase composition markedly influenced the migration and resolution of the phytochemical constituents. The progressive increase in methanol concentration resulted in greater migration of polar components, indicating the presence of constituents with a broad range of polarities within the methanolic extract. Based on the observed separation patterns, solvent systems 4, 10, and 12 were selected for subsequent column chromatographic separation.

Column Chromatography

Column chromatography was performed using silica gel as the stationary phase and the three optimized solvent systems identified from the TLC analysis. The selected solvent systems represented progressively increasing mobile-phase polarity, enabling differential elution of constituents according to their interactions with the stationary and mobile phases.

Batch 1 was eluted using 95% ethyl acetate and 5% methanol. The relatively lower polarity of this solvent system favored the elution of moderately polar and semi-polar constituents. The collected fractions exhibited limited but distinguishable separation of compounds, indicating the presence of constituents that could be effectively mobilized under relatively low-polarity conditions.

Batch 2 was eluted using 65% ethyl acetate and 35% methanol. The increased proportion of methanol resulted in enhanced mobile-phase polarity and facilitated the elution of more polar constituents. The fractions obtained from this batch exhibited broader elution patterns compared with Batch 1, suggesting the presence of a wider range of polar constituents within the extract.

Batch 3 was eluted using 55% ethyl acetate and 45% methanol, representing the most polar solvent system among those selected for column chromatography. This solvent composition facilitated

the migration of highly polar constituents. The elution profile was comparatively more continuous, with less sharply defined fraction boundaries, which may be attributed to the greater mobility of polar constituents under the increased methanol concentration.

The overall column chromatographic profiles were consistent with the TLC observations, demonstrating that increasing the methanol content progressively enhanced the elution of polar constituents. Among the three solvent systems, the fraction obtained using solvent system 12 showed a prominent separation profile and was therefore selected for subsequent chemical characterization by GC–MS.

GC-MS Analysis

The selected fraction from Batch 3 was subjected to gas chromatography–mass spectrometry (GC–MS) analysis to characterize its chemical constituents. The GC–MS chromatographic profile revealed the presence of multiple chemical constituents within the analyzed fraction. Based on the GC–MS identification profile and consideration of their reported pharmacological relevance, Pinoresinol and Neophytadiene were selected as candidate compounds for subsequent molecular docking analysis. The selection of these compounds was based on their detection in the analyzed extract and their reported biological/pharmacological potential in the literature. The selected compounds were subsequently subjected to *silico* molecular docking analysis to investigate their potential interactions with the predetermined protein targets.

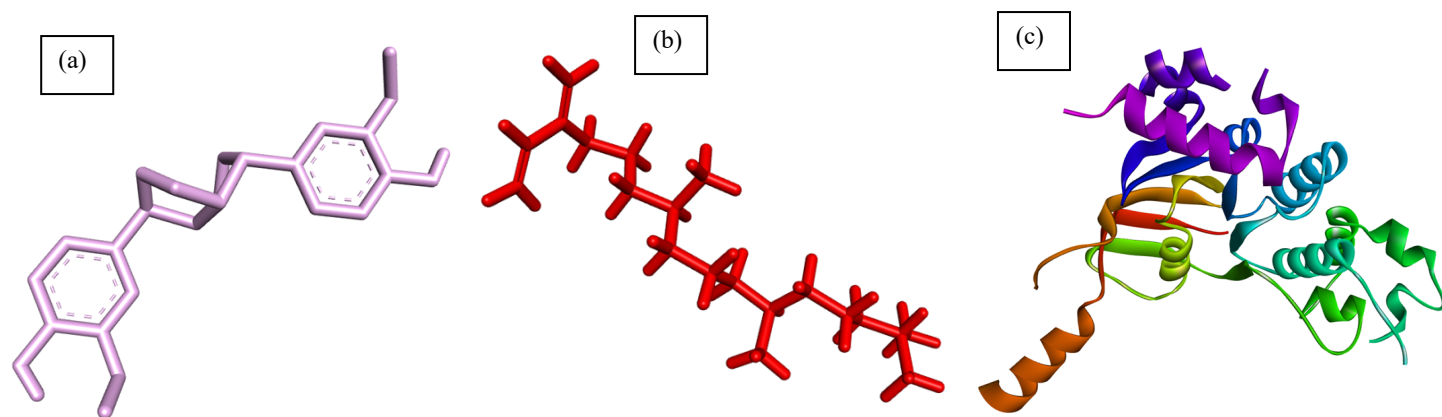


Figure 1. 3D Structure of (a) Pinoresinol (Compound 1), (b) Neophytadiene (Compound 2) and (c) CFTR-NBD1 Protein

