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IN-SILICO COMPARATIVE STRUCTURAL ANALYSIS OF CARBONIC ANHYDRASE FROM CORAL AND SYMBIOTIC ALGAE AND ITS INTERACTION WITH COSMETIC AGENTS THROUGH MOLECULAR DOCKING

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Keywords:

Acid Violet 43, Carbonic anhydrase, Coralalgal symbiosis, Coral reef conservation, Cosmetic agents, Molecular docking

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ABSTRACT: Coral reefs are dynamic ecosystems sustained by complex metabolic interactions between corals and their symbiotic algae, where Carbonic anhydrase (CA) plays a key role in biomineralization and carbon cycling. In the current study, the CA sequence of *Symbiodinium microadriaticum* (Zooxanthellae) was retrieved from NCBI and its three-dimensional structure was predicted using trRosetta. The CA structure of *Acropora millepora* (Coral) was obtained from the UniProt database. Both protein models were structurally validated using PROCHECK, which confirmed that 90.0% and 90.5% of residues were in the most favored regions. Comparative sequence alignment using the EMBOSS Water tool revealed conserved functional domains, supporting structural and functional preservation. Physicochemical analyses *via* ProtParam indicated that the algal CA is hydrophilic but relatively unstable, while the coral CA demonstrated enhanced stability and solubility. Molecular docking studies, conducted using PyRx and BIOVIA Discovery Studio, evaluated the binding potential of three standard CA inhibitors: Acetazolamide, Ethoxzolamide, and Dorzolamide, alongside 29 cosmetic agents. Notably, Acid Violet 43 exhibited the strongest binding affinity, with docking scores of -10.0 kcal/mol against zooxanthellae CA and -7.6 kcal/mol against coral CA, surpassing the standard inhibitors. This study lays a solid foundation for future investigations into Carbonic anhydrase inhibition within coralalgal symbiotic systems. However, extensive *in-vitro* and *in-vivo* studies are required to further validate and elucidate the potential effects on coral reefs and their associated symbiotic algae.

INTRODUCTION: Coral reefs are among the most ecologically valuable and biologically diverse marine ecosystems, providing essential services such as coastal protection, nutrient cycling, and habitat for countless marine species [1].

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The health and resilience of these ecosystems heavily depend on the intricate symbiotic relationship between reef building scleractinian corals and photosynthetic dinoflagellates of the genus Symbiodinium, commonly known as Zooxanthellae [2].

This mutualistic association is crucial for coral survival, with dinoflagellates supplying photosynthetically fixed carbon and enhancing the coral's calcification processes, while the coral provides a protected environment and access to

inorganic nutrients [3]. Carbonic anhydrase (CA) enzyme plays a crucial role in regulating carbon transport, photosynthesis, and biomineralization within the coralalgal symbiosis [4]. This enzyme catalyzes the reversible hydration of carbon dioxide, facilitating the efficient transfer of inorganic carbon to algal symbionts and contributing to coral skeleton formation. However, emerging environmental pollutants, including personal care products and synthetic dyes, pose significant threats to coral reef health [5]. Cosmetic agents have garnered significant attention due to their environmental persistence, potential for bioaccumulation, and suspected toxicity to marine organisms, including corals [6]. Despite growing concerns, the direct molecular interactions between cosmetic agents and key metabolic enzymes of coral and symbiotic algae remain largely unexplored. Understanding these interactions is critical for assessing the potential biochemical and ecological risks posed by such contaminants.

The present study employs an *in-silico* approach to investigate the binding potential of 29 widely used cosmetic related compounds and three established CA inhibitors against Carbonic anhydrase of*Symbiodinium microadriaticum* and *Acropora millepora*. Protein structures were modeled and validated using advanced bioinformatics tools, including trRosetta and PROCHECK, and the stereochemical quality, sequence conservation, and physicochemical properties of both enzymes were comprehensively analyzed. Subsequent molecular docking simulations using PyRx and BIOVIA Discovery Studio were performed to assess ligand binding affinities and key interaction patterns. This work aimed to provide foundational insights into the potential inhibitory effects of cosmetic agents on coralalgal carbonic anhydrases, contributing to the broader understanding of anthropogenic impacts on coral reef ecosystems and informing future environmental risk assessments.

MATERIALS AND METHODS:

Target Sequence Retrieval, Structure Prediction and Validation: The Carbonic anhydrase (CA) of *Symbiodinium microadriaticum* (Zooxanthellae) and *Acropora millepora* (Coral reef) was selected as a target protein for this study. The protein sequence of Zooxanthellae CA (OLP92365.1) was obtained from the NCBI database, and its 3D structure was predicted using the trRosetta webserver [7, 8]. The structural integrity and stereochemical quality of the predicted model we reassessed using the PROCHECK server[9]. Similarly, the crystal structure of coral CA (B7T143_ACRMI) was obtained from the UniProt database and validated through PROCHECK analysis to ensure structural stability. To identify the conserved regions within the Carbonic anhydrase (CA) of *Symbiodinium microadriaticum* and *Acropora millepora*, local sequence alignment was performed using the EMBOSS Water tool, which applies the Smith Waterman algorithm for optimal local alignment [10]. The alignment results were evaluated based on percentage identity and similarity to determine the conserved regions, which are critical for maintaining the enzyme's structural and functional integrity. Furthermore, to analyze the amino acid distribution and physicochemical properties of the selected CA, the sequences were subjected to analysis using the ProtParam tool available on the ExPASy server [11].

This tool enabled the computation of key physical and chemical parameters, including theoretical isoelectric point, molecular weight, instability index, amino acid composition, grand average of hydropathicity (GRAVY), and aliphatic index, offering a comprehensive overview of the structural characteristics of both enzymes. Both protein structures were subsequently refined using BIOVIA Discovery Studio to prepare them for docking analysis [12]. During the refinement, heteroatoms and water molecules were removed to expose the binding sites and polar hydrogens were added to facilitate potential hydrogen bonding interactions. The validated and optimized protein structures were then employed in *in-silico* molecular docking studies.

Ligand Selection and Preparation: Three well known Carbonic anhydrase (CA) inhibitors, Ethoxzolamide (3295), Acetazolamide (1986), and Dorzolamide (5284549), along with twenty nine additional ligands, were selected for this study. All compounds were retrieved from the PubChem database. The selected 29 ligands are chemical agents extensively used in commercial hair dyes and personal care products, known for their potential environmental persistence and bioactivity [13, 14].

The ligand preparation was conducted using PyRx virtual screening software to ensure appropriate energy minimization and file format conversion for docking analysis [15]. The complete list of ligands with their respective PubChem IDs is as follows: p-Phenylenediamine (7814), N, N′-Diacetyl-1, 4-phenylenediamine (67324), N - Phenyl – p - phenylenediamine (7564), Hydroxypropyl bis (N-hydroxyethyl-p-phenylenediamine) hydrochloride (21932462),2-Chloro-p-phenylenediamine (11998), 4-Methoxy-m-phenylenediamine (11976), p-(Methylamino) phenol (5931), 2-Methyl-5-hydroxyethylaminophenol (134396), 2, 4-Diaminophenol (7266), Hydroquinone (785), t-Butylhydroquinone (16043), Toluene-2,5-diamine (7252), Toluene-3, 4-diamine (10332), Disperse Blue 7 (18514), Disperse Violet 1 (1420), Disperse Yellow 3 (17811), Acid Violet 43 (2366962), Basic Blue 99 (93377), HC Blue No. 2 (36383), HC Yellow No. 5 (3648504), HC Red No. 7 (5484089), 3-Nitro-p-hydroxy ethyl amino phenol (5488102), 4-Amino-3-nitrophenol (3758882), 4-Amino-2-hydroxytoluene (17818), 1-Naphthol (7005), Resorcinol (5054), o-Phenylenediamine (7243), 4-Chloro-o-phenylenediamine (7263), and Di-n-butyl phthalate (3026).

