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Research Article

UNVEILING THE CYTOTOXIC POTENTIAL OF *HYLOCEREUS COSTARICENSIS* (F.A.C. WEBER) BRITTON AND ROSE LEAF: PHYTOCHEMICAL PROFILING AND MOLECULAR DOCKING INSIGHTS

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ABSTRACT

Objectives: *Hylocereus costaricensis* (F.A.C. Weber) Britton and Rose, a member of the *Cactaceae* family, is a well-known plant for its anticancer properties. This study evaluates the gas chromatography-mass spectrometry (GC-MS) analysis, molecular docking and *in vitro* anticancer activity of ethanolic (ETH) and aqueous (Aq.) extracts derived from its leaves.

Methods: GC-MS analysis was utilized to screen plant extracts and identify pharmacologically active compounds. The physicochemical and pharmacokinetic properties of n-hexadecanoic acid, octadecanoic acid, and 17-pentatriacontene were analyzed using SwissADME. Molecular docking studies were also conducted using AutoDock software to evaluate the binding interactions of these three phytoconstituents with the glucose regulatory protein 78 (GRP78) receptor. In addition, the brine shrimp lethality bioassay was performed to assess the *in vitro* cytotoxic activity of the ETH and Aq. extracts.

Results: GC-MS analysis of the ETH extract identified 19 bioactive compounds. Molecular docking showed strong binding of n-hexadecanoic acid, octadecanoic acid, and 17-pentatriacontene to the GRP78 receptor. *In vitro* cytotoxicity assay revealed dose-dependent effects, with the Aq. extract displayed greater toxicity ($LC_{50:}$ 6.6 µg/mL; mortality: 50±0.94% to 86.66±0.33%) compared to the ETH extract (LC_{50}: 18.57 µg/mL; mortality: 43.33±0.66% to 83.33±0.66%), both showed significant cytotoxicity at higher concentrations ($p<0.05$, $p<0.01$).

Conclusion: The bioactive compounds present in *H. costaricensis* may contribute to its pharmacological properties and hold significant potential for the development of novel therapeutic targets. Among the three studied phytoconstituents, n-hexadecanoic acid exhibited the strongest cytotoxic potential, as indicated by its highest binding affinity in molecular docking analysis.

Keywords: *Cactaceae*, *Hylocereus costaricensis*, Gas chromatography-mass spectrometry, SwissADME, Brine shrimp lethality bioassay.

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INTRODUCTION

Cancer is one of the most prevalent diseases worldwide, contributing significantly to mortality and disability. It is the second leading cause of death in developed countries. According to the World Health Organization, by 2050, the number of new cancer cases is expected to exceed 35 million, marking a 77% increase from the estimated 20 million cases in 2022 [1,2]. Cancer can develop in various organs due to the uncontrolled growth and spread of abnormal cells, commonly affecting the lungs, breast, colon, liver, and brain. Liver and breast cancers are among the most common types, often associated with factors such as alcohol consumption, metabolic disorders, and hormonal changes. Hepatocellular carcinoma is the most prevalent form of liver cancer, primarily arising in individuals with cirrhosis or chronic liver disease [3,4].

Cancer development is influenced by multiple factors, including immune system depletion, genetic mutations, abnormal protein production, obesity, smoking, and tissue damage [5]. Breast and colorectal cancers remain the leading causes of malignancy and cancer-related deaths globally. Standard cancer treatments include chemotherapy, surgical interventions, radiotherapy, and hormone-based therapy. However, these conventional treatments are often associated with severe side effects, toxicities, and other risk factors [6,7].

Due to these limitations, there has been a growing interest in herbal formulations for cancer treatment. Ayurveda, one of the most widely used traditional medicinal systems, plays a significant role in healthcare, particularly in rural India, where nearly 70% of the population relies on Ayurvedic remedies. These traditional medicines are not only essential for healthcare in resource-limited developing countries but are also increasingly used alongside modern medicine in developed nations [8,9]. Medicinal plants serve as a primary source of pharmaceutical raw materials, offering affordable and effective healthcare solutions, especially for rural communities [10,11].

One such medicinal plant is *Hylocereus costaricensis*, commonly known as red dragon fruit. This climbing cactus belongs to the *Cactaceae* family and is widely cultivated in tropical regions. The plant produces different varieties of dragon fruit, including red, yellow, purple, and white types [12,13]. *H. costaricensis* is characterized by aerial roots that allow it to grow up to 30 feet, with three-sided, notched stems measuring 1–2 inches in thickness. While its exact native origin is unknown due to its global cultivation as an ornamental and fruit-bearing plant, it is believed to have originated in the tropical rainforests of Central and South America. It is also known by names such as "Belle of the Night" and "Cinderella Plant," while its fruit is commonly referred to as red dragon fruit, red pitaya, pitahaya fruit, or strawberry pear [14,15].

This plant contains various phytoconstituents, including alkaloids, flavonoids, tannins, steroids, glycosides, saponins, and terpenoids, which contribute to its medicinal properties [16,17]. Studies have

reported its antidiabetic, antimalarial, anticancer, anticonvulsant, and anti-nephrolithiasis effects. The fruit's flesh has demonstrated antioxidant, antidiabetic, hepatoprotective, anti-hypercholesterolemic, and anticancer properties. Nutritionally, dragon fruit is rich in proascorbic acid, fats, and carbohydrates, making it a valuable dietary component [18-22].

