RESEARCH PAPER

Chemical Fingerprinting and In-Silico Targeting of Uterine Cancer Proteins Using Bioactives from Smilax Species

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Abstract:

Uterine cancer, also known as endometrial cancer, is a significant worldwide health burden that highlights the need for safer and more effective natural treatment alternatives. In order to find bioactive compounds with potential anti-uterine cancer activity, this study investigates the phytochemical composition, chromatographic profiling, and molecular docking potential of two medicinal plants: *Smilax zeylanica* and Smilax *china*. Preliminary phytochemical analysis of ethanolic extracts indicated the existence of many secondary metabolites, such as alkaloids, flavonoids, phenols, glycosides, tannins, and terpenoids. Preliminary phytochemical analysis of ethanolic extracts of rhizomes and leaves made by Soxhlet extraction indicated the existence of many secondary metabolites, such as alkaloids, flavonoids, phenols, glycosides, tannins, and terpenoids. Several notable phytoconstituents such as rutin, Sinapicacid, Cafteric acid,quercetin, catechin, gallic acid, and chlorogenic acid were identified by High-Performance Liquid Chromatography (HPLC) analysis, which produced distinctive fingerprints. Using the Schrödinger Glide XP algorithm, identified compounds were further assessed through in silico molecular docking against important uterine cancer-related protein targets, such as JNK-1, caspase-3, MAPK3, prolidase, PI-kinase, MEK-1, and GSK-3. Strong binding affinities between catechin, rutin hydrate, and proteins controlling apoptosis and cellular signalling pathways were shown by docking analysis. Interestingly, rutin hydrate interacted favourably with JNK-1, whereas catechin demonstrated a strong affinity for MAPK3. These results provide a scientific basis for additional pharmacological validation and drug development against uterine cancer and demonstrate the therapeutic potential of Smilax species as promising sources of anticancer phytochemicals.

Keywords: Endometrial cancer, HPLC profiling, Medicinal plants, Molecular docking.

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1. Introduction

In wealthy nations, endometrial cancer is the sixth most common cancer among women, and it ranks fourth overall. It is the second most common gynecologic cancer in developing countries, with 5.9 incidences per 100,000 women. It is 4.3 per 100,000 women in India [1]. The Global Cancer Observatory (GLOBOCAN) projects that there will be 19.3 million cancer cases worldwide in 2020. By 2040, India's population would have grown by 57.5% from 2020 to 2.08 million, ranking third behind China and the US. [2]

An estimated 13,328 new cases and 5010 fatalities from endometrial cancer were reported in India in 2018. In India, endometrial cancer has an age-standardised incidence rate (ASIR) of 2.3 per 100,000 females. The primary cause of the growth in endometrial cancer in India is women's evolving reproductive and lifestyle patterns, particularly in metropolitan areas.The mean age of diagnosis is 60 years old and most instances occur in the sixth and seventh decades of life. [3]

More than 70% of deaths from cancer occur in low- and middle-income countries, and the disease currently accounts for one in six deaths globally (Organisation, 2018). This may be caused by dietary and behavioural concerns, such as smoking, drinking alcohol, not exercising, and eating a poor diet low in fruits and vegetables.[4]

Chemotherapyis considered a main mode of cancer treatment. Many medications are available to treat cancer, but the majority of these medications have serious side effects. Chemotherapy drugs are becoming resistant to their use. As a result, cancer treatment and prevention continue to be very difficult. On the other hand, medicinal plants contain amazing anticancer compounds that have a lot of applications in the pharmaceutical sector. Researchers are becoming more interested in medicinal plants as a potential cancer cure because of their many benefits and minimal to nonexistent negative effects. Scientific research is expanding to use these products because they are widely available and less expensive than other therapeutics.

WHO suggests the use of medicinal plants as they are effective and safe in the treatment of cancer. According to the WHO, the death rate and cancer incidence in Asian and African countries are increasing. The lack of knowledge, resources, diagnosis and treatment facilities in some of these developing countries results in more cases and deaths as compared to the developed world.

