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Antimicrofouling activity of *Calotropis gigantea* (L). R. Br.

E. Suresh & P. Sureshkumar*
CAS in Marine Biology, Faculty of Marine Sciences, Annamalai University, Parangipettai-608502
*[E-mail: sure2004@gmail.com]

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Milkweed or *Calotropis gigantea* belongs to *Asclepiadaceae* family having many curative principles in it. This present work aimed to study the phytochemicals prevailing in the *Calotropis gigantea* during the summer season by GCMS method and some of these phytochemicals tested against the collagen-binding matrix protein (4CN8) produced by the bacterial foulant through computational method. The result of GCMS analysis revealed that the prevalence of stigmasterol, alpha-amyrin, urs-12-en-24-oic acid, 3-oxo-, methyl ester, (+)-, 2(1H) Naphthalenone, 3,5,6,7,8, 8a-hexahydro-4, 8a-dimethyl-6-(1-ethylethenyl)-, Beta.-Amyrin, Bicyclo [3.1.1] heptane,2,6,6-trimethyl-, 1R-(1.alpha., 2.beta., 5.alpha.) -and 1H-Indene, 5-butyl-6-hexyloctahydro-, 2-[3-(4-tert-Butyl-phenoxy)-2-hydroxy-propylsulfanyl]-4,6-dimethyl-nicotinonitrile and cyclopropane carboxamide, 2-cyclopropyl-2-methyl-N-(1-cyclopropylethyl)- and pyridine-3-carboxamide, oxime, N-(2-trifluoro methyl phenyl). The *in silico* study exhibited that all the screened phytochemicals are having remarkably good interaction with the tested 4CN8 and possessing-8 to-11 Kcal/mol docking energy except pyridine-3-carboxamide, oxime, N-(2-trifluoromethylphenyl). Hence, the phytochemicals of Calotropis is a right candidate for further elaborate study to establish an eco-friendly alternative to existing toxic antifouling chemicals.

[**Keywords**: Antibiofouling; Biofouling; *Calotropis gigantea;* Computational; Docking; *In silico*; Microfoulers; Phytochemicals]

Introduction

Marine engineered structures of harbour, jetties, ship hulls and other marine industries are subjected to severe bio-fouling. The bio-fouling increase the fuel consumption of marine vessels by collapsing the hydrodynamics of hulls and deteriorate the metallic substrate by corrosion leading to severe economic loss. To evade these bio-fouling evils antifouling paints are formulated by using a variety of biocides as active compounds. These antifouling compounds are creating numerous problems to the marine life and its ecosystem[1]. Though, the gathering and bio amplification of these amalgams in the marine environ created hefty problems in this habitat[2] with detrimental outcome on a wide range of other non-targeted organisms[3]. Recent antifouling approaches are investigated with living organisms to provide novel antifouling applications[4]. Antifoulants generated from natural origins have been proposed as one of the best ecologically pertinent antifouling panacea[5-8]. Even though studies have been done extensively over the past 20 years, works with the terrestrial plants are very few. Thus, in the current study, an attempt was made to investigate the antifouling trait of *Calotropis gigantea*, a widely distributed milkweed plant possessing numerous active compounds.

Materials and Methods

Plant materials collection, processing and extraction

The aerial parts of the plant *Calotropis gigantea* were collected during the 2018 summer season from the coastal area of Puthupettai, Cuddalore district, Tamil Nadu, India. The specimen was vouched by the Department of Botany, Bishop Heber College, Tiruchirappalli, Tamil Nadu, India and a kind specimen was submitted to the herbarium. The collected materials were washed, dried under shade and powdered and extracted with methanol through soxlet apparatus.

GC-MS analysis

In this study, an Agilent GC 6890 N was used in conjunction with a 5973 N mass detector. The methanol extract was transferred to the vial of GC-MS and inserted into the port of GCMS. Analysts were separated on the HP-5MS capillary column (30 m X 0.25 mm X 1.0 µl) by applying the following temperature program: 40 °C for 5 min, 40-70 °C for 2 °C / min, 70 °C for 2 min, 70-120 °C for 3 °C / min, 120-150 °C for 5 °C /min, 150-220 °C for 10 °C / min and 220 °C for 2 min. The temperature of the transfer line was 280 °C. Mass detector conditions were: 70 eV electronic impact (EI) mode; 230 °C source temperature; 2.88S-1 scan rate; 29-540 m / z mass scan range. The carrier gas was

helium at 1.0 ml per minute. The tentative identification of volatile chemicals was executed with the aid of comparing the mass spectra with the data system library[9] and different published spectra[10] supported through retention index data, which had been in contrast with handy literature retention indices[9]. All compounds had been quantified as 3-octanol equivalents.