Our current research focuses on the gas chromatography-mass spectrometry (GC-MS) analysis of the ethanolic (ETH) extract of *H. costaricensis* leaves to identify its bioactive compounds [23]. In addition, SwissADME was used to evaluate the pharmacokinetics, drug-likeness, and medicinal chemistry properties of small molecules. Computational approaches, including *in silico* studies and bioinformatics, play a crucial role in accelerating drug discovery by reducing time and costs. Molecular docking, a well-established computational technique, was employed to predict the interaction energy between molecules, providing insights into potential drug-receptor interactions [24].

This study aimed to explore the bioactive potential of *H. costaricensis* through GC-MS analysis, SwissADME evaluation, molecular docking, and *in vitro* cytotoxic activity, contributing to the search for novel therapeutic agents with anticancer properties.

METHODS

Collection and authentication of plant material

Fresh leaves of *H. costaricensis* were collected from a farm in Solapur, Maharashtra, India. The plant was identified and authenticated by Dr. R.F. Suryvunashi, Head of the Botany Department at Dr. Ganpatrao Deshmukh Mahavidyalaya, Sangola, Solapur, India. The voucher specimen has been deposited in the herbarium of the Department of Botany at Ganpatrao Deshmukh Mahavidyalaya, Sangola for future reference. The leaves were shade-dried at room temperature for 4 weeks. The dried material was then coarsely powdered using an electric grinder (mixer) and passed through sieve no. 20 to obtain a uniform coarse powder. The resulting powder was stored in a container at room temperature till its further use.

Extraction of plant material

Preparation of ETH extract

The extraction process was performed using a Soxhlet apparatus with ethanol as a solvent. A total of 50 g of coarse powder were subjected to successive extraction in 250 mL of ethanol using the Soxhlet method, continuing until a clear solvent was obtained. The ETH extract was then obtained using a vacuum rota evaporator and it was stored for subsequent study [25].

Preparation of aqueous (Aq.) extract

A 500 mL maceration flask containing 50 g of powdered drug was filled with 250 mL of water as the extraction solvent. To prevent fungal growth, 5 mL of chloroform was added. The cold maceration method was employed for Aq. extraction. After 24 h, Aq. extract was obtained using a vacuum rota evaporator and stored for future study [26].

Qualitative phytochemical test

Preliminary chemical tests were conducted on the ETH and Aq. extracts using standard protocols [27,28].

GC-MS analysis

The ETH extract of *H. costaricensis* was analyzed using GC-MS on a Shimadzu GC-MSQP2020 plus instrument equipped with an RTX-5 MS capillary column (0.25 mm × 30 m × 0.25 μm). Helium gas (99.999%) was used as the carrier gas at a constant flow rate of 16.3 mL/min, with a column flow rate maintained at 1.2 mL/min. The temperature program began with an initial hold at 50°C for 2 min, followed by a ramp to 250°C for 6 min, and then further increased to 280°C for 22 min. Before analysis, the extract was prepared using the described procedure and filtered through syringe filters. A 2 mL sample injection volume was used for GC-MS analysis [29,30].

SwissADME

The absorption, distribution, metabolism, and excretion (ADME) properties of the compounds from *H. costaricensis* were predicted using the SwissADME software (www.swissadme.ch) [31].

In silico docking study

Molecular docking analysis was performed using Auto Dock software [32,33].

Brine shrimp lethality (BSL) bioassay [34,35]

Preparation of sea-water

A total of 38 g of non-iodized sea salt and 6 mg of dried yeast were weighed, dissolved in 1 L of distilled water, and then filtered to obtain a clear solution.

Hatching of brine shrimp

Fifty milligrams of *Artemia salina* (brine shrimp eggs) were added to one side of a container containing 1 L of artificial seawater. The container was then covered with aluminum foil and placed in a dark area for 48 h to facilitate hatching. A continuous oxygen supply was maintained throughout the hatching period.

Preparation of stock and test solution

To prepare a 1000 μg/mL concentrated stock solution, 1 g of plant extracts (ETH and Aq.) was dissolved in 200 μL of pure dimethyl sulfoxide and seawater. From this stock solution, varying concentrations (10, 20, 30, 40, and 50 μg/mL) were serially diluted using seawater. The same procedure was followed for the standard drug 5-fluorouracil, with serial dilutions at concentrations of 0.5, 1, 1.5, 2, and 2.5 μg/mL.

Experimental procedure

Ten live *Brine shrimp nauplii* were carefully transferred using a micropipette into a test tube containing 2.5 mL of simulated seawater. Then, 2.5 mL of the plant extract solution (ETH, Aq.) was added to each test tube, which had been prepared with serial dilutions. A control test tube was also prepared, containing 10 live nauplii in 5 mL of simulated seawater, and was kept in a dark area. After 24 h, the test tubes were examined under a magnifying glass, and the number of surviving nauplii was recorded. The lethal concentration (LC_{50}) value was then determined.

