



IN-VITRO AND IN-SILICO EVALUATION OF ANTI-INFLAMMATORY POTENTIAL OF A PHYTOCHEMICAL-ENRICHED TOPICAL HERBAL CREAM DEVELOPED FROM ACANTHOCEREUS TETRAGONUS

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ABSTRACT

Inflammation is a protective biological response against tissue injury and infection; however, prolonged inflammation can lead to chronic disorders such as arthritis and dermatitis. The present study aimed to formulate and evaluate a polyherbal topical cream containing *Acanthocereus tetragonus* extract and to scientifically validate its anti-inflammatory potential using in vitro and in silico approaches. The objectives included extraction of phytoconstituents by Soxhlet extraction, phytochemical screening, formulation of a water-in-oil (W/O) cream, physicochemical evaluation, and assessment of anti-inflammatory activity through protein denaturation and HRBC membrane stabilization assays, along with molecular docking against protein target 4H3X. Phytochemical analysis confirmed the presence of alkaloids, flavonoids, phenolic compounds, and triterpenoids. The formulated creams (F1 and F2) exhibited acceptable physical characteristics, homogeneity, pH (6.46–6.56), spreadability, washability, and satisfactory acid and saponification values. In vitro studies demonstrated significant

anti-inflammatory activity, with maximum protein denaturation inhibition (66.07% at 300 µg/mL) and membrane stabilization (74.38% at 100 µg/mL), comparable to diclofenac. Molecular docking revealed strong binding affinities of selected phytoconstituents toward protein 4H3X, particularly luteolin-O-rutinoside (−10.83 kcal/mol), indicating stable ligand–protein interactions. The integrated findings confirm that *Acanthocereus tetragonus* possesses promising anti-inflammatory activity and supports its potential application in safe and effective topical herbal formulations.

KEYWORDS: *Acanthocereus tetragonus*, Anti-inflammatory activity, Polyherbal cream, Protein denaturation assay, HRBC membrane stabilization, Molecular docking.

1. INTRODUCTION

The process of inflammation is a critical bodily defensive reaction to damages, illness and adverse stimulus. It assists in clearing of pathogens and starting the repair of tissues. Nonetheless, prolonged inflammation or severe inflammation may result in chronic diseases, such as arthritis, dermatitis, and other skin diseases. To manage such problems, doctors tend to prescribe traditional anti-inflammatory medications that have side effects such as skin rash, allergy, and other complications when used in the long term. Due to this reason, people are more interested in safer, plant-based alternatives that have the potential to contain the inflammation with few side effects and enhanced patient adherence. Bioactive compounds in medicinal plants have a number of health effects. The second one is *Acanthocereus tetragonus* a significant medicinal cactus, which has attracted interest due to its excellent contents of secondary metabolites, such as flavonoids, phenolic compounds, alkaloids, sterols, and polysaccharides. These are anti-inflammatory, antioxidant and wound-healing compounds. They might act through the inhibition of pro-inflammatory compounds, stabilisation of the cell membranes, and inhibition of oxidative damage of tissues. These bioactive compounds should be added to a topical herbal cream to enhance stability, increase skin penetration, as well as deliver specific therapeutic benefits. Researchers apply in vitro and in silico studies in order to scientifically validate its anti-inflammatory properties. In vitro research looks at the mechanisms of inhibiting protein denaturation and membrane stabilisation. Molecular docking experiments using the protein target 4H3X are useful in predicting the affinity of interactions between selections of compounds and the protein target. The integration of both experimental and computational techniques will provide a

comprehensive analysis of the therapeutic potential of the herbal products made of *Acanthocereus tetragonus*.

2. MATERIALS AND METHOD

2.1. Soxhlet Extraction

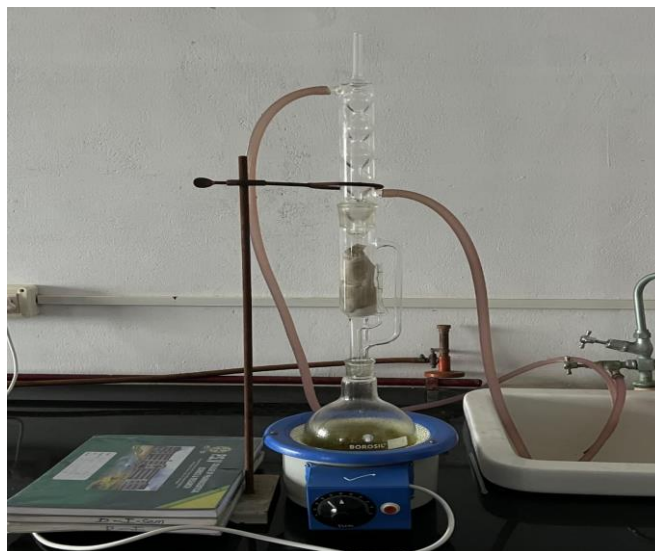


Figure 1: Soxhlet Extraction.

STEP 1: The fresh plant material is collected. It needs to be crushed, using a mortar and pestle, to provide a greater surface area. The plant material should be sufficient to fill the porous cellulose thimble (in our experiment, we use an average of 30 g of sample).

STEP 2: Assemble the apparatus used for extraction.

STEP 3: Following this, the solvent (150-200 ml of ethanol) is added to a round-bottom flask, which is attached to a Soxhlet extractor and condenser on an isomantle.

STEP 4: The crushed plant material is loaded into the thimble, which is placed inside the Soxhlet extractor. The side arm is lagged with glass wool.

STEP 5: The solvent is heated using the isomantle and will begin to evaporate, moving through the apparatus to the condenser. The condensate then drips into the reservoir containing the thimble. Once the level of solvent reaches the siphon, it pours back into the flask, and the cycle begins again. The process should run for a total of 16 hours.

2.2. Creams

A semi-solid dosage form, typically of the oil-in-water (O/W) or water-in-oil (W/O) type, consisting of a continuous phase and a dispersed phase, and formulated for topical application to skin, mucosa, or wounds.

2.2.1. Formulation Of Acanthocereus Tetragonus

Table 1: Formulation of the Cream (W/O).