Protein Structure preparation

The focused protein collagen-binding matrix protein (ID: 4CN8) having the resolution of 2.45 Å was obtained from the protein data bank. Structural and active site studies of the protein was done via using CASTP (Computed Atlas of Surface Topography of Proteins) and pymol molecular visualization software.

Ligand structure preparation

Phytochemicals prevailed in the summer season of the coastal plant; namely stigmasterol, alpha-amyrin, urs-12-en-24-oic acid, 3-oxo-, methyl ester, (+)-,2(1H) naphthalenone, 3,5,6,7,8,8a-hexahydro-4,8a-dimethyl-6-(1-ethylethenyl)-, beta.-amyrin, bicyclo [3.1.1] heptane,2,6,6-trimethyl-, 1R-(1.alpha., 2.beta., 5.alpha.) -and 1H-indene, 5-butyl-6-hexyloctahydro-, 2-[3-(4-tert-Butyl-phenoxy)-2-hydroxy-propylsulfanyl]-4,6-dimethyl-nicotinonitri-leand cyclopropane carboxamide, 2-cyclopropyl-2-methyl-N-(1-cyclopropylethyl)- and pyridine-3-carboxamide, oxime, N-(2-trifluoromethyl phenyl)- were screened against the envelope protein, 4CN8 produced by the fouling bacteria. The 2-D molecular structures of these photochemical were obtained from the Pubchem database and the chemical structures were generated from SMILES (simplified molecular input line entry specification) notation by using the Chemsketch Software[11-13].

Molecular Docking Analysis

The docking analysis was done using Argus Lab 4.01 software, which is extensively used for public domain molecular docking analysis. The inhibitor and target protein were geometrically optimized and docked using docking engine Argus dock. The docking simulations in the active sites of 4CN8 were performed with the aid of the Argus lab program, which has been proven to efficaciously reproduce experimentally located binding modes in terms of lowest docking energy. The target protein structure of 4CN8 used to be docked with plant-derived compounds which furnished splendid consequences as were seen by means of the least values of the binding energy. The best viable binding modes of the plant-derived compounds at focused protein's active sites are displayed.

Results

Phytochemical Assessment

The GCMS chromatogram of *Calotropis gigantea* (Fig. 1) shows the presence of about 27 phyto-chemicals (Table 1).