The percentage mortality was calculated using the following formula:

$$\% \text{ Mortality} = \frac{\text{No. of Naupalii taken} - \text{No. of live Naupalii}}{\text{No. of Naupalii taken}} \times 100$$

Statistical analysis

All the data were analyzed using GraphPad Prism 8 and were expressed as mean±standard deviation. The difference between experimental groups was compared by one-way analysis of variance followed by Dunnette's test. The $p<0.05$ values were considered statistically significant.

RESULTS

Phytochemical evaluation

Evaluation of phytochemicals represented the presence of carbohydrates, alkaloids, flavonoids, tannins, proteins, and steroids in ETH and Aq. Extracts, respectively, as represented in Table 1.

GC-MS analysis

The GC-MS analysis of the ETH extract of *H. costaricensis* leaves identified a total of 19 bioactive compounds, each characterized by their retention time, molecular weight (MW), and molecular formula (Table 2). The presence of these compounds indicates the potential pharmacological and therapeutic properties of the extract.

SwissADME

The characterization and analysis of the phytoconstituents of *H. costaricensis* provide valuable insights into their potential biological activities and pharmacokinetic properties as represented in Tables 3-9.

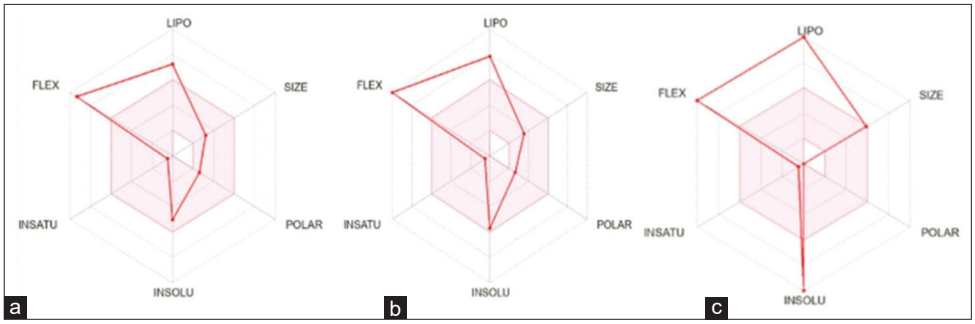


Fig. 1: Bioavailability radar for (a) n-hexadecanoic acid, (b) octadecanoic acid, (c) 17-pentatriacontene

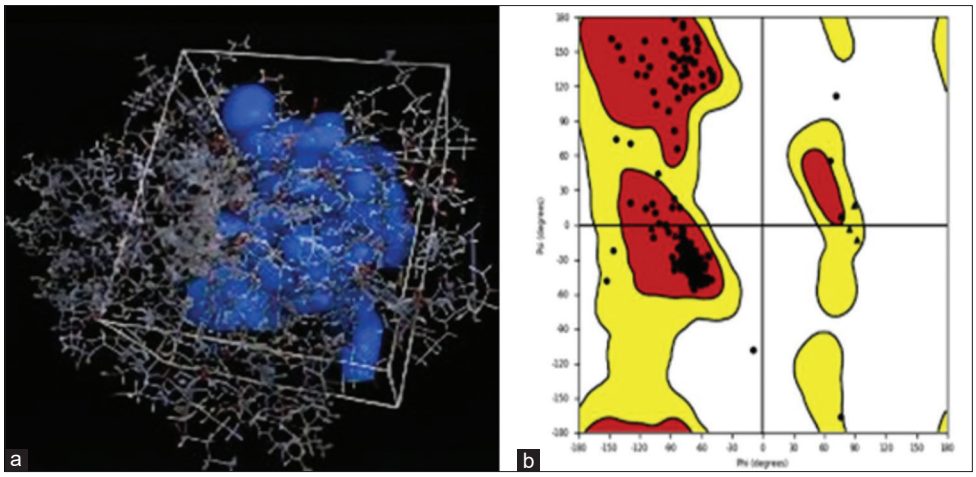


Fig. 2: (a) Amino acid cavity of glucose regulatory protein 78 receptor (b) Ramachandran plot

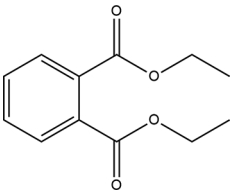
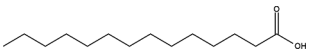
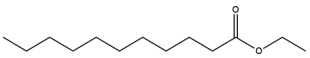
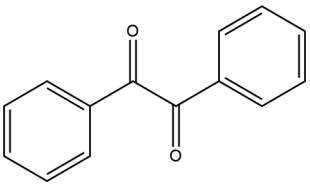
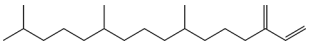
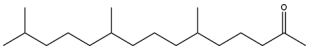
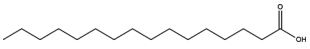
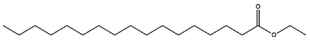
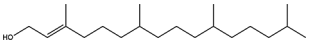
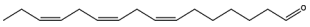
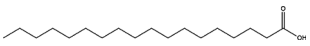
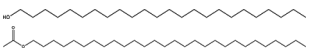
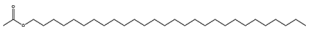
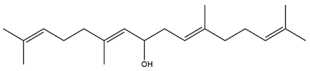
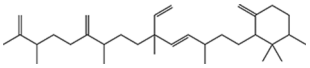
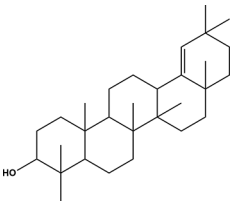
Table 1: Phytochemical screening of ethanol and aqueous extracts of *Hylocereus costaricensis* leaf