Various medicines, made out of naturally found products, are actively used in treating cancer. Such drugs are lupeol, emodin, furanocoumarins, kaempferol, allicin, colchicine, quercetin, betulinic acid and rutin. Numerous studies have been done in India on the significance of medicinal herbs in the treatment of cancer. Such studies have affirmed the claim that around 24% to 39% of Indian cancer patients use traditional medicine (India).(5)

On this basis, two plants of Smilax species, Smilax zeylanica and Smilax china, were selected to unveil the anti-uterine cancer properties of *Smilax zeylanica* and *Smilax china*.

Smilax zeylanica is *Smilax zeylanica* is a large climbing shrub with a thick, dioecious stem that is slender and slender, covered with prickles and angular branches. Leaves are simple, alternating, and vary in shape from broadly ovate or elliptic to oblong-lanceolate, with a sharp or bluntly cuspidate tip. The plant is found in tropical regions of India and is prevalent in woods. Countries such as India, Bangladesh, and Nepal have traditionally used *S. zeylanica.* Throughout the world individual plant parts of *S. zeylanica*, such as tuber, leaves, root and stem, are applied alone or in combination with other plants to treat a variety of human and animal diseases in different formulations, such as paste or soup. It is an herb, which has traditionally been used to heal piles, wounds, sexual diseases, toothache and dysentery [6].

Smilax china is a perennial climbing Liliaceae plant. It has extensive distribution across the world's tropical and temperate climates, particularly in Eastern Asia and North America. Smilax Linnaeus is the main genus of Smilacaceae, with 350 species found mostly in Asia and America. The family is made up of 225 species organised into ten genera.17 of the 33 species found in Northeast India have been reported in the eight states of "Assam, Arunachal Pradesh, Meghalaya, Manipur, Mizoram, Tripura, Nagaland, and Sikkim[7]. This plant has a high edible value and is rich in nutrients, including lipids, organic acids, amino acids, and seven types of amino acids that the human body needs, as well as one type of important amino acid (histidine) for the developing body.*S. China's* soft buds and leaves can be consumed as tasty vegetables, and the rhizome can be used to manufacture brewing ingredients, extract starch, and cure ailments. *S. China* is widely promoted as a medicinal herb in traditional medicine[8].

For a number of years, *S. china*, often known as China root, has been implemented in a variety of tribal and folk remedies. *Smilax china* root is used in Chinese medicine to treat "syphilis, psoriasis, rheumatoid arthritis, gout, urinary tract infections", and as an energy booster. Rhizomes and roots were decocted to treat syphilis and herpetism. Leprosy, scrofula, and a variety of skin infections that develop into ulcers have all been proven to respond favourably to its treatment. Roots have been used to heal burns, ulcers produced by pus-forming bacteria, and abscesses [9].

This article focuses on the phytochemicals and High-Performance Liquid Chromatography (HPLC) profiling of rhizomes of *Smilax zeylanica*, *Smilax china*. In addition the eluted compounds were docked against selected proteins and enzymes responsible for uterine cancer and promoting apoptosis etc.

2. Materials and methods

2.1 Plant material

The rhizomes of *Smilax china* and *Smilax zeylanica* were obtained from a herbal drug store and were gathered and verified by Dr.D Ramakanth Raju,Retd.Professor, Department of Botany, Andhra University, Visakhapatnam.

2.2 Preparation of extracts

Smilax zeylanica and *Smilax china* rhizomes were chopped, crushed into a fine powder, and then extracted using a Soxhlet apparatus for 72 hours using 99.9% ethanol as the solvent. With less pressure, the extracts were dried off by evaporation. For future research, the extracted materials were gathered and kept apart.

Preliminary phytochemical Analysis

Standard qualitative techniques were used to test for main elements in phytochemicals. "Carbohydrates, tannins, saponins, flavonoids, cardiac glycosides, terpenoids, triterpenoids, phlobatannins, anthraquinones, alkaloids, quinones, phenols, coumarins, glycosides, proteins, steroids, and phytosteroids" were all evaluated for.

