Ind. J. Pharm. Edu. Res., 2026; 60(3):1081-1095.
https://www.ijper.org

Original Article

Phytochemical Profiling and Biological Assessment of *Curcuma aromatica* Using UPLC-MS, *in silico*, and *in vitro* Approaches for Acne Treatment

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ABSTRACT

Background: Acne vulgaris is a dermatological condition involving sebaceous gland dysfunction and is characterized by excess sebum secretion, follicular hyperkeratinization, bacterial growth, and inflammation. *Curcuma aromatica* (*C. aromatica*), commonly known as wild turmeric, is a medicinal rhizome rich in bioactive phytochemicals. It is a potential source of anti-acne agents due to its antimicrobial, anti-inflammatory, and antioxidant properties. **Materials and Methods:** Extraction of *C. aromatica* was carried out using successive solvent extraction, and the phytochemical constituents were analyzed through both qualitative and quantitative methods. Untargeted metabolite profiling of the ethyl acetate extract was performed using HRLC-MS/MS. In addition, an *in silico* molecular docking study was conducted against the *P. acnes* hyaluronate lyase enzyme, followed by ADMET evaluation of the identified compounds and assessment of their *in vitro* antioxidant activity. **Results:** HRLC-MS/MS profiling identified 191 positive and 67 negative ionizations of secondary metabolites, predominantly flavonoids, phenols, and terpenoids, in the ethyl acetate extract of *C. aromatica*. The extract exhibited potent antioxidant activity (IC_{50}:168.92 µg/mL), comparable to that of ascorbic acid. Molecular docking studies revealed strong binding affinities of key metabolites sesaminol diglucoside, morusimic acid E, and Floralginsenoside O against hyaluronate lyase enzyme of *P. acnes*, suggesting potential anti-acne activity. **Conclusion:** Our findings highlight the therapeutic potential of *C. aromatica* for the development of novel anti-acne agents with improved safety profiles, and demand future studies focusing on clinical trials.

Keywords: Acne Vulgaris, Antioxidant, *Curcuma aromatica*, HRLC-MS/MS, *In silico*, Propionibacterium acnes.

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Received: 15-12-2025;
Revised: 23-02-2026;
Accepted: 08-04-2026.

INTRODUCTION

Acne Vulgaris (AV) is a common chronic inflammatory skin condition that primarily affects adolescents and young adults and has a higher prevalence among females.[1,2] As of 2024, the global prevalence of acne is 20.5%, with the highest rates observed in individuals aged 16-24.[3] Acne develops mainly during puberty, menstruation, pregnancy, certain medical conditions, and constitutes significant hormonal changes.[4-6] It affects the pilosebaceous unit of hair follicles their accompanying sebaceous glands is characterized by the formation of comedones, papules, pustules, nodules, and, in severe cases, cysts.[7] The clinical condition of AV varies from closed plugged pores to painful pus-filled lumps, depending on sebum deposition in infected areas.[8]

Circulating and cutaneously derived androgens, mainly testosterone and Insulin Growth Hormone (IGH-1), are often considered the primary inciting factors in the development of acne.[9,10] They alter pilosebaceous gland function, leading to hyperseborrhea (excessive sebum secretion) and inducing variations in the sebum fatty acid composition.[11,12] Excess sebum causes abnormal differentiation of follicular epithelial cells, forming tight intracellular adhesions that reduce shedding. This combines with the sebum to form clogged pores, leading to the development of hyperkeratotic plugs or microcomedones that can form closed or whitehead comedones.[13,14] It further expands with the accumulation of oxidized lipids and skin pigment melanin, contributing to an open or blackhead comedone, eventually leading to follicular rupture and the development of inflammatory lesions such as papules, pustules, nodules, and cysts (Figure 1).[15,16] This provides an anaerobic and lipid-rich environment for bacteria

DOI: 10.5530/ijper.20263322

to flourish.[17] Several bacterial species have been implicated in acne pathogenesis, including *Propionibacterium acnes* (*P. acnes*), *Staphylococcus aureus, Staphylococcus pyogenes,* and *Klebsiella pneumoniae.*[18-20] Among these, *P. acnes* is the most prominent and well-characterized owing to its abundance in sebaceous-rich areas and its ability to trigger innate immune responses, modulate sebaceous activity, and contribute to inflammation.[21]

Cutibacterium acnes, formerly known as *P. acnes*, is an anaerobic bacterium that resides in the sebaceous regions of the skin, such as the face, scalp, chest, and back.[22] It produces lipases that facilitate the breakdown of sebum into free Short-Chain Fatty Acids (SCFAs).[23,24] These SCFAs and bacterial components activate pattern recognition receptors, such as Toll-Like Receptors (TLRs), Nucleotide Oligomerization Domain (NOD)-like receptors, and NLRP3 inflammasome, leading to IL-1β production, a key cytokine in acne.[25-27] Hyaluronate Lyase (HYL), produced by *P. acnes*, degrades Hyaluronic Acid (HA) and other Glycosaminoglycans (GAG) present in the extracellular matrix of the epidermis and dermis.[28] Two *P. acnes* hyaluronidase variants, HylA and HylB, show distinct clinical associations with acne and health. HylA is strongly pro-inflammatory and degrades HA into large fragments that trigger the TLR2-dependent inflammatory pathway.[29] This degradation also facilitates bacterial invasion into deeper tissues, and the resulting HA degradation products may serve as nutrients for the bacteria.[30] Furthermore, *P. acnes* promotes a pro-inflammatory adaptive immune response by inducing IL-17 production via activation of the Th17 and Th1 pathways.[31] It also enhances inflammation by triggering the release of antimicrobial peptides and Matrix Metalloproteinases (MMPs), contributing to tissue degradation and sustained immune activity in acne lesions.[32] Oxidative stress, often driven by porphyrins released by *P. acne*, produces Reactive Oxygen Species (ROS), such as hydroxyl, superoxide, and nitrous oxide, further worsening inflammation resulting from the oxidant-antioxidant imbalance.[33-35]

Acne treatment involves various approaches tailored according to the severity and type. Mild-to-moderate acne is treated with topical application of benzoyl peroxide, antibiotics, retinoids, or a combination of these. In contrast, severe acne is treated with oral antibiotics (Tetracycline, Minocycline, Erythromycin), hormonal therapies (estrogen-containing oral contraceptives), and isotretinoin.[36] Physical treatments for acne include comedone extraction, chemical peels, microdermabrasion, laser and light therapies, and injectable fillers.[37-39] Most topical formulations cause irritation, redness, and dryness of the skin, whereas the long-term use of antibiotics is often associated with the development of resistance.[40,41] Although effective, oral antibiotics may cause gastrointestinal disorders and increase the risk of venous thromboembolism.[42] Additionally, there are rising concerns about the potential dangers of carcinogenesis associated with treatments such as chemical peeling and laser therapy.[43,44]

Owing to the side effects and safety concerns associated with current treatment strategies, there is a growing need for safer, more efficient, and affordable acne medications. Herbal compounds have emerged as promising alternatives, offering a relatively safer profile and greater patient compliance. Their therapeutic potential primarily stems from their antibacterial, anti-inflammatory, and anti-erythema activities. Additionally, certain herbal compounds also possess antioxidant and anti-androgenic activities, further enhancing their effectiveness in acne management.[45,46] *C. aromatica* (wild turmeric) rhizomes are the gold standard in traditional medicine and have been prioritized for their potent therapeutic properties and extensive historical use. They are rich in flavonoids, triterpenoids, and polyphenols, which have been proven to be antioxidant, antimicrobial, and anti-inflammatory for skin infections. They have been used since ancient times as a remedy for skin infections, depigmentation, burns, and scars have been widely incorporated into face packs, soaps, etc.[47,48] Our study aimed to extract active constituents from *C. aromatica* using the percolation technique, identify the constituents responsible for their anti-acne activity using High Resolution Liquid Chromatography-TademMass spectrometry (HRLC-MS/MS), and evaluate their activity through *in silico* docking studies.