Table 1. Solvent systems evaluated for TLC separation

Ratio	Chloroform (μL)	Ethyl acetate (μL)	Methanol (μL)
1	1000	—	—
2	500	500	—
3	—	1000	—
4	—	950	50
5	—	900	100
6	—	850	150
7	—	800	200
8	—	750	250
9	—	700	300
10	—	650	350
11	—	600	400
12	—	550	450
13	—	500	500
14	—	450	550

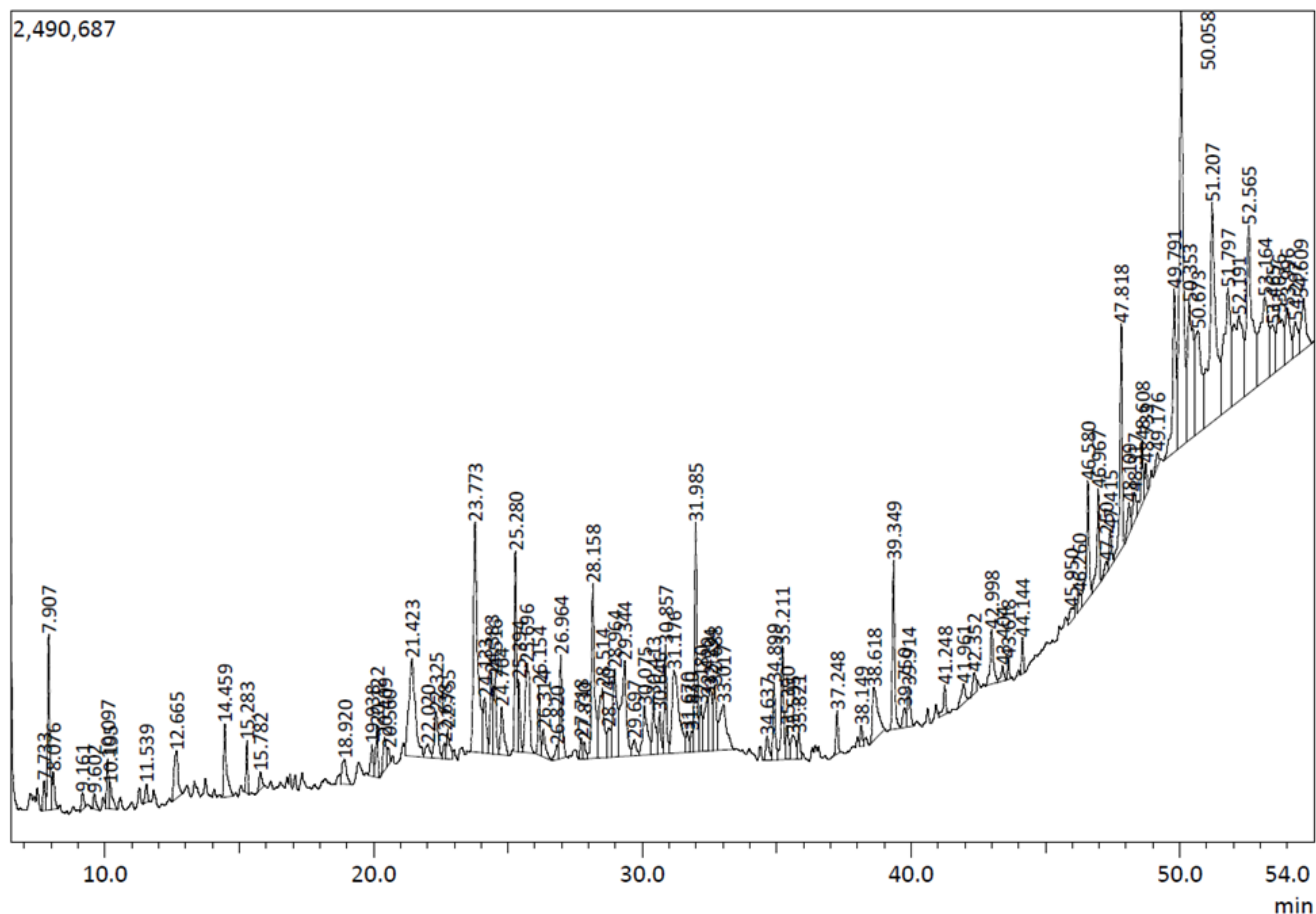


Figure 2. Chromatogram for selected bioactive compounds

Table 2. GC-MS Analysis for selected bioactive compounds

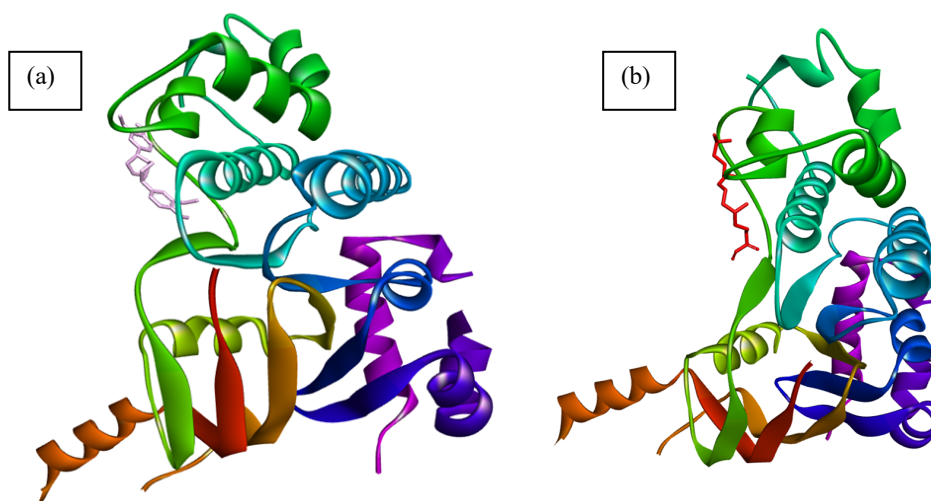
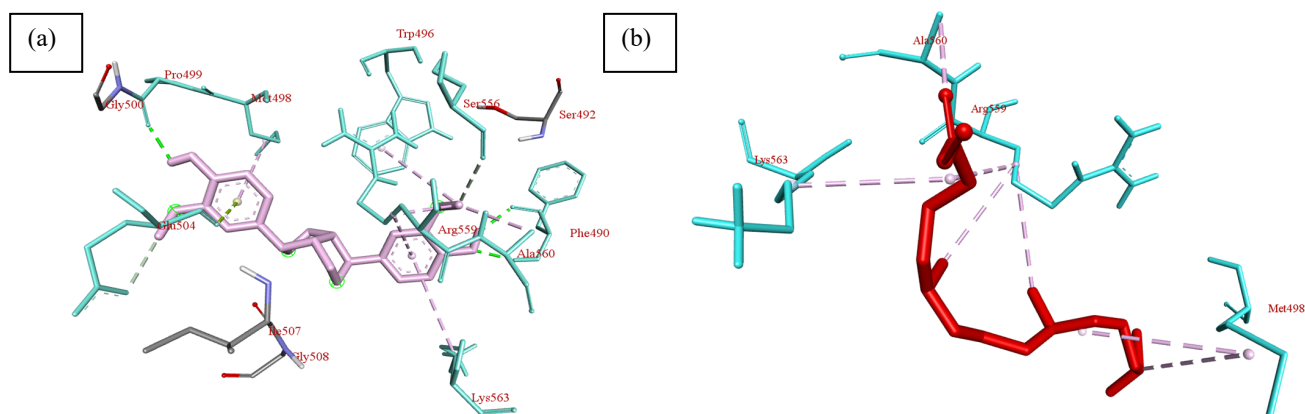
S. No.	Compound identified	Chemical class	Retention time (min)	Relative abundance (%)	Reported biological activity
1.	Methyl Linoleate	Fatty Acids	19.938	0.32%	Anti-inflammatory, emollient, melanogenic effects
2.	Methyl Palmitoleate	Fatty acids	31.176	2.37%	Anti-inflammatory, lipid metabolism regulator
3.	Eicosapentaenoic Acid-TMS	Esters and Alcohols	52.191	3.31%	Anti-inflammatory and Cardio protective effects
4.	Methyl Linolenate	Esters and Alcohols	51.797	3.24%	Anti-inflammatory and Cardio protective effects
5.	Docosapentanoic acid-TMS	Esters and Alcohols	52.565	0.98%	Anti-inflammatory and Cardio protective effects
6.	Phenol-TMS	Phenolic and Aromatic compounds	49.791	0.19%	Anti-septic and Anti-bacterial effects
7.	Pinoresinol	Phenolic and aromatic compounds	50.058	6.61%	Anti-oxidant and anti-cancer effects
8.	Neophytadiene	Terpenes and Hydrocarbons	25.280	0.24%	Anti-oxidant, Anti-pathogenic, Anti-inflammatory effects
9.	Cis- 2,3- Epoxy- 4S-(Hydroxy methyl)-isophorone	Terpenoid derivative	35.211	1.16%	Anti-bacterial and Anti-tumour effects