INGREDIENTS	QUANTITY (20gm)	% W/W	FUNCTION
Acanthocereus tetragonus extract	2.0ml	10.0 %	anti-inflammatory, antioxidant and analgesic activity
GINGER	0.5gm	2.5 %	Anti-inflammatory, improves blood circulation
TURMERIC	0.4gm	2.0 %	Anti-inflammatory, antioxidant
FIG	0.3gm	1.5%	Soothing, moisturizing, supports wound healing
ALOE VERA	1.5gm	7.5%	Cooling agent, anti-inflammatory, skin moisturizer
BETEL LEAVES	0.3gm	1.5%	Anti-inflammatory, antimicrobial
HIBISCUS	0.3gm	1.5%	Anti-inflammatory, emollient, skin conditioning
LIQUID PARAFFIN	3.0ml	15.0%	Emollient, occlusive moisturizer
BEESWAX	1.2gm	6.0 %	Stiffening agent, emollient
CETYL ALCOHOL	0.8gm	4.0%	Co-emulsifier, emollient, improves consistency
BORAX	0.2gm	1.0%	Emulsifying agent (with beeswax)
METHYL PARABEN	0.04gm	0.2%	Preservative
LEMON GRASS OIL	0.10ml	0.5%	Anti-inflammatory, antimicrobial, fragrance
DISTILLED WATER	10ml	50%	Vehicle, forms aqueous phase

2.2.2. Procedure For Cream Formulation

- The W/O type of cream was prepared using 2 phases.
- All the active pharmaceutical ingredients and excipients were measured accurately.
- The Oil phase was prepared by Melt beeswax, cetyl alcohol and liquid paraffin at 70–75 °C.
- Similarly, Aqueous phase was prepared by heat distilled water separately, dissolve borax and methyl paraben. Add aloe vera and all herbal extracts including Acanthocereus tetragonus extract (0.6 mL).
- When two phase attain 70⁰c temperature aqueous phase, gradually introduced into the oil phase while continuous stirring until the cream consistency is attained.
- Cooling At about 40 °C, add lemongrass oil (0.06 mL) and mix uniformly.
- Make up the weight to 20 g, cool, and pack.

2.3. Phytochemical Tests

Table 2: Phytochemical Tests.

S.NO	PHYTOCHEMICAL TESTS				
1.	TEST FOR ALKALOIDS	Bouchardat's test: 6mL plant extract, evaporated completely + 6mL ethanol at (60 °C) + few drops of Bouchardat's reagent (dilute iodine solution). A reddish-brown colour is present.	Dragendorff test: Few mL filtrate + 1-2 mL Dragendorff's reagents A reddish-brown precipitate formed.	Wager's test: Few mL filtrate + 1-2 drops of Wagner's reagent (Along the sides of test tube). A brown/reddish precipitate is formed.	Mayer's test: Few mL filtrate + 1-2 drops of Mayer's reagent (Along the sides of test tube) A creamy white/yellow precipitate is formed.
2.	TEST FOR FLAVONOIDS	Alkaline reagent test: 1mL extract + 2mL of 2% NaOH solution + (few drops dil. HCl). An intense yellow colour becomes colourless on addition of diluted acid.	Ferric chloride test: Extract aqueous solution + few drops 10% ferric chloride solution. A green precipitate is formed.	Lead acetate test: 1mL plant extract + few drops of 10% lead acetate solution. A yellow precipitate is formed.	Conc. sulphuric acid test: Plant extract + conc. H ₂ SO ₄ . An orange colour is present.
3.	TEST FOR PHENOLIC COMPOUNDS	Iodine test: 1mL extract + few drops of dil. Iodine sol. A transient red colour is produced	Ferric chloride test: Extract aqueous solution + few drops 5% ferric chloride sol. Dark green/bluish black colour appeared.	Gelatin test: Plant extract is dissolved in 5mL distilled water + 1% gelatin solution + 10% NaCl. A white precipitate is formed.	Lead acetate test: Plant extract is dissolved in 5mL distilled water + 3mL of 10% lead acetate sol. A white precipitate is formed.
4.	TEST FOR TRITERPENOIDS	Salkowski's test: Filtrate + few drops of conc. H ₂ SO ₄ (Shaken well and allowed to stand). A Golden yellow layer is formed (at the bottom).			

3. RESULT AND DISCUSSION

3.1. Phytochemical Test

Table 3: Phytochemical Tests.

S.No	Phytochemical Test	Present (+)	Absent(-)
1.	Test For Alkaloids	+	+
2.	Test For Flavanoids	+	+
3.	Test For Phenolic Compounds	+	+
4.	Test For Triterpenoids	+	

3.2. Evaluation Test

3.2.1. Physical Appearance

The morphology evaluation of cream is shown in the table. The produced formulation is pleasant and well acceptable flavour makes the ideal for cosmetic formulations. Smoothness and texture was suitable for cosmetic formulation.

Table 4: Organoleptic Parameters.

Formulation(F)	Colour	Odour	Texture
F1	Greenish-Brown	Aromatic	Smooth
F2	Greenish-Brown	Aromatic	Smooth



Figure 2: Physical Appearance of Cream.

3.2.2. HOMOGENEITY

All formulations produced uniform distribution of extracts in cream. This was confirmed by visual appearance and by touch. The appearance and touch of the cream was good.

3.2.3. WASHABILITY

Washability test was carried out by applying small amount of cream on slide then washing with tap water. The observation is as follows.

Table 5: Washability Parameters.

Formulation (F)	Washability
F1	Easily Washable
F2	Easily Washable

3.2.4. AFTER FEEL

The cream was applied to as certain whether it is greasy or not.

Table 6: After Feel Effects.

Formulation (F)	Results
F1	Greasy
F2	Non-Greasy

3.2.5. MEASUREMENTS OF pH

pH of the formulations was measured using digital pH meter. The poly herbal cream formulations show the pH in the range from 6.46 to 6.55.

Table 7: Measurements of Ph.

Formulation (F)	Ph		Average Ph
	Trial 1	Trial 2	
F1	6.55	6.42	6.50
F2	6.46	6.57	6.56

**Figure 3: pH of cream formulation.****3.2.6 DYE TEST**

The dye test confirms that all formulations were W/O type emulsion cream. All the formulation F1 to F2 were mixed with amaranth dye (water- soluble dye) individually and examined under a microscope, it is found that all 2- formulations showed dispersed globules which confirms the formulated creams as W/O type.