2.3 HPLC instrumentation

HPLC analysis under chromatographic conditions was carried out using a Shimadzu LC-20AD system that has a PDA detector. At 30°C, separation was accomplished using a reverse-phase C18 column (250 × 4.6 mm, 5 µm). Acetonitrile: water (30: 70, v/v) containing 0.1% formic acid (final) was supplied at a flow rate of 1.0 mL/min to create the isocratic mobile phase. 20 µL was the injection volume, and 254 nm was the detection wavelength. The entire run took 25 minutes. Using LC-Solution software, chromatograms were captured and processed. By comparing retention periods and UV spectra with those of genuine standards and by co-injection, peaks were identified. Rutin, Polydatin, gallicacid, Betulinic acid, scirpusin A, quercetin, Caftaric acid, epicatechin, reservesterol are examples of standard reference chemicals that were utilized to identify prominent peaks.

3. Results

3.1 Preliminary phytochemical screening of the Samples

The preliminary phytochemical screening of the natural extract of *Smilax zeylanica* revealed alkaloids, flavonoids, glycosides, saponins, tannins, triterpenoids, sterols, and phenolic compounds.

Table-1 Preliminary phytochemical screening of *Smilax zeylanica*

S.no	Phyto constituents	Test performed	Result
1	Alkaloids	Dragendroff's test	+
2	Glycosides	Salkowski's test	+
3	Flavonoids	Alkaline tests	+
4	Phenols	Ferric chloride test	+
5	Sterols	Liebermann-Burchard's test	+
6	Triterpenoids	Salkowski's test	+
7	Saponins	forth test	+
8	Tannins	Ferric chloride test	+

Initial phytochemical analysis of the crude ethanolic extract of *Smilax china* in this investigation indicated the presence of red ucing sugars, alkaloids, steroids, glycosides, carbonydrates, saponins, and tannins.

Table-2 Preliminary phytochemical screening of *Smilax china*.

S.no	Phyto constituents	Test performed	Result
1	Alkaloids	Dragendroff's test	+
2	Steroids	Molisch test	+
3	Glycosides	Salkowski'sTests	+
4	Saponins	Froth test	+
5	Tannins	Ferric Chloride Test	+
6	Reducing sugars	Benedict's test	+
7	Carbohydrates	Molisch test	+

3.2 HPLC analysis of *Smilax zeylanica* and *Smilax china*

HPLC of Smilax zeylanica

The HPLC chromatogram of the ethanolic extract of *Smilax zeylanica* showed distinct and well-resolved peaks, showing the presence of several phytoconstituents (Figure 1). Thirteen main peaks were found between 3.0 and 22.0 minutes of retention time. The existence of rutin (Rt 3.029 min), gallic acid (Rt: 4.064 min), and polydatin (Rt: 5.05 min) was established by comparison with genuine standards. The relative peak area analysis revealed that rutin, gallic acid, and caffeic acid were present in substantial concentrations, accounting for 35.9%, 10.22%, and 12.6% of the total peak area, respectively. Peaks were identified by comparing retention duration and UV spectra to standards, as well as by co-injection.