Molecular Docking and Interaction Analysis: Molecular docking studies were conducted to assess the binding affinities of three established Carbonic Anhydrase (CA) inhibitors and29 selected cosmetic agents against the target CA. All ligands were initially processed using Open Babel, where they were subjected to energy minimization and subsequently converted to the pdbqt format required for docking using the AutoDock Vina Wizard of PyRx [16, 17, 18, 19]. Docking grids were defined specifically for each target protein to ensure optimal coverage of the binding pocket. For the CA enzyme of *Symbiodinium microadriaticum*, the grid center coordinates were set to X: -156, Y: 82.6, Z: 30.6. For the CA enzyme of *Acropora millepora*, the grid was centered at X: 44.72, Y: 48.69, Z: 49.59. Following the docking simulations, ligand protein complexes with the most favorable binding energies were selected for further analysis. The top ranked docking poses were subsequently analyzed for key molecular interactions, including hydrogen bonding and hydrophobic contacts, using BIOVIA Discovery Studio Visualizer.

RESULTS AND DISCUSSION:

Stereochemical Validation of *Symbiodinium microadriaticum* Carbonic Anhydrase: The stereochemical validation of the predicted Carbonic anhydrase (CA) structure from *Symbiodinium microadriaticum* was systematically performed using PROCHECK. The Ramachandran plot analysis provided critical insights into the conformational quality of the model.

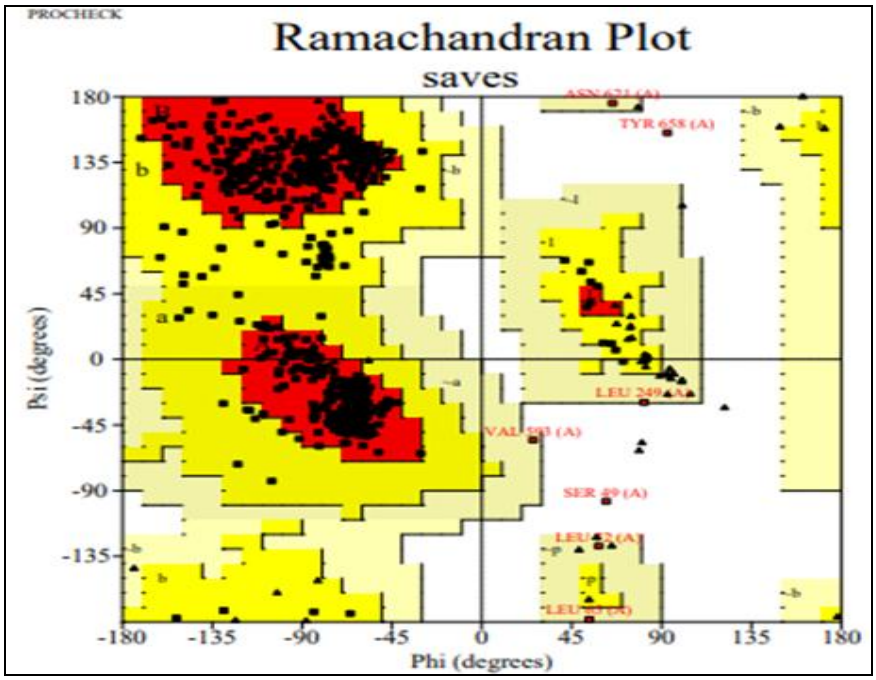

FIG. 1: RAMACHANDRAN PLOT OF *SYMBIODINIUM MICROADRIATICUM* CARBONIC ANHYDRASE

Notably, 90.0% of the residues (651 residues) were positioned within the most favored regions of the plot, indicating a high degree of structural reliability and proper backbone dihedral angle distribution. Additionally, 9.0% of the residues (65 residues) occupied the additional allowed regions, while 0.7% (5 residues) were located within generously allowed regions. Importantly, only 0.3% of the residues (2 residues) were identified in disallowed regions **Fig. 1**.

The outlier residues identified in the disallowed regions included Ser49 and Tyr658, which may represent regions of local flexibility but do not significantly compromise the overall structural integrity. The protein sequence comprised a total of 847 amino acid residues, including 723 nonglycine, nonproline residues, 62 glycine residues, and 60 proline residues. The dataset also accounted for two endresidues, excluding glycine and proline. Collectively, the high proportion of residues in favorable regions and the minimal occurrence of disallowed conformations confirm the predicted CA structure as stereochemically stable and suitable for further computational and functional analyses.

Stereochemical Validation of *Acropora millepora* Carbonic Anhydrase: The stereochemical quality of the *Acropora millepora* Carbonic anhydrase (CA) structure was evaluated using PROCHECK, and the Ramachandran plot was used to assess backbone dihedral angle distributions. The analysis demonstrated that 199 residues (90.5%) were located within the most favored regions, indicating excellent stereochemical quality. Additionally, 20 residues (9.1%) were situated in the additional allowed regions, while only 1 residue (0.5%) was found in the generously allowed regions. Importantly, no residues (0.0%) were located in disallowed regions **Fig. 2**, reflecting a highly reliable and structurally stable protein model.

The total number of nonglycine and nonproline residues analyzed was 220, accounting for 100% of the primary structural evaluation. The protein sequence included 18 glycine residues and 20 proline residues, with two endresidues excluded from the dihedral angle analysis. The absence of residues in disallowed regions strongly supports the structural integrity and stereochemical correctness of the predicted protein model. This highquality Ramachandran plot profile confirms that the predicted structure is suitable for downstream computational studies, including molecular docking and interaction analysis.

