RESEARCH PAPER

In Vitro Assessment of Anticancer and Antiulithiatic Potential of Canna Indica L. Flowers

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ABSTRACT

The present study was designed to evaluate the anticancer and antiurolithiatic potential of *Canna indica* L. along with molecular docking analysis to identify possible bioactive interactions. Anticancer activity was assessed using in vitro cytotoxicity assays against selected cancer cell lines, while antiurolithiatic activity was evaluated through inhibition of calcium oxalate crystallization. The extract exhibited significant anticancer activity with an IC_{50} value of 60.24 µg/mL and a maximum inhibition of 60% at 100 µg/mL concentration. It also showed considerable antiurolithiatic activity with 75% inhibition of calcium oxalate crystal nucleation and aggregation and 50% dissolution activity. Molecular docking studies revealed strong binding affinities of phytoconstituents such as rutin and oleanolic acid with target proteins, showing docking scores ranging from −7.0 to −9.4 kcal/mol. The findings suggest that *Canna indica* L. possesses promising anticancer and antiurolithiatic therapeutic potential.

KEYWORDS: Anticancer activity, Antiurolithiatic activity, *Canna indica* L., MTT assay, MCF-7 cell line.

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INTRODUCTION

A collection of illnesses known as cancer are distinguished by the unchecked proliferation and dissemination of aberrant cells. Breast, lung, liver, and colorectal cancers are among the main cancer kinds that occur often[1]. Stones that develop in the urinary tract, either in the upper tract means kidneys, ureter or lower tract means bladder, urethra, are referred to as urolithiasis. Plant-based diets are associated with a lower risk of stone development and recurrence. After infections and problems with the prostate, it is the third most prevalent urinary tract ailment[2]. Although conventional medications are frequently used to treat conditions including cancer and urolithiasis, their adverse effects have raised interest in ethnobotanical treatments. Secondary plant metabolites, such as alkaloids, terpenes, flavonoids, sterols, anthocyanins, and phenolics, are the source of many contemporary medications and represent crucial leads in drug discovery[3]. Since medicinal plants are thought to be safer and less damaging to the human body, research into their antiurolithiatic and anticancer properties is growing[1].

Fig. 1: *Canna indica* L.

The tropical herb *Canna indica* L., (Fig. 1) which is a member of the Cannaceae family, it is recognized by the common names Indian shot, African arrowroot, and purple arrowroot. *C. indica* is although originally from Mexico, the West Indies, Central America, and South America, they have naturalized in Europe, sub-Saharan Africa, Southeast Asia, and Oceania[4].

Taxonomical classification was shown in Table1.

Table 1: Taxonomical classification of *Canna indica* L.[5].

Kingdom:	**Plantae**
Subkingdom:	Tracheobiont
Superdivision:	Spermatophyta
Division:	Magnoliophyta
Class:	Liliopsida
Subclass:	Zingiberidae
Order:	Zingiberales
Family:	Cannaceae
Genus:	Canna
Species:	*indica*

Table. 2: Vernacular names[5]

Language	**Name**
Andes	Achira
English	Canna
French	Balisier
Hindi	Saka siri, Devkali
Marathi	Kardal
Sanskrit	Vankelee
Spanish	Chupa flor

Canna indica L. is a perennial herb that grows up to 1.5 m in height and flowers from August to October. The plant bears hermaphrodite flowers containing both female and male reproductive parts[6]. Traditionally, *C. indica* has been used for the treatment of malaria, diarrhea, dysentery, fever, dropsy, cuts, and bruises. Different plant parts were also reported to possess diuretic and digestive properties[7].

Chemical Constituents:

A phytochemical analysis revealed that the *C. indica* flower included a variety of phytochemicals, including as alkaloids, carbohydrates, proteins, flavonoids, terpenoids, cardiac glycosides, steroids, tannins, saponins, and phlobatannins. The fruit's red color is caused by flavonoids, anthocyanins, and phenols. These anthocyanins include cyanidin-3-O-(600-O-a-rhamnopyranosyl)-b-glucopyranoside, cyanidin-3-O-(600-O-a-rhamnopyranosyl)-b-galactopyranoside, cyanidin-3-O-b-glucopyranoside, and cyanidin-O-b-galactopyranoside. Flowers were shown to contain rutin, neochlorogenic, chlorogenic, and caffeic acids[4,8,9].

MTT Assay

The MTT assay is the cell viability method that is widely used worldwide. Tim Mosmann originally described it in 1983. This colorimetric technique measures cellular metabolic activity as an indicator of cell viability by reducing a yellow tetrazolium salt (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide, or MTT). The reagent is reduced to formazan, an insoluble crystalline product with a deep purple color, by NAD(P)H-dependent oxidoreductase enzymes found in viable cells. A solubilizing solution is then used to dissolve the formazan crystals, and a plate reader is used to measure the absorbance at 500–600 nm. There are more live and metabolically active cells in a darker solution[10].

MATERIALS AND METHODS

Collection and authentication of plant

Fresh flowers of *Canna indica* L. (500 g) were collected in October 2025 from Sangli, Maharashtra, India, during the flowering season. The plant material was authenticated by a qualified botanist, Dr. Sanjay S. Sathe. The collected flowers were washed thoroughly with distilled water to remove adhering impurities and shade-dried at room temperature until a constant weight was attained. The dried flowers were pulverized to a coarse powder using a mechanical grinder and stored in an airtight, light-resistant container at room temperature until further use.

Organoleptic evaluation

The flowers of *Canna indica* L. were subjected to macroscopic evaluation for the determination of organoleptic and morphological characteristics. The study included observation of color, odor, taste, surface characteristics, shape, and size of the crude drug[11].

Microscopic characteristics:

Transverse section

Fresh flowers of *Canna indica* L. were washed with distilled water, and thin transverse sections were prepared manually using a sharp razor blade. The sections were stained with phloroglucinol–hydrochloric acid reagent and mounted in glycerin

on glass slides. The prepared slides were examined under a compound light microscope at different magnifications, and photomicrographs were recorded for anatomical evaluation.

Powder characteristics

The shade-dried flowers of *Canna indica* L. were powdered and subjected to powder microscopy according to standard pharmacognostic procedures. A small quantity of the powder was treated with suitable reagents, including phloroglucinol–hydrochloric acid and glycerin, mounted on a glass slide, and examined under a compound light microscope. Characteristic diagnostic features were observed and photographed for identification of the crude drug.

Physiochemical evaluation:

The ash values and extractive values of the dried leaves and stems were determined using standard procedures[11].

