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ORIGINAL ARTICLE

Cinnamaldehyde, Cinnamic Acid, and Cinnamyl Alcohol, the Bioactives of *Cinnamomum cassia* Exhibit HDAC8 Inhibitory Activity: An *In vitro* and *In silico* Study

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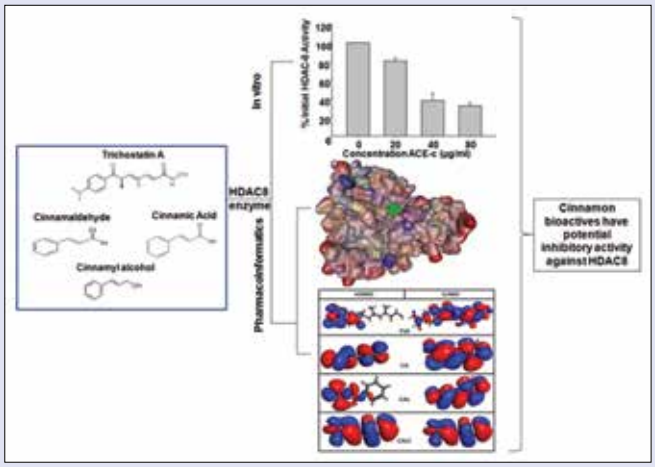
ABSTRACT

Background: The altered expression of histone deacetylase family member 8 (HDAC8) has been found to be linked with various cancers, thereby making its selective inhibition a potential strategy in cancer therapy. Recently, plant secondary metabolites, particularly phenolic compounds, have been shown to possess HDAC inhibitory activity. **Objective:** In the present work, we have evaluated the ability of cinnamaldehyde (CAL), cinnamic acid (CA), and cinnamyl alcohol (CALC) (bioactives of Cinnamomum) as well as aqueous cinnamon extract (ACE), to inhibit HDAC8 activity *in vitro* and *in silico*. **Materials and Methods:** HDAC8 inhibitory activity of ACE and cinnamon bioactives was determined *in vitro* using HDAC8 inhibitor screening kit. Trichostatin A (TSA), a well-known anti-cancer agent and HDAC inhibitor, was used as a positive control. *In silico* studies included molecular descriptor Analysis molecular docking absorption, distribution, metabolism, excretion, and toxicity prediction, density function theory calculation and synthetic accessibility program. **Results:** Pharmacoinformatics studies implicated that ACE and its Bioactives (CAL, CA, and CALC) exhibited comparable activity with that of TSA. The highest occupied molecular orbitals and lowest unoccupied molecular orbitals along with binding energy of cinnamon bioactives were comparable with that of TSA. Molecular docking results suggested that all the ligands maintained two hydrogen bond interactions within the active site of HDAC8. Finally, the synthetic accessibility values showed that cinnamon bioactives were easy to synthesize compared to TSA. **Conclusion:** It was evident from both the experimental and computational data that cinnamon bioactives exhibited significant HDAC8 inhibitory activity, thereby suggesting their potential therapeutic implications against cancer.

Key words: Absorption, cinnamon, density function theory, distribution, excretion, HDAC8, metabolism, toxicity prediction, molecular docking, synthetic accessibility

SUMMARY

- Pharmacoinformatics studies revealed that cinnamon bioactives bound to the active site of HDAC8 enzyme in a way similar to that of TSA
- The molecular descriptors of cinnamon compounds successfully correlated with TSA values. The binding interactions and energies were also found to be close to TSA
- Synthetic accessibility values showed that cinnamon bioactives were easy to synthesize compared to TSA.



Abbreviations used: ACE: Aqueous Cinnamon Extract; DFT: Density Function Theory; CAL: Cinnamaldehyde; CA: Cinnamic Acid; CALC: Cinnamyl Alcohol; MW: Molecular Weight; ROTBs: Rotatable Bonds; ROF: Lipinski's Rule of Five; TSA: Trichostatin A; PDB: Protein Data Bank; RMSD: Root Mean Square Deviation; HBA: Hydrogen Bond Acceptor; HBD: Hydrogen Bond Donor; ADMET: Absorption, Distribution, Metabolism, Excretion and Toxicity; FO: Frontier Orbital; HOMOs: Highest Occupied Molecular Orbitals; LUMOs: Lowest Unoccupied Molecular Orbitals; BE: Binding Energy.