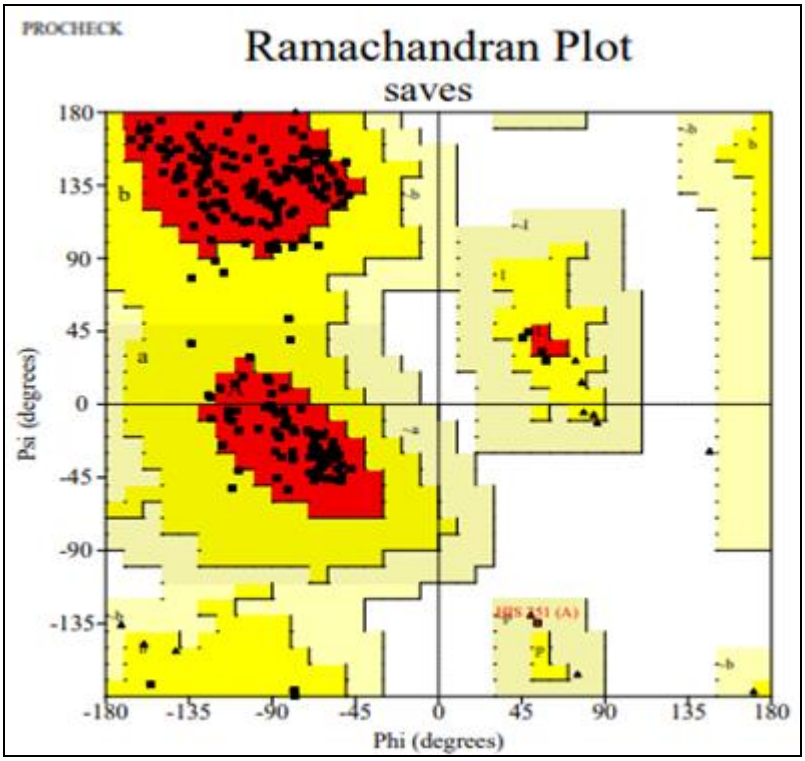

FIG. 2: RAMACHANDRAN PLOT OF *ACROPORA MILLEPORA* CARBONIC ANHYDRASE

Comparative Carbonic Anhydrase Sequence Analysis between *Symbiodinium microadriaticum* and *Acropora millepora*: The local sequence alignment between the Carbonic anhydrase (CA) of *Symbiodinium microadriaticum* and *Acropora millepora* was performed using the EMBOSS Water tool, which employs the SmithWaterman algorithm for optimal local alignment. The alignment was executed with the BLOSUM62 substitution matrix, a gap penalty of 10.0, and an extension penalty of 0.5 to ensure rigorous assessment of sequence similarity and conserved regions. The alignment spanned a length of 276 amino acids and revealed an identity of 25.0% (69 out of 276 residues) and a similarity of 34.1% (94 out of 276 residues). The alignment also indicated the presence of gaps accounting for 25.0% (69 out of 276 residues), reflecting evolutionary divergence between the two CA enzymes **Fig. 3**. Despite the moderate sequence identity, the observed similarity suggests that key functional domains may be conserved, supporting the possibility of structural and functional retention across species. Detailed sequence alignment illustrated specific regions of sequence conservation interspersed with variable and divergent segments, indicative of evolutionary adaptation while maintaining critical catalytic or binding features. The obtained alignment offers valuable insights into the structural conservation and evolutionary relationship between the coral and symbiont CA enzymes, which could be crucial for understanding their cooperative roles in carbon metabolism and biomineralization within the coral holobiont.

```
B7T143_ACRMI       3 WGYDKENGPANWYKSCPIAFIGKR-----QSPIDLIDNEVKRDSSLEPLV     47
                     |.||...  ..::|:    .:|..     |||||:            |..
OLP92365.1       447 WTYDYPQ--QQYWKN----IVGTECGANYQSPIDI-----------PKA    478

B7T143_ACRMI      48 VSYPPFSKGKFLNNGHSVQFVPDASNNLSE-----LSGGPLAKKYKF---     89
                     :||...:....|..|...:..|.|.:..::     |..|||..:.|.
OLP92365.1       479 LSYVDHANLTKLATGIGERLGPFAWSLAAQTVNVTLHPGPLTWEVKLVNP    528

B7T143_ACRMI      90 EQFHFHWGDKD--------SEGSEHRVNGKMYSAELHFVHWDCETHASFP    131
                     |......|..|        ...|||.|.|.....|:...|
OLP92365.1       529 EAIVVQIGGLDYSLQSMSFKSPSEHTVEGAHNGMEIQMRH----------    568

B7T143_ACRMI     132 ETVKANKRNGLCVLGFFL------KVGAENAALKPITDMLPKVKKAGSSE    175
                      ..|:....|..||...:      .|...||.|.||...:|...:...|.
OLP92365.1       569 -VAKSPFGGGEKVLMVSISLRLAENVNVGNAFLSPIFSTMPLDGQGAPSV    617

B7T143_ACRMI     176 PLAIEFDMRSVMPKDLTDYYTYDGSLTTPPLLECVKWIVFKEPLEVSAAQ    225
                     .:|..:  ..:.|.| ..|..|.||.|.|| .|...||||.||..:...|
OLP92365.1       618 FIANPY--LDLAPPD-KSYVRYSGSTTAPP-CEHADWIVFMEPGYLGFQQ    663

B7T143_ACRMI     226 MEAFRAVENG---------NGEPLVG    242
                     :|.||:..:|         |..|.:|
OLP92365.1       664 LEQFRSSISGYQPSRLAPANSTPPLG    689
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FIG. 3: SEQUENCE ALIGNMENT OF *SYMBIODINIUM MICROADRIATICUM* AND *ACROPORA MILLEPORA* CARBONIC ANHYDRASE