S. No.	Phytoconstituents	Test	ETH	Aq.
1	Carbohydrate	Molish test	+	++
		Fehling's test	++	++
		Benedict's test	+	++
2	Alkaloids	Dragendorff's test	++	+
		Mayer's test	+	+
		Hanger's test	++	++
		Wagner's test	++	+
3	Flavonoids	Shinoda's test	++	++
		Lead acetate test	+	+
4	Tannins and phenols	Ferric chloride test	++	++
		Lead acetate solution	+	+
5	Proteins	Millions test	+	++
		Xanthoprotein test	+	++
		Ninhydrin test	+	++
6	Steroids	Salkowski's reaction	++	+
7	Test for gums	Fehling test	+	+
		Benedict's test	+	+

ETH: Ethanolic, Aq.: aqueous. (– absent, +present, ++ strongly present)

The GC-MS analysis identified three major compounds: n-hexadecanoic acid, octadecanoic acid, and 17-pentatriacontene. These compounds possess distinct molecular structures and properties, with MWs ranging from 256.42 g/moL to 490.53 g/moL. The molecular formulas and canonical SMILES representation confirm their chemical structures, which are known to contribute to their biological functions as shown in Table 3. The physicochemical properties of these compounds reveal their structural characteristics. All three compounds exhibit a high fraction of Csp3 (0.94), indicating a significant aliphatic nature. Their hydrogen bond acceptor and donor counts suggest their ability to participate in biochemical interactions, influencing their bioavailability and absorption (Table 4) [36,37]. The lipophilicity assessment using multiple log P models (iLOGP, XLOGP3, WLOGP, etc.) indicated that 17-pentatriacontene has the highest lipophilicity (Consensus Log p=13.13), suggesting it is highly hydrophobic and may have limited Aq. Solubility. In contrast, n-hexadecanoic acid and octadecanoic acid exhibit moderate lipophilicity, making them more likely to interact with lipid membranes (Table 5) [38]. Solubility predictions classify n-hexadecanoic acid and octadecanoic acid as moderately soluble, while 17-pentatriacontene is categorized as insoluble due to its highly non-polar nature. This aligns with its high log p-values, indicating its tendency to partition into lipid environments rather than Aq. media (Table 6). Both n-hexadecanoic acid and octadecanoic acid show high gastrointestinal (GI) absorption, suggesting good oral bioavailability. However, 17-pentatriacontene exhibits low GI absorption, likely due to its large molecular size and high lipophilicity [39]. n-hexadecanoic acid is blood–brain barrier permeant, indicating potential central nervous system activity. These compounds exhibit varied inhibition of CYP enzymes, with n-hexadecanoic acid inhibiting CYP1A2 and CYP2C9, potentially affecting drug metabolism. The negative values for n-hexadecanoic acid and octadecanoic acid suggest moderate skin penetration, while 17-pentatriacontene shows enhanced skin permeability (Log kP=3.66), indicating potential topical applications (Table 7). Lipinski's rule of five and other drug-likeness filters suggest that n-hexadecanoic acid and octadecanoic acid mostly comply with drug-likeness rules, whereas 17-pentatriacontene violates multiple parameters (e.g., high MW, excessive rotatable bonds), reducing its potential as an orally active drug. However, all three compounds exhibit a moderate to high bioavailability score (0.55–0.85) (Table 8). The pan-assay interference compounds (PAINS) and Brenk filters indicate that all three compounds have zero PAINS alerts, meaning they are less likely to interfere with biological assays. However, 17-pentatriacontene has a Brenk alert (isolated alkene group), which may affect its reactivity. Synthetic accessibility scores suggest that n-hexadecanoic acid and

Table 2: GC-MS analysis of the ethanolic extract of *Hylocereus costaricensis* leaf