Preparation of plant extract

The dried leaves of *Canna indica* L. were powdered using a mechanical grinder. 30 g of the powdered material were extracted with 300 mL of ethanol using a Soxhlet apparatus for 2 h. The obtained extract was filtered and concentrated by evaporation of the solvent under reduced pressure. The concentrated extract was further dried to obtain a crude ethanolic extract. Extracted percent yield of the ethanol fraction (*C. indica* L.) was 18%. The dried extract was stored in an airtight container at 4°C until further use.

Preliminary phytochemical screening

Several biochemical tests were used to perform the initial phytochemical assessment of the extracts in accordance with the described procedure[12].

Molecular docking:

Molecular docking is a computational method used to predict the binding orientation and interaction of a ligand within the active site of a target receptor. It helps to evaluate the binding affinity of compounds, identify key molecular interactions, and support the design of more effective therapeutic agents. The docking process involves the generation and evaluation of multiple ligand conformations to determine the most favorable binding pose within the receptor binding site[13].

Receptor Preparation

The crystal structures of the Progesterone receptor (PDB ID: 4OAR) and human uromodulin/Tamm–Horsfall protein (PDB ID: 4WRN) were retrieved from the Protein Data Bank. The protein structures were prepared using BIOVIA Discovery Studio by removing water molecules, co-crystallized ligands, and other heteroatoms. Polar hydrogen atoms were added, and the proteins were further processed in AutoDock Tools by assigning Gasteiger charges. The prepared receptor structures were saved in PDBQT format and used for molecular docking studies[14].

Ligand preparation

The structures of Rutin, Betulinic acid, Oleanolic acid, Cyanidin-3-O-β-glucopyranoside, and Quercetin were retrieved from the PubChem database in SDF format. The ligands were converted into three-dimensional structures and energy minimized to obtain stable conformations. The optimized structures were imported into AutoDock Tools, where Gasteiger charges were assigned and rotatable bonds were defined. The prepared ligands were then saved in PDBQT format for molecular docking studies using AutoDock Vina.

Docking analysis

Molecular docking studies were performed using AutoDock Vina to evaluate the binding interactions of selected phytoconstituents with the target proteins 4OAR and 4WRN. Rutin, Betulinic acid, Oleanolic acid, Cyanidin-3-O-β-glucopyranoside, and Quercetin were docked into the active sites of the prepared receptors. Multiple binding poses were generated, and the best conformation was selected based on the lowest binding energy and favorable orientation within the binding site. The docked complexes were visualized using BIOVIA Discovery Studio Visualizer, and the interactions, including hydrogen bonds, hydrophobic interactions, and electrostatic interactions, were analyzed[15].

Anticancer assay (MTT assay)

Procedure

The National Centre for Cell Sciences (NCCS) in Pune provided the human mammary gland breast cancer cell line (MCF-7), which was kept in Minimum Essential Medium (MEM) supplemented with 10% fetal bovine serum (FBS). The cells underwent incubation. In a humidified environment with 5% CO2 at 37°C. Cells were cultured for 24 hours after being seeded at a density of 1×10^3 cells/mL for the test. 96-well tissue culture plates were then filled with 70 µL of the cell suspension, which contained roughly 1×10^3 cells/well. 100 µL of culture media was then added. The wells were filled with test samples at different concentrations (10–100 µg/mL). The cell line and 0.2% DMSO in PBS were added to the control wells. Every treatment was carried out in triplicate, including the controls. After that, the plates were kept in an incubator with 5% CO2 for a whole day at 37°C.

Following incubation, each well received 20 µL of MTT reagent (5 mg/mL in PBS) after the culture media was properly removed. The plates were then incubated at 37°C for a further four hours. Under a

microscope, dark purple formazan crystals were seen to develop as a result of metabolically active cells reducing yellow MTT. The formazan crystals were dissolved by adding 200 µL of DMSO to each well after the media was entirely removed after incubation. Aluminium foil was used to shield the plates from light while they were incubated for ten minutes at 37°C.

An ELISA microplate reader was used to measure each well's absorbance at 570 nm. By comparing the absorbance of treated samples with that of control cells, cell viability was ascertained[16-18].

Percentage viability can be calculated using the formula:

$$\%viability = 100 \frac{O.D\ Test\ item}{O.D\ of\ Control}$$

$$\%\ Activity = 100 - \%\ Viability$$

Antiurolithiatic activity

Preparation of synthetic kidney stones

A homogeneous precipitation approach was used to create the calcium oxalate (CaOx) experimental kidney stones. First, 100 mL of distilled water were used to dissolve 1.47 grams of calcium chloride dihydrate. In a different experiment, 100 mL of 1 M sulfuric acid was used to dissolve 1.34 g of sodium oxalate. To enable the precipitation of calcium oxalate, these two solutions were combined in a beaker and agitated. To get rid of the sulfuric acid and free the crystals, ammonia solution was added. After that, the crystals were cleaned with distilled water and dried at 60°C. The artificially created kidney stone model was used for additional research [19].

Nucleation assay

Aqueous solutions of sodium oxalate (7.5 mM) and calcium chloride (5 mM) were used for the calcium oxalate crystallization test. In short, 1 mL of the test extract at varying concentrations (5, 7, and 10 mg/mL) was combined with 9 mL of calcium chloride solution. 950 µL of sodium oxalate solution was added to start the crystallization process. For thirty minutes, the reaction mixture was incubated at 37 °C (Fig. 2). A spectrophotometer was used to detect the optical density at 620 nm after incubation. By comparing the induction time in the presence of the test samples with the control, the rate of nucleation was ascertained. (absence of extract)[20]. It was anticipated that crystal formation would result from the following reaction:

$$CaCl_2 + Na_2C_2O_4 \rightarrow CaC_2O_4 + 2NaCl$$

$$\%\ inhibition = \frac{absorbance\ of\ control\ - absorbance\ of\ test}{absorbance\ of\ control} \times 100$$

Egg membrane assay

Preparation of Semi-Permeable Membrane:

An egg was used to create a semipermeable membrane (Fig. 2). A glass rod was used to penetrate the egg's apex, and the contents were extracted entirely. After giving the empty shells, a thorough rinse with distilled water, they were submerged in a solution that contained 4.0 mL of 200 mL of pure water were mixed with strong hydrochloric acid (HCl). To fully decalcify the shells, they were left in this solution for the entire night.

The next day, the membranes were carefully removed from the shells, thoroughly cleaned with distilled water, and treated with an ammonia solution to neutralize any remaining acid. Then, they were washed once more with distilled water and kept moist in a refrigerator with a pH between 7 and 7.4. As a negative control for the experiment, 10 mg of calcium oxalate was dissolved in 10 mL of distilled water. Aqueous extracts of the test samples were also made in 5 mL. Absolute ethanol was used to remove the coating off a 500 mg Cystone pill. leaving about 400 milligrams of the active ingredient. After that, the tablet was ground into a powder, combined with 100 mL of purified water, and filtered. Antiurolithiatic activity was assessed in vitro using the resultant filtrate as a positive control.