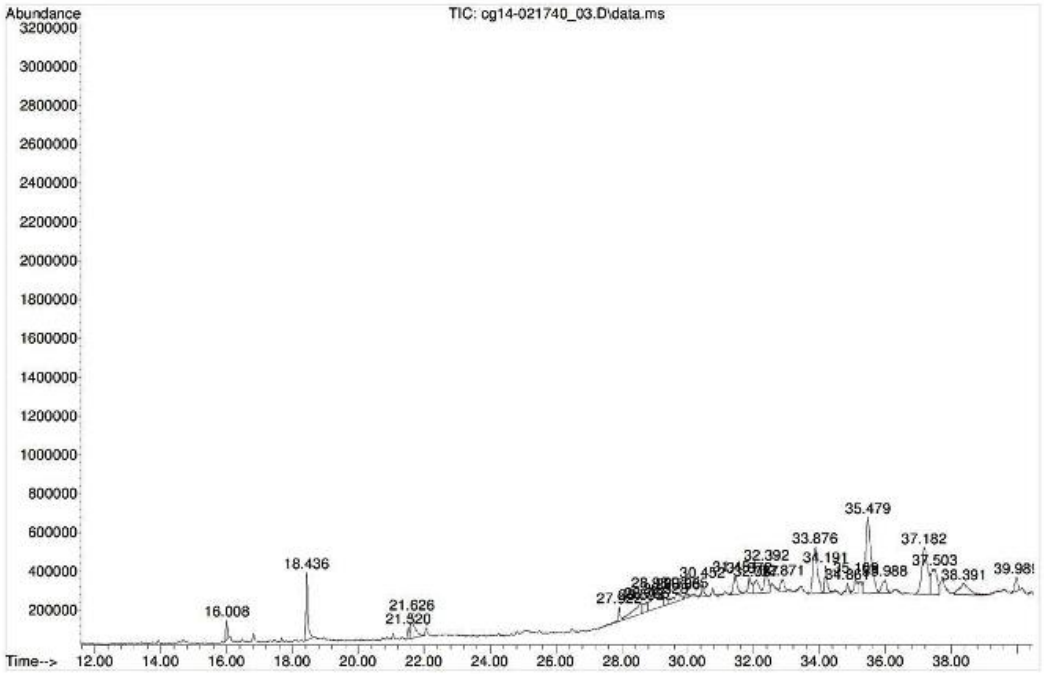

Fig. 1 — GCMS chromatogram of *Calotropis gigantean*

Table 1 — The composition of methanolic extract of Calotropis gigantea through GCMS

Si. No	RT	Class of Compound	Compound name	Peak area %
1	16.006	Terpenoids	Bicyclo[3.1.1]heptane,2,6,6-trimethyl-,[1R-(1.alpha.,2.beta.,5.alpha.)]-	1.23
2	18.439	Fatty acid	n-Hexadecanoic acid	5.07
3	21.521	Fatty acid	9,12-Octadecadienoic acid (Z,Z)-	0.80
4	21.622	Fatty acid	9,12-Octadecadienoic acid (Z,Z)-	3.60
5	27.918	Hologens	Oxirane, hexadecyl-	1.12
6	28.597	Alkane	Cyclopropane carboxamide, 2-cyclopropyl-2-methyl-N-(1-cyclopropylethyl)-	3.62
7	28.770	Fatty acid	2-[3-(4-tert-Butyl-phenoxy)-2-hydroxy-propylsulfanyl]-4,6-dimethyl-nicotinonitrile	1.73
8	28.983	Other metabolites	Benzoquinoline, 2,4-dimethyl-	6.93
9	29.327	Steroid	Pyridine-3-carboxamide, oxime, N-(2-trifluoromethylphenyl)-	0.94
10	29.702	Sterol	Stigmasterol	2.61
11	29.956	Steroid	Pyridine-3-carboxamide, oxime, N-(2-trifluoromethylphenyl)-	0.58
12	30.453	Aldehyde	Cyclopropaneoctanal, 2-octyl-	1.01
13	31.456	Alcohol	7b-Phenyl-2a,7b-dihydro-3H-cyclobuta[a]indene	2.92
14	31.872	Hydrocarbon	Tetracosane	2.40
15	32.085	Ketones	Pyridine-3-carboxamide, oxime, N-(2-trifluoromethylphenyl)-	2.58
16	32.389	Tri-terpenes	Alpha.-Amyrin	3.87
17	32.876	Ketones	Pyridine-3-carboxamide, oxime, N-(2-trifluoromethylphenyl)-	1.86
18	33.879	Tri-terpenes	Beta.-Amyrin	8.48
19	34.193	Heterocyclic compound	L.beta. – Tocopherol	2.85
20	34.863	Heterocyclic compounds	Barbituric acid, 5-allyl-5-(cyclohex-2-en-1-yl)-	1.09
21	35.106	Heterocyclic compounds	Eicosane	1.86
22	35.481	Heterocyclic compounds	2(1H)Naphthalenone, 3,5,6,7,8,8a-hexahydro-4,8a-dimethyl-6-(1-ethylethenyl)-	17,48
23	35.988	Ketones	Pyridine-3-carboxamide, oxime, N(2-trifluoromethylphenyl)-	2.42
24	37.184	Tri-terpenes	Urs-12-en-24-oic acid, 3-oxo-, methyl ester, (+)-	10.93
25	37.498	Esters	1H-Indene, 5-butyl-6-hexyloctahydro-	6.18
26	38.391	Heterocyclic compounds	Cyclopentenol[4.3-b]tetrahydrofuran, 3-[(4-methyl-5-oxo-3-phenylthio)	4.67
27	39.992	Sterol	Gamma.-Sitosterol	1.74