MATERIALS AND METHODS

Extraction of *C. aromatica*

Shade-dried *C. aromatica* rhizome powder was packed into the column of a Soxhlet apparatus. Extraction was performed using various solvents (petroleum ether, chloroform, ethyl acetate, methanol, and ethanol) to optimize the process. Subsequently, the extracts were dried using a rotary evaporator, maintaining a suitable condition of 40-50°C, 50 rpm, and vacuuming at 20 mmHg.[49] The resultant dried extract was stored in a closed airtight container and used for further studies. The extract was subjected to a comprehensive preliminary phytochemical analysis for the qualitative analysis of the phytoconstituents present.

Quantitative analysis

Determination of total phenolic content

Briefly, 100 mg of gallic acid (reference drug) and *C. aromatica* rhizome ethyl acetate extract were dissolved in 10 mL of methanol to obtain a stock solution of 10 mg/mL, which was then diluted with methanol to obtain various concentrations ranging from 10 to 100 µg/mL. Folin-Ciocalteu reagent (1.5) was added to the above dilutions and incubated in the dark for 5 min. After incubation, 4 mL of Na_2CO_3 solution (20% w/v) was added to a final volume of 25 mL using distilled water. The resultant mixture was set aside for 30 min until it stabilized at room temperature.[50] The absorbance of the standard, blank, and test solutions was measured at 765 nm using a UV-visible spectrophotometer (Shimadzu UV, 1900). The study was performed in triplicates to minimize errors.

Determination of total flavonoid content

The total flavonoid content was determined using spectrophotometry. Quercetin was used as a reference standard. A stock solution (10 mg/mL) of *C. aromatica* rhizome ethyl acetate extract was prepared in methanol and subsequently diluted to obtain concentrations ranging from to 10-100 µg/mL. To each sample, 100 µL of a 5% sodium nitrate solution was added and allowed to stand for 6 min. This was followed by the addition of 150 µL of a 10% $AlCl_3$ solution and 200 µL of 1M NaOH.[50] After a 5-min incubation period, the absorbance of the reaction mixture was measured at 510 nm using a UV-visible spectrophotometer (Shimadzu UV 1900).

Fourier Transform Infrared (FT-IR) Analysis

Fourier Transform Infrared (FT-IR) spectroscopy was performed to analyze the emission spectra of the extract. The *C. aromatica* rhizome ethyl acetate extract was stored at 25 ± 2°C and 60 ± 5% relative humidity for seven days prior to analysis. FT-IR spectra were recorded using a Bruker Alpha-one spectrophotometer (Bruker Optik, Germany) in the wavenumber range of 4000-400 cm^{-1}. The resulting spectra were analyzed for significant spectral features and compared to identify the characteristic functional groups in the sample.

HR Liquid Chromatography Mass Spectrometry (HRLC-MS/MS)

HRLC-MS/MS of the ethyl acetate extract of *C. aromatica* was performed at the Central Facility for Research at NITK Surathkal HRLC-MS/MS, using a XevoQToF instrument from Waters, USA. The compatibility of the extracts was assessed through filtration and dilution. The extract was prepared, and the sample was loaded automatically and injected (0.1 µL) into the LC system using an autosampler. The metabolite was separated from the extract by passing through a C18 chromatographic column at an average system pressure of 6391 psi, average sample temperature of 22.80°C, and average column temperature of 22.90°C. The metabolite separation was based on the difference and affinity towards the mobile phase (Solvent A: water +0.1% formic acid and Solvent B: Acetonitrile +0.1 % formic acid) and the stationary phase (C18 column). The metabolites were eluted from the column at different times (0.17, 2.26, 2.61, 2.95, and 10 min) at a flow rate of 0.40 µL/min, depending on their interactions with the stationary and mobile phases (Solvent A 70% and Solvent B: 30%). The metabolite profile was recorded as a chromatogram, which typically showed peaks corresponding to individual analytes. The eluted metabolite profile from the LC column enters the MS, where positive and negative ionization techniques, including Electrospray Ionization (ESI) and Atmospheric-Pressure Chemical Ionization (APCI), are used. The ions were then separated based on their mass-to-charge ratio (m/z) within the mass analyzer, which includes quadrupoles, Time-of-Flight (TOF), ion traps, and orbitraps. The separated ions were detected using a detector with an electron-multiplier. The resulting data were recorded as a mass spectrum, which shows the intensity of the ions at different m/z values. The raw data from the HRLC-MS/MS analysis were processed using specialized software to extract relevant information such as peak intensities, RT, and m/z. The processed data were compared with reference databases or standards to identify and quantify the analytes present in the sample by matching retention times, mass spectra, and other characteristic properties. Thorough tandem HR LC-MS/MS was performed in both positive and negative ionization modes to qualitatively investigate the metabolites present in the ethyl acetate extract of *C. aromatica* rhizomes. The identification was further validated by analyzing the fragmentation patterns of detected metabolites. An untargeted metabolomic approach using tandem Mass Spectrometry (MS/MS) was employed to identify the signature metabolites in *C. aromatica* extract. Data were acquired in both positive and negative modes and total ion chromatograms. MZmine was used for data alignment from the technical replicates of their respective polarities. The precursor m/z, retention time, and fragment mass ratios were obtained. The identified and quantified metabolites were interpreted and reported using the UNIFI software.

In silico study of the extracts

Protein Preparation

Previous studies have shown that selective inhibition of HylA alleviates acne-associated inflammation, highlighting it as a promising therapeutic target for acne treatment. Therefore, the X-ray crystal structure of hyaluronate lyase A from *P. acnes* was obtained from the RCSB Protein Data Bank (PDB ID: 8FYG).[51] The Protein was prepared using the Protein Preparation Wizard in Schrodinger Maestro 2024. During preprocessing, all nonessential water molecules and heteroatoms were removed, and the structure was subjected to energy minimization and optimization using the default parameters. Because this structure lacks a co-crystallized inhibitor, the binding site was inferred by superimposing the crystal structure of *Streptococcus pneumoniae* hyaluronate lyase (PDB ID: 1F9G) co-crystallized with ascorbic acid. The resulting optimized structure was saved in PDB format, and the aligned inhibitor-binding region was used as a reference for grid generation in subsequent docking studies.

Ligand preparation

The bioactive compounds of *C. aromatica* obtained by HRLC-MS/MS were selected for further study. Their 3-Dimensional (3D) structures in SDF format were downloaded, and ligand preparation was carried out using the LigPrep module in Schrödinger Maestro (version 14.1.138). During the preparation process, the OPLS4 force field was employed for energy minimization at physiological pH (7.0 ± 2.0).

Molecular Docking

Docking of the ligands and the protein was performed using Schrodinger Maestro. A receptor grid was generated using the Receptor Grid Generation tool in Maestro. The grid box was centered on the inferred binding site of hyaluronate lyase A from *P. acnes*. The van der Waals scaling factor for the receptor was maintained at the default value of 1.0. Docking studies were conducted using the Glide module of Maestro. Extra-precision (XP) docking was applied to obtain accurate binding poses and interaction profiles. Ligands were docked flexibly, allowing internal conformational adjustments to better fit the active site during the docking process.

ADMET analyses

The top three phytochemicals from *C. aromatica* with the highest XP Glide docking scores were selected to test their drug-likeness using ADMETlab 3.0.[52] The SMILES structures were obtained from PubChem database.[53] The key parameters, including Molecular Weight (MW), Topological Polar Surface Area (TPSA), and Hydrogen Bonding features (nHD, nHA), lipophilicity (logP), aqueous solubility (logS), and molecular flexibility (nRot) were analysed to assess drug-likeness.