Fig. 2: Experimental setup for the egg membrane assay.

Estimation of calcium oxalate by using dissolution method:

In this experiment, the extract dissolves in the internal system of the already-formed stone nucleus. The synthetic calcium oxalate was created in a lab using a normal procedure. Using conventional techniques, a semipermeable membrane was created from an egg and synthetic calcium. After weighing the oxalate crystals, 1 ml of extract (10 mg/ml solution) was combined with them in a different egg membrane.

Every therapy was carried out three times. A secondary conical flask holding 100 mL of 0.1 M Tris buffer was suspended by the material packed in a semipermeable membrane. wrapped the membrane in aluminium foil after tying it with

thread. As a negative control, one group was used. For two hours, every flask was kept in an incubator at 37°C. Membrane was gathered in several test tubes. Next, two mL of 0.5M sulfuric acid were added to each tube. Titrate each test tube with 0.1 N KMnO4 until a pale pink hue is attained. Subtract the amount of calcium oxalate that is still undissolved to find the total amount of dissolved calcium oxalate by different extracts. calcium oxalate from the initial total amount used in the experiment. 0.1369 mg of calcium oxalate is equivalent to one mL of 0.02 M KMnO4[21].

Statistical analysis:

The IC_{50} values were calculated by linear regression using Excel Microsoft.

RESULT

Organoleptic evaluation

Table 3: Macromorphological Description of *Canna indica* L. flowers

S. No	Character	Observation
1	Colour	Bright red to orange-red (occasionally yellow varieties observed)
2	Inflorescence	White to pale yellow reproductive structures within bracts
3	Odour	Mild, slightly aromatic
4	Taste	Slightly bitter and tongue-sensitizing
5.	Width	0.6 – 0.8 cm
6.	Length	1.2 – 1.8 cm
7.	Shape	Obovate
8.	Texture	Soft, smooth, and delicate

Transverse section

The microscopic examination of the transverse section (T.S.) of the petal revealed distinct anatomical characteristics of the floral tissue. The outermost layer consisted of a single-layered epidermis composed of compactly arranged cells that provide protection to the internal tissues. Numerous glandular trichomes were observed arising from the epidermal surface, indicating the presence of secretory structures that may be associated with the production of secondary metabolites.

The internal region was mainly composed of loosely arranged parenchymatous cells with intercellular spaces. These parenchyma tissues serve as storage and supportive tissues within the petal. Several starch granules or cellular inclusions were also identified within the parenchymatous region, suggesting the accumulation of reserve food materials (Fig 3).

Distinct spiral vascular bundles containing coiled xylem vessel elements were observed embedded within the ground tissue. The spiral thickening of the xylem vessels is characteristic of conducting tissues involved in water transport and mechanical support. The presence of well-developed vascular bundles confirms the active transport system within the floral organ.

Overall, the anatomical features such as epidermal layers, glandular trichomes, parenchyma tissue, starch inclusions, and spiral xylem vessels contribute to the diagnostic characteristics of the petal and may aid in the pharmacognostic identification and authentication of the plant material.

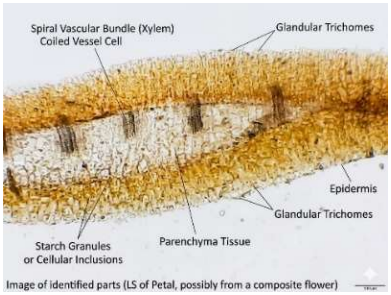


Fig. 3: TS of *Canna indica* L. Flower petal (Camera: SAGLO Digital Camera, Resolution: 800DPI, Software: SAGLOsoft version 2, Magnification: 100×)

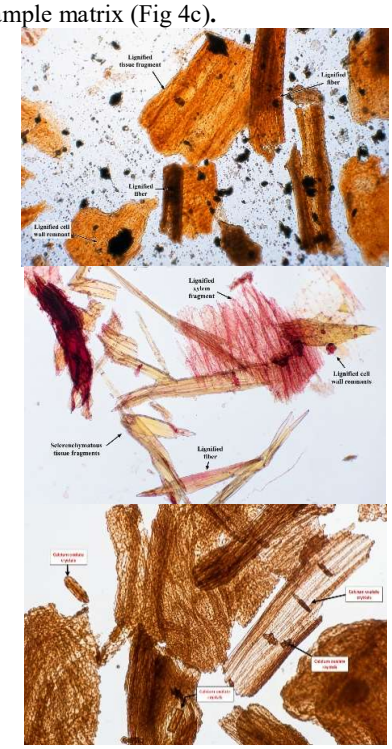
Powder characteristics

Sudan Red stained microscopy revealed prominent orange-red stained tissue fragments indicating the presence of lipophilic constituents. The preparation exhibited elongated fibrous elements, irregular parenchymatous fragments, and densely stained lipid rich regions. Several tissue components demonstrated intense Sudanophilic staining suggestive of intracellular lipid deposition and waxy materials. The distribution of stained regions confirmed the presence of hydrophobic constituents within the sample matrix (Fig 4a).

Phloroglucinol stained microscopy revealed distinct reddish-pink stained lignified tissues within the sample. The preparation exhibited elongated lignified fibers, sclerenchymatous fragments, xylem-like vascular elements, and thick-walled cell remnants. Intense staining of fibrous structures confirmed the presence of lignin-containing secondary cell walls. The observed histochemical characteristics indicated the occurrence of lignified structural tissues within the investigated sample (Fig 4b).

Acetic acid–stained microscopy revealed fragmented fibrous and membranous tissue elements along with elongated crystal-like deposits distributed throughout the preparation. Several refractile structures exhibited morphology suggestive of calcium oxalate crystal aggregates. The preparation also demonstrated irregular translucent tissue fragments and granular deposits. The observed microscopic characteristics indicated

the presence of crystal-associated materials within the sample matrix (Fig 4c).



a. **b.** **c.**

Fig. 4: a. Fibers, b. Sclerenchyma, c. Calcium oxalate crystal

7.4 The physicochemical evaluation of *Canna indica* L. flower

Table no. 4: The physicochemical parameters of *Canna indica* L. flower

S. No	Parameter	Observation (%) by weight	Pharmacopoeial limit(%)
1	Moisture content (Loss on drying)	7.25	Not more than 12
2	Total ash value	8.40	Not more than 15
3	Acid insoluble ash	1.12	Not more than 5
4	Water soluble ash	2.85	Not more than 18
5	Alcohol soluble extractive value	10.64	Not more than 15
6	pH (1% solution)	6.4	5.0-7.0
7	pH (10% solution)	6.8	5.0-7.0

Phytochemical evaluation

The qualitative estimation of the extracts showed the presence of the tested phytochemicals, (Table 5)

Table 5: Phytochemical evaluation of *Canna indica* L.