Docking of dominant molecules

Some of these selective phytochemicals derived from coastal strands have been docked with metalloprotease of the marine fouling bacteria. The 3Dcrystal protein structure (Fig. 2) was retrieved from protein information bank (PDB) and the protein tying locales of the trial blends were recognized. The ligands were selected based on docking strength and appropriate interaction with the active site residues and the outcomes are shown in the Table 2. From the 10 ligand molecules, 7 confirmed the binding strength higher than -10 Kcal/mol which are stigmasterol, alpha-amyrin, urs-12-en-24-oic acid, 3-oxo-, methyl ester, (+)-,2(1H) naphthalenone, 3,5,6,7,8,8a-hexahydro-4,8a-dimethyl-6-(1-ethylethenyl)-, beta.-amyrin, bicyclo[3.1.1]heptane,2,6,6-trimethyl-,[1R-

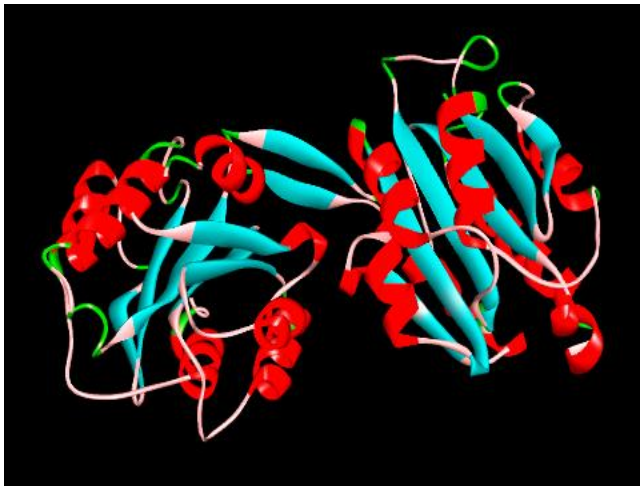

Fig. 2 — 3D structure of the protein 4CN8

Table 2 — Phytochemicals of Calotropis and protein binding interaction energy values

Sl. No.	Compound Name	Molecular Formula	Molecular Weight g/mol	Hydrogen donor and accepter	Docking values
1	Bicyclo[3.1.1]heptane,2,6,6-trimethyl-,[1R-(1.alpha.,2.beta.,5.alpha.)]-	$C_{10}H_{18}$	138.2499	1/1	-10.3198
2	Cyclopropanecarboxamide, 2-cyclopropyl-2-methyl-N-(1-cyclopropylethyl)-	$C_{13}H_{21}NO$	207.31194	1/1	-8.31477
3	2-[3-(4-tert-Butyl-phenoxy)-2-hydroxy-propylsulfanyl]-4,6-dimethyl-nicotinonitrile	$C_{21}H_{26}N_2O_2S$	370.50834	1/5	-8.4756
4	Stigmasterol	$C_{29}H_{48}O$	412.69082	1/1	-11.4268
5	Alpha.-Amyrin	$C_{30}H_{50}O$	426.7174	1/1	-11.4246
6	Pyridine-3-carboxamide, oxime, N-(2-trifluoromethylphenyl)-	$C_{13}H_{10}F_3N_3O$	281.23321	2/6	Nil
7	Beta.-Amyrin	$C_{30}H_{50}O$	426.7174	1/1	-11.1617
8	2(1H)Naphthalenone, 3,5,6,7,8,8a-hexahydro-4,8a-dimethyl-6-(1-ethylethenyl)-	$C_{15}H_{22}O$	218.33458	0/1	-11.2467
9	Urs-12-en-24-oic acid, 3-oxo-, methyl ester, (+)-	$C_{31}H_{48}O_3$	468.71102	0/3	-11.2556
10	1H-Indene, 5-butyl-6-hexyloctahydro-	$C_{19}H_{36}$	264.48914	0/0	-10.2494