Assessment of antioxidant activity by DPPH assay

Accurately weighed 100 mg of *C. aromatica* rhizome ethyl acetate extract and ascorbic acid was dissolved in 10 mL of methanol to obtain the stock solution of 10 mg/mL concentration and diluted using methanol to obtain various concentrations ranging from 20-100 μg/mL. To the above solution, 2 mL of methanolic DPPH solution (0.0024% w/v, 24 mg of DPPH dissolved in 100 mL of methanol to make the stock solution) was added. The solutions were stabilized by placing them in the dark for 30 min at room temperature.[54-56] Absorbance was measured at 517 nm using a UV-visible spectrophotometer (Shimadzu UV 1900).

% of antioxidant activity= [(Ac-As) /Ac] × 100

where Ac is the absorbance of the control sample, and As is the absorbance of the test sample.

RESULTS

Extraction of Yield

C. aromatica rhizome was powdered and percolated using a Soxhlet extractor with various solvents (based on polarity). The percentage yield of each extract was calculated. The ethyl acetate solvent had the highest yield (20.24 % w/v) compared to the other solvents.

Qualitative phytochemical test

Crude extracts of *C. aromatica* were obtained using various solvents, such as methanol, ethanol, and ethyl acetate. Phytochemical analyses revealed the presence of alkaloids, phenols, tannins, flavonoids, fixed oils, and lipids. Notably, the ethyl acetate extract contained triterpenoids, flavonoids, polyphenols, and alkaloids, whereas the extracts obtained from methanol and ethanol did not. The results are presented in Table 1.

Total Flavonoid and Phenol content

The total flavonoid and phenol contents of *C. aromatica* were calculated and found to be 5.25±1.4 mg QUE/g and 6.31±0.04 mg GA/g, respectively. The solvent-dependent differences in yield and composition, but the evidence here, support the

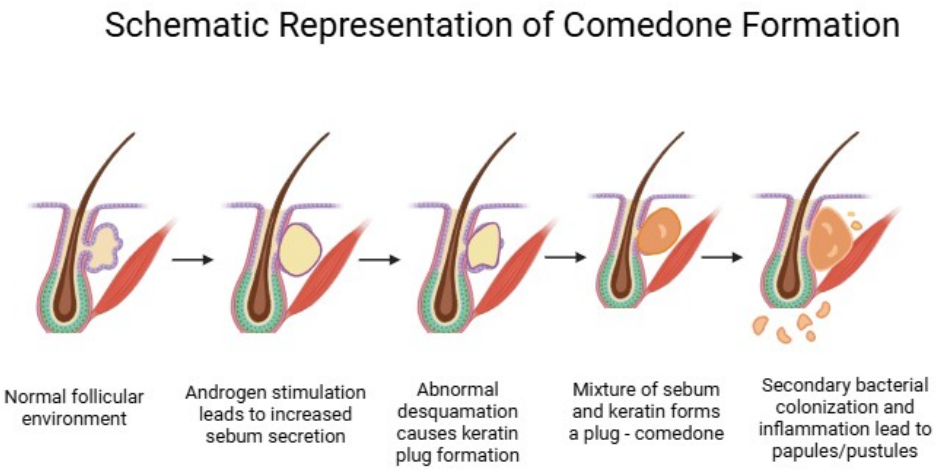

Figure 1: Schematic representation of comedone formation.

applicability of ethyl acetate as a selective extraction agent. The phytochemical profile of the ethyl acetate extract justified its use in HRLC-MS/MS and biological activity studies. These findings lead to future investigation of the therapeutic uses of *C. aromatica* rhizome ethyl acetate, especially for dermatological preparations.

FTIR spectral analysis of the ethyl acetate of *C. aromatica*

FTIR spectral analysis of ethyl acetate of *C. aromatica* rhizome extract revealed the presence of multiple functional groups, suggesting the presence of a range of phytocompounds, including alkaloids, flavonoids, aldehydes, and polyphenols. The peaks and their respective functional groups shown in Figure 2 and Table 2 provide significant insights into the potential bioactivity of the extract. A total of ten distinct peaks were formed at 3789.47 cm^{-1}, 3699.29 cm^{-1}, 3661.43 cm^{-1}, 2922.10 cm^{-1}, 1725.08 cm^{-1}, 1585.71 cm^{-1}, 1513.77 cm^{-1}, 1446.68 cm^{-1}, 1032.38 cm^{-1} and 813.65 cm^{-1} each corresponding to specific functional groups.

HR LC-MS/MS metabolic profiling of *C. aromatica* rhizome ethyl acetate extract

The major classes of identified compounds were triterpenoids, phenolic acids, flavonoids, and alkaloids. They were tentatively identified by comparing their exact masses with the reported values in established databases, such as UNIFI, as reported in the literature. A total of 191 and 67 metabolites were identified in the positive and negative ionization modes, respectively (Figures 3 (a) and (b), Supplementary Material 1). The profiling revealed a wide range of bioactive compounds, including Kansuiphorin A (Figure 4a), Octadecanamide (Figure 4b), and Rubixanthin (Figure 4c), along with others such as Polyporusterine A, Dehydropachymic acid, Ellipticine, Isosilchristin, 5-dehydeokarounidiol, Cauloside A, Oroxidin, Cimiside E, and Cinobufagin (Table 3).

Table 1: **Phytochemical analysis of the *C. aromatic*.**

Sl. No.	Phytocompound	Petroleum ether	Chloroform	Ethyl Acetate	Methanol	Ethanol
1	Alkaloids	-	-	+++	+	+
2	Flavonoids	-	-	+++	+	+
3	Steroids and triterpenoid	-	-	+++	-	-
4	Glycosides	-	-	+++	-	-
5	Carbohydrate	-	-	-	-	-
6	Phenol	-	-	+++	-	-
7	Saponin	-	-	-	-	-
8	Tannins	-	-	+++	+	+

The symbol indicates the presence (+) or absence (-) of phytoconstituents.

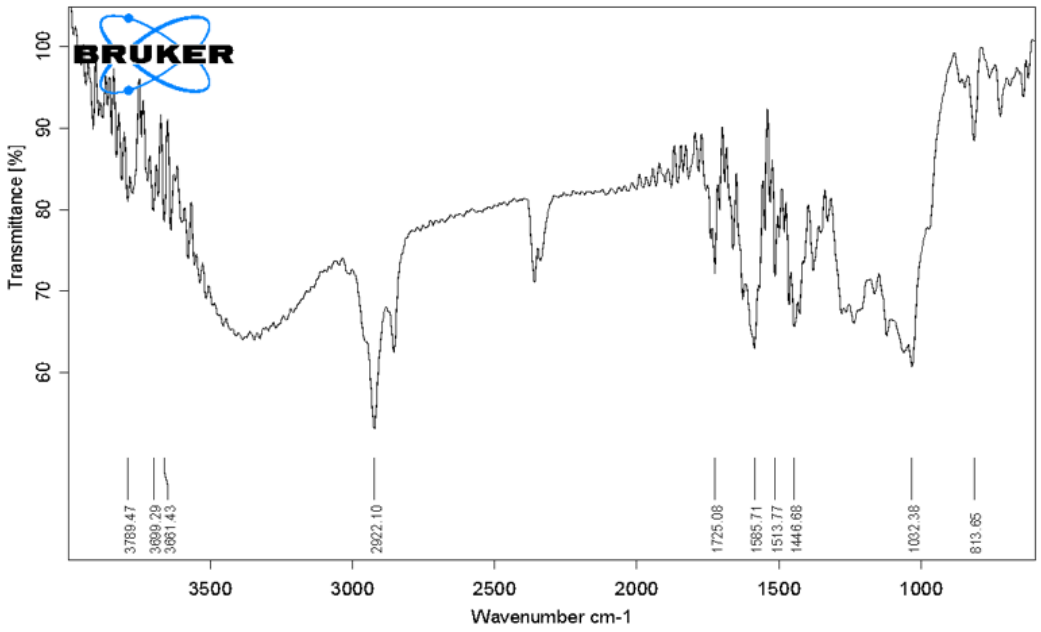

Figure 2: FT-IR peaks representing potential bands corresponding to various functional groups in the ethyl acetate extract of *C. aromatica* rhizome.