Sr. No.	Phytoconstituents	Ethanolic extract
1.	Alkaloids	+
2.	Flavonoids	+
3.	Glycosides	+
4.	Tannins	+
5.	Steroids	+
6.	Carbohydrates	+
7.	Proteins	+
8.	Amino acids	-
9.	Phenolic compound	+

Pharmacological screening

1. Anticancer activity

Table 7: Effects Of 5-Flurouracil against MCF-7 (Human Mammary gland Breast Adenocarcinoma Cell line) MTT Assay

Sr.no	Concentration (µg/mL)	Absorbance (O D) at 570nm				Cell viability (%)	IC50 (µg/mL)
		1	2	3	Average		
1.	Control	2.107	2.114	2.11	2.110		
2.	Standard-FU 20	1.112	1.113	1.116	1.113	52.78	
	40	0.785	0.786	0.781	0.784	37.15	16.04
	60	0.623	0.625	0.629	0.625	29.65	
	80	0.521	0.526	0.524	0.523	24.81	
	100	0.422	0.426	0.423	0.423	20.04	

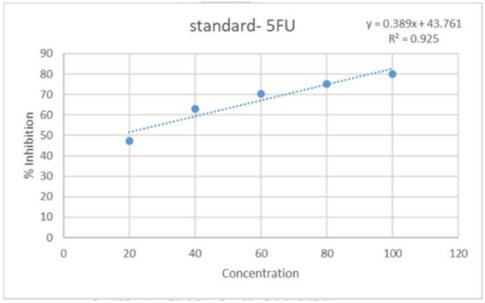


Fig. 5: Dose–response curve of standard 5-Fluorouracil

The IC50 value of 5-Fluorouracil was found to be 16.04 µg/mL, indicating significant cytotoxic activity against the tested cell line (Table 7). The reduction in absorbance values confirms inhibition of cell growth and increased anticancer potential of the standard drug at higher concentrations.

Table 8: Effects of Sample against MCF-7 (Human Mammary gland Breast Adenocarcinoma Cell line) by MTT assay

Sr. no	Concentration (µg/mL)	Absorbance (O D) at 570nm				Cell Viability (%)	IC50(µg/mL)
		1	2	3	Average		
1.	Control	2.107	2.114	2.11	2.110		
2.	Sample20	1.963	1.965	1.968	1.965	93.14	
	40	1.856	1.852	1.859	1.855	87.94	
	60	1.754	1.753	1.759	1.755	83.19	60.24
	80	1.452	1.453	1.456	1.453	68.89	
	100	0.844	0.842	0.846	0.844	40	

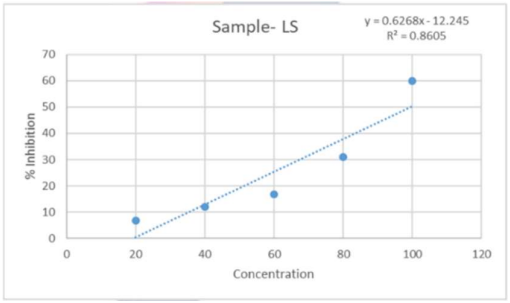


Fig. 6: Dose–response curve of Sample LS

The IC50 value of the sample extract was found to be 60.24 µg/mL, indicating moderate cytotoxic and anticancer activity against the tested cell line (Table 8). The progressive decrease in absorbance and cell viability confirms the concentration-dependent inhibitory effect of the sample extract on cancer cell growth.

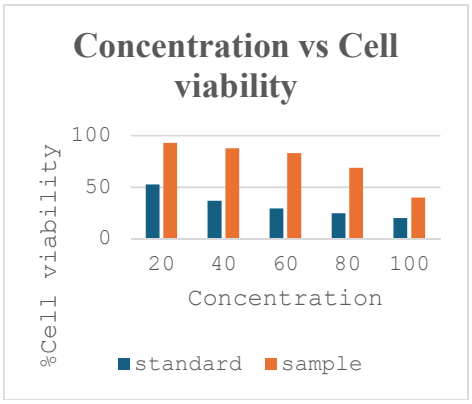
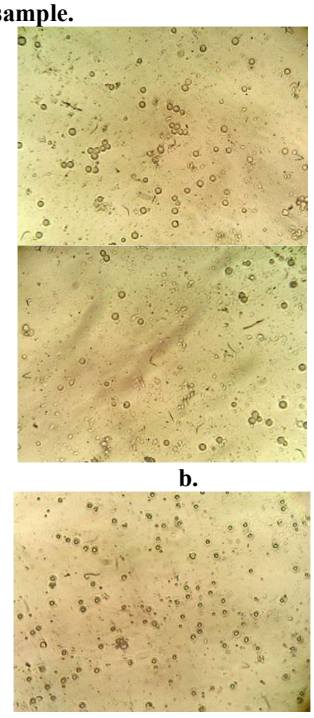


Fig. 7: Effect of increasing concentrations on cell viability (%) of standard and test sample.



a. **b.**

c.

Figure no. 8: Microscopic Observation of Cancer Cells after Treatment with Test Extract a. Sample, b. Standard, c. Control. (Camera: SAGLO Digital Camera, Date: 8 Feb 2026,

Resolution: 800DPI, Software: SAGLOsoft version 2, Magnification: 100×)

2. Assays for in vitro antiurolithiatic activity of flower of *Canna indica* L.

The results of the calcium oxalate crystal nucleation assay are presented in Table 9. The ethanolic extract showed a considerable inhibitory effect on calcium oxalate crystal formation under in vitro conditions. Furthermore, the inhibitory activity increased with increasing concentrations of the extract. The extent of inhibition observed with the ethanolic extract was found to be comparable to that of the standard antiurolithiatic drug cystone.

According to the current investigation, the ethanolic extract exhibits strong antiurolithiatic activity by successfully inhibiting the growth and development of kidney stones by lowering stone-forming ingredients.

Antiurolithiatic study

Turbidity method

Table 9: Shows percentage reduction in turbidity by plant extract compared with controlled drugs.