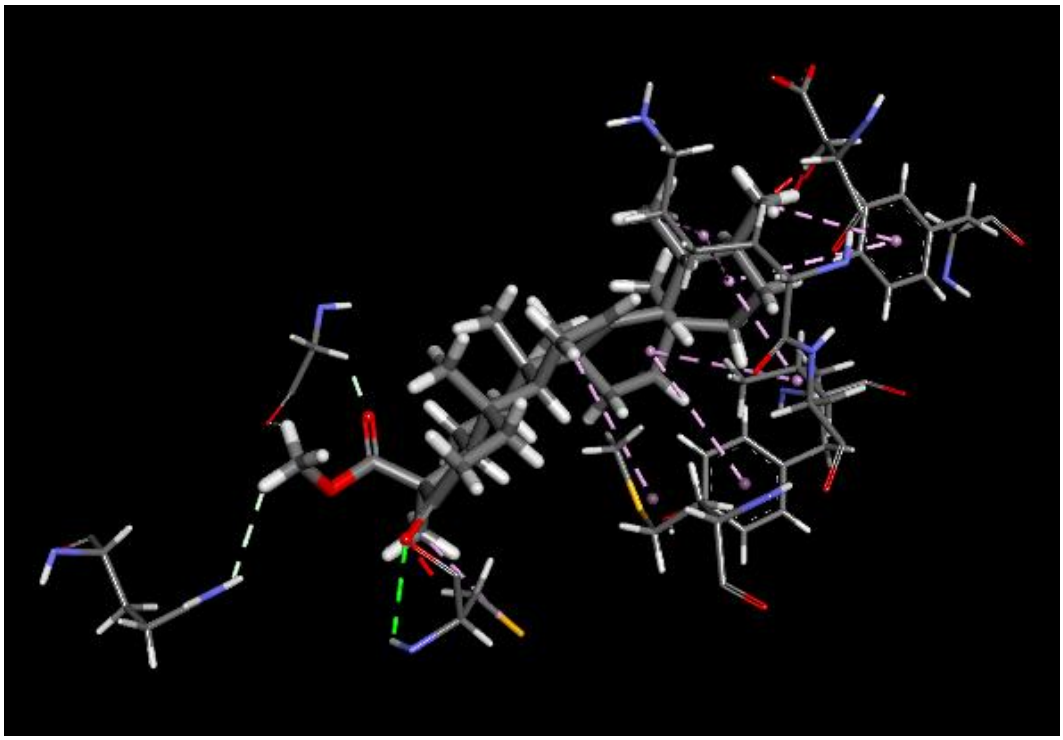

Fig. 3 — Protein-ligandinteraction: 4CN8 (Collagen-Binding Matrix Protein) - Urs-12-en-24-oic acid, 3-oxo-, methyl ester, (+)-

(1.alpha.,2.beta.,5.alpha.)]- and 1H-Indene, 5-butyl-6-hexyloctahydro- with binding energies -11.4268, -11.4268, -11.2556, -11.2467, -11.1617, -10.3198 and -10.2467 Kcal/mol, respectively while two compounds exhibited the values less than -10 kcal/mol i.e. –8.47456 and -8.31477 Kcal/mol were the lowest values observed in 2-[3-(4-tert-Butyl-phenoxy)-2-hydroxy-propylsulfanyl]-4,6-dimethyl-nicotinonitrile and cyclopropanecarboxamide, 2-cyclopropyl-2-methyl-N-(1-cyclopropylethyl)(Table 2). Figure 3 and 4 illustrates the interaction of the highest docking energy of -11.2556 Kcal/mol observed for urs-12-en-24-oic acid, 3-oxo-, methyl ester, (+)- and the minimum docking energy of -8.31477 Kcal/mol observed for cyclopropanecarboxamide, 2-cyclopropyl-2-methyl-N-(1-cyclopropylethyl)-.