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INTRODUCTION

Histone deacetylases (HDACs) are enzymes that have specific role in development and tissue homeostasis.[1] Based on phylogenetic similarity, HDACs have been categorized into four classes, Class I (HDAC1, 2, 3, and 8); II (HDAC4, 5, 6, 7, 9, and 10); and IV (HDAC11).[2] Biochemical analysis has revealed that Class I HDACs hold majority of HDAC activity, whereas purified recombinant Class II HDACs possess only minimal activity.[3-5] In the recent years, HDACs have emerged as important therapeutic targets for various diseases, such as cancer, cardiovascular

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diseases, autoimmunity, and neurodegenerative diseases.[6-9] In cancer, deregulation of HDAC activity due to aberrant expression or recruitment to promoter regions results into uncontrolled growth.[2,10] HDAC family member 8 (HDAC8) has been found to play a crucial role in the physiology of both hematological and solid malignancies.[11] Knockdown studies of HDAC8 have been shown to alter the growth of human lung, colon, and cervical cancer cells,[12] and induce cell cycle arrest as well as differentiation of neuroblastoma cells.[13]

HDAC inhibitors have emerged as potent anticancer agents that can restore programmed cell death in malignant cells.[14,15] Compared to other Class I isoforms, HDAC8 could be an attractive target due to the presence of unique second metal binding site in proximity to the main catalytic domain, which displays a therapeutic potential with greater efficacy for selective inhibition.[16] Recently, there has been growing interest in plant secondary metabolites, particularly phenolic compounds that possess HDAC inhibitory activity.[17] Many studies have indicated that cancer prevalence could be significantly modulated by an increased intake of diet rich in polyphenols such as fruits and vegetables.[18-20] Various polyphenols such as green tea catechin (−)-epigallocatechin-3-gallate, curcumin, resveratrol, soy isoflavones, quercetin, and caffeic acid have been reported to exhibit HDAC inhibitory potential.[20-22] A few studies have demonstrated synergistic activity of plant-based histone modifiers, when combined with ionizing irradiation or DNA-damaging drugs.[21]

Cinnamon, a commonly used food spice, contains several bioactive components such as cinnamic acid, cinnamyl aldehyde, tannin, mucus, and carbohydrates.[22] Cinnamon has been known to exhibit various pharmacological activities including antioxidant,[23-26] anti-inflammatory,[27,28] antimicrobial,[29-32] antipyretic,[33] antiulcer,[34,35] antidiabetic,[36-40] and antitumor.[41,42] We have previously reported chemopreventive potential of aqueous cinnamon extract (ACE) in cervical cancer, wherein it induced apoptosis in SiHa cell line through loss of mitochondrial membrane potential.[41] To evaluate whether the apoptosis induced by ACE was due to inhibition of HDAC8, we examined its activity in a cell-free system. Further, various pharmacoinformatics techniques such as structure activity relationship,[43,44] molecular docking,[45] density function theory (DFT),[46] toxicity prediction,[47] and synthetic accessibility[47] were adopted to elucidate HDAC8 inhibitory potential of the test materials.