Treatment groups	Dose	Absorbance	% inhibition (Mean ± SD)
Control	-	0.820 ± 0.010	0.0
Cystone	10mg	0.470 ± 0.015	80
Ethanolic extract	5mg	0.700 ± 0.020	57
	7mg	0.610 ± 0.018	69
	10mg	0.520 ± 0.012	75

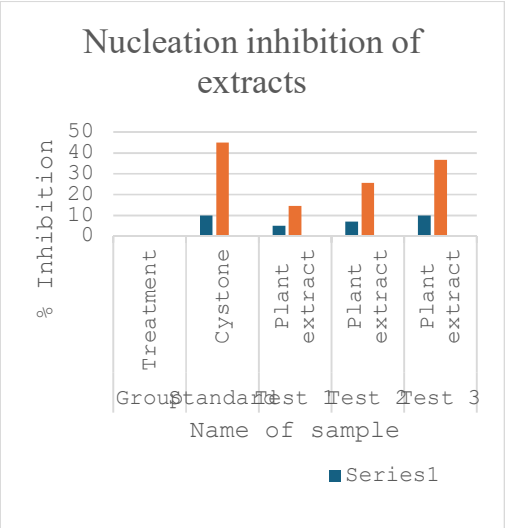


Fig. 9: Comparative graph showing percentage nucleation inhibition of standard and plant extracts

Dissolution:

Table 10: Shows dissolution of CaOx by plant extracts compare

Dose (mg)	Initial Weight (mg)	Final Weight of Standard (mg)	% Dissolution of Standard	Final Weight of Sample (mg)	% Dissolution of Sample
Control	10	10	–	10	–
100	10	6.6 ± 0.1	34 ± 1.0	7.7 ± 0.1	23 ± 1.0
200	10	5.9 ± 0.2	41 ± 2.0	5.9 ± 0.1	41 ± 1.0
400	10	4.4 ± 0.1	56 ± 1.0	5.0 ± 0.2	50 ± 2.0