Table 2: Results of potential bands, compounds functional groups, and possible compounds identified in the ethyl acetate extract by using FT-IR.

Band Range (literature) (cm^{-1})	Band Range (Experimental)	Band no.	Band interaction	Band assignments	Possible compounds
1000-650	813.65	1	Bend	C=C	Alkene
1400-1000	1032.38	2	Bend	C-N/F	Amine and fluoro Compound
1600-1400	1446.68	3	Stretch	C-H	Alkane
	1513.77		Stretch	C=O	Conjugated acid
	1585.71		Bend	C=O	Ester
2000-1650	1725.08	4	Stretch	C=0	Aldehyde
4000-2500	2922.10	5	Bend	C-H	Alkane
	3661.43		Stretch	N-H	Amine
	3699.29		Bend	0-H	Alcohol
	3789.47		Bend	C-H	Carboxylic acid

The FTIR spectra, potential bands, corresponding functional groups, and possible compounds of *C. aromatica* rhizome ethyl acetate extract are listed above.

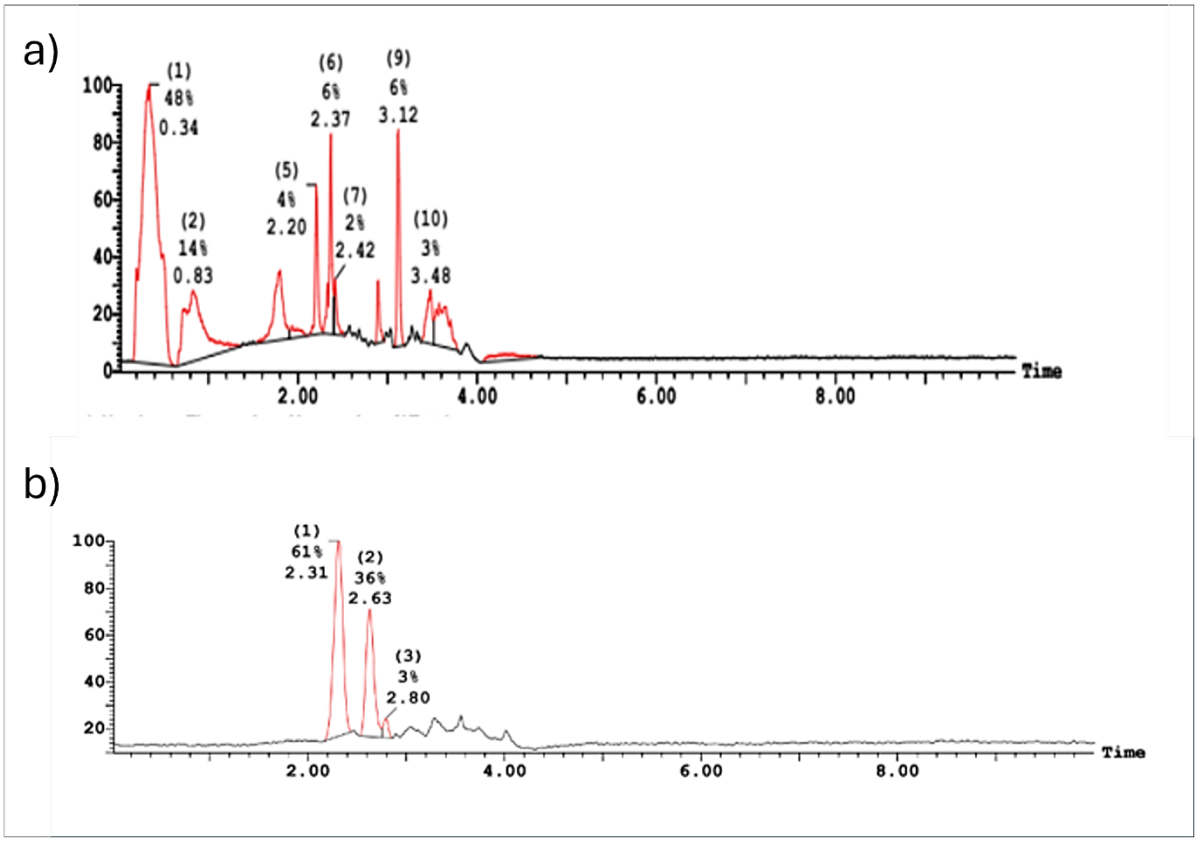

Figure 3: HR LC MS/MS profile of identified phytoconstituents in the (a) positive and (b) negative ionisation mode of the ethyl acetate extract from *C. aromatica* rhizome.

Molecular docking studies

A total of 213 compounds obtained from the HRLC-MS/MS analysis of *C. aromatica*, along with ascorbic acid, were docked against the target protein. Among the screened compounds, sesaminol diglucoside exhibited a strong binding affinity, with a docking score of -10.019, followed by morusimic acid E (-9.696) and Floralginseonoside O (-9.069). Notably, these scores were higher than those of the control (ascorbic acid). A list of compounds and their docking scores is provided in Supplementary Material 2. The top five compounds with a dock score higher than ascorbic acid are listed in Table 4.

Protein-ligand interaction

The protein-ligand binding poses of the top-ranking compounds in both two dimensions (Figure 5) and three dimensions (Figure 6), along with the control ascorbic acid, were generated using the target protein 8FYG. The three-dimensional docking poses

of the bound ligands revealed the optimal orientation of each ligand within the binding site of the enzyme. The 2D interaction diagram of Sesaminol diglucoside shows extensive interaction between binding site residues and ligands, suggesting its potential inhibitory role against hyaluronate lyase A.

ADMET evaluation of the phytochemicals

Among the top three phytochemicals, floralginsenoside O was excluded from further analysis because its high MW exceeded the acceptable range for drug-like compounds, which could limit its permeability and bioavailability. The ADMET results are shown in Figure 7 and Table 5. Compared to the control, ascorbic acid, which exhibited high polarity and low lipophilicity, resulting in limited skin permeation and minimal absorption, sesaminol diglucoside showed a higher MW and stronger polarity with multiple hydrogen-bonding groups and low-to-moderate lipophilicity, indicating similarly low systemic absorption and low toxicity. In contrast, morusimic acid E displayed more balanced properties, including moderate MW, TPSA, and hydrogen-bond counts, along with an optimal logP to logS ratio, suggesting better dermal penetration while maintaining minimal toxicity relative to the control.

Antioxidant potential of *C. aromatica*

The 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assay demonstrated the antioxidant potential of the ethyl acetate extract of the *C. aromatica* rhizomes. The ethyl acetate extract of the *C. aromatica* rhizome exhibited a lower IC_{50} value (168.92 µg/mL) than ascorbic acid (217.18 µg/mL) in the DPPH assay, as shown in Figure 8. This finding suggests that the extract possesses antioxidant activity comparable to or potentially higher than that of the reference compound.

DISCUSSION

Acne is a skin inflammatory disorder, and its treatment varies according to severity. The prescribed drugs include topical antibodies, oral antibodies, hormonal therapy, and laser therapy. The present study focused on identifying alternative herbal compounds for acne treatment with anti-inflammatory and antioxidant properties. *C. aromatica* rhizome has been traditionally used for various skin infections and is mentioned in various studies. The high extraction efficiency of ethyl acetate indicates that the target compounds in *C. aromatica* are more soluble in moderately polar solvents.[57] The phytoconstituents and extraction yields of *Curcuma longa* depend on the solvent and occurrence and can be similar for *C. aromatica* as well. Although the effects of solvents on extraction yield and content have been debated by various authors, the unique fact that the ethyl acetate extract contains triterpenoids and their possible synergistic action with other bioactive compounds against acne makes the subject worthy of further investigation.[58-63] The extraction yield and phytochemical composition of *C. aromatica* rhizomes primarily depend on the polarity of the solvent used for extraction. Phytochemical screening of important secondary metabolites, such as alkaloids, phenols, tannins, flavonoids, and lipids, was confirmed in all extracts. The ethyl acetate extract contained triterpenoids, which were absent in the methanol and ethanol extracts. This shows that ethyl acetate is particularly efficient for the isolation of bioactive triterpenoid compounds from *C. aromatica.*[64] The concurrent presence of flavonoids, polyphenols, and alkaloids in the extract further adds to its pharmacological significance. These blends of phytoconstituents could exhibit synergistic action, leading to a more active biological performance, particularly in diseases such as acne,

Table 3: Secondary metabolites identified in positive ionization mode in ethyl acetate extract using HRLC-MS/MS.