Figure 4: Dye Test For Cream.

3.2.7. SPREADABILITY

The sample was taken between two glass slides and a weight of 100gm is applied on the slides for 5 mins and by using the formula, the value of spreadability for the formulations was calculated. The spreadability value for the formulation was found to be in the range of.

Table 8: Spreadability of Cream.

Formulation (F)	Spreadability (G.Cm/5min)			Average Spreadability (G.Cm/5cm)
	Trail 1	Trail 2	Trail 3	
F1	24	26	25	25
F2	27	26	28	27

3.2.8. Acid Value

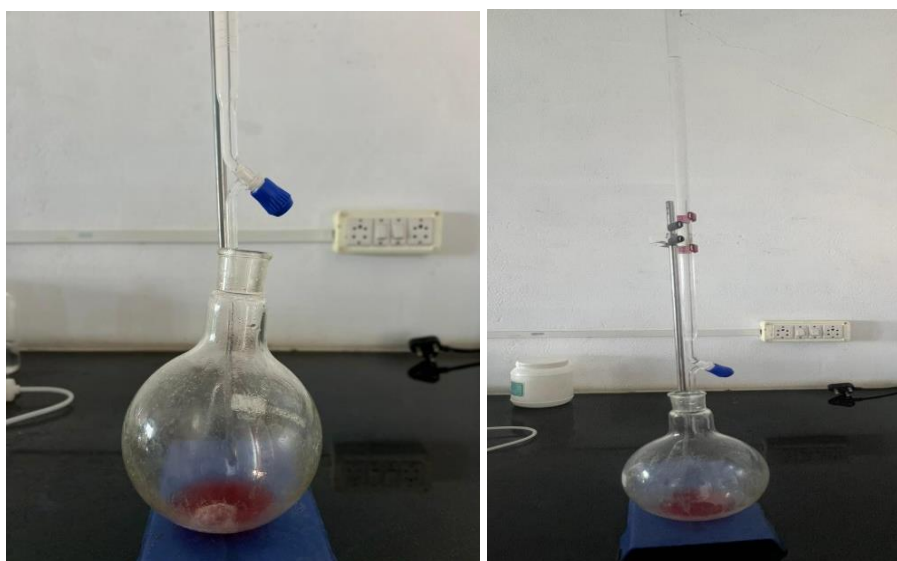


Figure 5: Acid value.

Acid value of the formulated cream was found to be in satisfactory values. Acid value is defined as the number of milligrams of KOH required to neutralize 1gm of oil or fat. It indicates the amount of free fatty acid present in sample. If there are no fatty acids present, the oil or fat is of good quality, if there are more fatty acids present, it indicates the fat or oil has become rancid (hydrolysis of oil or fat). The result of acid value of all formulation of cream is presented in below table and showed satisfactory values.

Table 9: Acid Value.

Formulation(F)	Acid Value (Mg Koh/G)
F1	3.5 Mg Koh/G
F2	3.1 Mg Koh/G

3.2.9. SAPONIFICATION VALUE

Saponification value for the formulated cream was found to be in satisfactory values. Saponification value is the number of mg of potassium hydroxide (KOH) or sodium hydroxide (NaOH) required to Saponify one gram of fat under the condition specified. It is the measure of the average molecular weight (or chain length) of all the fatty acids present in the sample in the form of triglycerides. The higher the saponification value, the lower the fatty acids average length, the lighter the mean molecular weight of triglycerides and vice versa. The result saponification value of all formulation of cream is presented in below table and showed satisfactory value.

Table 10: Saponification Value.

Formulation (F)	Saponification Value (mg Koh/G)
F1	133.24 mg Koh/G
F2	129.03 mg Koh/G

3.2.10. PROTEIN DENATURATION TEST

The protein denaturation assay was performed using fresh egg albumin. The reaction mixture containing albumin, phosphate buffer, and plant extract at various concentrations was incubated, heated to induce denaturation, cooled, and absorbance measured spectrophotometrically. Percentage inhibition of protein denaturation was calculated against a control.

$$\% \text{ Inhibition} = \frac{(\text{Absorbance of control} - \text{Absorbance of Test})}{\text{Absorbance of control}} \times 100$$

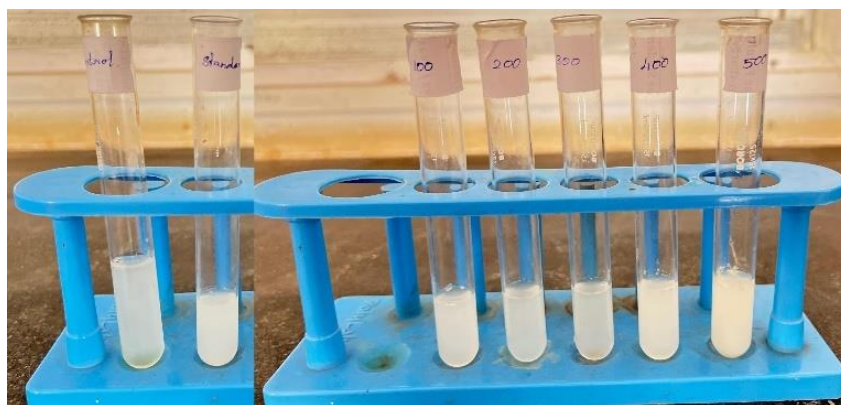


Figure 6: Protein denaturation test.

Calculation

Table 11: Protein Denaturation Test.