MATERIALS AND METHODS

Preparation of extract and determination of its histone deacetylase inhibitory activity

The bark of *Cinnamomum cassia* was purchased from Shivam Ayurvedics, Pune, Maharashtra, India, with voucher specimen number 104. The sample was authenticated from Regional Research Institute (AY) Kothrud, Pune (ref no. 1045). The ACE was prepared as described earlier,[41] and HDAC8 inhibitor screening assay was performed as per the manufacturer's instructions (Cayman chemical, USA). Briefly, the reaction was initiated in a 96-well plate which contained 25 µl assay buffer, 5 µl HDAC8 enzyme, 5 µl of extract/inhibitor at various concentrations (0–80 µg), 15 µl substrate, i.e., Arg-His-Lys-Lys (ε-acetyl)-AMC p53 sequence (100 µM) and incubated at 37°C for 30 min. Following the incubation, 50 µl of developer/stop solution was added, and the fluorescence was analyzed with an excitation wavelength of 350–360 nm and an emission wavelength of 450–465 nm using a microplate reader (BMG, Fluostar Omega). Percentage inhibition activity was calculated by the formula:

% inhibition = ([Initial activity − inhibitor]/initial activity) × 100.

RESULTS AND DISCUSSION

Effect of aqueous cinnamon extract and its bioactives (cinnamaldehyde, cinnamic acid, cinnamyl alcohol) on histone deacetylases family member 8 inhibitory activity

HDAC8 activity was significantly inhibited at 40 (~62%) and 80 µg/ml (~67%) concentrations of ACE. IC_{50} value of ACE was 25.24 µg/ml [Figure 1]. We further analyzed whether ACE and its bioactives (cinnamaldehyde [CAL], cinnamic acid [CA], cinnamyl alcohol [CALC]) exhibited HDAC8 inhibitory activity. Before this, we performed high-performance liquid chromatography analysis of ACE to confirm cinnamon bark identity by detecting the presence of marker molecules, CAL, CA, and CALC have been provided in Figure 2. This was conducted using a Phenomenex C18 (4.6 mm × 250 mm, 5 µm; Phenomenex, Torrance, CA, USA) column whose temperature was set at 40°C. Gradient flows for the two solvent systems (solvent A, 0.1% phosphoric acid in water; solvent B, acetonitrile) were: 0 min, 10% B; 12 min, 20% B; 35 min, 50% B; 40 min, and 100% B and hold at 100% B for 5 min. The standard marker compounds, CAL, CA, and CALC, were used. The flow rate of the mobile phase was 1.0 ml/min. The injection volume was 10 µl, and the chromatogram was monitored at a wavelength of 265 nm throughout the experiment. It was observed that 80 µg/ml of ACE contained 310.25, 60.75, and 24.2 µM concentrations of CAL, CA, and CALC, respectively [Figure 2]. At these particular concentrations, CAL, CA, and CALC showed 18.5, 9.3, and 5.7% inhibition of HDAC8 activity, respectively [Table 1].

Moreover, combination of three (CAL + CA + CALC) inhibited HDAC8 activity by 37.7% [Table 1]. Thus, compared to the whole ACE, the combination (CAL + CA + CALC) contributed to almost half of HDAC8 inhibition. These results suggested that besides the selected bioactives of cinnamon, there may be other compounds in the extract that might have contributed to HDAC8 inhibition.

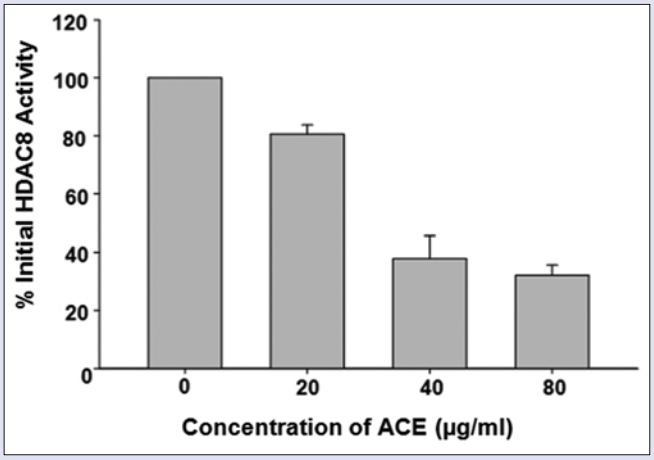

Figure 1: HDAC8 inhibition by aqueous cinnamon extract in a cell free system. ACE was examined for its HDAC8 inhibitory activity in a cell free system at concentrations of 20–80 µg/ml. The results have been expressed as mean ± standard deviation of three independent experiments