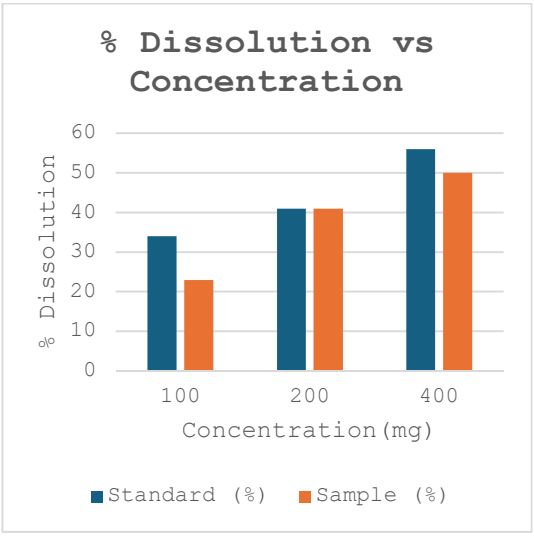


Fig. 10: Effect of Concentration on % Dissolution of Standard and Sample

Molecular Docking

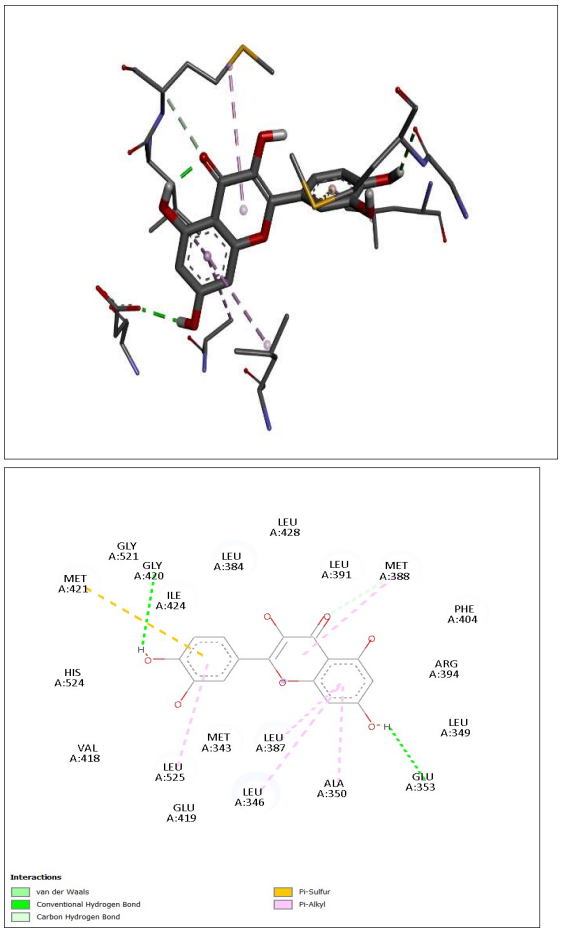


Fig. 11(a) 3D and 2D interaction of Quercetin with 4OAR receptor

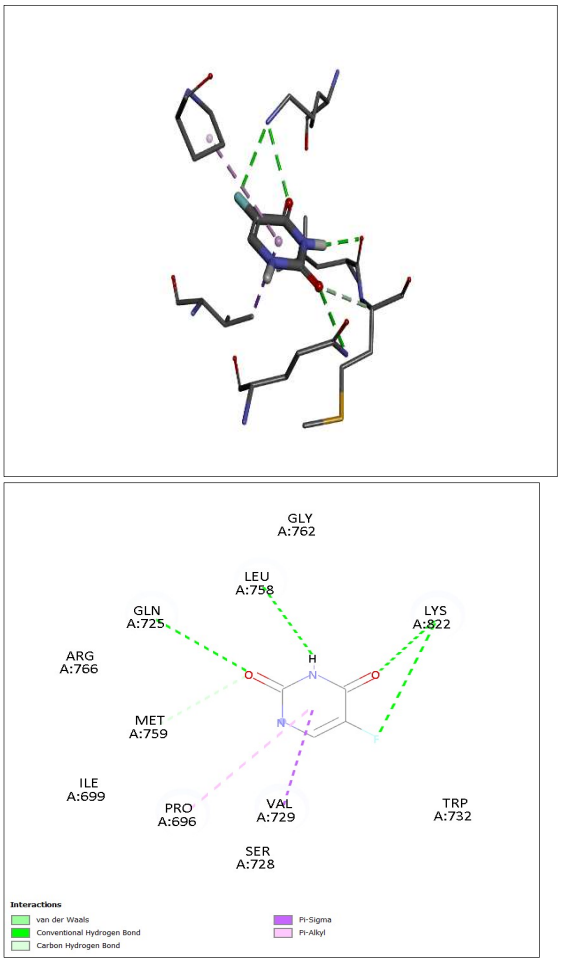


Fig. 11(b) Fig. 11(a) 3D and 2D interaction of Rutin with 4OAR receptor

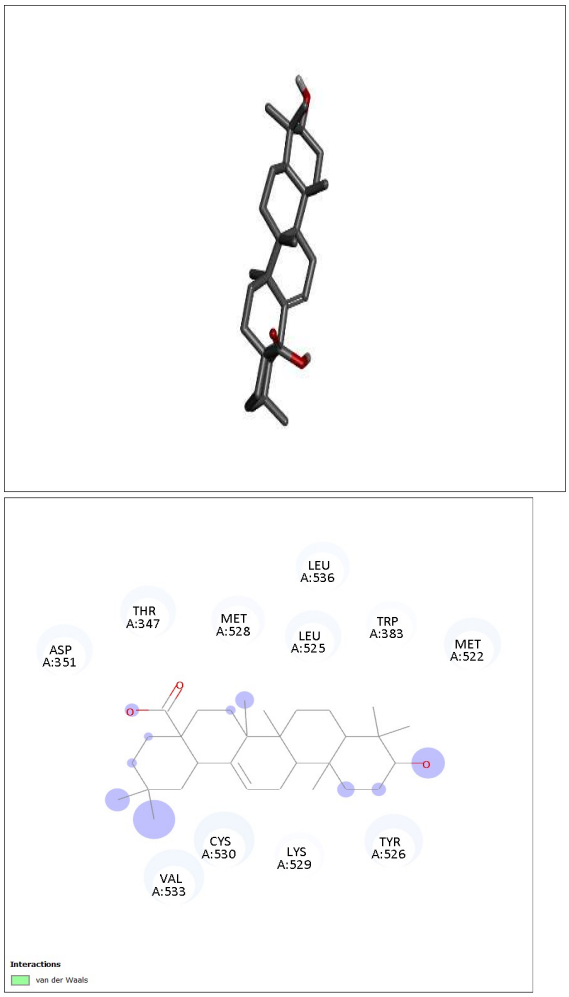


Fig. 11(c) 3D and 2D interaction of Oleanolic acid with 4OAR receptor

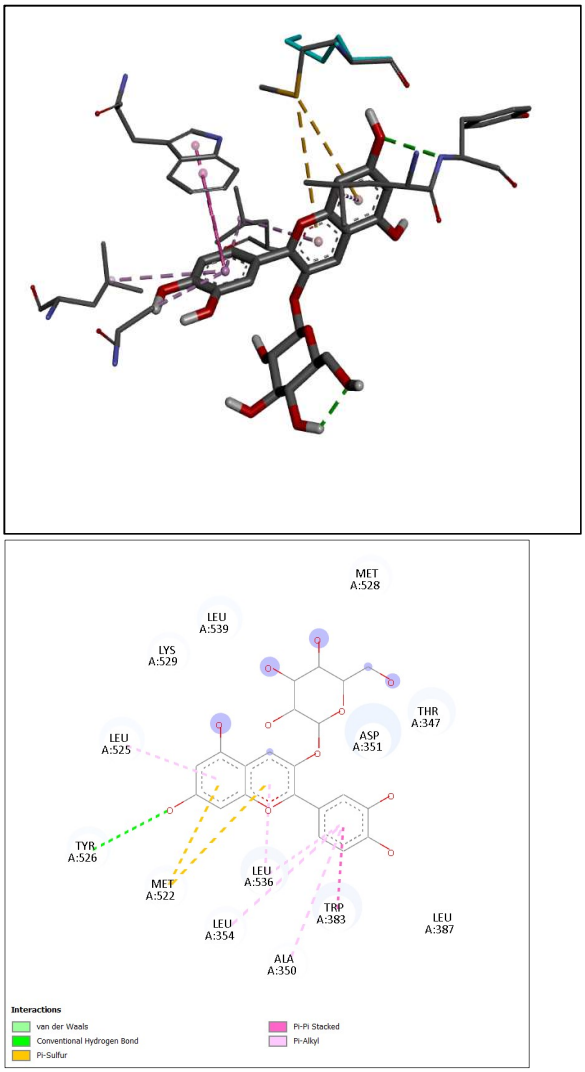


Fig. 11(d) 3D and 2D interaction of Cyanidin-3-O-b-glucopyranoside with 4OAR receptor