S.No	Sample	Concentration (µg/ML)	Absorbance (660nm)	% Inhibition
1	Control	----	1.68	----
2	Diclofenac	100 µg	1.46	13.09
3	Acanthocereus Tetragonus Extract	100	0.79	52.97
4		200	0.59	64.88
5		300	0.57	66.07
6		400	1.29	23.21
7		500	1.53	8.928

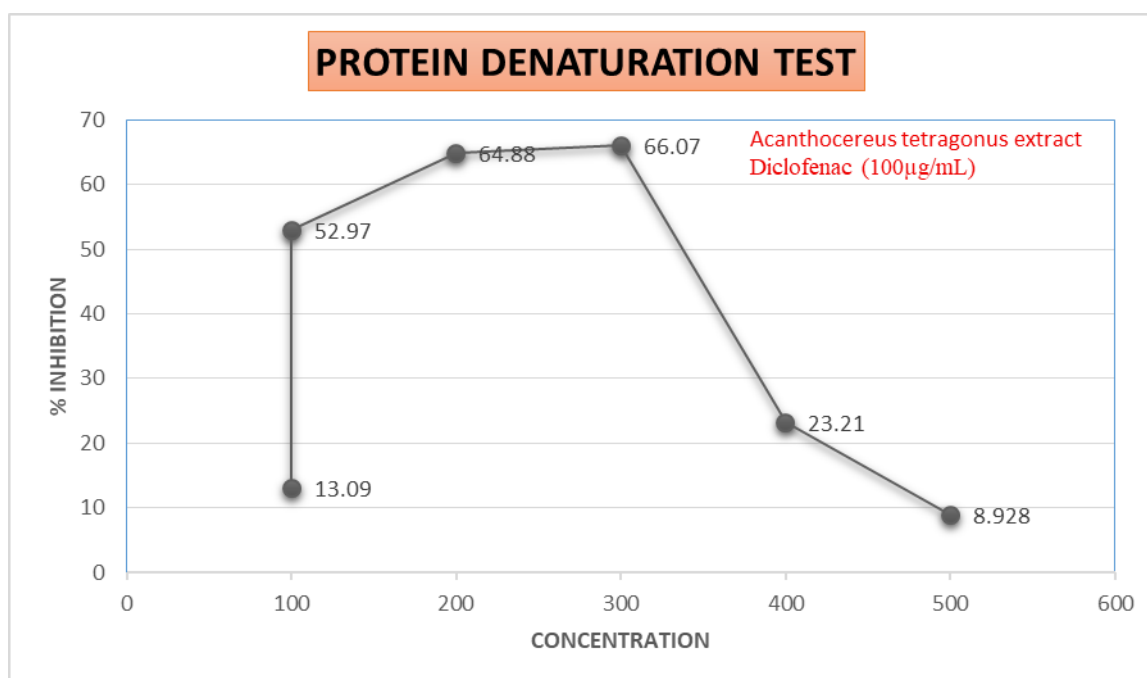


Figure 7: Graph for Protein denaturation test.

The graph shows an increase in percentage inhibition of protein denaturation with increasing concentration of *Acanthocereus tetragonus* extract up to 300 µg/mL, indicating maximum inhibitory activity. Beyond this concentration, a decline in inhibition was observed, suggesting reduced effectiveness at higher doses. The extract exhibited significantly higher inhibition compared to the standard drug diclofenac at 100 µg/mL.

3.2.11. HRBC Membrane Stabilization Test

The HRBC membrane stabilization assay was carried out using human red blood cells. The reaction mixture containing HRBC suspension, hypotonic solution, phosphate buffer, and plant extract was incubated and centrifuged. The absorbance of the supernatant was measured spectrophotometrically, and percentage membrane stabilization was calculated compared to the control.

$$\% \text{ Membrane stabilization} = \frac{(\text{Absorbance of Control} - \text{Absorbance of Test})}{\text{Absorbance of control}} \times 100$$



Figure 8: HRBC Membrane Stabilization test.

Calculation

Table 12: HRBC Membrane Stabilization Test.

S.No	Sample	Concentration (µg/Ml)	Absorbance (560nm)	% Membrane Stabilization
1	Control	----	0.367	----
2	Diclofenac	100 µg	0.090	75.47
3	Acanthocers Tetragonus Extract	20	0.295	19.61
4		40	0.249	32.15
5		60	0.196	46.59
6		80	0.152	58.58
7		100	0.094	74.38

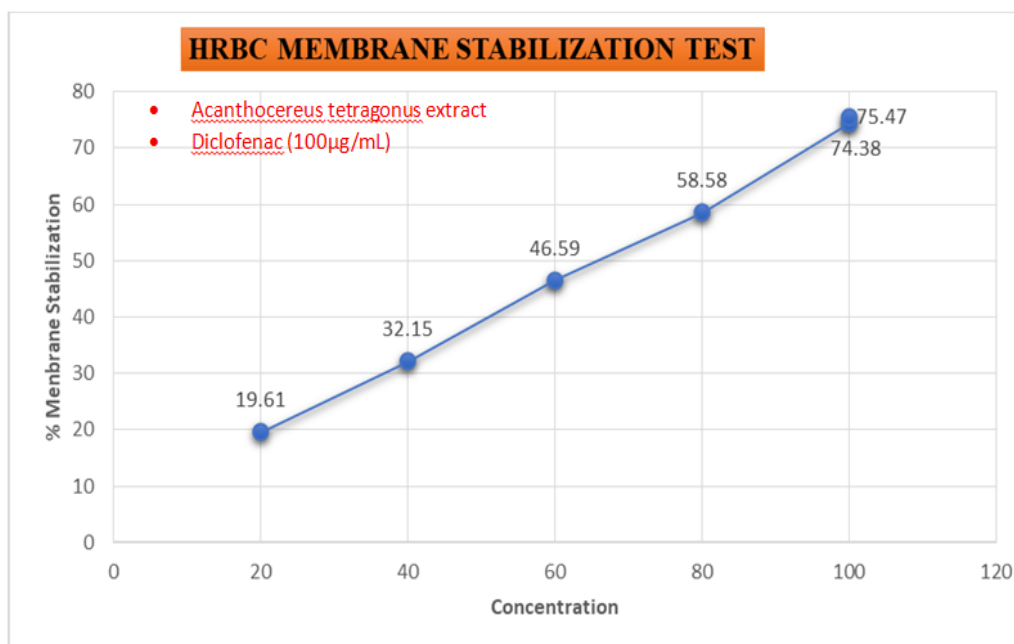


Figure 9: Graph for HRBC Membrane stabilization test.

The graph shows a concentration-dependent increase in percentage membrane stabilization with *Acanthocereus tetragonus* extract. At 100µg/mL, the extract exhibited 74.38% membrane stabilization, which is comparable to the standard drug diclofenac (75.47%). This confirms significant in-vitro anti-inflammatory activity of the extract.