Sl. No.	Compound identified	Observed neutral mass (Da)	Observed RT (min)	Observed m/z	Mass error (mDa)	Mass error (ppm)	Detector counts	Response	Adducts
1	Kansuiphorin A	882.6610	4.97	921.6242	2.5	2.7	510420	355119	+K
2	Octadecanamide	283.2879	4.51	283.2874	0.4	1.5	320746	291973	-e
3	Rubixanthin	552.4348	4.13	591.3979	1.7	2.8	204538	178740	+K
4	Polyporusterone A	478.3291	4.45	479.3364	-0.3	-0.7	180878	180878	+H
5	Dehydropachymic acid	526.3684	4.67	527.3757	2.6	4.9	120087	103716	+H
6	Ellipticine	246.1164	3.31	269.1057	0.7	2.7	46306	42249	+Na
7	Isosilychristin	482.1223	4.59	482.1217	1.0	2.1	34578	34578	-e
8	5-Dehydrokarounidiol	438.3485	4.98	461.3377	-1.3	-2.9	33330	29427	+Na
9	Cauloside A	604.3976	4.51	643.3607	0.1	0.1	23178	15467	+K
10	Oroxindin	460.1011	4.68	460.1005	0.5	1.1	22570	22570	-e
11	Cimiside E	602.3827	4.40	641.3458	0.8	1.2	21547	14802	+K
12	Cinobufagin	442.2336	2.43	465.2228	-1.9	-4.1	20795	19884	+Na

RT-retention time; MW-molecular weight; (m/z)-mass divided by charge number.

Table 4: Dockscore of phytochemicals from *C. aromatica* against Hyaluronate Lyase A.

Compound	XP GScore (Kcal/mol)	H-bond forming residues	Hydrophobic residues
Ascorbate (control)	-3.882	ASP 229, ASN 226	PHE 285, VAL 288, ALA 225, MET 228, TRP 162
Sesaminol diglucoside	-10.019	SER 284, ASP 229, ARG 343, TRP 162,	PHE 285, VAL 288, TRP 162, TRP 161, ALA 225, TRP 507
Morusimic acid E	-9.696	ASN 226, GLN 275, ASP 229, TRP 162, ARG 113	VAL 288, PHE 285, ALA 225, TRP 162, TRP 161
Floralginsenoside O	-9.069	TRP 162, ASP 229, ARG 113, ARG 170, ARG 635, GLU 453,	PHE 285, VAL 288, TRP 161, TRP 162, ALA 225, ALA 634

The above table tabulates the extra precision Glide Score (XP GScore, kcal/mol) for the ascorbic acid and phytochemicals with corresponding amino acid residues involved in both hydrogen bonding and hydrophobic interactions within the active site of the enzyme. Higher score for XP GScore results in stronger predicted binding affinity.

Table 5: ADMET parameters of the top-scoring compounds.

Properties	Ascorbic acid	Sesaminol diglucoside	Morusimic acid E
MW	176.0	694.21	507.3
TPSA	107.22	233.91	189.17
nHD	4	7	8
nHA	6	17	11
logP	-1.706606622	-0.8471731	0.081113707
logS	0.231997079	-2.880642967	-2.437011844
nRot	2	8	16

* MW- Molecular Weight; TPSA-Topological Polar Surface Area; nHD-Number of Hydrogen Bond Donors; nHA-Number of Hydrogen Bond Acceptors; LogP-Partition Coefficient; LogS-Logarithm of Solubility; nRot- Number of Rotatable Bonds

where anti-inflammatory, antimicrobial, and sebum-control activities are desirable.[65]

The FTIR peak of *C. aromatica* rhizome extract was at 3789.47 cm^{-1} corresponds to the O-H stretching vibrations typically associated with alcohols and phenolic compounds.[66-68] Peaks at 3699.29 cm^{-1} and 3661.43 cm^{-1} are likely due to the N-H stretching vibrations of amines, suggesting the presence of alkaloids. A peak at 1725.08 cm^{-1} indicates C=O stretching of aldehydes. Peaks at 1585.71 cm^{-1}, 1513.77 cm^{-1}, and 1446.68 cm^{-1} fall within the region characteristic of C=C stretching in aromatic rings, consistent with flavonoids and polyphenols. The peaks at 1032.38 cm^{-1} and 813.65 cm^{-1} represent amine and fluoro compounds and alkene. The OH group plays a crucial role in antibacterial activity. This peak indicates the occurrence of aldehydes at 1725.08 cm^{-1}. The presence of these functional groups is consistent with the reported antibacterial and antioxidant activities of the ethyl acetate *C. aromatica* rhizome extract. Phenolic compounds and flavonoids are antioxidants that may exert antibacterial activity through various mechanisms. The hydroxyl groups within these compounds, as evidenced by the O-H stretching vibration at 3789.47 cm^{-1}, are critical to these activities through hydrogen donation and free radical scavenging.[69] FTIR was used to identify menthol obtained from *Mentha longifolia*, illustrating the application of FTIR in identifying antimicrobial compounds in plant extracts. Although FTIR readings are informative regarding the functional groups present, more studies are required to determine the actual compounds responsible for the reported bioactivity. HRLC-MS/MS, as utilized in,[70] can be used to detect the individual constituents of an extract. In addition, highlighting certain terpenes and terpenoids in the extract and examining their mechanisms of action can further elucidate the reported range of antibacterial activity.[70] A finer analysis will further consolidate the evidence for the application of ethyl acetate *C. aromatica* rhizome for its therapeutic uses. These include saponins, mono-, di-, and tri-terpenoids, alkaloids, flavonoids, and phenolic compounds, which are characterized by antimicrobial, anti-inflammatory, and antioxidant activities that promote healthy skin. Polyporusterone and Kansuiphorin A are the diterpenoids with immunomodulatory and anti-inflammatory properties that can modulate the excessive immune response of *P. acnes*-induced inflammation.[71,72]

Octadecanamide, a fatty acid amide, has been reported to exert anti-inflammatory and antimicrobial activity, possibly through the modulation of lipid metabolism, which plays a very important role in sebum production and the development of acne.[73-76.] Rubixanthin, a carotenoid xanthophyll, has been shown to possess antioxidant activity and to neutralize the Reactive Oxygen Species (ROS) produced during the inflammatory reaction in acne lesions.[77-80] Dehydropachymic acid, a triterpenoid of the lanostane-type, and Cauloside A, a steroidal saponin, are involved in the lysis of bacterial membranes and the inhibition of inflammatory cytokines like IL-1β and TNF-α, demonstrating

their anti-inflammatory and antibacterial activity. Ellipticine, a carbazole alkaloid, possesses highly active antimicrobial and cytostatic effects and is a prime candidate for the topical treatment of acne, in which microbial growth needs to be controlled.[67]

Isosilychristin, a flavonolignan, possesses free radical scavenging and anti-inflammatory effects that can be used to decrease oxidative stress and inflammation in acne lesions.[81-84] 5-Dehydrokarounidiol, an oxygenated triterpenoid, has revealed cytotoxic and anti-inflammatory activities, further validating the therapeutic potential of the extract.[85] Oroxindin, a flavone glycoside compound, possess prominent antibacterial and wound-healing activity, relevant to infection prevention as well as the promotion of healing in acne lesions.[86,87] Cimiside E, another saponin derivative compound, contributes dermato-protective activity, with cinobufagin, a bufadienolide steroid, also possessing significant antimicrobial and anti-proliferative activities.[88-90]