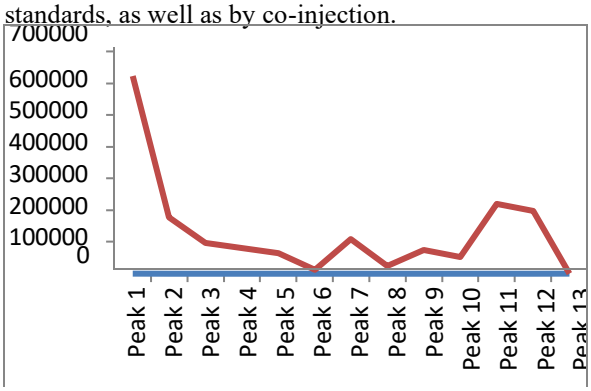


Fig.1: Chromatogram of *Smilax zeylanica*

Table 5: Peaks of *Smilax zeylanica* and its identified phytoconstituents

Peak	Retention time	Area	Area %	Identified compound
Peak 1	3.029	622744	35.984	Rutin hydrate
Peak 2	4.064	176892	10.221	Gallic acid
Peak 3	5.056	96678	5.586	Polydatin
Peak 4	6.389	81336	4.700	Protocatechuic acid
Peak 5	6.784	63442	3.666	3,4-dihydroxy phenol acetic acid
Peak 6	7.296	12260	0.708	Epigallocatechin
Peak 7	8.213	109758	6.342	Chlorogenic acid
Peak 8	10.101	24066	1.391	2,5 di hydroxy benzoic acid
Peak 9	11.755	75368	4.355	Betulinic acid
Peak 10	13.120	52086	3.010	Scirpusin A
Peak	15.125	21881	12.64	Caftaric acid

k 11		6	4	
Pea k 12	18.912	19706 3	11.38 7	Sinapic acid
Pea k 13	20.395	85	0.005	Rosmarinic acid

HPLC of Smilax china

After a 30-minute run, the isocratic technique (ACN:water 32:68, 0.1% FA) produced a repeatable chromatographic fingerprint of the ethanolic extract of *Smilax china* (Figure 2). Under these exemplary settings, fifteen well-resolved peaks were found between 3.0 and 27.0 minutes. Caftaric acid (Rt≈3.029 min), quercetin (Rt≈4.085 min), polydatin (Rt≈4.331min), gallic acid (Rt≈5.33 min), resveratrol (Rt≈8.213min), and catechin (Rt≈14.2- 19 min) were recognised by comparison with genuine standards. Caftaric acid, quercetin, polydatin, and gallic acid were identified as significant ingredients using relative peak area analysis. Caftaric acid peak areas were 38.532%, gallic acid 22.845%, polydatin 8.99%, and quercetin 8.33%. The HPLC fingerprint may be used to identify and monitor the quality of S. china materials and preparations.

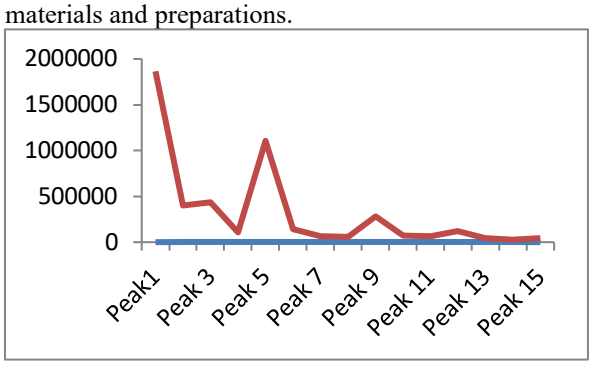


Fig 2: Chromatogram of *Smilax china*

Table 6: Peak table of *Smilax china* and its identified compounds.

Peak	Retention time	Area	Area %	Identified compounds
Peak 1	3.029	1865443	38.532	Caftaric acid
Peak 2	4.085	403255	8.330	Quercetin
Peak 3	4.331	435651	8.999	Polydatin
Peak 4	5.045	108430	2.240	Friedelin
Peak 5	5.333	1105956	22.845	Gallic acid
Peak 6	6.379	142543	2.944	Chlorogenic acid
Peak 7	8.213	63776	1.317	Resveratrol
Peak 8	8.811	58012	1.198	p-coumaric acid
Peak 9	10.112	278580	5.754	Betulinic acid
Peak 10	11.829	70961	1.466	Cedrol
Peak 11	12.587	66135	1.366	o-hydroxybenzoic acid
Peak 12	14.219	123140	2.544	Catechin
Peak 13	18.464	45393	0.938	Taxifolin
Peak 14	21.803	27167	0.561	Cinchonin
Peak 15	27.168	46784	0.966	Epicatechin

3.3 Molecular Docking Studies:

3.3.1 Insilico docking of biomolecules

Structure-bespoke drug design was the primary approach employed in this experiment. The target proteins were retrieved in three dimensions using the RCSB PDB, and the potential ligand compounds' binding affinities to these specific proteins were evaluated. The Schrodinger collection of applications was the sole instrument used during the whole procedure. [10,11] Tyrosine kinases, JNK 1, MAPK3, phosphatidylinositol 4-kinase (PI kinase), MEK-1, Glycogen synthase kinase-3 (GSK-3), caspase 3, prolidase, and other proteins and enzymes were among those affected by the docking of all eluted medicines against endometrial cancer.