3.3. Molecular Docking

3.3.1. Molecular Docking Analysis of Selected Phytoconstituents Against Anti-Inflammatory Protein (4H3X)

Molecular docking studies were carried out to evaluate the anti-inflammatory potential of selected phytoconstituents identified from *Acanthocereus tetragonus*. The compounds selected for the study included luteolin-O-rutinoside, sinapic acid, linolenic acid, citric acid, 4-hydroxycinnamic acid, di-tert-butyl 4-amino-4-(3-(tert-butoxy)-3-oxopropyl) heptanedioate, and malic acid. The docking analysis was performed against the anti-inflammatory target protein 4H3X to predict binding affinity, interaction modes, and stability of ligand–protein complexes. The docked conformations were evaluated based on binding energy values, hydrogen bond formation, hydrophobic interactions, electrostatic contacts, and overall molecular stability. The results provided insight into the possible inhibitory mechanisms of these phytoconstituents at the molecular level and supported their potential role in inflammation modulation.

Table 13: Molecular Docking Analysis of Selected Phytoconstituents Against Anti-Inflammatory Protein (4H3X).

S.No	Name Of the Ligands	Binding Energy (ΔG) With 3nt1 (Kcal/Mol)	Types Of Interactions	Amino Acids
1.	Luteolin-O-Rutinoside	-10.83	Conventional Hydrogen Bond	ALA B : 189 LEU B : 188 MET B : 247
			Pi-Pi Stacked	HIS B: 226
			Pi-Alkyl	VAL B :223
2.	Sinapic Acid	-9.17	Conventional Hydrogen Bond	TYR B: 245 HIS B: 226 LEU B: 188
			Carbon Hydrogen Bond	GLU B: 227
			Alkyl	HIS B: 230
			Pi-Alkyl	HIS B: 236
3.	Linolenic Acid	-8.97	Alkyl	PRO A: 255 LEU A: 222 LEU A: 243 ARG A: 249
			Pi-Alkyl	VAL A: 223 TYR A: 248 HIS A: 226 LEU A: 188
4.	Citric Acid,	-8.28	Conventional Hydrogen Bond	HIS A: 226 TYR A: 245 MET A: 247
5.	4-Hydroxycinnamic Acid	-8.13	Pi-Pi Stacked	HIS B: 226
			Pi-Pi T-Shaped	TYR B: 248
			Pi- Alkyl	VAL B: 223
6.	Di-Tert-Butyl 4-Amino-4-(3-(Tert-Butoxy)-3-Oxopropyl) Heptanedioate	-5.78	Conventional Hydrogen Bond	HIS B: 190
			Pi- Sigma	TYR B: 179 PHE B: 192
			Pi- Alkyl	HIS B: 226
7.	Malic Acid	-2.30	Conventional Hydrogen Bond	MET A: 247 ARG A: 249 TYR A: 245 LEU A: 243

3.3.2. Docking Results of Luteolin-O-Rutinoside with Protein 4H3X

Luteolin-O-rutinoside exhibited the strongest binding affinity among all tested phytoconstituents against protein 4H3X. The docking analysis revealed the formation of multiple hydrogen bonds between the hydroxyl groups of luteolin-O-rutinoside and key amino acid residues within the active site of the protein. Additionally, π - π stacking interactions and van der Waals forces contributed significantly to the stability of the ligand–

protein complex. The polyphenolic flavonoid structure, along with the sugar moiety, enabled extensive molecular interactions, enhancing binding strength and stability. The strong docking score and interaction profile suggest that luteolin-O-rutinoside may effectively inhibit inflammatory pathways by occupying and stabilizing the active site of the target protein. These findings strongly support its reported anti-inflammatory and antioxidant activities.

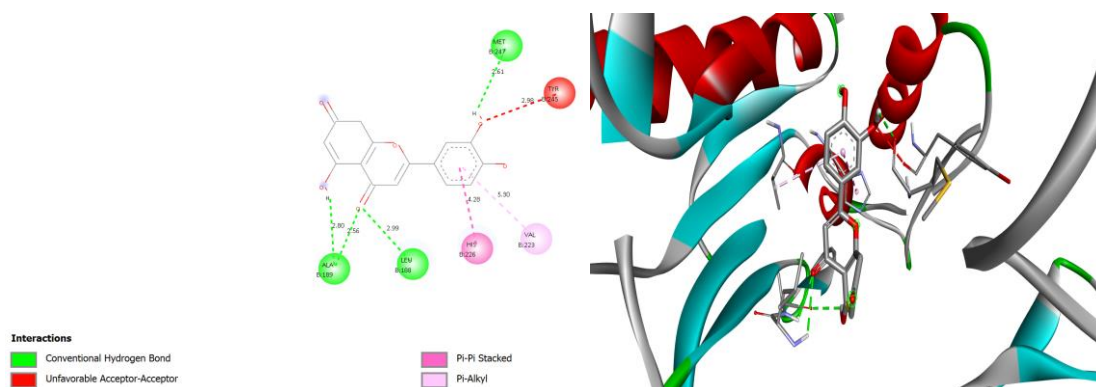


Figure 10: 2D and 3D structure of Luteolin-O-Rutinoside with Protein 4H3X.

3.3.3. Docking Results of Sinapic Acid with Protein 4H3X

Sinapic acid demonstrated good binding affinity toward protein 4H3X. The docking results showed that sinapic acid interacted primarily through hydrogen bonds involving its phenolic hydroxyl and carboxylic acid functional groups. These interactions were complemented by hydrophobic contacts within the binding pocket, contributing to moderate-to-strong complex stability. The presence of conjugated aromatic rings facilitated effective accommodation within the active site. The interaction pattern suggests that sinapic acid may inhibit inflammatory mediators by interfering with enzyme activity at the molecular level. The results align well with previously reported anti-inflammatory properties of phenolic acids.