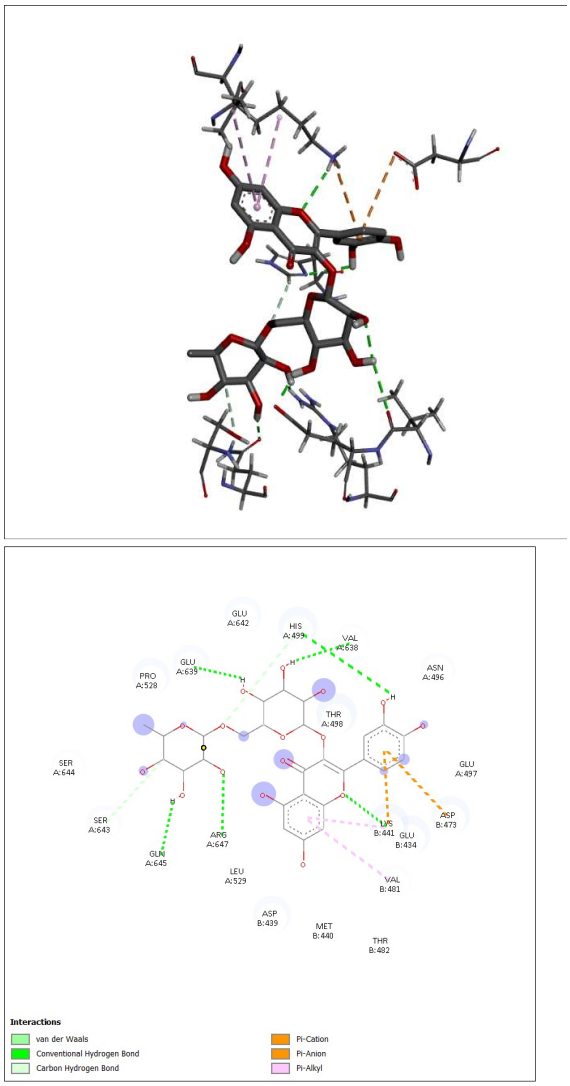


Fig. 12(a) 3D and 2D interaction of Rutin with 4WRN receptor

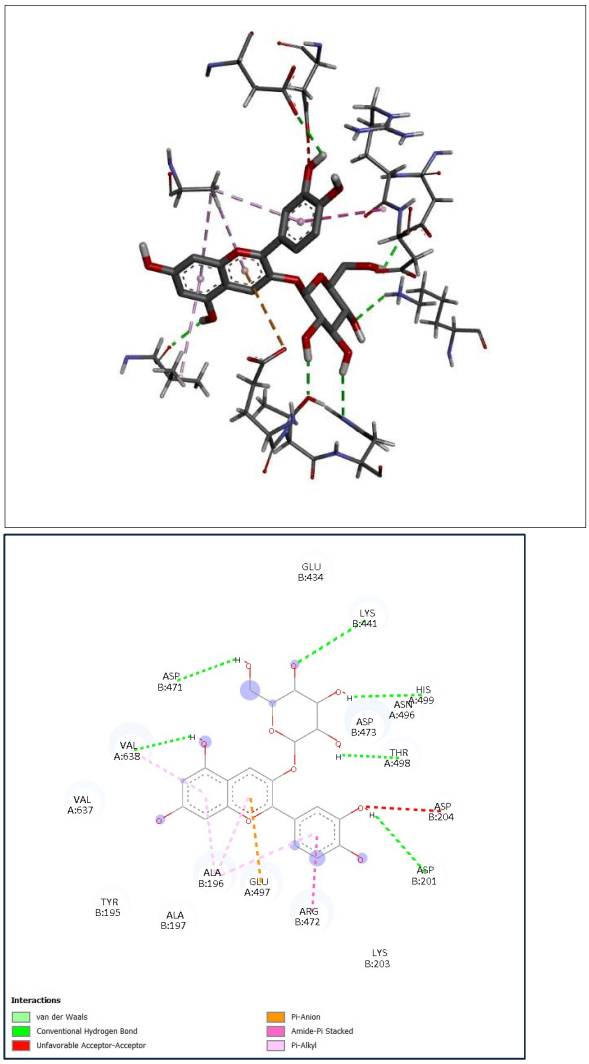


Fig. 12(b) 3D and 2D interaction of Cyanidin-3-O-b-glucopyranoside with 4WRN receptor

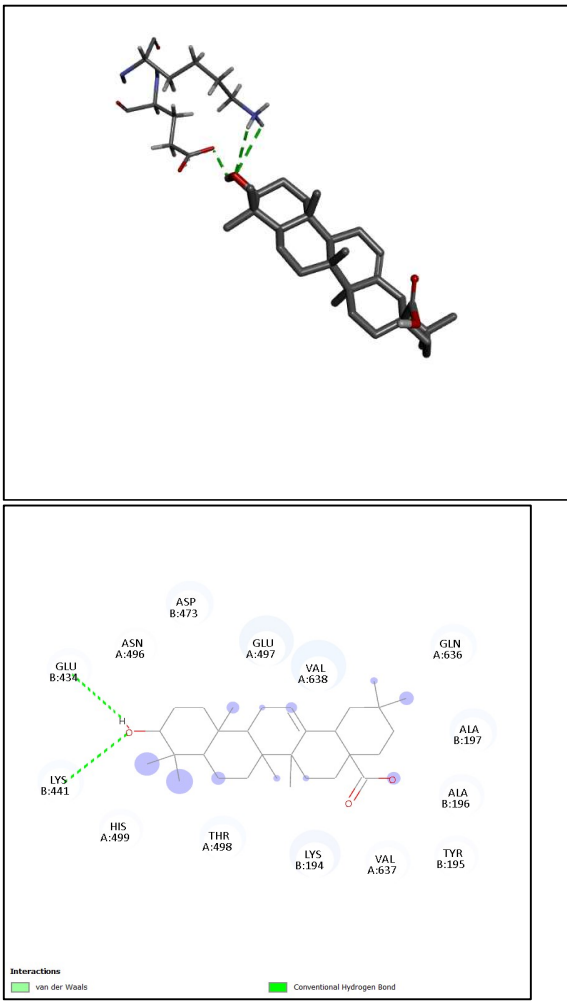


Fig. 12(c) 3D and 2D interaction of Oleanolic acid with 4WRN receptor

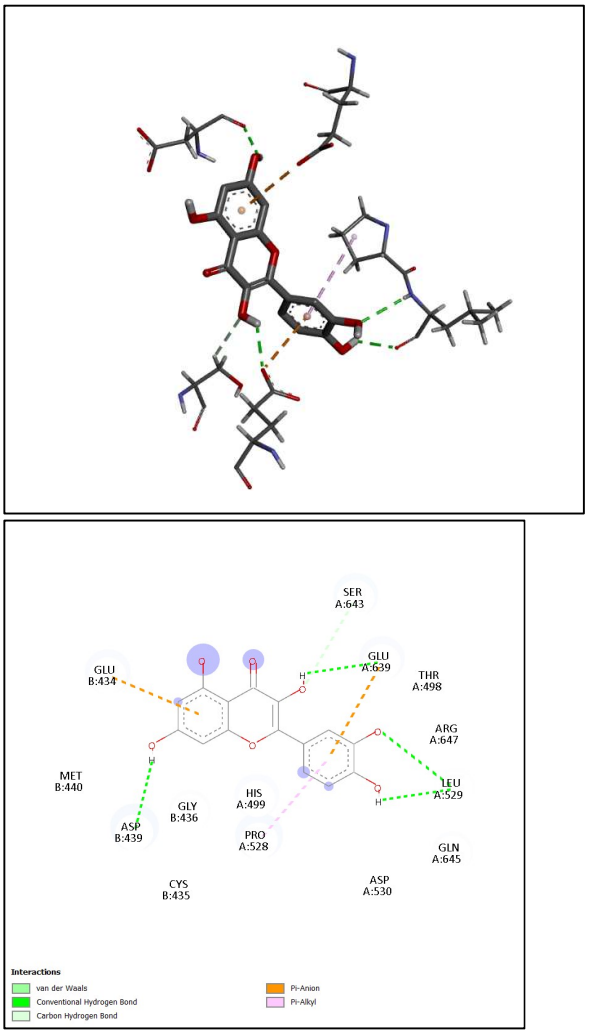


Fig. 12(d) 3D and 2D interaction of Quercetin with 4WRN receptor

Table 11: Molecular Docking Interactions of Phytoconstituents with Target Protein 4OAR

Compound name	Binding Affinity	Conventional H-bond Interaction	Pi Interaction	Other
Rutin	-8.3	THR 34, ASP 35	TRP 38	MET 52
Betulinic acid	-7.1	-	-	-
Oleanolic acid	-8.3	LEU 52, SER 64	GLU 63, GLU 43	-
Cyanidin-3-O-b-glucopyranoside	-7.9	TYR 52,	TRP 38	MET 52

Quercetin	-7.9	MET 38	MET 42	MET 42

Results: Molecular docking result examination of bioactive substances of *Canna indica* L. against the protein 4WRN

Table 12: Molecular Docking Interactions of Phytoconstituents with Target Protein Showing

Compound name	Binding Affinity	Conventional H-bond Interaction	Pi Interaction
Rutin	-9.4	THR 85, GLY 71	LEU 71, THR 79
Betulinic acid	-6.9	-	-
Oleanolic acid	-7.0	LEU 52, SER 64	GLU 63, GLU 43
Cyanidin-3-O-b-glucopyranoside	-7.7	LYS 44,	GLU 49
Quercetin	-7.0	LEU 52, SER 64	GLU 63, GLU43

DISCUSSION:

The cytotoxic activity of standard 5-Fluorouracil (5-FU) was evaluated by MTT assay using different concentrations ranging from 20–100 µg/mL, and absorbance was measured at 570 nm. The results demonstrated a concentration-dependent decrease in cell viability with increasing concentration of the standard drug (Table 7).