Table 1: HDAC8 inhibition by aqueous cinnamon extract and its bioactives

Test material	Concentration (µg/ml) (%)	Concentration (µM/ml)	HDAC8 inhibitory activity (%)
ACE	80.00 (100)*	-	67
CAL	40.69 (50.08)	310.25	18.5
CA	9.00 (11.25)	60.75	9.3
CALC	3.96 (4)	24.2	5.7
Combination (CAL + CA + CALC)	53.65 (67.06)	310.25+60.75+24.2	37.7

*Assuming 80 µg=100%. HDAC8: Histone deacetylase family member 8; CAL: Cinnamaldehyde; CA: Cinnamic acid; ACE: Aqueous cinnamon extract; CALC: Cinnamyl alcohol

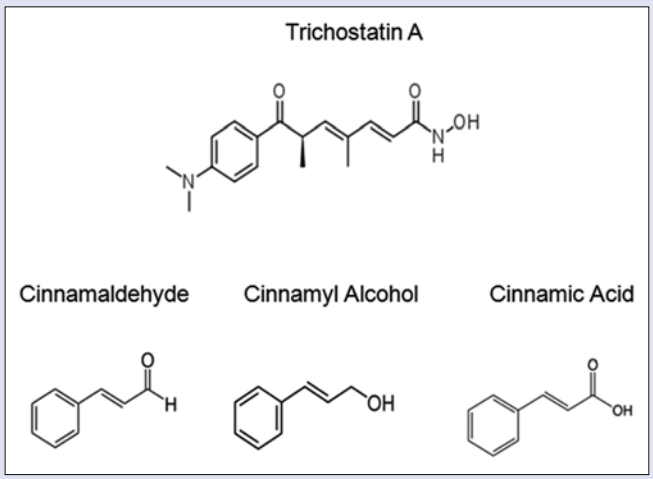

Figure 2: Chemical structures of trichostatin A, cinnamaldehyde, cinnamyl alcohol, and cinnamic acid

Descriptor analysis, absorption, distribution, metabolism, excretion, and toxicity, density function theory, molecular docking, and synthetic accessibility studies of aqueous cinnamon extract, cinnamaldehyde, cinnamic acid and cinnamyl alcohol, and trichostatin A

Descriptor analysis included physicochemical properties, molecular weight (MW), number of rotatable bonds (ROTBs), molecular volume, polar surface area, and bioavailability score. The drug likeness was also analyzed by Lipinski's Rule of Five (ROF). To understand how the ligands (CAL, CALC, and CA along with trichostatin A [TSA]) bind to HDAC8 enzyme, molecular docking was carried out using AutoDock 4.2 tool, Molecular Graphics Lab, Scrrips Research Institute, California tool that uses the Lamarckian Genetic algorithm.[14,59] 3D crystal structure of HDAC8 protein (Protein Data Bank [PDB] ID: 1T67)[14] was collected from RCSB-PDB based on bound ligand, date of deposition, and resolution. For molecular docking, twenty conformations of each ligand were generated, and best conformer was considered for further study. The grid preparation was carried out based on the information of the bound ligand B3N (4-(dimethylamino)-N-[7-(hydroxyamino)-7-oxoheptyl] benzamide) at the active site of 1T67.[14] From the docking results, the best docked pose was checked to evaluate whether it was able to form potential hydrogen-bonding interactions with the critical amino residues. Further, root mean square deviation (RMSD) between the best pose and the co-crystal was calculated by superimposing to govern whether the parameters used in the docking were able to replicate a conformation similar to that of the co-crystal at the active site cavity. The ligands were docked using similar parameters that were used for the co-crystal docking. Drug-like descriptors were calculated to evaluate drug likeness and to determine whether the bioactives of cinnamon exhibited properties similar to HDAC8 inhibitors. MW, *logP* value, hydrogen bond acceptor (HBA), and hydrogen bond donor (HBD) for each ligand were generated using molinspiration [Table 2].