3.3.2 Software and software elements employed in the research

The Maestro interface from Schrödinger was used to execute all software and software components, unless otherwise noted. Using SiteMap and Receptor Grid Generation, ligand-binding sites were located. The ligand preparation procedure made use of LigPrep. Glide was needed to perform ligand docking; the specifics are covered in a different section.

3.3.3 Identifying ligand-binding sites

RCSB protein data bank was used to download proteins such as caspase-3 (3KJF), prolidase (1PV9), tyrosine kinases (6L8L), JNK-1 (2N03),

MAPK3 (2Z0Q), PI kinase (5E3S), MEK-1 (3SLS), GSK-3 (8DJD), and NAIP-neuronal apoptosis inhibitory protein (3U0R).To locate ligand-binding sites for the selected proteins, the Site Map was used with its default parameters. The sole change was that six sites were selected for Site Map's site-point group reporting instead of the usual five. This was done in order to improve the chances of discovering more ligand-binding sites, which the Site Map software component identified as site-point groups.Receptor Grid Generation could be used to create ligand-binding sites. The default option was utilised in every grid made for this project. The binding sites of the ligand sets were represented by each grid.

3.3.4 Docking of ligands

Educational or pharmaceutical investigators doing structurally driven electronic screening investigations can easily use PubChem, a free library of 13 million freely accessible chemicals, provided the ligand structures of the chosen compounds are used in this experiment. The ligands were received as SDF files [12].

3.3.5 Developing ligands

To confirm that the selected ligand had the required stereochemistry for its structure and was in a relatively low-energy state, Schrödinger's LigPrep was utilised [13]. Brooks et al. discussed the importance of the field of stereochemistry in simulations and said that it was crucial to include all stereoisomers of all potential lead compounds in order to minimise erroneous findings and avoid losing a sizable number of potential leads. [14]. A 2D sketched structure may also be transformed into a 3D shape using LigPrep. The 3D ligand is subsequently produced through a sequence of processing procedures. Making sure the ligands were in the proper ionisation states, tautomers, ring conformations, molecular weights, and amount and kind of functional groups were among the preparation techniques.The preparation procedures included ensuring that the ligands had the appropriate ionisation states, tautomers, ring conformations, molecular weights, and quantity and kind of functional groups. LigPrep has been utilised in several studies that use structure-based virtual screening to find possible lead drugs for various illnesses. [15-2]

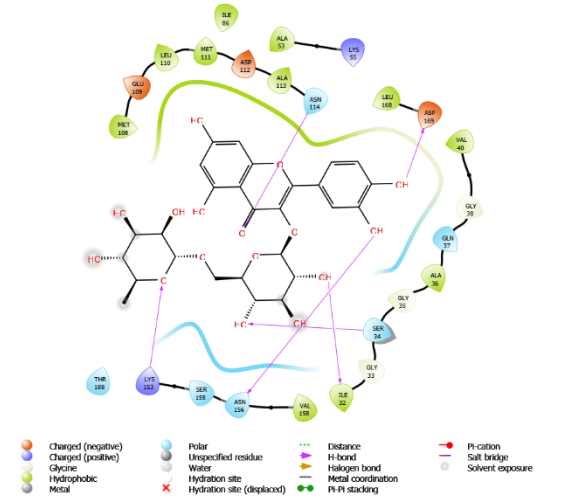
3.3.6 Ligand docking

All ligands made by PubChem were submitted to Extra Precision. [17] By examining the highest-scoring ligand poses, XP mode was designed to detect incorrect positives and substances of interest that may bind to a specific receptor conformation. Every docked activity for the specified ligands was evaluated and classified into several distinct tasks that could manage the computer's limited number of central processing units.(CPUs).

Table-7 Docking scores for the selected compounds towards the proteins involved in uterine cancer.