S. No.	Ret. time	Name of compound	Molecular weight	Molecular formula	Chemical structures
1	18.959	Diethyl phthalate	222	$C_{12}H_{14}O_4$	
2	20.887	Tetradecanoic acid	228	$C_{14}H_{28}O_2$	
3	21.247	Undecanoic acid, ethyl ester	214	$C_{13}H_{26}O_2$	
4	21.605	Ethanedione, diphenyl-	210	$C_{14}H_{10}O_2$	
5	21.729	Neophytadiene	278	$C_{20}H_{38}$	
6	21.783	2-Pentadecanone, 6,10,14-trimethyl-	268	$C_{18}H_{36}O$	
7	22.954	n-Hexadecanoic acid	256	$C_{16}H_{32}O_2$	
8	23.299	Heptadecanoic acid, ethyl ester	298	$C_{19}H_{38}O_2$	
9	24.451	3,7,11,15-Tetramethyl-2-hexadecen-1-ol	296	$C_{20}H_{40}O$	
10	24.663	cis, cis, cis-7,10,13-Hexadecatrienal	234	$C_{16}H_{26}O$	
11	24.875	Octadecanoic acid	284	$C_{18}H_{36}O_2$	
12	26.058	n-Nonadecanol-1	284	$C_{19}H_{40}O$	
13	27.722	n-Tetracosanol-1	354	$C_{24}H_{50}O$	
14	28.636	Octacosyl acetate	452	$C_{30}H_{60}O_2$	
15	29.467	17-Pentatriacontene	490	$C_{35}H_{70}$	
16	30.620	Octacosyl acetate	452	$C_{30}H_{60}O_2$	
17	30.705	trans-Geranylgeraniol	290	$C_{20}H_{34}O$	
18	31.611	1,1,6-trimethyl-3-methylene-2-(3,6,9,13-tetramethyl-6-ethenye-10,14-dimethylene-pentadec-4-enyl) cyclohexane	452	$C_{33}H_{56}$	
19	32.068	Germanicol	426	$C_{30}H_{50}O$	

GC-MS: Gas chromatography-mass spectrometry

Table 3: General characteristics of the phytoconstituents of *Hylocereus costaricensis* leaf

S. No.	Name of compound	PubChem ID	Molecular formula	Canonical SMILES	Molecular weight g/moL
1	n-Hexadecanoic acid	985	$C_{16}H_{32}O_2$	CCCCCCCCCCCCCCCC(=O) O	256.42
2	Octadecanoic acid	5281	$C_{18}H_{36}O_2$	CCCCCCCCCCCCCCCCCC(=O) O	284.48
3	17-Pentatriacontene	97997	$C_{35}H_{70}$	CCCCCCCCCCCCCCCCCC=CCCCCCCCCCCCCCCCC	490.53

octadecanoic acid are relatively easy to synthesize (scores 2.3–2.5), while 17-pentatriacontene is more complex (score=5.57) (Table 9).

Molecular docking

To assess the binding affinities and interactions of phytoconstituents derived from *Hylocereus costaricensis* with the GRP78 receptor, a molecular docking analysis was performed. Important residues involved in ligand binding were discovered when the amino acid cavity of the GRP78 receptor was identified (Fig. 2a). Additionally, the protein's structural quality was validated by the Ramachandran plot (Fig. 2b), confirming its suitability for docking studies. The docking scores and interaction profiles of the top three compounds are compiled in Table

Table 4: Phytochemical properties of the phytoconstituents of *Hylocereus costaricensis* leaf

S. No.	Name of compound	Num. heavy atom	Num. arom. heavy atoms	Fraction Csp3	Num. rotatable bonds	Num. H-bond acceptors	Num. H-bond donors	Molar refractivity	TPSA ($^0A^2$)
1	n-Hexadecanoic acid	18	0	0.94	14	**2**	1	80.80	37.30
2	Octadecanoic acid	20	0	0.94	16	2	1	9041	37.30
3	17-Pentatriacontene	35	0	0.94	31	0	0	169.89	0.00

Table 5: Lipophilicity characteristic of the phytoconstituents of *Hylocereus costaricensis* leaf

S. No.	Name of compound	iLOGP	XLOGP3	WLOGP	MLOGP	SILICOS-IT	Consensus Log Po/w
1	n-Hexadecanoic acid	3.85	7.17	5.55	4.19	5.25	5.20
2	Octadecanoic acid	4.30	8.23	6.33	4.67	6.13	5.93
3	17-Pentatriacontene	9.06	18.24	13.68	10.23	14.45	13.13

Table 6: Water solubility characteristic of the phytoconstituents of *Hylocereus costaricensis* leaf

S.No.	Name of molecules	ESOL				Ali				SILICOS-IT			
		LOG S (ESOL)	Solubility		Class	Log s (Ali)	Solubility		Class	Log s SILICOS -IT	Solubility		Class
			mg/mL	mol/L			mg/mL	moL/L			Mg/ML	moL/L	
1	n-Hexadecanoic acid	−5.02	2.43e−03	9.49e−06	MS	−7.77	4.31e−06	1.68e−08	PS	−5.31	1.25e−0.3	4.88e−06	MS
2	Octadecanoic acid	−5.73	5.26e−04	1.85e−06	MS	−8.87	3.80e−07	1.33e−0.9	PS	−6.11	2.19e−04	7.71e−07	PS
3	17-Pentatriacontene	−12.33	2.30e−10	4.69e−13	IS	−18.48	1.63e−16	3.32e−19	IS	−13.12	3.69e−11	7.52e−14	IS

Table 7: Pharmacokinetic parameters of the phytoconstituents of *Hylocereus costaricensis* leaf

S No.	Name of molecules	GI absorption	BBB permeant	P-gp substrate	CYP1A2 inhibitor	CYP2C19 inhibitor	CYP2C9 inhibitor	CYP2D6 inhibitor	CYP3A4 inhibitor	LOG k_p (skin permeation) (cm/s)
1	n-Hexadecanoic acid	High	Yes	No	Yes	No	Yes	No	No	−2.77
2	Octadecanoic acid	High	No	No	Yes	No	No	No	No	−2.19
3	17-Pentatriacontene	Low	No	Yes	No	No	No	No	No	3.66