ProtParam Analysis of *Symbiodinium microadriaticum* Carbonic Anhydrase: The physicochemical properties of the Carbonic anhydrase (CA) enzyme from *Symbiodinium microadriaticum* were analyzed using the ProtParam tool. The enzyme comprises 847 amino acids with a calculated molecular weight of 94,076.05 Da and a theoretical isoelectric point (pI) of 6.61, indicating that the protein is slightly acidic. The amino acid composition analysis revealed that alanine (10.2%), leucine (9.1%), glycine (7.3%), proline (7.1%), and serine (7.1%) are the most abundant residues in the sequence **Fig. 4.** The total number of negatively charged residues (Asp + Glu) was 83, while the positively charged residues (Arg + Lys) totaled 79, suggesting a near balance between acidic and basic amino acids. The instability index was calculated to be 55.74,

classifying the protein as unstable under *in-vitro* conditions (a value above 40 generally indicates instability). The aliphatic index of 72.99 suggests that the protein possesses moderate thermostability, as higher aliphatic index values are typically associated with greater thermal stability. The Grand Average of Hydropathicity (GRAVY) was -0.310, indicating that the protein is overall hydrophilic, which may contribute to its solubility in aqueous environments. These physicochemical characteristics provide essential baseline information for understanding the structural properties and potential stability of the target protein, particularly relevant for *in-silico* docking and functional studies.

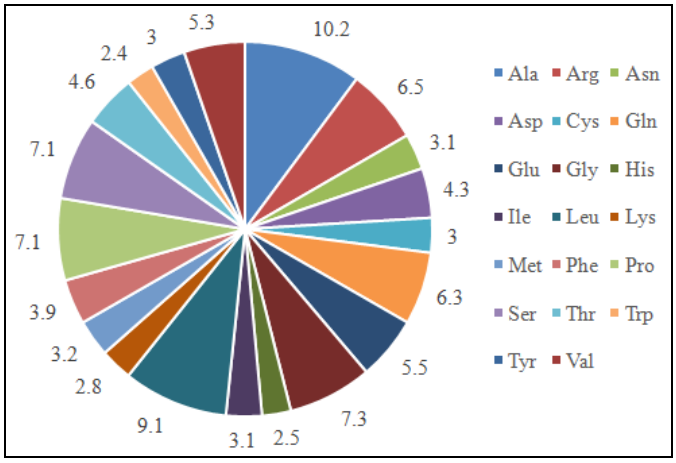


FIG. 4: AMINO ACID DISTRIBUTION IN *SYMBIODINIUM MICROADRIATICUM* CARBONIC ANHYDRASE

ProtParam Analysis of *Acropora millepora* Carbonic Anhydrase: The Carbonic anhydrase (CA) enzyme from *Acropora millepora* was analyzed for its physicochemical properties using the ProtParam tool. The enzyme consists of 260 amino acids with a molecular weight of 29,078.07 Da and a theoretical isoelectric point (pI) of 6.46, suggesting the protein is slightly acidic under physiological conditions. The amino acid composition indicates a higher abundance of serine (8.1%), lysine (8.5%), leucine (7.7%), proline (7.7%), and valine (7.7%), which are likely to contribute to the protein's structural stability and potential functional regions **Fig. 5**. The total number of negatively charged residues (Asp + Glu) was 32, while the positively charged residues (Arg + Lys) totaled 30, reflecting a nearly balanced charge distribution. The instability index was counted to be 36.34, classifying the protein as stable (proteins with an instability index below 40 are typically considered stable *in-vitro*). The aliphatic index was found to be 69.73, indicating moderate thermostability, which could support the enzyme's functional adaptability in the coral reef environment. The Grand Average of Hydropathicity (GRAVY) value of -0.425 suggests that the protein is predominantly hydrophilic, indicating good solubility in aqueous conditions, which is important for its enzymatic activity within the coral system. These physicochemical features provide foundational information regarding the stability, solubility, and potential environmental resilience of the coral CA enzyme, supporting its selection for further docking and functional interaction studies.

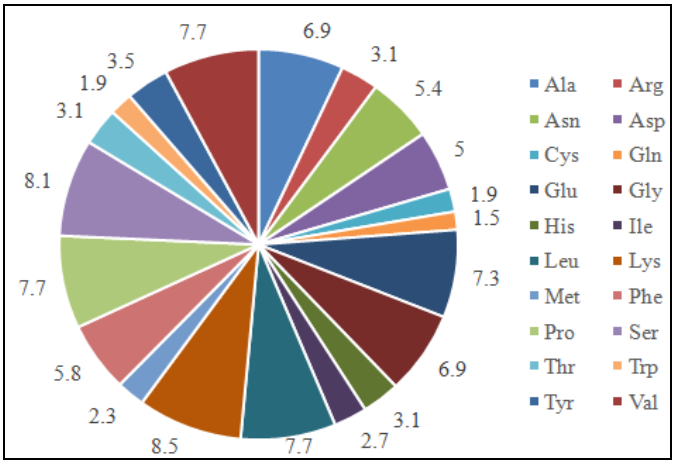


FIG. 5: AMINO ACID DISTRIBUTION IN *ACROPORA MILLEPORA* CARBONIC ANHYDRASE

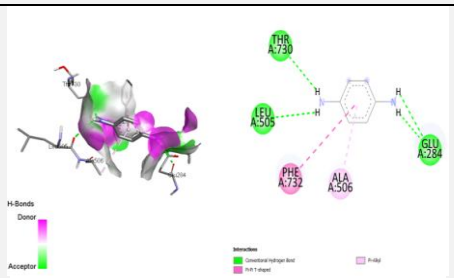
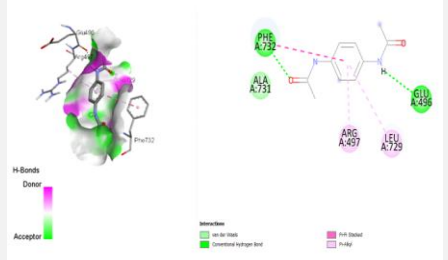
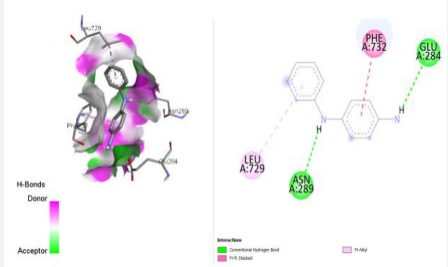