Table 2: Molecular descriptors of trichostatin A, cinnamaldehyde, cinnamic acid, and cinnamyl alcohol

Descriptors	TSA	CAL	CA	CALC
logP	2.683	2.484	1.91	2.032
MW	302.374	132.162	148.161	134.178
TPSA	69.635	17.071	37.299	20.228
Number of atoms	22	10	11	10
Number of ON	5	1	2	1
Number of OHNH	2	0	1	1
Number of violations of ROF	0	0	0	0
ROTBs	6	2	2	2
Volume	293.12	130.444	138.462	136.28
Binding energy	−7.77	−5.27	−5.14	−5.8
Bioactivity score	0.63	−0.46	−0.3	−0.24

CAL: Cinnamaldehyde; CA: Cinnamic acid; ACE: Aqueous cinnamon extract; CALC: Cinnamyl alcohol; MW: Molecular weight; ROF: Rule of five; ROTBs: Rotatable bonds; TPSA: Total polar surface area

Except for MW, volume, and surface area, rest of the descriptors showed comparable values to TSA. This indicated that all the three bioactives had induced HDAC8 inhibition. The hydrophobicity (*logP*) of TSA was 2.683 and that of CAL, CA, and CALC was found to be 2.484, 1.910, and 2.032, respectively. There was no violation of ROF for any compounds. ROF rules[51] have been applied for the majority of molecules with good oral absorption and include that *logP* should be <5; MW should not be more than 500 kDa; number of HBD should be <5; and number of HBA should be <10. Number of ROTBs was found to be two for all the compounds of interest, while it was 6 for TSA. The data for all the descriptors were found to be within the proposed limit, thereby confirming their drug likeness.

Higher the bioactivity score of a compound, greater would be its probability to be active. In general, a compound with bioactivity score > 0.00 would have considerable biological activity; with values between − 0.50 and 0.00, the compound could be moderately active; and with score below − 0.50, the compound could be inactive.[50] Bioactivities of all the test compounds have been depicted in Table 2. TSA, a known enzyme inhibitor, showed highest (0.63) bioactivity score, whereas CALC, CA, and CAL showed score of − 0.46, −0.3, and − 0.24, respectively. This indicated that all the compounds were moderately active and may possess enzyme inhibition activity.

Molecular docking is one of the crucial techniques in drug design and validation process. It gives accurate and preferred orientations of the ligand at the active site of the enzyme.[52,53] The docked complexes of CAL, CA, and CALC were assessed for their optimal orientation and binding abilities. The crystal structure of HDAC8 (PDB ID: 1T67) was collected from RCSB-PDB.[50] In the present study, self-docking approach was considered to validate the docking procedure, and the freshly docked complex of bound compound with the receptor was superimposed with

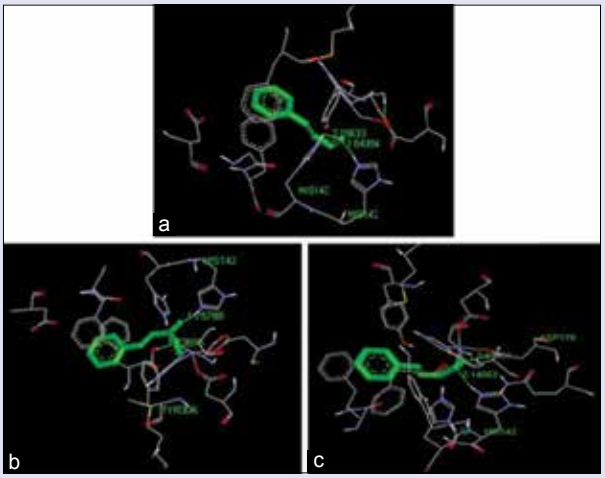

Figure 3: Representation of binding interaction between histone deacetylases family member 8 and (a) cinnamaldehyde, (b) cinnamyl alcohol, and (c) cinnamic acid. Molecular docking simulations indicating interaction of histone deacetylases family member 8 with cinnamaldehyde, cinnamic acid, and cinnamyl alcohol