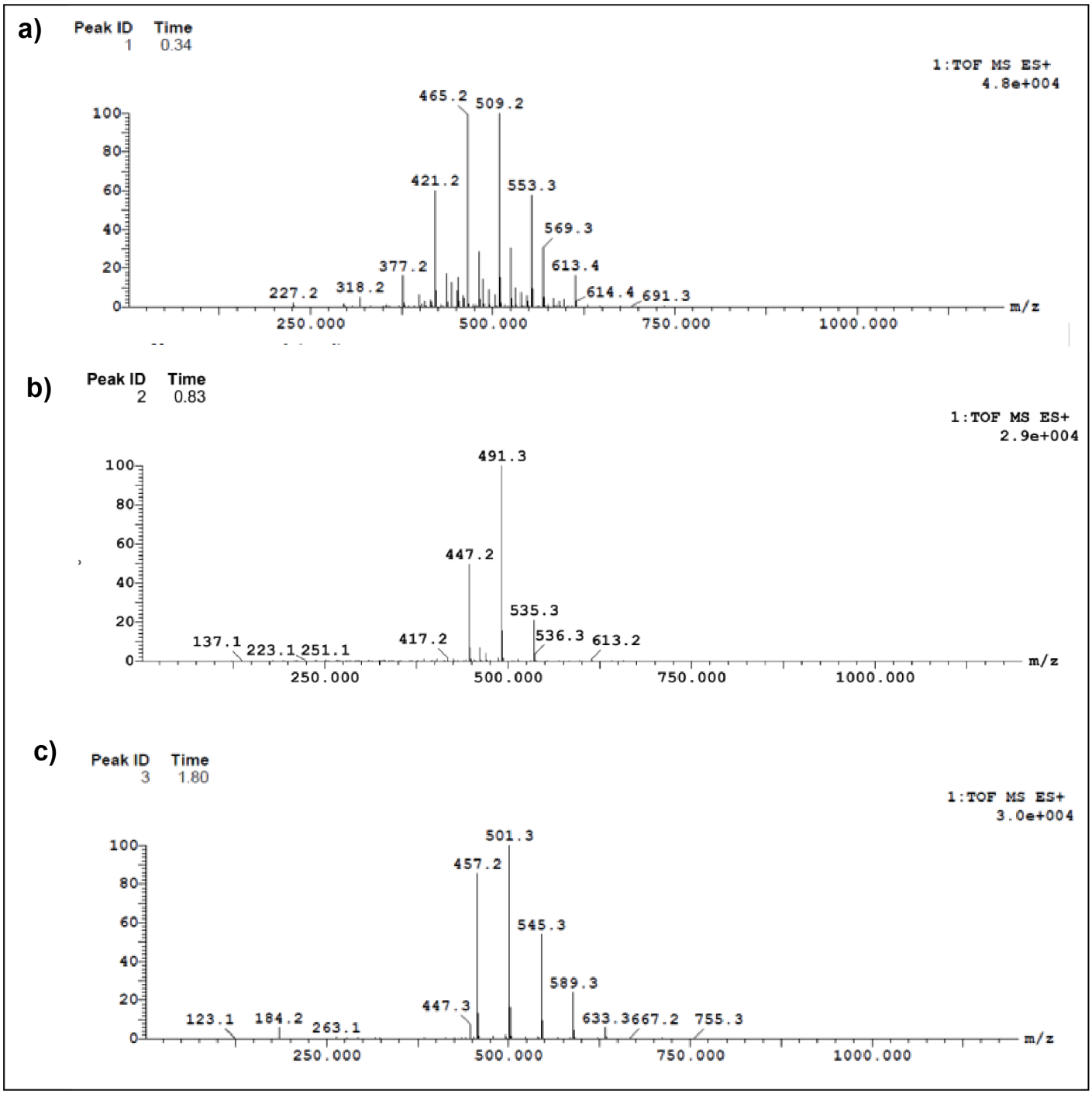

Figure 4: HR LC MS/MS based chromatograms of identified phytoconstituents in the ethyl acetate extract of *C. aromatica* in positive ionization mode. (a) Kansuiphorin A; (b) Octadecanamide and (c) Rubixanthin.

The docking study with 213 metabolites identified by HRLC-MS/MS from *C. aromatica* along with control against 8FYG demonstrated a promising binding affinity towards *P. acne* hyaluronate lyase. Among the identified metabolites, sesaminol diglucoside, morusimic acid E, and floralginseonoside O were identified as potential hyaluronate lyase A inhibitors. Notably, sesaminol diglucoside had the highest docking score of -10.019, indicating a strong binding affinity for the target enzyme. The ADMET results indicated that sesaminol diglucoside and morusimic acid E possess favorable profiles for topical applications against acne. This is supported by previous studies reporting the antioxidant and hyaluronate lyase inhibitory properties of sesaminol diglucoside, which contribute to maintaining the integrity of the extracellular matrix and preventing tissue degradation.[91] This aligns with the high binding affinity observed in our docking study against *P. acnes* hyaluronate lyase, thereby supporting its therapeutic potential in treating skin-related disorders, including acne. The strong binding affinity suggests stable complex formation with the protein 8FYG and the bioactive compounds present in the extract might be capable of targeting the protein more effectively. The strong binding energy and high dock score can be attributed to the structural complexity,