Table 3. Binding affinities and energies of the screened ligand with the protein

S.no	Lowest Binding energy (kcal/mol)	kI (micromolar)	Intramolecular energy (kcal/mol)	Internal energy (kcal/mol)	Torisional energy (kcal/mol)	Unbound extended energy (kcal/mol)
Compound 1	-6.12	32.48	-7.19	-2.09	1.79	-2.09
Compound 2	-3.90	1.38	-7.78	-0.97	3.38	-0.97

Table 4. Interaction profile of screened ligands with the protein

Interaction profile of the ligands with the protein				
S.No.:	Ligand	Binding affinity	Nature of interactions	Amino acids interacted with
1	Compound 1	-6.12	Hydrogen Bond, Pi-Lone pair, Hydrophobic.	PRO A:499, MET A:498, GLU A:504, ALA A:560, SER A:556, PHE A:490, LYS A:563, ARG A:559, TRP A:496
2	Compound 2	-3.90	Hydrophobic	MET A:498, ARG A:559, LYS A:563, ALA A:560

**Figure 3. (a) Compound 1 and (b) Compound 2 bound with the protein****Figure 4. Interaction of (a) Compound 1 and (b) Compound 2 with the protein**

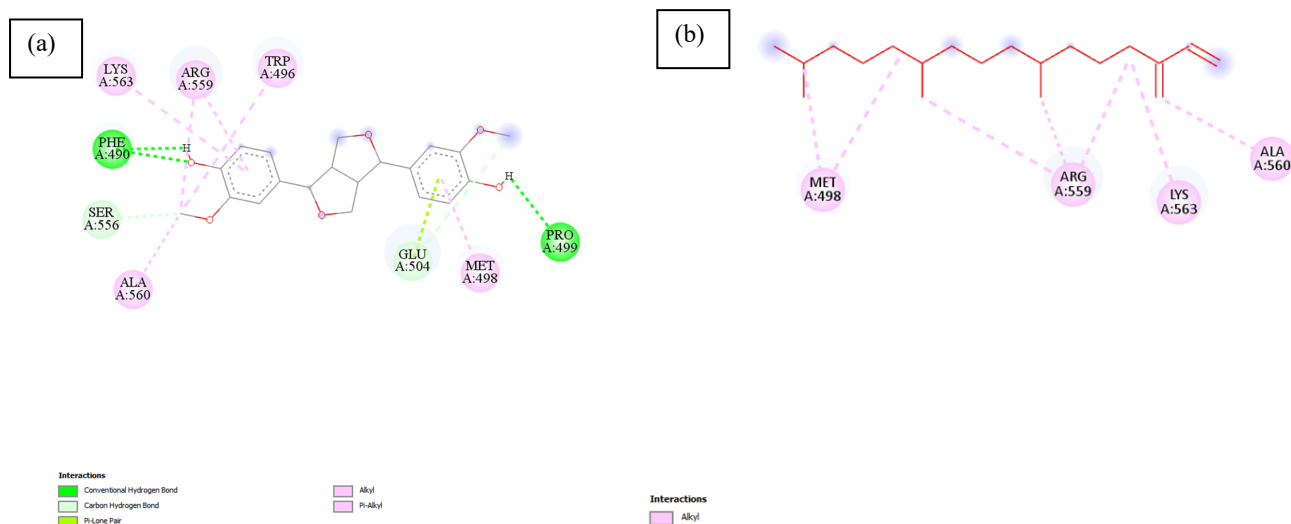


Figure 5. 2D interaction profile of (a) Compound 1 and (b) Compound 2 bound with the protein

DISCUSSION

Cystic fibrosis (CF) is a progressive autosomal recessive genetic disorder primarily caused by pathogenic variants in the cystic fibrosis transmembrane conductance regulator (CFTR) gene. Dysfunctional CFTR-mediated ion transport results in altered epithelial fluid homeostasis, accumulation of viscous mucus, recurrent respiratory infections, chronic inflammation, and progressive deterioration of pulmonary function. Although the development of CFTR modulator therapies, including ivacaftor and combination regimens such as *Orkambi*, *Symkevi*, and *Trikafta*, has substantially improved clinical outcomes for many individuals with CF, therapeutic response remains dependent on the underlying CFTR genotype. Consequently, patients carrying mutations that are not amenable to currently available modulators continue to represent an important unmet therapeutic need. In addition, persistent inflammation and oxidative stress may contribute to progressive tissue damage despite correction of CFTR dysfunction in responsive patients. (Van *et al.*, 2011; Burgel *et al.*, 2023; Sian *et al.*, 2022; Griesenbach *et al.*, 2024; Bell *et al.*, 2023; Ahmad *et al.*, 2023).