Table 3: Hydrogen bond interactions of histone deacetylase family member 8 proteins with trichostatin A, cinnamaldehyde, cinnamic acid and cinnamyl alcohol

Ligands	Ligand atoms	Residue atoms	Distance Å
TSA	O1	HIS 142 HE2	1.838
	O1	HIS 143 HE2	2.215
	H	ASP 178 OD2	2.091
CALC	O1	HIS 142 HE2	2.141
	H	ASP 178 OD1	2.079
CAL	O1	HIS 142 HE2	1.844
	O1	HIS 143 HE2	2.186
CA	O1	HIS 142 HE2	1.758
	O1	TYR 306 HH	1.737

CAL: Cinnamaldehyde; CA: Cinnamic acid; ACE: Aqueous cinnamon extract; CALC: Cinnamyl alcohol

Table 4: Metal ion Zn-interactions of histone deacetylase family member 8 proteins with trichostatin A, cinnamyl alcohol, cinnamaldehyde, and cinnamic acid

Ligands	Zn-interactions	Distance Å
TSA	ASP 267 OD2	2.0004
	ASP 178 OD2	1.9825
	HIS 180 ND1	2.0624
	O1, N1	2.1301, 2.1278
CALC	HIS 180 ND1	2.0624
	ASP 267 OD2	2.0004
	ASP 178 OD2	1.9825
	O1, N1	2.1341, 2.1229
CAL	ASP 178 OD2	1.9825
	HIS 180 ND1	2.0624
	ASP 267 OD2	2.0004
CA	ASP 267 OD2	2.0004
	ASP 178 OD2	1.9825
	HIS 180 ND1, O1	2.0624, 1.9243

CAL: Cinnamaldehyde; CA: Cinnamic acid; ACE: Aqueous cinnamon extract; CALC: Cinnamyl alcohol

original complex downloaded from RCSB PDB (PDB ID: 1T67).[49,52] This was done to calculate RMSD values. Low RMSD (<2 Å) value of original bound ligand validates the docking procedure. RMSD values were found to be 1.492Å, which indicated that the protocol selected in the docking method was validated.

Molecular docking simulations of HDAC8 proteins with CALC, CA, and CAL were performed using AutoDock 4.2.[48] The accuracy of the AutoDock 4.2 results was confirmed by considering clusters of twenty runs of conformations/orientations with RMSD value 0.0 in addition to the lowest binding free energy and hydrogen bonds between macromolecules.[48] Further, the docked conformations were energetically and statistically validated. Potential binding interactions were observed between ligand and catalytic residues at the active site. The ligand receptor binding interactions are given in Figure 3.

Molecular docking between HDAC8 and TSA showed [Figure 3 and Table 3] high negative binding energy (BE; −7.18 kcal/mol) along with the formation of three strong hydrogen bond interactions with the catalytic residues (His142, His143, and Asp178). Similarly, docking of HDAC8 with CAL, CALC, and CA showed BEs of − 7.18, −5.8, and − 5.14 kcal/mol, respectively. It was also observed that all the ligands maintained two hydrogen bond interactions within the active site.

The metal ion, Zn interactions with the ligand, and active site residues were also taken into consideration during the docking study [Table 4].

1T67-TSA complex showed five interactions with zinc ion as well as active site residues Asp267 OD2, Asp178 OD2, His180 ND1, and O1 and N1 of TSA. The proposed inhibitor group compounds, CALC-1T67 complex, showed four interactions with Asp267 OD2, His180 ND1, Asp178 OD2, and O1 of CALC. CAL-1T67 complex showed three metal ion interactions with active site residues, mainly Asp267 OD2, His180 ND1, and Asp267 OD2. CA-1T67 complex showed four Zn ion interactions with Asp267 OD2, Asp178 OD2, His180 ND1, and O1 of CA.