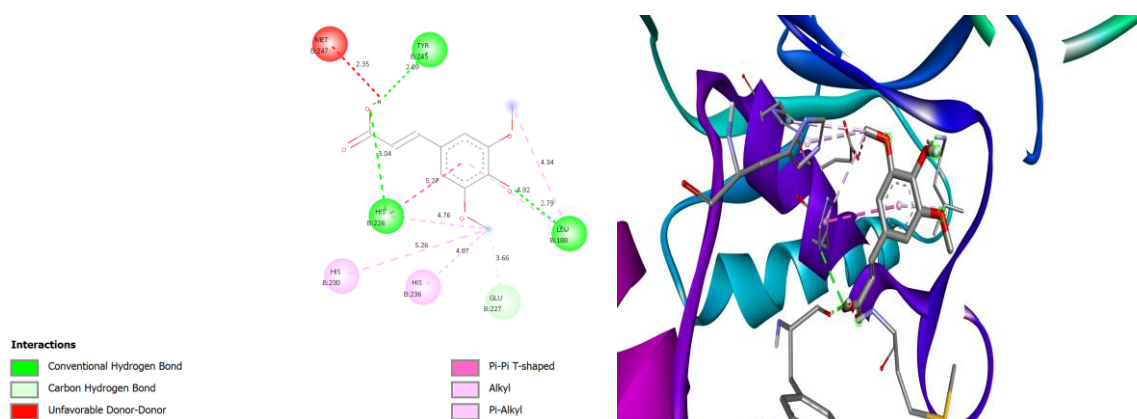


Figure 11: 2D and 3D structure of Sinapic Acid with Protein 4H3X.

3.3.4. Docking Results of Linolenic Acid with Protein 4H3X

Linolenic acid exhibited favorable binding affinity, primarily mediated through hydrophobic interactions. The long aliphatic carbon chain of linolenic acid allowed deep penetration into the hydrophobic regions of the protein's active site. Van der Waals forces and hydrophobic stabilization played a dominant role in maintaining the ligand–protein complex. Although hydrogen bond formation was minimal, the extensive hydrophobic contacts compensated for this limitation, resulting in a stable docked conformation. These findings suggest that linolenic acid may exert anti-inflammatory effects by modulating protein activity through lipid–protein interactions, supporting its known role in inflammation regulation.

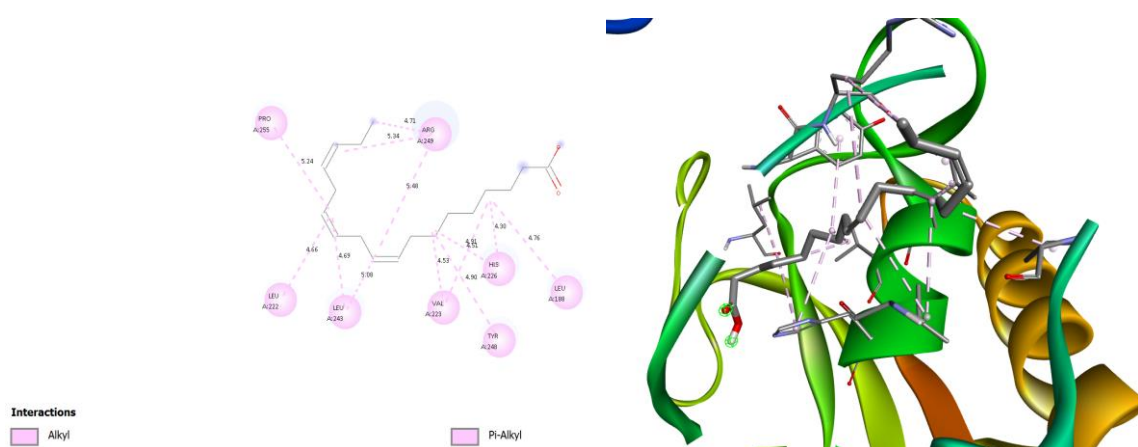


Figure 12: 2D and 3D structure of Linolenic Acid with Protein 4H3X.

3.3.5. Docking Results of Citric Acid with Protein 4H3X

Citric acid showed moderate binding affinity toward protein 4H3X. The docking analysis revealed the formation of multiple hydrogen bonds between the carboxyl groups of citric acid and polar amino acid residues present in the active site. These interactions contributed to ligand stabilization within the binding pocket. Despite its small molecular size, citric acid demonstrated effective interaction through electrostatic and hydrogen bonding mechanisms. The results indicate that citric acid may support anti-inflammatory activity by interacting with key residues involved in inflammatory signaling.

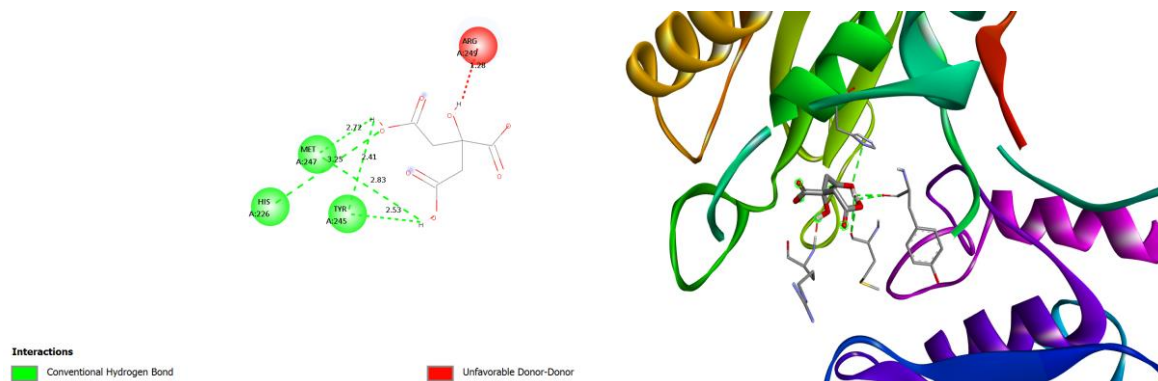


Figure 13: 2D and 3D structure of Citric Acid with Protein 4H3X.

3.3.6. Docking Results of 4-Hydroxycinnamic Acid with Protein 4H3X

4-Hydroxycinnamic acid exhibited moderate to good binding affinity against protein 4H3X. The ligand formed hydrogen bonds via its hydroxyl and carboxyl functional groups, along with hydrophobic interactions involving the aromatic ring. These interactions collectively contributed to the stability of the docked complex. The molecular interaction profile suggests that 4-hydroxycinnamic acid can effectively interact with inflammation-related proteins, thereby inhibiting inflammatory mediator activity. The docking results support its reported anti-inflammatory potential.