GI: Gastrointestinal, BBB: Blood-brain barrier

Table 8: Drug likeness rule and bioavailability of the phytoconstituents of *Hylocereus costaricensis* leaf

S. No.	Name of molecules	Lipinski	Ghose	Veber	Egan	Muegge	Bioavailability score
1	n-Hexadecanoic acid	Yes; 1 violation; MLOGP>4.15	Yes	No; 1 violation; Rotors>10	Yes	No; 1 Violation; XLOGP3>5	0.85
2	Octadecanoic acid	Yes; 1 violation: MLOGP>4.15	No; 1 violation: WLOGP>5.6	No; 1 violation: Rotors>10	No; 1 violation: WLOGP>5.88	No; 2 violations: XLOGP3>5, Rotors>15	0.85
3	17-Pentatriacontene	Yes; 1 violation: MLOGP>4.15	No; 4 violations: MW>480, WLOGP>5.6, MR>130, #atoms>70	No; 1 violation: Rotors>10	No; 1 violation: WLOGP>5.88	No; 3 violations: XLOGP3>5, Heteroatoms<2, Rotors>15	055

Table 9: Medicinal chemistry properties of the phytoconstituents of *Hylocereus costaricensis* leaf

S. No.	Name of molecules	Pains	Brenk	Leadlikeness	Synthetic accessibility
1	n-Hexadecanoic acid	0 alert	0 alert	No; 2 violations; Rotors>7; XLOGP3>3.5	2.31
2	Octadecanoic acid	0 alert	0 alert	No; 2 violations: Rotors>7, XLOGP3>3.5	2.54
3	17-Pentatriacontene	0 alert	1 alert isolated _alkene	No; 2 violations: MW-350, Rotors>7, XLOGP3>3.5	5.57

10. With a docking score of −12.248 kcal/mol, n-hexadecanoic acid showed the strongest binding affinity among them, suggesting a very positive interaction with the GRP78 receptor. With a docking score of −10.408 kcal/mol, octadecanoic acid also demonstrated a strong binding affinity, while 17-Pentatriacontene had a lower score of −7.135 kcal/mol.

Table 10: Molecular docking analysis of *Hylocereus costaricensis* leaf

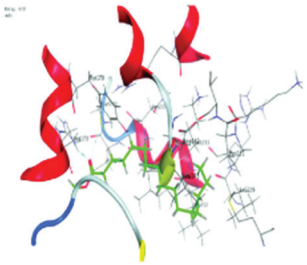
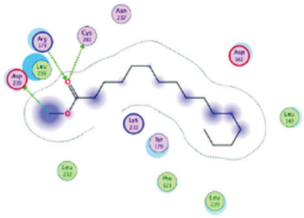
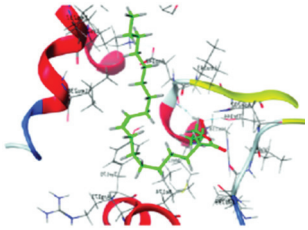
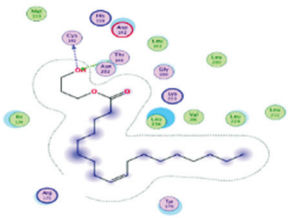
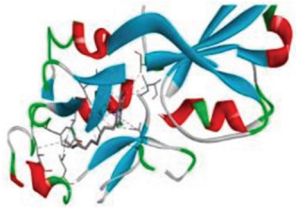
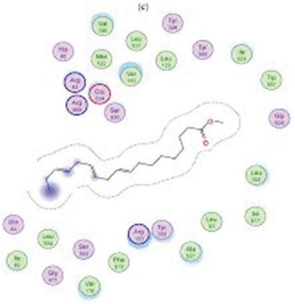
S. No.	Docking score (KcaL/mo)	3D Interaction of compound	2D Interaction of compound
1	−12.248		
		n-Hexadecanoic acid	
2	−10.408		
		Octadecanoic acid	
3	−7.135		
		17-Pentatriacontene	

Table 11: Effect of ethanolic and aqueous extract of *Hylocereus costaricensis* leaves on percentage mortality by using brine shrimp cytotoxic assay

S. No.	Sample	Concentration (µg/mL)	No. of dead nauplii out of 10			Dead nauplii out of 30	Live nauplii out of 10	% Mortality mean±SD	LC_{50} (µg/mL)
			T1	T2	T3				
1	Ethanolic	10	5	5	3	13	17	43.33±0.66	18.57
2	extract	20	5	5	4	14	16	46.66±033	
3		30	7	7	5	19	11	63.33±0.66	
4		40	7	7	9	23	7	76.66±0.66*	
5		50	8	9	9	26	4	83.33±0.66**	
1	Aqueous	10	6	5	4	16	14	50±0.94	6.6
2	extract	20	4	8	7	19	11	63.33±0.78	
3		30	7	7	7	21	9	70±0.29	
4		40	8	8	7	23	7	76.66±0.36*	
5		50	8	9	8	25	5	86.66±0.33**	
1	5-Fluorouracil	0.5	4	5	5	14	16	46.66±0.33	0.594
2		1	5	6	7	18	12	60±0.57	
3		1.5	6	7	8	21	9	70±0.57	
4		2	7	8	9	24	6	80±0.57	
5		2.5	8	9	10	27	3	90±0.57	

Values are represented as (Mean±SD) (n=3), where (*p<0.05, **p<0.01, ***p<0.001). Bioassay was done in triplicate; each concentration was performed for 3 replicates

According to their individual 3D and 2D interaction diagrams, each compound showed distinct interactions within the receptor's;s active site. These illustrations show how van der Waals forces, hydrogen bonds, and hydrophobic interactions all contribute to the overall stability of the binding.