To obtain compounds with good pharmacokinetic properties, absorption, distribution, metabolism, excretion, and toxicity (ADMET) descriptors were calculated using Discovery Studio (DS) [Computer program]. Version Release 4.0. San Diego: Biovia Accelrys Software Inc.; 2015.[54,55] ADMET of the selected compounds was applied to verify whether the molecules were able to cross the blood–brain barrier (BBB) and exhibit better solubility, human intestinal absorption (HIA), and low toxicity. In the present study, main focus was on oral bioavailability, hepatotoxicity, and the capacity to infiltrate the BBB. ADMET properties compute the values of BBB penetration, solubility, cytochrome P450 (CYP450) 2D6 inhibition, hepatotoxicity, HIA, plasma protein binding (PPB), and an extensive range of ligand toxicity. It has been reported that a drug should not cross BBB level 3. In the present study, TSA showed BBB value of 2 [Table 5] while CAL, CA, and CALC showed values of 1, 2, and 1, respectively, showing that the values fall within the range that describe a drug candidate. The value of 0 in CYP26 [Table 5] hepatotoxicity for all the compounds, indicated that they exhibited low toxicity. ADMET_solubility, ADMET_solubility_level, ADMET_EXT_PPB, and ADMET_Alogp98 of all three cinnamon bioactive compounds were comparable to TSA [Table 5], suggesting that CAL, CA, and CALC could behave as HDAC8 inhibitors.

Frontier orbital (FO) theory states that the shape and symmetries of the highest occupied molecular orbitals (HOMOs) and lowest unoccupied molecular orbitals (LUMOs) are essential to predict the reactivity and the stereochemistry of a compound.[56] For quantum mechanical simulation, DFT is an effective and promising approach of periodic systems, and it offers an accurate elucidation of the electronic and structural behavior of small molecules by calculating the electronic structure of the substance.[57] DFT was performed on CAL, CA, CALC, and TSA to analyze the electronic behavior of the molecules. For this, calculate

Table 5: Absorption, distribution, metabolism, excretion, and toxicity values of trichostatin A, cinnamic acid, cinnamaldehyde, and cinnamyl alcohol

Molecule	ADMET solubility	ADMET solubility level	ADMET BBB	ADMET BBB level	ADMET extension of PPB	ADMET_AlogP98	CYP26_hepato toxicity
TSA	−3.226	3	−0.343	2	0.709	3.051	0
CA	−1.867	4	−0.161	2	0.797	1.927	0
CAL	−2.298	3	0.175	1	0.885	1.949	0
CALC	−1.456	4	0.040	1	−0.457	1.693	0

ADMET: Absorption, distribution, metabolism, excretion, and toxicity; CAL: Cinnamaldehyde; CA: Cinnamic acid; ACE: Aqueous cinnamon extract; CALC: Cinnamyl alcohol; BBB: Blood-brain barrier; EXT: PPB: Plasma protein binding

Table 6: Highest occupied molecular orbital, lowest unoccupied molecular orbital and binding energies of cinnamic acid, cinnamaldehyde, cinnamyl alcohol, and trichostatin A

Compounds	BE	HOMO	LUMO
CA	−3.874	−0.223	−0.107
CAL	−3.667	−0.195	−0.105
CALC	−3.876	−0.195	−0.054
TSA	−8.746	−0.171	−0.073

HOMO: Highest occupied molecular orbital; LUMO: Lowest unoccupied molecular orbital; CAL: Cinnamaldehyde; CA: Cinnamic acid; ACE: Aqueous cinnamon extract; CALC: Cinnamyl alcohol; BE: Binding energy