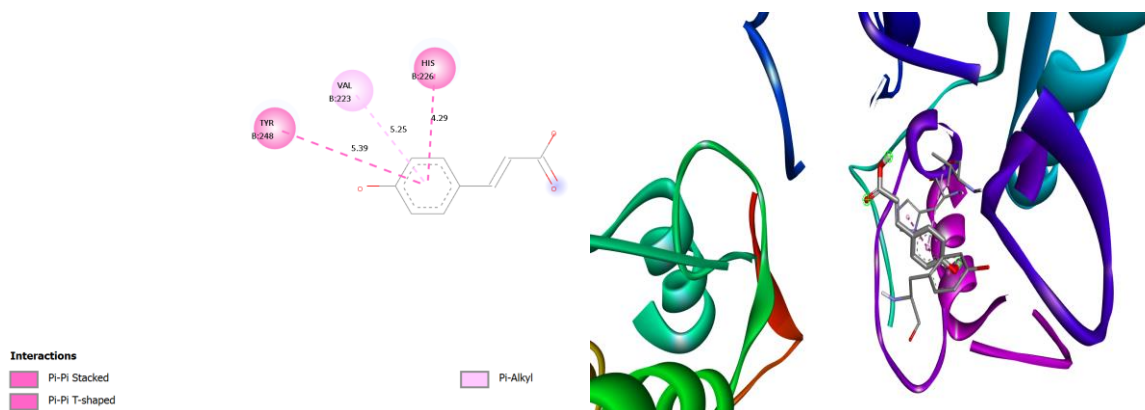


Figure 14: 2D and 3D structure of 4-Hydroxycinnamic Acid with Protein 4H3X.

3.3.7. Docking Results of Di-tert-butyl 4-amino-4-(3-(tert-butoxy)-3-oxopropyl) Heptanedioate with Protein 4H3X

This compound exhibited stable binding affinity with protein 4H3X, predominantly through hydrophobic interactions and steric fitting within the active site. The bulky tert-butyl groups contributed to strong van der Waals interactions, enhancing ligand–protein stability. Limited hydrogen bonding was observed; however, the extensive hydrophobic contacts compensated for this, resulting in an energetically favorable docked complex. The results suggest that this

3.3.8. Docking Results of Malic Acid with Protein 4H3X

The present molecular docking and ADMET studies collectively demonstrate the significant anti-inflammatory potential of selected phytoconstituents derived from *Acanthocereus tetragonus*. Docking analysis against the target protein 4H3X revealed that all investigated compounds could bind within the active site, exhibiting distinct interaction patterns involving hydrogen bonding, hydrophobic interactions, π - π stacking, and van der Waals forces. Among

the tested ligands, luteolin-O-rutinoside showed the strongest binding affinity and the most stable interaction profile, supported by multiple hydrogen bonds and aromatic interactions. Despite violating certain Lipinski parameters, its excellent safety profile and strong molecular interactions suggest high relevance, particularly for topical anti-inflammatory applications. Sinapic acid and 4-hydroxycinnamic acid displayed favorable binding energies along with good compliance to Lipinski's rule of five, indicating balanced pharmacokinetic behavior, safety, and effective interaction with inflammation-related residues. Linolenic acid demonstrated strong hydrophobic binding within the protein pocket, supporting its role in inflammation modulation, although its higher lipophilicity and associated toxicity indicate limitations for systemic use. Citric acid and malic acid showed moderate binding affinities but excellent safety and solubility profiles, suggesting their supportive contribution to anti-inflammatory formulations through hydrogen bonding and electrostatic interactions. The di-tert-butyl derivative also exhibited acceptable drug-likeness and stable binding through hydrophobic and steric interactions. Overall, the integrated docking and ADME results confirm that the phytoconstituents of *Acanthocereus tetragonus* possess promising anti-inflammatory potential at the molecular level. These findings strongly support their further exploration through in vitro and in vivo studies to validate their therapeutic efficacy and mechanism of action in inflammation management.

CONCLUSION

The present study successfully demonstrated the potential of *Acanthocereus tetragonus* as a promising natural source for anti-inflammatory therapy through an integrated experimental and computational approach. Soxhlet extraction yielded bioactive constituents, and preliminary phytochemical screening confirmed the presence of alkaloids, flavonoids, phenolic compounds, and triterpenoids, which are well known for their anti-inflammatory and antioxidant properties. The formulated polyherbal water-in-oil cream showed satisfactory physicochemical characteristics, including appropriate pH, homogeneity, spreadability, washability, acceptable acid and saponification values, and overall cosmetic acceptability, indicating its suitability for topical application. In vitro anti-inflammatory evaluation revealed significant inhibition of protein denaturation and effective stabilization of the HRBC membrane in a concentration-dependent manner. Notably, the extract exhibited activity comparable to the standard drug diclofenac at higher concentrations, supporting its therapeutic relevance. Furthermore, molecular docking studies against the anti-inflammatory target protein 4H3X provided mechanistic insight into the interaction of selected

phytoconstituents. Among them, luteolin-O-rutinoside demonstrated the strongest binding affinity, supported by multiple hydrogen bonds and hydrophobic interactions, suggesting its key role in inflammation modulation. Other compounds such as sinapic acid, linolenic acid, and 4-hydroxycinnamic acid also showed favorable binding profiles, reinforcing the synergistic potential of the extract. Overall, the combined in vitro and in silico findings validate the anti-inflammatory efficacy of *Acanthocereus tetragonus* and support its application in topical herbal formulations. Further in vivo and clinical investigations are warranted to confirm its safety, efficacy, and therapeutic applicability in the management of inflammatory disorders.