The bioavailability radar profiles of the compounds n-hexadecanoic acid, octadecanoic acid, and 17-Pentatriacontene from *Hylocereus costaricensis* are shown in Fig. 1. Key physicochemical characteristics that affect drug-likeness and oral bioavailability are assessed by each radar chart, including lipophilicity (LIPO), size, polarity, solubility (INSOLU), saturation (INSATU), and flexibility (FLEX). Moderate drug-likeness was indicated by the balanced profiles of n-hexadecanoic acid and octadecanoic acid, which had acceptable lipophilicity and flexibility

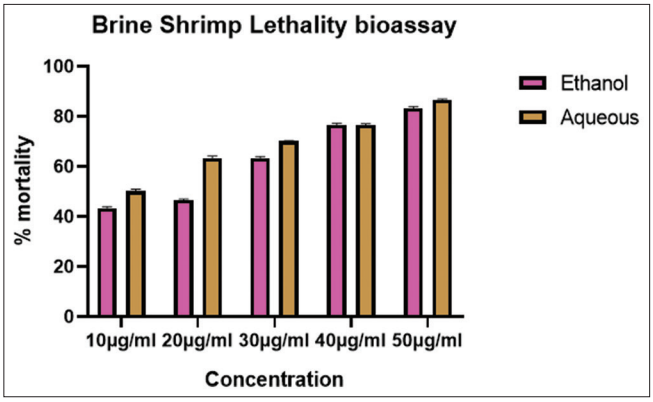


Fig. 3: Effect of ethanolic and aqueous extracts of *Hylocereus costaricensis* on percent mortality by using brine shrimp lethality bioassay

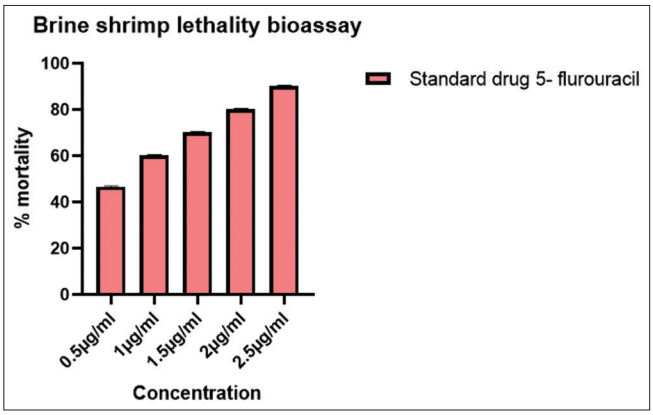


Fig. 4: Effect of standard drug (5-fluorouracil) on *Hylocereus costaricensis* on percent mortality by using brine shrimp lethality bioassay

but somewhat lower solubility and high saturation. Despite having good lipophilicity and flexibility, 17-Pentatriacontene showed extreme insolubility and poor polarity, which may limit its bioavailability. These radar plots help rank candidates for additional pharmacokinetic analysis and offer preliminary information about the compounds' drug-like characteristics.

BSL bioassay

The brine shrimp cytotoxic assay was performed to evaluate the cytotoxic potential of ETH and Aq. extracts of *H. costaricensis* leaves. The results, as presented in Table 11, Figs. 3 and 4, demonstrate a dose-dependent increase in the percentage mortality of brine shrimp nauplii upon exposure to both extracts. 5-fluorouracil, a known chemotherapeutic agent, was used as a positive control. It exhibited potent cytotoxicity, with mortality rates increasing from 46.66±0.33% at 0.5 µg/mL to 90±0.57% at 2.5 µg/mL. The LC_{50} value was 0.594 µg/mL, confirming its significant cytotoxic activity.

ETH extract

The ETH extract exhibited significant cytotoxicity, with mortality rates ranging from 43.33±0.66% at 10 µg/mL to 83.33±0.66% at 50 µg/mL. The LC_{50} value for the ETH extract was determined to be 18.57 µg/mL, indicating moderate toxicity. Notably, at higher concentrations (40 and 50 µg/mL), the mortality rates were significantly increased ($p<0.05$ and $p<0.01$, respectively), suggesting a significant cytotoxic potential.

Aq. extract

The Aq. extract also demonstrated cytotoxicity, with percentage mortality ranging from 50±0.94% at 10 µg/mL to 86.66±0.33% at 50 µg/mL. The LC_{50} value was 6.6 µg/mL, indicating a higher toxicity compared to the ETH extract. Similar to the ETH extract, a dose-dependent increase in mortality was observed, with significant mortality at 40 and 50 µg/mL ($p<0.05$ and $p<0.01$, respectively).