Molecular Docking and Interaction Analysis of Cosmetic Agents and Standard Inhibitors with Zooxanthellae Carbonic Anhydrase: Molecular docking simulations were systematically performed using PyRx software to investigate the binding affinities of the *Symbiodinium microadriaticum* and *Acropora millepora* Carbonic anhydrase (CA) enzyme with three well-established CA inhibitors: Ethoxzolamide, Acetazolamide, and Dorzolamide, as well as 29 chemical compounds commonly associated with cosmetic and hair dye formulations. Following docking, BIOVIA Discovery Studio Visualizer was used to elucidate the key proteinligand interactions and hydrogen bonding patterns that contribute to the observed binding affinities. The docking analysis revealed that among the standard inhibitors, Dorzolamide exhibited the maximum binding affinity to the CA of *Symbiodinium microadriaticum*, with a docking

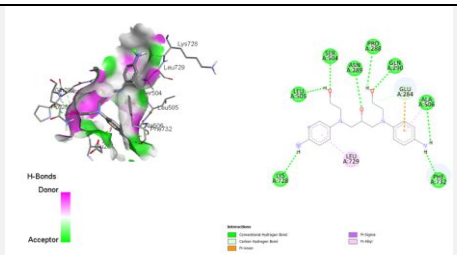
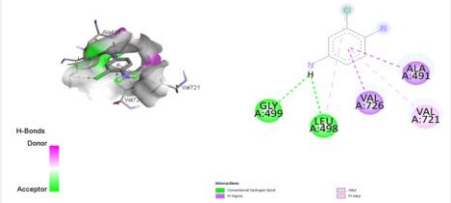
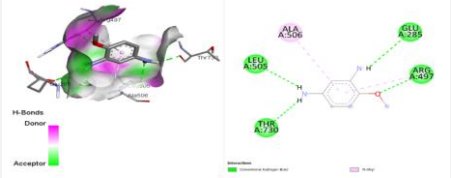
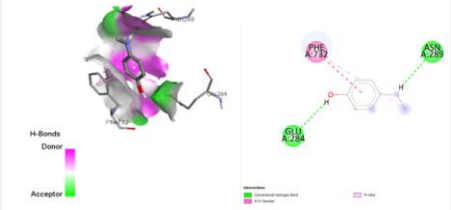
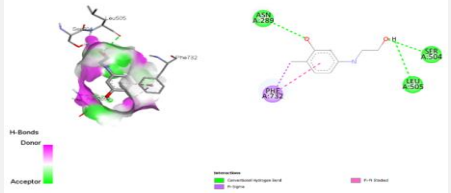
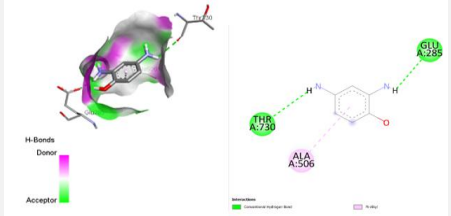
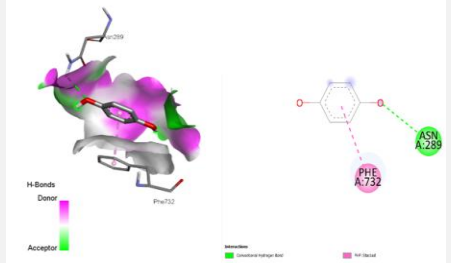
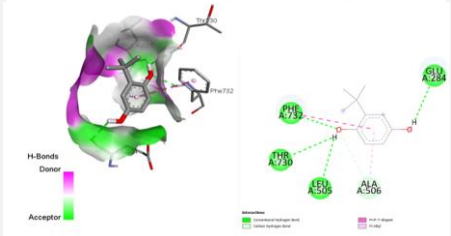
score of -6.9 kcal/mol, engaging critical residues such as Glu284, Asn289, Gln290, and Trp741. Ethoxzolamide and Acetazolamide displayed comparable binding scores of -6.4 kcal/mol and -6.3 kcal/mol, respectively, forming hydrogen bonds with essential active site residues, including Glu284, Arg497, Thr730, Glu285, Gln290, Arg497, and Ser504. Among the tested cosmetic agents, Acid Violet 43 demonstrated the strongest binding affinity, achieving a docking score of -10.0 kcal/mol.

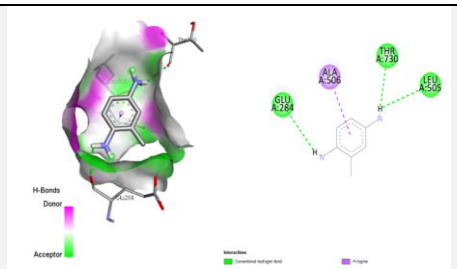
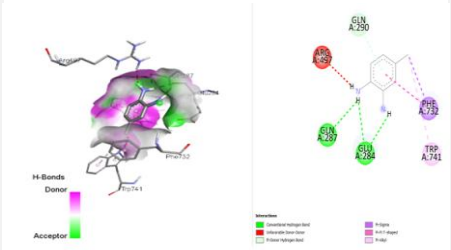
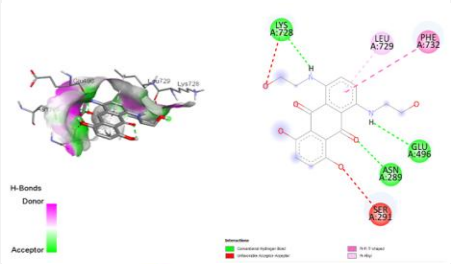
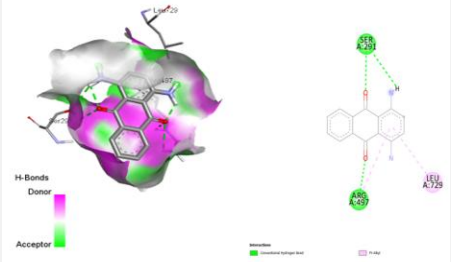
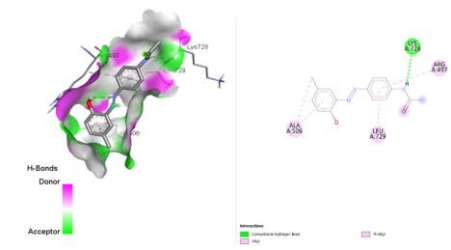
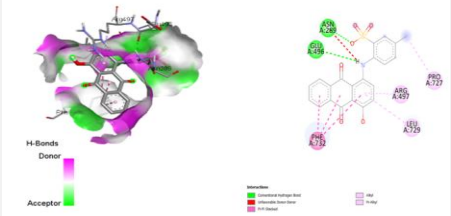
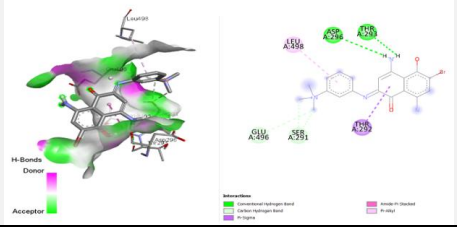
This interaction was stabilized through contacts with Asn289 and Glu496, indicating a potentially robust inhibitory interaction. Disperse Violet 1 and Hydroxypropyl bis (N-hydroxyethyl-p-phenylenediamine) hydrochloride also exhibited favorable binding affinities of -7.9 kcal/mol and -7.4 kcal/mol, respectively, with notable interactions involving Ser291, Arg497, Pro288, Asn289, Gln290, Ser504, Leu505, Ala506, Lys728, Phe732 and other key residues within the binding pocket. Other notable compounds demonstrating robust binding affinity include Disperse Yellow 3 (-7.4 kcal/mol), Di-n-butyl phthalate (-6.8 kcal/mol), Basic Blue 99 (-6.6 kcal/mol), and N-Phenyl-p-phenylenediamine (-6.5 kcal/mol). These ligands exhibited multiple hydrogen bonds, frequently engaging residues like Lys728, Asn289, Ser291, Thr293, Asp296, Arg497, Phe732, and Glu496, suggesting their capacity to form stable complexes with the protein.

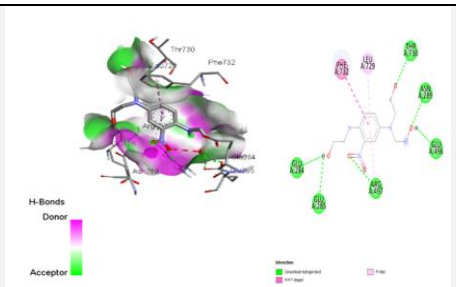
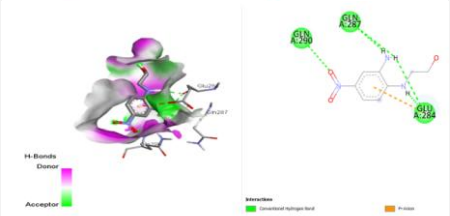
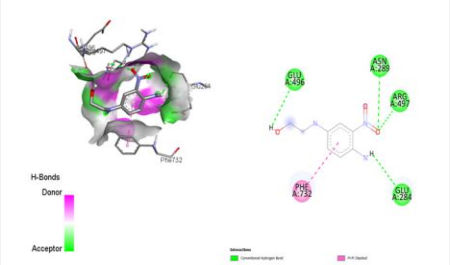
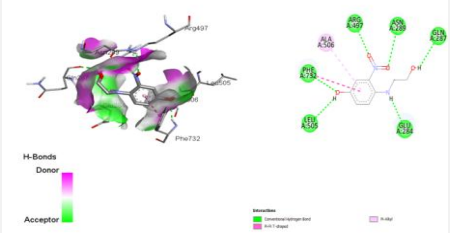
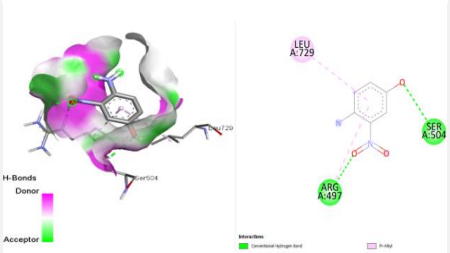
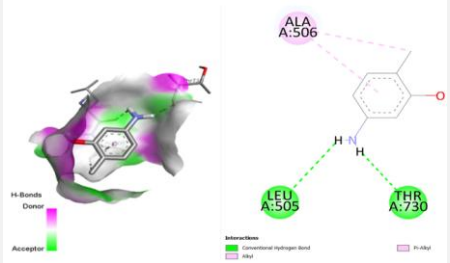
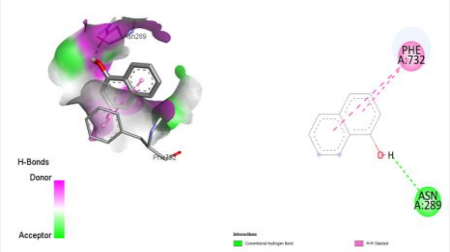
Comparative analysis of all compounds suggests that several cosmetic agents exhibited binding affinities surpassing the standard inhibitors, implying potential inhibition and possible biological impacts if bioaccumulated. The detailed binding scores and interacting residues for each compound are summarized in **Table 1**. This comprehensive interaction analysis provides valuable insights into the molecular recognition patterns of the CA enzyme and highlights the potential bioactivity of widely used cosmetic agents, warranting further biochemical validation.