Natural products have attracted considerable interest as potential sources of complementary therapeutic agents because of their structural diversity and their ability to interact with multiple molecular pathways. Several naturally occurring compounds, including genistein, curcumin, resveratrol, pinoresinol, and neophytadiene, have been investigated for antioxidant, anti-inflammatory, or other pharmacological activities. Previous studies have suggested that certain phytochemicals may influence pathways associated with CFTR function, oxidative stress, and inflammatory signaling. However, the biological effects of individual natural compounds can vary considerably depending on their concentration, bioavailability, molecular target, and experimental model. Therefore, identification of such compounds in a plant extract represents an initial step toward evaluating their therapeutic potential rather than evidence of clinical efficacy. (Atanasov *et al.*, 2021; Singh *et al.*, 2016; Ren *et al.*, 2013; Mathew, 2015; Sharma *et al.*, 2023; Adedapo *et al.*, 2011; Banu *et al.*, 2024).

In the present study, methanolic extracts of *Calotropis gigantea* and *Calotropis procera* were investigated using chromatographic and spectrometric approaches to characterize their phytochemical constituents. The use of methanol as the extraction solvent enabled the recovery of a broad range of polar and moderately polar constituents. Subsequent TLC optimization demonstrated that

changes in mobile-phase polarity substantially influenced the migration and resolution of extracted components. Among the 14 solvent systems evaluated, ratios 4, 10, and 12 produced comparatively distinct separation patterns. The progressive increase in methanol concentration from ratio 4 to ratio 12 resulted in greater migration of polar constituents, consistent with the increased polarity of the mobile phase.

The TLC observations were further supported by the column chromatographic separation. The solvent system containing 95% ethyl acetate and 5% methanol preferentially facilitated the elution of relatively less polar constituents, whereas increasing the methanol proportion enhanced the migration of more polar components. The 55% ethyl acetate and 45% methanol system produced a prominent separation profile and was therefore selected for subsequent GC–MS characterization. These observations indicate that preliminary TLC optimization can be effectively used to guide the selection of mobile-phase conditions for subsequent fractionation of complex plant extracts. (Castro *et al.*, 2020; Ong *et al.*, 2020; Jain *et al.*, 2023; Arora *et al.*, 2024; Yang *et al.*, 2024; Kunle *et al.*, 2012; Gaikwad *et al.*, 2025; Nichols *et al.*, 2015; Pranke *et al.*, 2019; Mathew, 2015).