S.No	Name of the compound	Docking Scores								
		3KJF	1PV9	6L8L	2NO3	2ZOQ	5E3S	3SLS	8DJD	3UOR
1	Rutin hydrate	-3.999	--	-7.4668	-7.9144	-12.5556	-5.557	-7.0955	-4.9266	-3.5122
2	Catechin	-5.7761	-5.8955	-7.4667	-7.1996	-7.8887	-5.1339	-5.4335	-5.882	-5.2255

3KJF-Caspase 3;1PV9-Prolidase;6L8L-tyrosine kinases;2NO3-JNK 1;2ZOQ-MAPK3;5E3S-Phosphatidylinositol 4-kinase (PI kinase); 3SLS-MEK-1;8DJD-Glycogen synthase kinase-3 (GSK-3);3U0R-NAIP-neuronal apoptosis inhibitory protein.



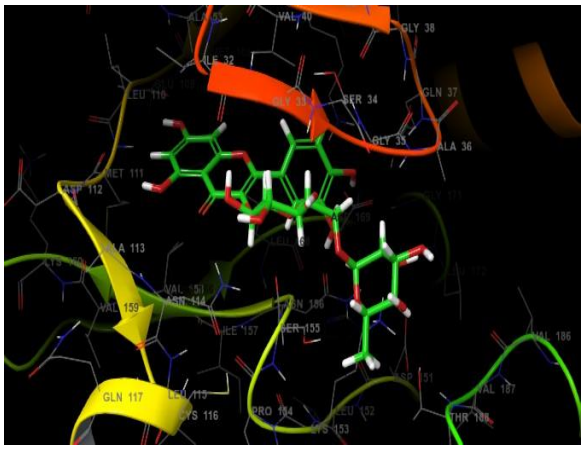
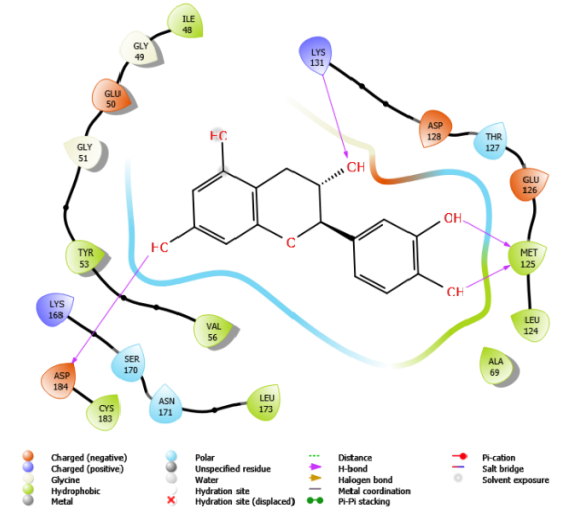


Fig. 3: 2D and 3D interactions of Rutin hydrate with 2NO3 Receptor



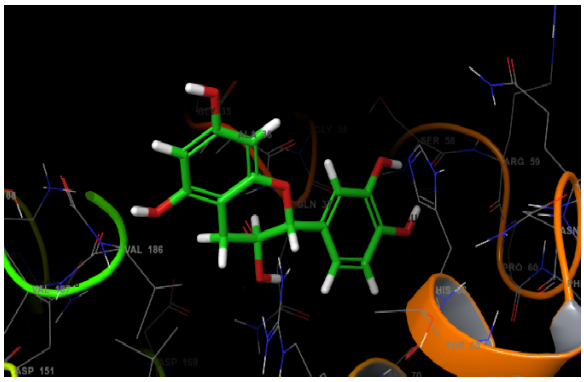
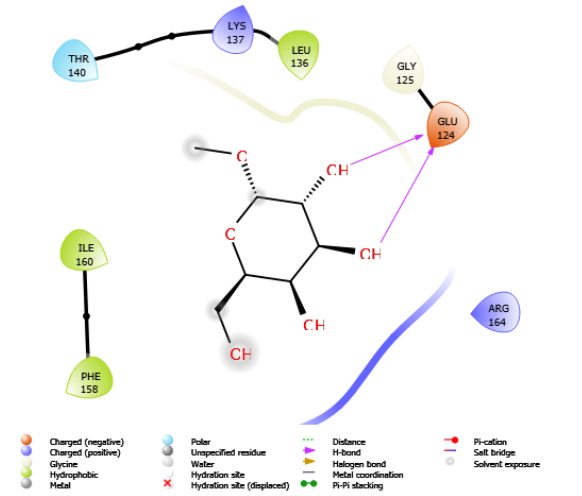