REFERENCES

1. Cornejo-Campos J, Gómez-Aguirre YA, Velázquez-Martínez JR, Ramos-Herrera OJ, Chávez-Murillo CE, Cruz-Sosa F, et al. Impact of the Cooking Process on Metabolite Profiling of *Acanthocereus tetragonus*, a Plant Traditionally Consumed in Mexico. *Molecules*, Jun. 9, 2022; 27(12): 3707. <https://doi.org/10.3390/molecules27123707>.
2. Sharma P, Modi N. INVESTIGATING THE QUALITATIVE AND QUANTITATIVE PHYTOCHEMICAL ANALYSIS OF ACANTHOCEREUS TETRAGONUS (CACTACEAE FAMILY) ALONG WITH THE ANTIOXIDANT ACTIVITY. *VIDYA - A JOURNAL OF GUJARAT UNIVERSITY*, May 15, 2023; 2(1): 176–82. <https://doi.org/10.47413/vidya.v2i1.169>.
3. GONZÁLEZ-ZAMORA P, CARRILLO-REYES P, SÁNCHEZ D. *Acanthocereus paradoxus* (Cactaceae), a new endemic species from Jalisco, Mexico. *Phytotaxa*, Nov. 3, 2020; 470(2). <https://doi.org/10.11646/phytotaxa.470.2.3>.
4. Lopez-Ojeda W, Pandey A, Alhajj M, Oakley AM. *Anatomy, Skin (Integument)*. StatPearls, Dec. 1, 2019; <https://www.ncbi.nlm.nih.gov/books/NBK441980/>
5. Lotfollahi Z. The anatomy, physiology and function of all skin layers and the impact of ageing on the skin. *Wound practice & research*, Mar. 1, 2024; 32(1): 6–10. DOI 10.33235/wpr.32.1.6-10.
6. Dayan, N. and Lambros Kromidas (2011). *Formulating, Packaging, and Marketing of Natural Cosmetic Products*. John Wiley & Sons. www.TheCosmeticSite.com
7. International journal of pharmaceutical sciences and research, Sep. 1, 2014; 5(9). [http://dx.doi.org/10.13040/IJPSR.0975-8232.5\(9\).3594-08](http://dx.doi.org/10.13040/IJPSR.0975-8232.5(9).3594-08)

8. Chauhan L, Gupta S. Creams: A Review on Classification, Preparation Methods, Evaluation and its Applications. *Journal of Drug Delivery and Therapeutics*, Oct. 15, 2020; 10(5-s): 281–9. <http://dx.doi.org/10.22270/jddt.v10i5-s.4430>
9. Redfern J, Verran J, Burdass D, Kinninmonth M. Using Soxhlet Ethanol Extraction to Produce and Test Plant Material (Essential Oils) for Their Antimicrobial Properties †. *Journal of Microbiology & Biology Education* [Internet], May 1, 2014; 15(1): 45–6. <https://doi.org/10.1128/jmbe.v15i1.656>
10. NAVINDGIKAR NN, KAMALAPURKAR KA, CHAVAN PS. FORMULATION AND EVALUATION OF MULTIPURPOSE HERBAL CREAM. *International Journal of Current Pharmaceutical Research*, May 14, 2020; 12(3): 25–30. <http://dx.doi.org/10.22159/ijcpr.2020v12i3.38300>.
11. Diana IH, Vanathi Sundarapandiyan. New Potential and Characterization of *Andrographis echinoides* L. Ness Stem Extract as an Anti-Arthritic Agent. *IntechOpen eBooks*, 2025 Jul 21. [10.5772/intechopen.1010925](https://doi.org/10.5772/intechopen.1010925).
12. Mohamed, Abdel S, Mohamed OA, Hassan A, Ibrahim, Reffat HM, et al. Synthesis, Anti-Inflammatory, and Molecular Docking Studies of New Heterocyclic Derivatives Comprising Pyrazole, Pyridine, and/or Pyran Moieties. *Pharmaceuticals* [Internet], Feb. 26, 2025; 18(3): 335–5. <https://doi.org/10.3390/ph18030335>.
13. Utami W, Aziz HA, Fitriani IN, Zikri AT, Mayasri A, Nasrudin D. In silico anti-inflammatory activity evaluation of some bioactive compound from *ficus religiosa* through molecular docking approach. *Journal of Physics: Conference Series*, Jun. 1, 2020; 1563(1): 012024. <https://iopscience.iop.org/article/10.1088/1742-6596/1563/1/012024/pdf>.
14. Tri MD, Phat NT, Minh PN, Chi MT, Hao BX, Minh An TN, et al. *In vitro* anti-inflammatory, *in silico* molecular docking and molecular dynamics simulation of oleanane-type triterpenes from aerial parts of *Mussaenda recurvata*. *RSC Advances* [Internet], 2023; 13(8): 5324–36. [10.1039/D2RA06870B](https://doi.org/10.1039/D2RA06870B)
15. Heath PV. THE TYPE OF *ACANTHOCEREUS* (ENGELMANN) BRITTON AND ROSE (CACTACEAE). *TAXON*, Aug. 1989; 38(3): 480–0. <https://doi.org/10.2307/1222296>.
16. MORALES-CANO KL, RIVERA-ALAMILLO YC, OLIART-ROS RM, PEÑA-MONTES C. Modulation of the gut microbiota by dietary intervention with *Acanthocereus tetragonus* improves the health status of Wistar rats with metabolic

- syndrome. Bioscience of Microbiota, Food and Health, 2025; 44(2): 100–9. doi: 10.12938/bmfh.2024-04.
17. GONZÁLEZ-ZAMORA P, RODRÍGUEZ-CONTRERAS A, SÁNCHEZ D. A new endemic species of *Acanthocereus* (Cactaceae) from southern Jalisco, Mexico. *Phytotaxa*, Oct. 7, 2021; 522(2): 131–8. [10.11646/phytotaxa.522.2.5](https://doi.org/10.11646/phytotaxa.522.2.5).
18. Niyaz AM, Ravikumar S. Antilarval and in vitro Anticancer efficacy of *Cladode* extracts of *Opuntia dillenii* (Ker Gawl.) Haw., *Cereus pterogonus* Lem. and *Acanthocereus tetragonus* (L.) Hummelinck. *Research Journal of Pharmacy and Technology*, Jul. 29, 2022; 2877–82. [10.52711/0974-360X.2022.00480](https://doi.org/10.52711/0974-360X.2022.00480).
19. Jain N, Nandal M. Effect of ecological parameters (Light, water, soil-mix, temperature & fertilizers) on growth of different cactus species (*Acanthocereus tetragonus*, *Mammillaria elongata* & *Opuntia* species). *International Journal of Multidisciplinary Trends*, Feb. 1, 2025; 7(2): 102–8. [10.22271/multi.2025.v7.i2b.604](https://doi.org/10.22271/multi.2025.v7.i2b.604).