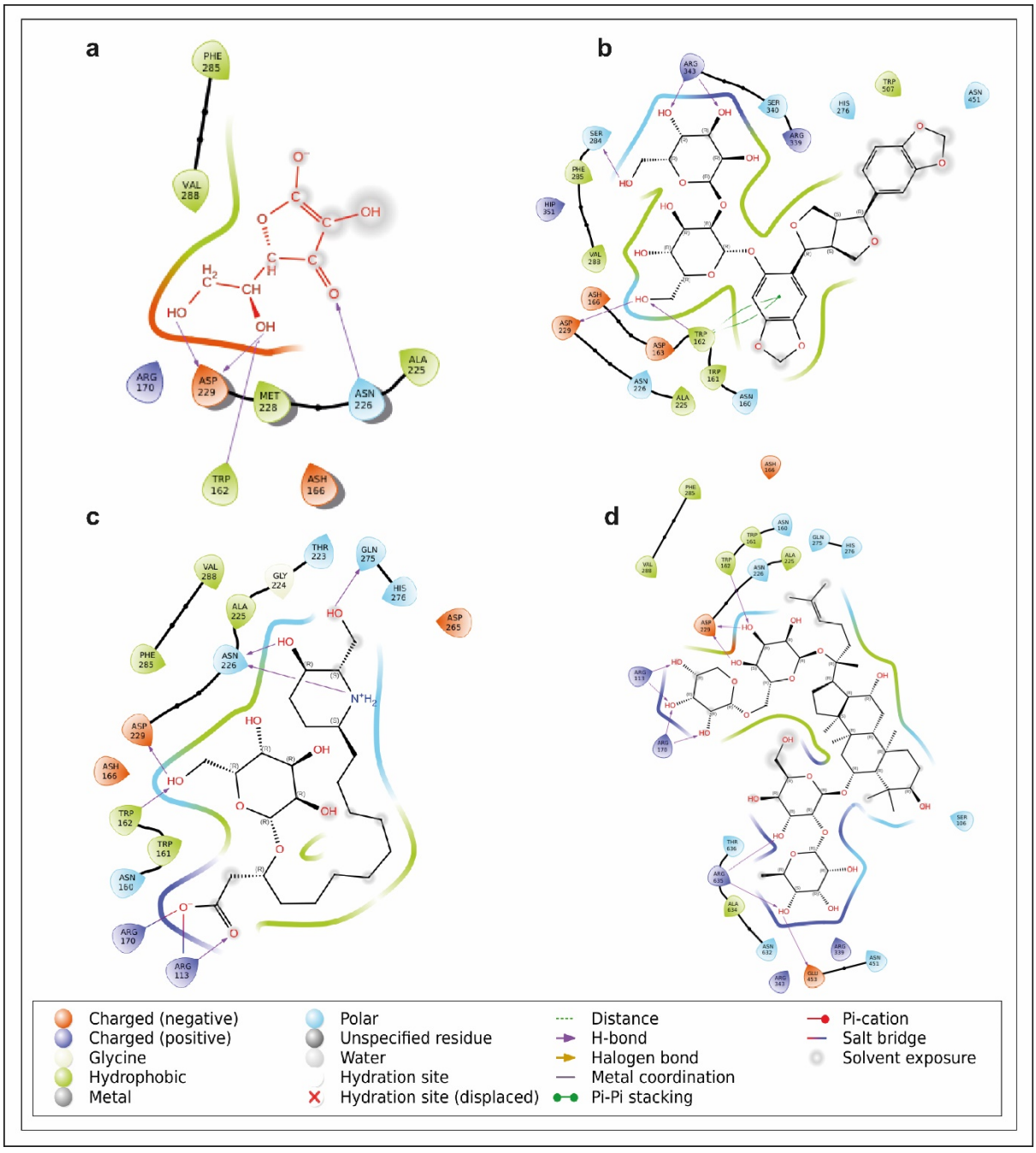

Figure 5: 2D diagram showing the likely interaction of *P. acnes* hyaluronate lyase A, with (a) ascorbic acid, (b) Sesaminol diglucoside, (c) Morusimic acid E, (d) Floralginsenoside O.

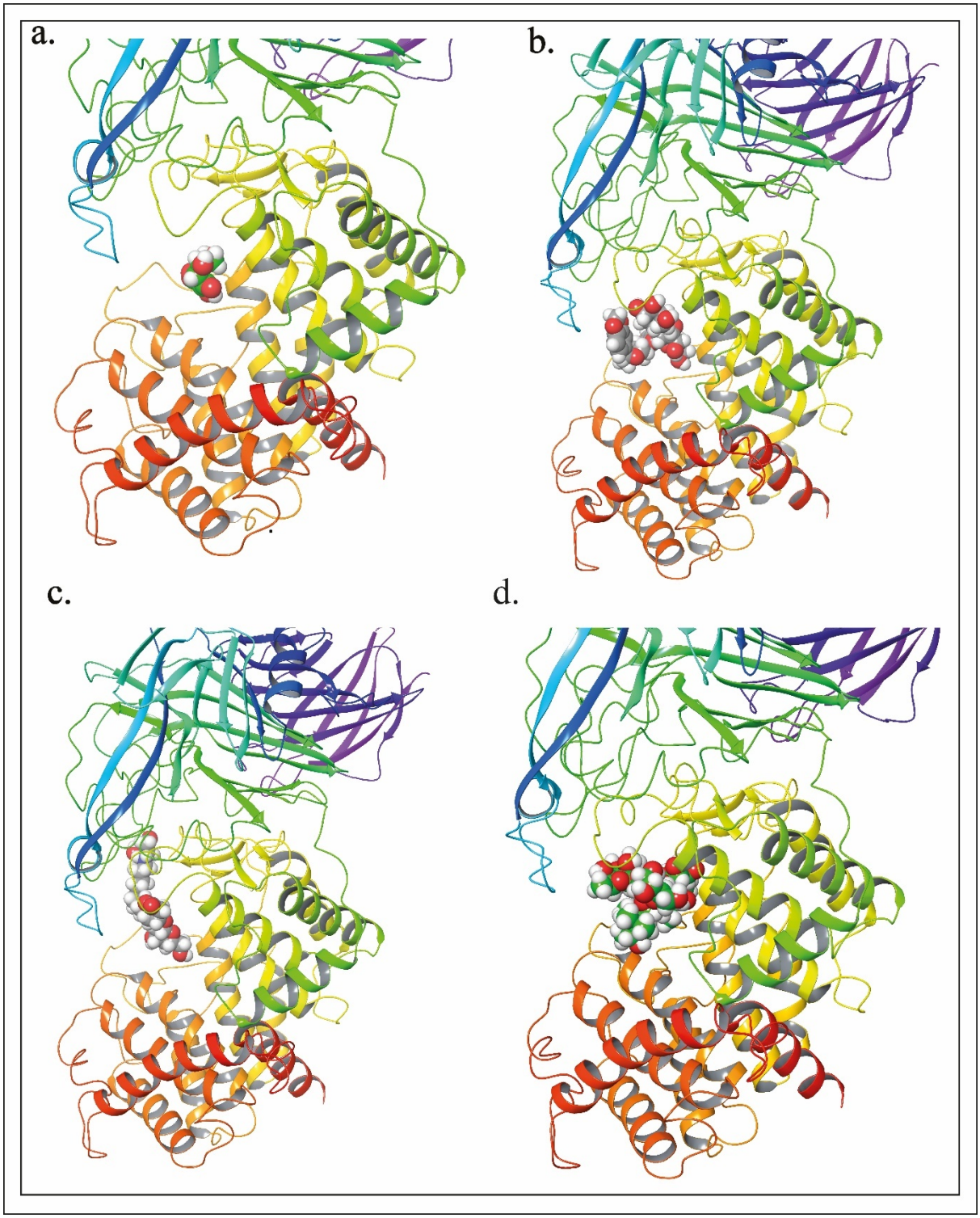

Figure 6: 3D diagram showing the likely interaction of *P. acnes* hyaluronate lyase A, with (a) Ascorbic acid, (b) Sesaminol diglucoside, (c) Morusimic acid E, (d) Floralginsenoside O.

the presence of functional groups capable of forming stable interactions, and enzyme binding against the target. Inhibition of hyaluronate lyase holds significant therapeutic potential, as it contributes to the degradation of the extracellular matrix, causes inflammatory diseases, and causes tissue injury. By inhibiting the function of this enzyme, such inhibitors are likely to prevent the depletion of hyaluronic acid from tissues, thus minimizing inflammation and ensuring tissue integrity. Although molecular docking cannot confirm biological activity, the high-affinity interactions observed in this study provide a strong rationale for further *in vitro* and *in vivo* validation. These findings not only reinforce the pharmacological interest of *C. aromatica* but also provide opportunities for the discovery of new anti-inflammatory or anti-virulence agents from natural sources. Antioxidant studies