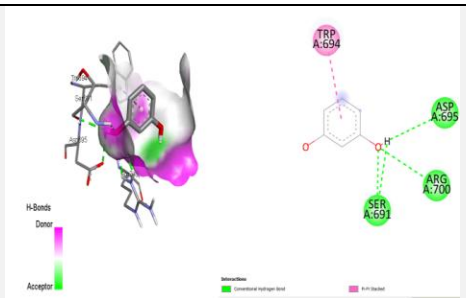
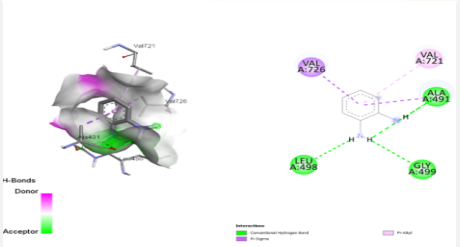
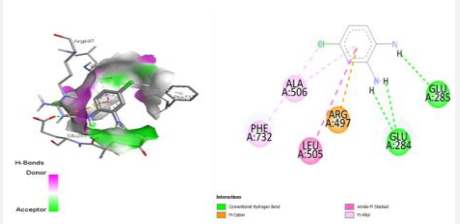
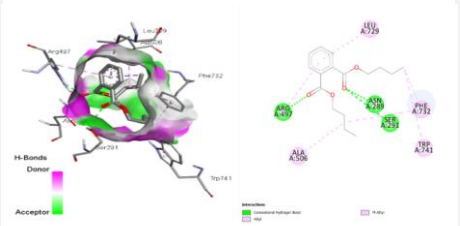
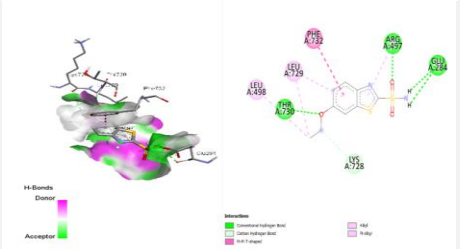
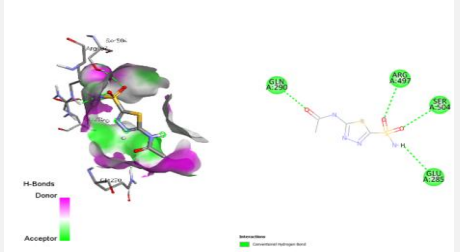
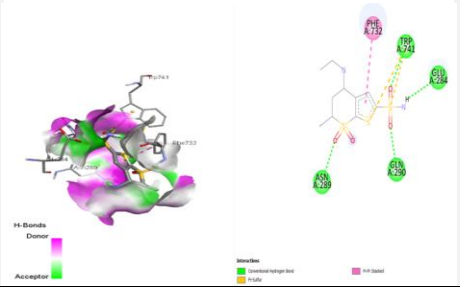
TABLE 1: MOLECULAR DOCKING RESULTS AND KEY INTERACTIONS OF COSMETIC AGENTS AND STANDARD INHIBITORS WITH ZOOXANTHELLAE CARBONIC ANHYDRASE

S. no.	Molecule Name	Binding Score Kcal/mol	Interacting Amino Acids	2D and Hydrogen Bond Interaction Diagram
1	p-Phenylenediamine	-4.2	Thr730, Lue505 and Glu284	
2	N, N'-Diacetyl-1,4-phenylenediamine	-6.2	Phe732 and Glu496	
3	N-Phenyl-p-phenylenediamine	-6.5	Glu284 and Asn289	

4	Hydroxypropyl bis(N-hydroxyethyl-p-phenylenediamine) hydrochloride	-7.4	Pro288, Asn289, Gln290, Ser504, Leu505, Ala506, Lys728 and Phe732	
5	2-Chloro-p-phenylenediamine	-4.3	Leu498 and Gly499	
6	4-Methoxy-m-phenylenediamine	-4.9	Glu285, Arg497, Leu505 and Thr730	
7	p-(Methylamino)phenol	-4.7	Asn289, and Glu284	
8	2-Methyl-5-hydroxyethylaminophenol	-5.5	Asn289, Ser504 and Leu505	
9	2,4-Diaminophenol	-4.8	Glu285 and Thr730	
10	Hydroquinone	-4.5	Asn289	
11	t-Butylhydroquinone	-5.8	Glu284, Leu505, Thr730 and Phe732	

12	Toluene-2,5-diamine	-4.7	Glu284, Leu505 and Thr730	
13	Toluene-3,4-diamine	-4.9	Glu284 and Gln287	
14	Disperse blue 7	-6.1	Asn289, Glu496 and Lys728	
15	Disperse violet 1	-7.9	Ser291 and Arg497	
16	Disperse yellow 3	-7.4	Lys728	
17	Acid violet 43	-10.0	Asn289 and Glu496	
18	Basic blue 99	-6.6	Thr293 and Asp296	

19	HC blue no.2	-6.3	Glu284, Glu285, Asn289, Glu496, Arg497 and Thr730	
20	HC yellow no.5	-6.0	Glu284, Gln287 and Gln290	
21	HC Red no.7	-5.8	Glu284, Asn289, Glu496 and Arg497	
22	3-Nitro-p-hydroxyethylamino phenol	-6.0	Gln287, Glu284, Asn289, Arg497, Leu505 and Phe732	
23	4-Amino-3-nitrophenol	-5.7	Ser504 and Arg497	
24	4-Amino-2-hydroxytoluene	-4.8	Leu505 and Thr730	
25	1-Naphthol	-5.8	Asn289	

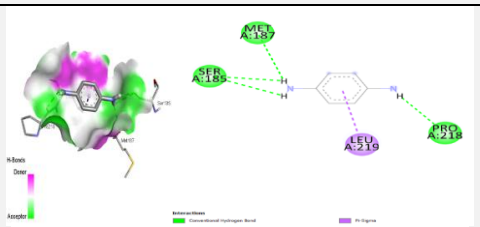
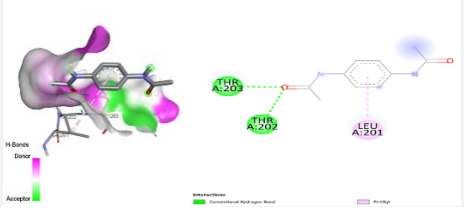
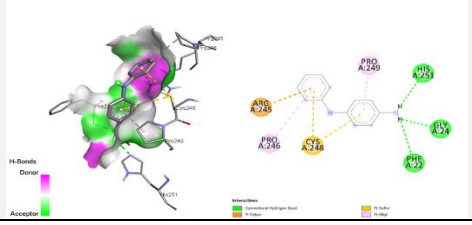
26	Resorcinol	-4.3	Ser691, Asp695 and Arg700	
27	o-phenylenediamine	-4.5	Ala491, Leu498 and Gly499	
28	4-chloro-o-phenylenediamine	-4.6	Glu284 and Glu285	
29	Di-n-butyl phthalate	-6.8	Asn289, Ser291 and Arg497	
30	Ethoxzolamide	-6.4	Glu284, Arg497 and Thr730	
31	Acetazolamide	-6.3	Glu285, Gln290, Arg497 and Ser504	
32	Dorzolamide	-6.9	Glu284, Asn289, Gln290 and Trp741	

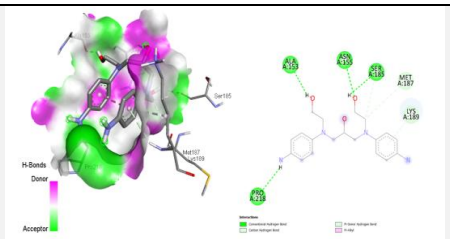
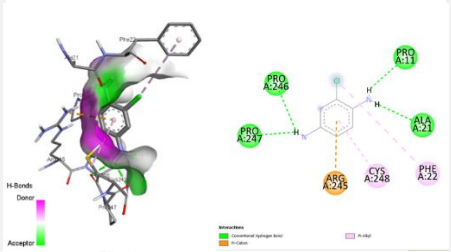
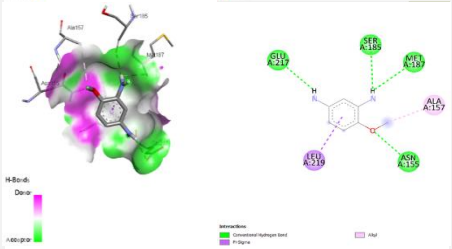
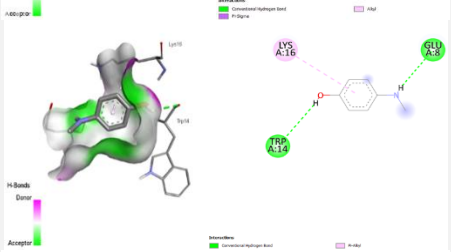
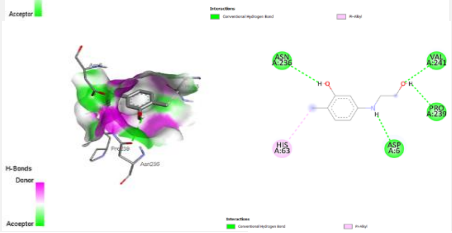
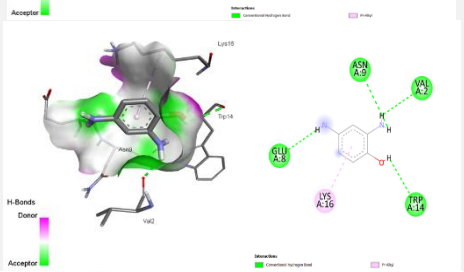
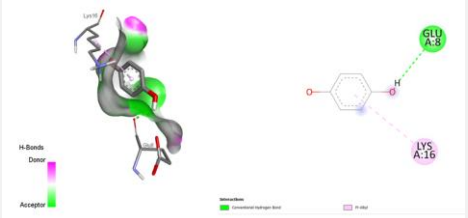
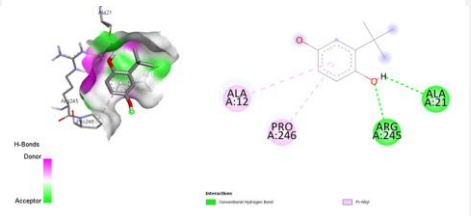
Molecular Docking and Interaction Analysis of Cosmetic Agents and Standard Inhibitors with Coral Carbonic Anhydrase: Upon molecular docking of coral carbonic anhydrase with the three standard inhibitors and 29 cosmetic agents, we noticed that, among the standard inhibitors, Acetazolamide exhibited the strongest binding affinity with a docking score of -6.1 kcal/mol, forming stable interactions with residues Thr202, Thr203, Gln66, and Asn61. Dorzolamide and Ethoxzolamide displayed docking scores of -5.7 kcal/mol and -5.4 kcal/mol, respectively, with Dorzolamide interacting with His63 and Thr203, while Ethoxzolamide engaged Pro11, Arg245, and Cys248. These findings reaffirm the established inhibitory potential of these compounds against CA. Among all the cosmetic agents, Acid Violet 43 demonstrated the highest binding affinity with a docking score of -7.6 kcal/mol, engaging in critical interactions with Arg184, Ser185, and Met187.