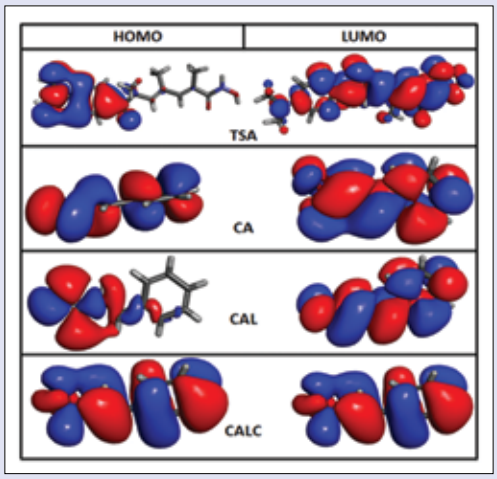

Figure 4: Plots of highest occupied molecular orbitals and lowest unoccupied molecular orbitals of trichostatin A, cinnamic acid, cinnamaldehyde, and cinnamyl alcohol. The quantum chemical descriptors (highest occupied molecular orbitals and lowest unoccupied molecular orbitals) showing map of molecular orbitals indicating the electron exchange and electron transfer ability of trichostatin A, cinnamic acid, cinnamaldehyde, and cinnamyl alcohol

energy (DFT) module of DS was used with B3 LYP algorithm that providing the information, concerning the capability of the molecules to transmission their energies from a HOMO, which can behave as an electron donor, to a LUMO, which can behave as an electron acceptor.[55] The quantum chemical descriptors (HOMO and LUMO) can help to discriminate the reactive or binding sites and substantial stimulus on electronic structure of the molecules. HOMO and LUMO maps have been plotted and depicted in Figure 4. HOMO, LUMO, and BE of CA, CAL, CALC, and TSA are given in Table 6. The molecular orbitals maps indicated that electron exchange and transfer capability of the tested molecules may have a pivotal influence in HDAC8 inhibitory activity. The maps explained that HOMO molecular orbitals were located towards the electron rich area for CAL, CA, and CALC, whereas aromatic rings were also found to be critical for HOMO in TSA, CA, and CALC. LUMO orbitals were found to be present around the aromatic ring and linear chain of the compounds. The functional groups of CAL, CA, and CALC were found to be important for both the types of orbitals. The results of DFT were fairly consistent with the molecular docking output that illustrated the important contribution of these scaffolds in the key ligand-receptor interactions.

To assess the synthetic feasibility of all the four compounds (TSA, CA, CAL, and CALC), the synthetic accessibility score was measured using SYLVIA v1.4 program.[48,58] This program provides values between 1 and 10 for compounds, suggesting that the synthesis becomes more complex with increasing values. A number of criteria are considered to calculate the synthetic accessibility that include complexity of the molecular structure, complexity of the ring structure, number of stereo centers, resemblance to commercially available compounds, and potential for using powerful synthetic reactions. The outcome of the SYLVIA program indicated that synthetic accessibility score of TSA was 4.81, whereas for CAL, CA, and CALC, the scores were 2.07, 2.09 and 2.05, respectively. Thus, the SYLVIA score for the CAL, CA, and CALC clearly illustrated that selected compounds were easy to synthesize compared to TSA.

CONCLUSION

We demonstrated HDAC8 inhibitory potential of cinnamon extract and its bioactives. In vitro studies confirmed that ACE significantly inhibited HDAC8 activity compared to the individual bioactives (CAL, CA, and CALC), suggesting that these compounds along with other phenolic compounds might be working synergistically to induce inhibition. Pharmacoinformatics studies revealed that cinnamon bioactives bound to the active site of HDAC8 enzyme in a way similar to that of TSA. The molecular descriptors of cinnamon compounds were successfully correlated with TSA values. The binding interactions and energies were also found to be close to TSA. In case of TSA, His142, His143, and Asp178 amino residues were found to be critical for binding, while His142, His143, Asp178, and Tyr306 were found to be crucial for binding with the cinnamon bioactives. ADMET and DFT calculations were carried out to check the toxicity and reactivity of the tested compounds. All the compounds showed minimum toxicity and values of HOMO and LUMO were comparable to TSA. Finally, synthetic accessibility data indicated that cinnamon bioactives had low synthetic complexity than TSA. Therefore, both experimental and computational studies clearly explained that cinnamon exhibited potential inhibitory activity against HDAC8 enzyme. The data suggested that herbal bioactives could be explored in future for their potential HDAC inhibition to target cancer cells.

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Conflicts of interest

There are no conflicts of interest.

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