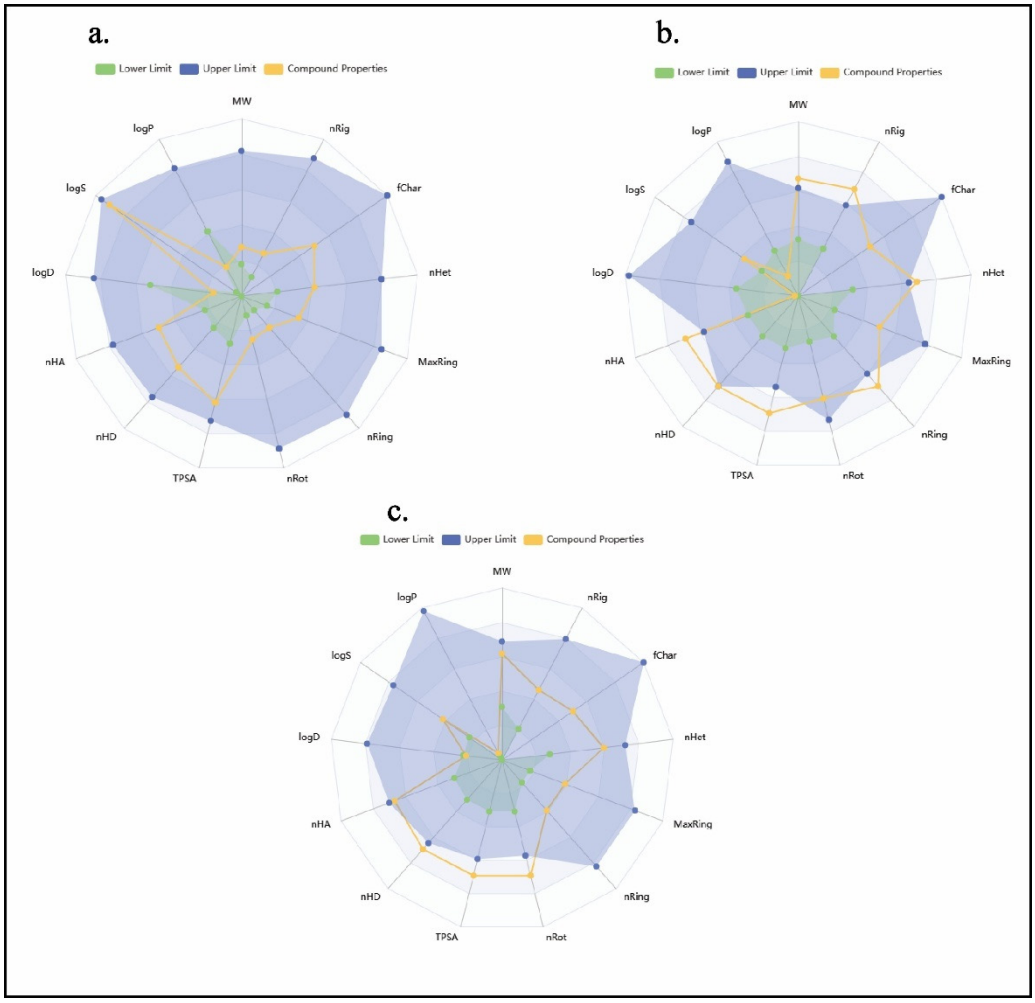

Figure 7: Radar plots representing the ADMET properties of (a) Ascorbic acid, (b) Sesaminol diglucoside, (c) Morusimic acid E.

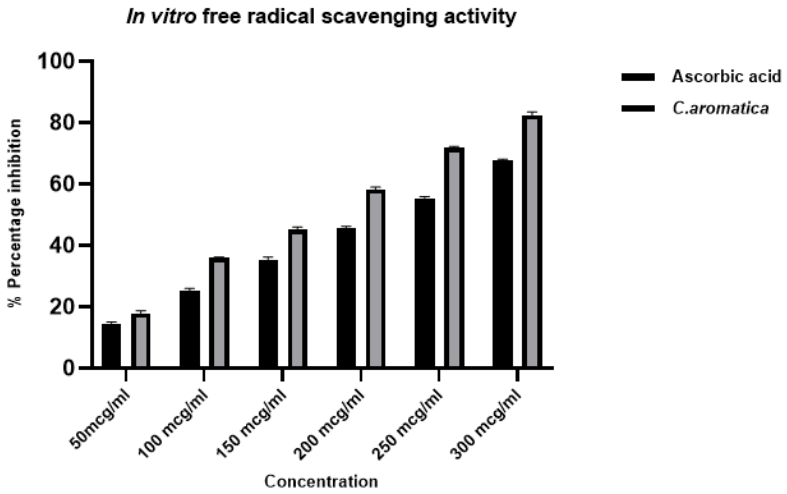

Figure 8: *In vitro* free radical scavenging activity of *C. aromatica* ethyl acetate extract and ascorbic acid. The absorbance was measured at 517 nm using a UV spectrophotometer (Shimadzu UV 1900). Data are presented as mean ± standard deviation of triplicate experiments (n=3).

using DPPH scavenging activity and corresponding IC_{50} values exhibited variability due to the diverse plant sources, extraction methodologies, and solvents employed.[91-93] The antioxidant activity of the *C. aromatica* extract may have potential therapeutic implications for acne management by mitigating oxidative stress, a known contributor to acne pathogenesis. These findings are particularly focused on novel therapies because many current acne treatments are associated with skin irritation, possibly due to their inability to effectively address cutaneous oxidative stress.[94] The incorporation of antioxidant formulations could potentially enhance the tolerability and efficacy of acne therapies. The ethyl acetate extract of *C. aromatica* rhizomes demonstrated promising *in vitro* antioxidant activity, complementing its previously reported biological properties.[95-100] Experimental confirmation through enzyme inhibition assays and mechanistic investigations is necessary to ascertain its efficacy and therapeutic utility. Further research is required to elucidate the molecular mechanisms underlying these effects.

CONCLUSION

Phytochemical screening of the ethyl acetate *C. aromatica* extract revealed the presence of bioactive constituents, mainly alkaloids, phenolic compounds, and flavonoids, which are likely responsible for the antimicrobial activity of *C. aromatica.* FTIR analysis confirmed the presence of key functional groups, including hydroxyl, amine, and aromatic groups, supporting the phytochemical richness of the extract. To support the antimicrobial potential, molecular docking was performed using the compounds identified by HRLC-MS/MS. Several phytochemicals, particularly sesaminol diglucoside, showed strong binding affinities against *P. acnes* hyaluronate lyase A. The findings of this study suggest that *C. aromatica* extract contains promising bioactive compounds. Further studies are needed to isolate and characterize these compounds and validate their mechanisms of action *in vivo* experiments.

ACKNOWLEDGEMENT

The author would like to thank Yenepoya (Deemed to be University) for supporting the work.

ABBREVIATIONS

***C. aromatica*:** *Curcuma aromatica;* ***P. acnes*:** *Propionibacterium acnes;* **FTIR:** Fourier-Transform Infrared; **HRLC-MS/MS:** High Resolution Liquid Chromatography–Tandem Mass Spectrometry; **HYL:** Hyaluronate Lyase; **SCFAs:** Short-Chain Fatty Acids; **TLRs:** Toll-Like Receptors; **NOD:** Nucleotide Oligomerization Domain; **NLRP3:** NOD-, LRR- and Pyrin Domain-Containing Protein 3; **HA:** Hyaluronic Acid; **GAG:** Glycosaminoglycans; **Th17:** T Helper 17 Cells; **MMPs:** Matrix Metalloproteinases; **ROS:** Reactive Oxygen Species; **ESI:** Electrospray Ionization; **APCI:** Atmospheric-Pressure Chemical Ionization; **TOF:** Time-of-Flight; **m/z:** Mass-to-Charge Ratio; **PDB:** Protein Data Bank; **3D:** Three-Dimensional; **XP:** Extra Precision; **DPPH:** 2,2-Diphenyl-1-picrylhydrazyl; **TNF-α:** Tumor Necrosis Factor-Alpha; **MW:** Molecular Weight; **TPSA:** Topological Polar Surface Area; **nHD:** Number of Hydrogen Bond Donors; **nHA:** Number of Hydrogen Bond Acceptors; **LogP:** Partition Coefficient; **LogS:** Logarithm of Solubility; **nRot:** Number of Rotatable Bonds.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

SUMMARY

- The present study had carried out the extraction, qualitative, quantitative analysis, and *in silico* activity of *C. aromatica* rhizome for acne vulgaris.
- *C. aromatica* rhizome ethyl acetate extract contains triterpenoids and it will provide the synergistic action with other bioactive compounds against acne.
- FTIR analysis further confirmed the presence of key functional groups, including hydroxyl, amine, and aromatic groups.
- HRLC-MS/MS identified 213 metabolites in both positive and negative ionization modes, which were mainly triterpenoids, phenols, and flavonoids. Docking of 213 metabolites against P. acne hyaluronate lyase revealed that sesaminol diglucoside, morusimic acid, and floralginseonoside exhibited the most favorable docking scores, indicating a strong binding potential with the target protein.
- Antioxidant activity of the *C. aromatica* extract may have potential therapeutic implications for acne management by mitigating oxidative stress.

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Cite this article: Vasanth S, Fahma A, Kanekar S, Raju R, Rehman N, Ahmed MG. Phytochemical Profiling and Biological Assessment of *Curcuma aromatica* Using UPLC-MS, *in silico*, and *in vitro* Approaches for Acne Treatment. Indian J of Pharmaceutical Education and Research. 2026;60(3):1081-95.