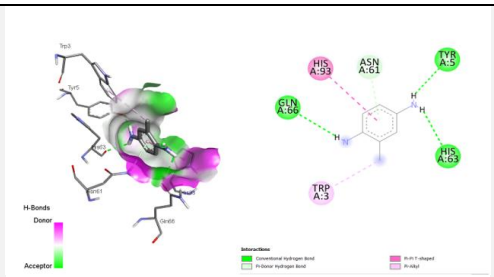
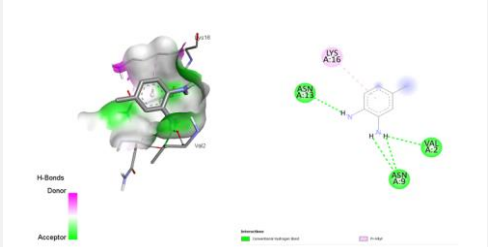
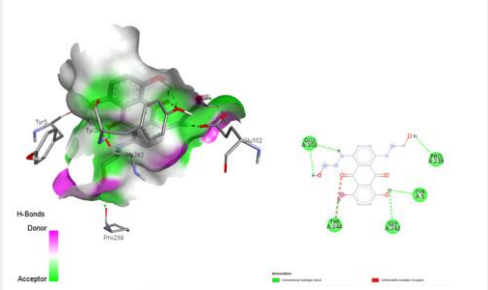
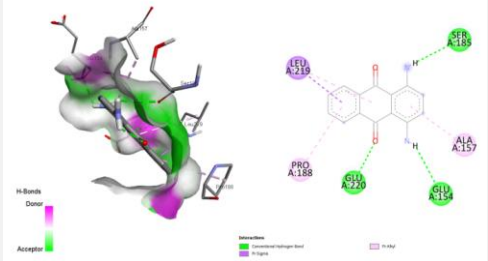
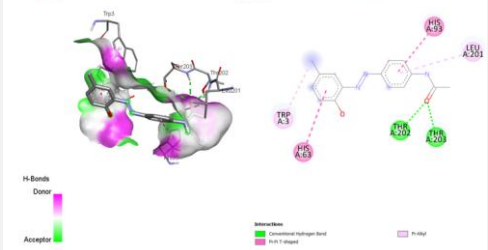
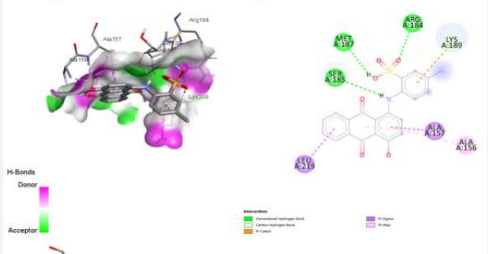
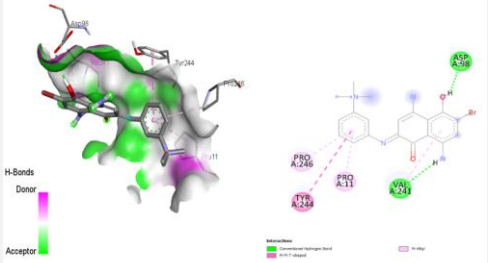
Disperse Violet 1 and Disperse Blue 7 also exhibited favorable binding profiles, with docking scores of -7.1 kcal/mol and -6.6 kcal/mol, respectively, stabilizing through contacts with residues such as Ser185, Glu220, Glu154, Glu102, Tyr244, Gly242, Pro239, and Tyr5. Disperse Yellow 3 and Basic Blue 99 further displayed significant affinities of -6.6 kcal/mol and -6.5 kcal/mol, respectively, suggesting potential inhibition. Additional compounds, including N-Phenyl-p-phenylenediamine (-5.7 kcal/mol), Di-n-butyl phthalate (-5.9 kcal/mol), and 3-nitro-p-hydroxyethylaminophenol (-5.3 kcal/mol), exhibited moderate binding energies, interacting with key residues such as Thr202, Thr203, Asp98, Val241, Gly24, His251, Phe22, Gln91, Asn74, Trp122 and Glu90. Interestingly, Hydroxypropyl bis (N - hydroxyethyl – p - phenylenediamine) hydrochloride and HC Red No. 7 demonstrated stable docking poses with key residues including Pro218, Asn155, Ser185, Ala153, Ala21, Cys248, Arg245, and Pro11.

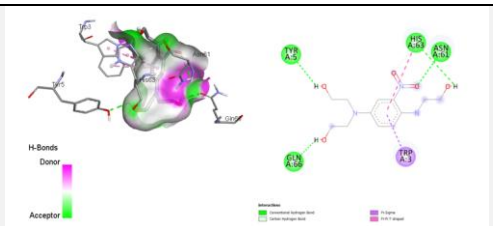
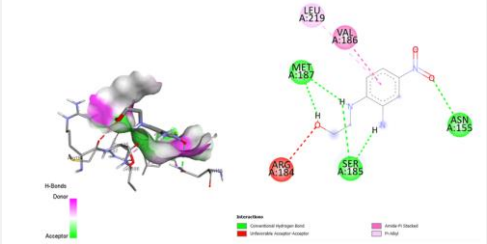
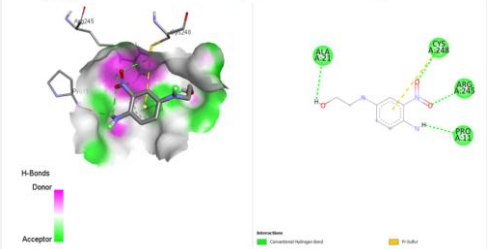
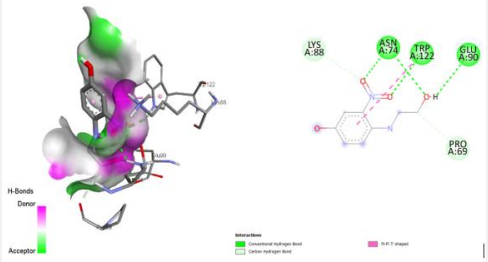
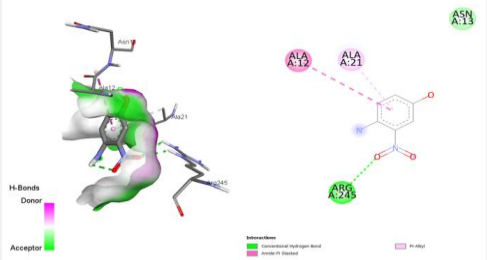
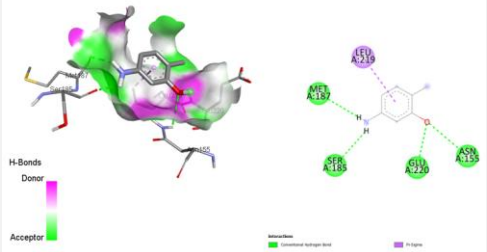
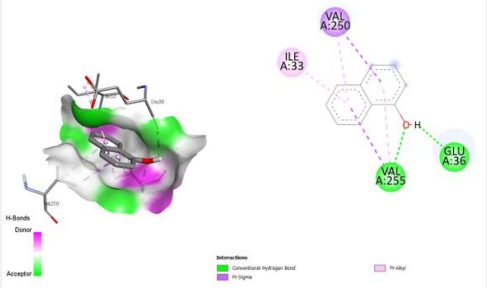
The interaction analysis revealed that several cosmetic agents formed superior binding affinities to those of the known CA inhibitors, with frequent engagement of active site residues such as Ser185, Thr203, Glu284, and Arg245. This suggests that these environmental cosmetic compounds may exhibit significant binding potential, potentially leading to the inhibition of CA. The detailed binding scores and amino acid interactions for each compound are summarized in **Table 2.** These findings underscore the need for further *in vitro* and *in-vivo* evaluations to assess the biological relevance and potential environmental risks associated with these widely used cosmetic agents.

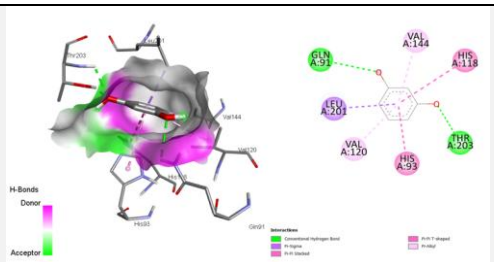
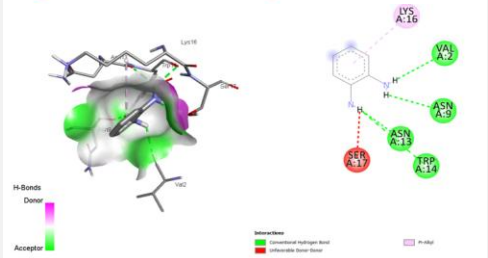
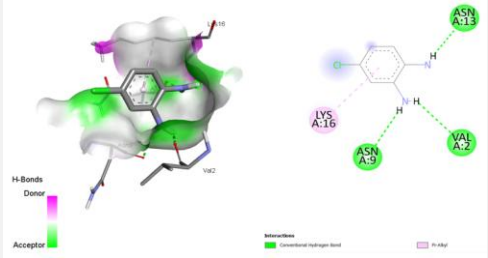
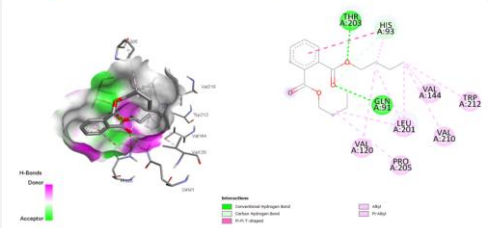
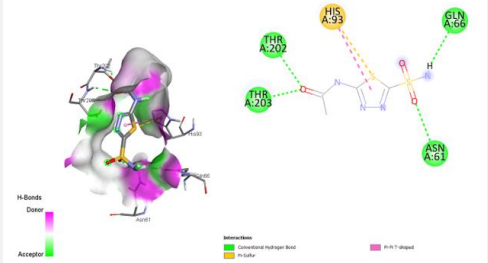
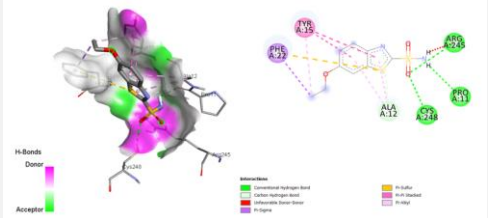
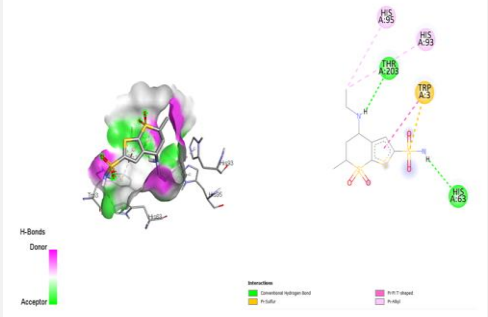
TABLE 2: MOLECULAR DOCKING RESULTS AND KEY INTERACTIONS OF COSMETIC AGENTS AND STANDARD INHIBITORS WITH CORAL CARBONIC ANHYDRASE

S. no.	Molecule Name	Binding Score Kcal/mol	Interacting Amino Acids	2D and Hydrogen Bond Interaction Diagram
1	p-phenylenediamine	-3.9	Met187, Ser185 and Pro218	
2	N,N-Diacetyl-p-phenylenediamine	-5.7	Thr203 and Thr202	
3	N-phenyl-p-phenylenediamine	-5.7	Gly24, His251 and Phe22	

4	Hydroxypropyl bis(N-hydroxyethyl-p-phenylenediamine) HCL	-5.6	Pro218, Asn155, Ser185 and Ala153	