GC–MS analysis of the selected fraction resulted in the detection of multiple chemical constituents. Among the identified compounds, pinoresinol and neophytadiene were selected for further computational investigation based on their detection in the analyzed fraction and their previously reported pharmacological relevance. Pinoresinol is a lignan-type phytochemical that has been investigated for several biological activities, including antioxidant and anti-inflammatory effects. Neophytadiene, a diterpene-related hydrocarbon reported in several medicinal plants, has likewise been associated with various biological activities in previous phytochemical investigations. The presence of these constituents in *Calotropis* extracts provides a basis for further evaluation of their possible molecular mechanisms.

Molecular docking was subsequently employed to investigate the potential interactions of pinoresinol and neophytadiene with the selected protein target(s) associated with CF. Docking provides a computational approach for estimating the preferred orientation of a ligand within a protein binding site and comparing predicted binding energies and residue-level interactions. In the present study, pinoresinol demonstrated a comparatively stronger predicted binding affinity and a more favorable interaction profile than neophytadiene against the selected target. The predicted interactions observed in the docking analysis suggest that pinoresinol may possess a greater capacity for interaction with the selected protein than neophytadiene under the computational conditions employed.

The comparatively favorable docking profile of pinoresinol is particularly noteworthy because lignan-type compounds have been investigated for antioxidant and anti-inflammatory activities

that may be relevant to chronic inflammatory disorders. However, the docking results should be interpreted cautiously. A favorable docking score indicates a predicted ligand–protein interaction and does not by itself establish CFTR activation, inhibition, correction of CFTR dysfunction, or therapeutic efficacy. Furthermore, docking does not account fully for factors such as cellular permeability, ligand metabolism, protein dynamics, aqueous solubility, bioavailability, and pharmacokinetic behavior. Therefore, the present findings should be considered preliminary evidence supporting further investigation rather than confirmation of a CF therapeutic mechanism.

The potential relevance of the identified compounds may also extend beyond direct interaction with CFTR. Oxidative stress and persistent inflammatory signaling are important components of CF pathology, and compounds possessing antioxidant or anti-inflammatory properties could potentially contribute to attenuation of secondary pathological processes. Nevertheless, establishing whether pinoselinol or neophytadiene directly modulates CFTR-dependent chloride transport or indirectly influences CF-associated inflammatory pathways requires dedicated functional experiments.

Overall, the present study demonstrates that chromatographic optimization followed by GC–MS profiling can be used to identify potentially relevant phytochemicals from *Calotropis* extracts, while molecular docking provides an initial computational assessment of their interactions with selected CF-associated targets. The comparatively favorable docking profile of pinoselinol identifies it as a candidate worthy of further investigation. However, further studies are required to determine whether these predicted interactions translate into meaningful CFTR-related or anti-inflammatory biological effects.

CONCLUSION

The present study investigated the phytochemical constituents of methanolic *Calotropis gigantea* and *Calotropis procera* extracts using TLC, column chromatography, and GC–MS, followed by molecular docking of selected compounds. TLC optimization identified solvent systems containing different proportions of ethyl acetate and methanol that provided comparatively effective separation of phytochemical constituents. Among the optimized systems, the 55% ethyl acetate and 45% methanol composition produced a prominent separation profile and was selected for subsequent GC–MS analysis.

GC–MS analysis of the selected fraction resulted in the identification of several chemical constituents, among which pinoselinol and neophytadiene were selected for computational investigation based on their reported biological relevance. Molecular docking indicated that pinoselinol exhibited a comparatively more favorable predicted interaction profile with the selected CF-associated protein target than neophytadiene.

These findings suggest that *Calotropis*-derived phytochemicals, particularly pinoselinol, may represent promising candidates for further investigation in the context of CF-associated molecular pathways. However, the present studies are based on chemical profiling and computational predictions and therefore cannot establish direct CFTR modulation or therapeutic efficacy. Further studies may help determine whether the identified phytochemicals have potential as complementary candidates for future CF drug-discovery research.

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