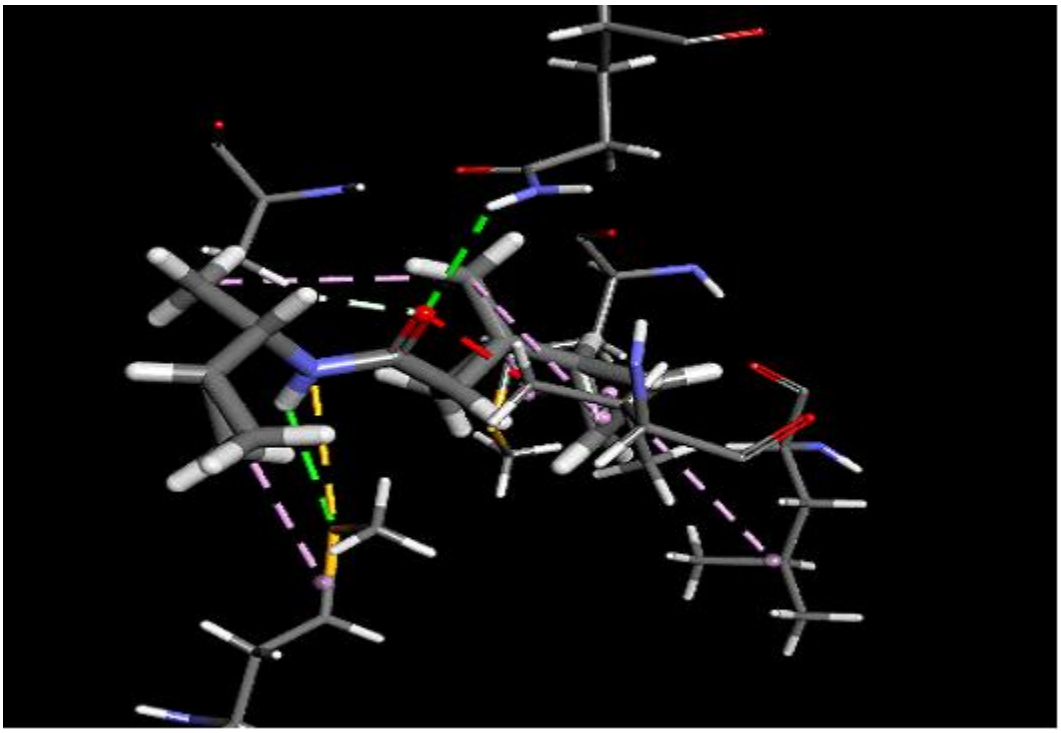

Fig. 4 — Protein ligand interaction: 4CN8 (Collagen-Binding Matrix Protein)- cyclopropanecarboxamide, 2-cyclopropyl-2-methyl-N-(1-cyclopropylethyl)-

Discussion

Docking is a calculation strategy that specimens corroborative of little atoms in protein tying destinations, scoring capacities are utilized to evaluate which of these corroborative best supplements the protein tying site[14]. In this present study, milkweed plant phytochemicals fit for obstructing the proteins discharged by the fouling organisms were studied. The beachfront marine inferred mixes were discovered equipped for hindering the protein, in charge of cervical carcinogenesis[15]. Sureshkumar et al.[16] reported that the presence of phytosterols in the prominent a fraction of the plant *Calotropis gigantea*. Notwithstanding this, *in silico* docking investigation was likewise done to accept the counter capability of these phytosterol mixes. Rutten and Statius Van Eps[17] recommended the cyto-poisonous quality of different concentrates of root, leaves, and blossoms of Calotropis. Past experiments with this plant, concentrates of the root and the leaves verified cytotoxic property towards human epidermal nasopharynx carcinoma[18]. The whole latex of *Calotropis gigantea* has anticancer and cytotoxic properties towards hepato cell carcinoma[19]. The results uncovered from the present study suggested that among 10 mixes, stigmasterol, alpha.- amyrin, urs-12-en-24-oic, 3-oxo-, methyl ester, (+)- ,2(1H) naphthalenone, 3,5,6,7,8,8a-hexahydro-4,8a-dimethyl-6-(1-ethylethenyl)-, beta.- amyrin, bicyclo [3.1.1] heptane,2,6,6-trimethyl-,[1R-(1.alpha.,2.beta.,5.alpha.)]- and 1H-indene, 5-butyl-6-hexyloctahydro-were discovered proficient in decimating the protein, 4CN8-biofouling. In the present study, as observed from among the 10 compounds tested; 7 compounds are having more than 10 Kcal/mol tying energies *in silico* docking study. This work was also upheld by Shahu et al.[20] who proposed that out of seven phytochemicals, three were discovered intense i.e. stigmasterol, lupeol and betulin displayed least docking score of - 15.0363, - 11.9573 and - 11.5012 Kcal/mol. Suresh kumar et al.[21] found that phytosterol was having the docking vitality between -12 to - 16.0235 Kcal/mol, while the maximum docking vitality was found in desmosterol (- 16.0235 Kcal/mol) and gammasitosterol (- 13.5785 Kcal/mol). Hence, Calotropis can be further studied to establish an eco-friendly less toxic substitute for existing toxic antifouling formulations.

Conclusion

The existing *in silico* docking consequences revealed that coastal strands derived compounds have the excellent potential towards inhibition of metalloprotease. Hence, *Calotropis* can be further

studied to set up an eco-friendly less toxic alternative for existing toxic antifouling formulations.

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