5	2-chloro-p-phenylenediamine	-4.0	Pro11,Pro246,Pro247 and Ala21	
6	4-Methoxy-m-phenylenediamine	-4.2	Glu217, Ser185, Met187 and Asn155	
7	p-Methylaminophenol	-4.3	Glu8 and Trp14	
8	2-Methyl-5-hydroxethyl aminophenol	-5.1	Asn236, Val241, Pro239 and Asp6	
9	2,4-Diaminophenol	-4.6	Asn9, Val2, Trp14 and Glu8	
10	Hydroquinone	-4.2	Glu8	
11	t-butyl hydroquinone	-5.3	Arg245 and Ala21	

12	Toluene-2,5-diamine	-4.2	Gln66, Tyr5 and His63	
13	Toluene-3,4-diamine	-4.4	Asn13, Asn9 and Val2	
14	Disperse blue 7	-6.6	Glu102, Tyr244, Gly242,Pro239 and Tyr5	
15	Disperse violet 1	-7.1	Ser185, Glu220 and Glu154	
16	Disperse yellow 3	-6.6	Thr202 and Thr203	
17	Acid violet 43	-7.6	Arg184, Ser185 and Met187	
18	Basic blue 99	-6.5	Asp98 and Val241	

19	HC blue no.2	-5.2	Tyr5, Gln66, His63 and Asn61	
20	HC yellow no.5	-5.2	Met187, Ser185 and Asn155	
21	HC red no.7	-5.5	Ala21, Cys248, Arg245 and Pro11	
22	3-nitro-p-hydroxyethylamino phenol	-5.3	Asn74, Trp122 and Glu90	
23	4-amino-3-nitrophenol	-5.0	Arg245	
24	4-amino-2-hydroxytoluene	-4.8	Met187, Ser185,Glu220 and Asn155	
25	1-napthol	-5.5	Val255 and Glu36	

26	Resorcinol	-4.4	Gln91 and Thr203	
27	o-phenylenediamine	-4.1	Val2, Asn9, Asn13 and Trp14	
28	4-chloro-o-phenylenediamine	-4.2	Asn13, Asn9 and Val2	
29	Di-n-butyl-pthalate	-5.9	Thr203 and Gln91	
30	Acetazolamide	-6.1	Thr202, Thr203, Gln66 and Asn61	
31	Ethoxzolamide	-5.4	Pro11, Arg245 and Cys248	
32	Dorzolamide	-5.7	His63 and Thr203	

CONCLUSION: This study systematically analyzed the structural integrity, sequence conservation, physicochemical characteristics, and ligand binding potential of Carbonic anhydrase (CA) from *Symbiodinium microadriaticum* and *Acropora millepora*. Stereochemical validation confirmed the high quality models, with over 90% of residues present in the most favored regions of the Ramachandran plot, affirming their reliability for computational analyses.

Although sequence alignment revealed limited overall identity, the presence of conserved functional domains suggests evolutionary retention of critical catalytic features essential for CA function. Physicochemical analysis further indicated that the algal CA is hydrophilic but relatively unstable, while the coral CA is structurally more stable and soluble, reflecting their distinct biological roles and environmental adaptations. Molecular docking results identified several cosmetic agents, particularly Acid Violet 43, with binding affinities significantly stronger than those of standard CA inhibitors, including Acetazolamide, Ethoxzolamide, and Dorzolamide. Acid Violet 43 demonstrated robust interactions with both algal and coral CA, indicating its potential as a potent CA inhibitor. Additional compounds, such as Disperse Violet 1, Disperse Yellow 3, and Basic Blue 99, also exhibited considerable binding potential, raising concerns regarding their possible ecological risks if these agents persist or bioaccumulate in marine environments. Collectively, these findings suggest that commonly used cosmetic agents may inadvertently interfere with coralalgal metabolic processes. Further *in-vitro* and *in-vivo* studies are essential to validate these interactions and to comprehensively assess their potential environmental impacts. This study provides a critical foundation for future research aimed at evaluating the ecological safety of cosmetic agents within coral reef ecosystems.

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