Comparing the LC_{50} values, the Aq. extract (6.6 µg/mL) demonstrated higher toxicity than the ETH extract (18.57 µg/mL), suggesting the presence of more potent bioactive compounds in the Aq. fraction. However, both extracts exhibited lower toxicity than 5-fluorouracil, which had the lowest LC_{50} value (0.594 µg/mL). The dose-dependent mortality observed in both extracts indicates their potential cytotoxic effects, which may be attributed to the presence of bioactive secondary metabolites such as flavonoids, tannins, and alkaloids.

DISCUSSION

Phytochemical screening of *H. costaricensis* leaf ETH and Aq. extracts revealed the presence of carbohydrates, alkaloids, flavonoids, tannins, proteins, and steroids. These bioactive compounds contribute to the plant's pharmacological properties, including antioxidant and cytotoxic potential [40]. The qualitative analysis indicated that the ETH extract contained a higher concentration of phytoconstituents than the Aq. extract (Aq.), prompting further GC-MS analysis of the ETH extract to identify its major components.

GC-MS analysis identified three major compounds in the ETH extract: n-Hexadecanoic acid, octadecanoic acid, and 17-pentatriacontene. These compounds are known to exhibit biological activities, including anticancer, anti-inflammatory, and antimicrobial properties. Among these, polyphenols and flavonoids present in the extract are known for their strong antioxidant and cytotoxic effects, primarily by scavenging free radicals and inhibiting tumor vascularization and metastasis [41-43].

To assess the drug-likeness and pharmacokinetic properties of the identified phytoconstituents, an *in silico* ADME study was conducted using SwissADME software. The evaluation included various parameters such as general characteristics, lipophilicity, water solubility, pharmacokinetics, bioavailability, and drug-likeness. Results showed that n-hexadecanoic acid and octadecanoic acid displayed high GI absorption, moderate lipophilicity, and good drug-likeness profiles, making them promising candidates for further drug development. In contrast, 17-Pentatriacontene exhibited low GI absorption and poor water solubility, limiting its potential for oral drug formulations. To further validate the cytotoxic potential, molecular docking studies were performed against the glucose regulatory protein 78 (GRP78) receptor, a key protein involved in cancer progression and drug resistance. AutoDock software was used to determine the binding affinity of n-hexadecanoic acid, octadecanoic acid, and 17-pentatriacontene with GRP78. The results revealed that n-hexadecanoic acid exhibited the highest binding energy (−12.248 kcaL/moL), followed by octadecanoic acid (−10.408 kcaL/moL) and 17-pentatriacontene (−7.135 kcaL/moL). These findings suggest that n-hexadecanoic acid has the strongest potential for cytotoxic activity, making it a promising lead compound for further investigation. In addition, protein-ligand interactions were analyzed to identify potential active binding sites (BS). The docking analysis effectively mapped these active sites (AS) and secondary BS, providing insights into ligand interactions. The Ramachandran plot analysis, which evaluates the conformational stability of protein structures, further supported the validity of the docking results.

The cytotoxic potential of the extract was further evaluated using the BSL bioassay. The standard anticancer drug 5-fluorouracil exhibited an LC_{50} value of 0.59 µg/mL, whereas the ETH extract demonstrated an LC_{50} of 18.57 µg/mL and the Aq. extract showed an LC_{50} of 6.6 µg/mL. The lower LC_{50} value of the Aq. extract suggests higher cytotoxic activity compared to the ETH extract, though both extracts exhibited potential cytotoxic effects.

The *in silico* ADME and molecular docking analyses identified n-hexadecanoic acid and octadecanoic acid as promising lead

compounds, with favorable pharmacokinetic properties and strong binding affinities toward the GRP78 receptor, a target associated with cancer progression. Among them, n-hexadecanoic acid showed the highest binding energy, indicating strong interaction and potential cytotoxicity. These computational findings are supported by the BSL bioassay, which demonstrated significant cytotoxicity for both ETH and Aq. extracts. Notably, the Aq. extract exhibited a lower LC_{50} (6.6 µg/mL) compared to the ETH extract (18.57 µg/mL), suggesting higher *in vitro* cytotoxic activity. Together, the *in silico* and *in vitro* results reinforce the therapeutic potential of these phytoconstituents, especially n-hexadecanoic acid, as candidates for anticancer drug development.

CONCLUSION

The phytochemical analysis, ADME studies, molecular docking investigations, and *in vitro* cytotoxicity assays, collectively indicated that *H. costaricensis* leaves contain bioactive compounds with potential cytotoxic properties. Among the identified compounds, n-hexadecanoic acid exhibited the most significant cytotoxic activity, as evidenced by its strong binding affinity with the GRP78 receptor. These findings support further *in vivo* and clinical investigations to develop potential anticancer therapeutics derived from *H. costaricensis* leaf extracts.

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AUTHORS' CONTRIBUTION

DS conceptualized and designed the study, with AT handling data collection. JS conducted data analysis and prepared the initial draft of the article. NT and KP supervised the study, contributed to data analysis and interpretation, and provided essential revisions. All authors have reviewed and approved the final version of the manuscript.

CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

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