The control group showed an average absorbance value of 2.110, indicating 100% viable cells. Treatment with 5-FU at 20 µg/mL showed an average absorbance of 1.113 with 52.78% cell viability. Further increase in concentration to 40, 60, 80, and 100 µg/mL resulted in gradual reduction of cell viability to 37.15%, 29.65%, 24.81%, and 20.04%, respectively.

The cytotoxic activity of the sample extract was evaluated by MTT assay at different concentrations ranging from 20–100 µg/mL, and the absorbance was measured at 570 nm (Table 8). The results indicated a gradual decrease in cell viability with increasing concentration of the sample extract, demonstrating dose-dependent cytotoxic activity.

The graph represents the percentage inhibition produced by standard 5-Fluorouracil (5-FU) at different concentrations ranging from 20–100 µg/mL. The percentage inhibition increased with increase in concentration, indicating dose-dependent cytotoxic activity against the cancer cell line. Maximum inhibition was observed at 100 µg/mL concentration (Fig. 5)

The graph illustrates the percentage inhibition produced by the sample extract (LS) at different concentrations ranging from 20–100 µg/mL. The percentage inhibition increased progressively with increase in concentration, indicating concentration-dependent cytotoxic activity of the sample against the tested cancer cell line (Fig. 6).

The graph shows the effect of increasing concentration on cell viability for both the standard and sample treatments. As the concentration increased from 20 to 100 µg/mL, the percentage of viable cells decreased in both groups, indicating a concentration-dependent cytotoxic effect. The standard exhibited greater cytotoxicity, reducing cell viability from approximately 52% to 20%, whereas the sample showed a comparatively lower effect, with cell viability decreasing from about 92% to 40%. These results suggest that the standard possesses stronger anticancer activity than the sample (Fig. 7). Maximum inhibition was observed at 100 µg/mL concentration (Fig. 8).

The antiurolithiatic activity of the ethanolic extract of *Canna indica* L. was evaluated using calcium oxalate crystal nucleation and egg membrane dissolution assays. The extract exhibited significant inhibition of calcium oxalate crystal formation, and the inhibitory effect increased with increasing concentration, indicating dose-dependent activity. The observed effect was comparable to that of the standard drug, Cystone (Table 9).

In the dissolution assay, the extract demonstrated appreciable calcium oxalate stone-dissolving potential. The percentage dissolution increased progressively with increasing dose (Fig. 9), with maximum dissolution observed at 400 mg. Although the activity was slightly lower than that of the standard, the extract showed considerable efficacy in reducing stone-forming constituents (Table 10).

The dissolution study demonstrated a dose-dependent increase in stone dissolution for both the standard and sample treatments (Fig. 10). As the dose increased from 100 mg to 400 mg, the percentage dissolution increased significantly. At 400 mg, the standard showed the highest dissolution (56%), while the sample exhibited 50% dissolution. The sample displayed appreciable antiurolithiatic activity, although its effect was slightly lower than that of the standard. These findings indicate that the sample possesses promising stone-dissolving potential.

These findings suggest that the ethanolic extract of *Canna indica* L. possesses promising antiurolithiatic activity by inhibiting calcium oxalate crystal nucleation and promoting stone dissolution, thereby supporting its potential use in the management of urolithiasis.

Molecular docking studies were carried out to evaluate the interaction of the identified phytoconstituents with the selected anticancer, Progesterone receptor with bound ulipristal acetate and a peptide from the co-repressor SMRT (PDB ID: 4OAR) and antiurolithiatic, Crystal structure of the

polymerization region of human uromodulin/Tamm-Horsfall protein (PDB ID: 4WRN) target proteins. The results revealed that all tested compounds exhibited favorable binding affinities, indicating their potential biological activity.

Among the compounds, Rutin and Oleanolic acid showed the highest binding affinity against the anticancer target 4OAR, with docking scores of −8.3 kcal/mol, suggesting stable ligand–receptor interactions (Table 11). Similarly, Rutin exhibited the strongest interaction with the antiurolithiatic target 4WRN, with a docking score of −9.4 kcal/mol, indicating excellent binding stability and potential antiurolithiatic activity (Table 12).

The 2D and 3D interaction analyses confirmed the involvement of hydrogen bonds and other non-covalent interactions within the active sites of both target proteins. The strong binding affinities observed for Rutin correlate with the biological activities of the extract and suggest that it may be one of the major phytoconstituents responsible for the anticancer and antiurolithiatic effects of *Canna indica* L. flower extract. These findings support further pharmacological investigations of the plant and its bioactive compounds.

CONCLUSION:

The MTT assay results demonstrate that Sample LS exhibits a concentration-dependent cytotoxic effect against the MCF-7 (human mammary gland breast adenocarcinoma) cell line. The untreated control cells showed 100% viability, confirming normal cell proliferation. Treatment with Sample LS resulted in a gradual decrease in cell viability with increasing concentrations. Cell viability remained high at lower concentrations, with 93.14% at 20 µL/mL, 87.94% at 40 µL/mL, and 83.19% at 60 µL/mL, indicating minimal cytotoxicity at these doses. A more pronounced reduction in viability was observed at higher concentrations, with cell viability decreasing to 68.89% at 80 µL/mL and markedly to 40% at 100 µL/mL. The calculated IC_{50} value of 93.07 µg/mL indicates a moderate antiproliferative activity of Sample LS against MCF-7 cells. Overall, these findings suggest that Sample LS possesses significant cytotoxic potential and may be considered a promising candidate for further anticancer investigations.

The results of the calcium oxalate (CaOx) inhibition assay indicate that the plant extract exhibits a dose-dependent antiurolithiatic activity. The gradual increase in percentage inhibition from Test 1 (5 mg) to Test 3 (10 mg) demonstrates that higher concentrations of the extract are more effective in reducing CaOx crystal formation.

Although the standard drug (Cystone) showed the highest inhibitory effect, the plant extract also produced a significant reduction in crystal formation compared to the control, suggesting a meaningful protective effect against urolithiasis.

Overall, the findings suggest that the plant extract possesses moderate but promising antiurolithiatic potential, likely due to its ability to inhibit nucleation and aggregation of calcium oxalate crystals. Further studies involving isolation of active phytoconstituents and in vivo evaluation are recommended to confirm its therapeutic usefulness.

CONFLICT OF INTEREST:

The authors declare no conflict of interest.

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