Fig. 4; 2D and 3D interactions of Catechin with 2NO3 Receptor



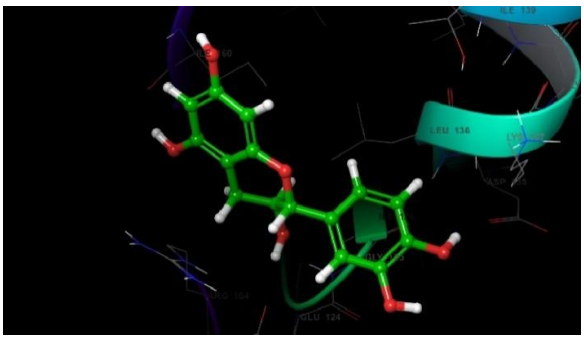
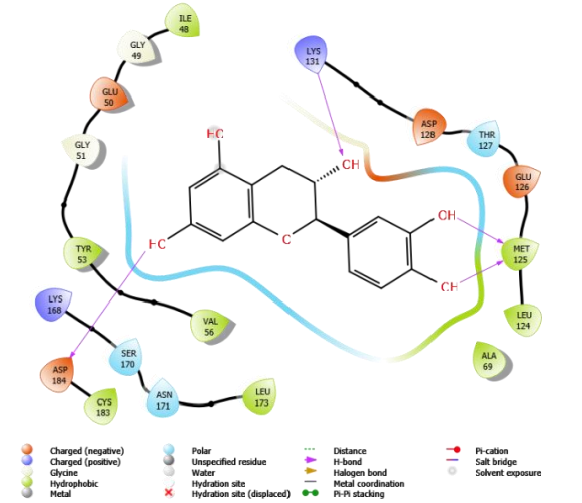


Fig. 5: 2D and 3D interactions of Catechin with 3KJF Receptor



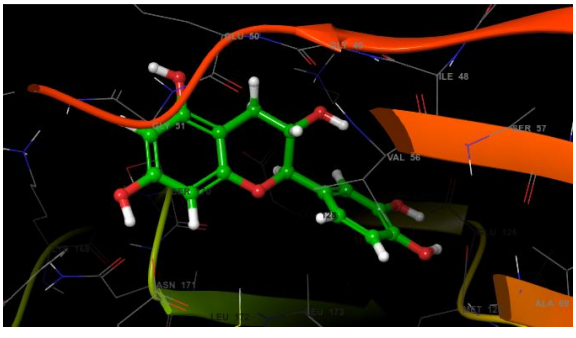


Fig. 6: 2D and 3D interactions of Catechin with 2ZOQ Receptor

The compounds Rutin hydrate and Catechin, were docked with various enzymes, including 2NO3 (JNK-1), 3KJF (Caspase-3), 2ZOQ (MAPK-3), and 1PV9 (Prolidase). Their capacity to interact with the selected proteins is obvious.

Applying the docking score analysis, Catechin has a significant binding affinity for 2ZOQ (MAPK3), a Serine/Theronine kinase that modulates signal transmission from the cell surface to the nucleus, influencing gene transcription related to cell cycle, apoptosis, and cell motility. Rutin strongly binds to 2NO3 (JNK-1).

3.4 Endometrial cancer activity of selected compounds

Rutin and catechin were selected from *Smilax zeylanica* and *Smilax china* respectively.

3.4.1 Acute toxicity studies results

1. Rutin: LD_{50} = 1.49 g/kg 1.49 g/kg = 1.49 × 1000 mg/g = **1490 mg/kg**
2. Catechin: LD_{50} > 10,000 mg/kg (treat conservatively as 10,000 mg/kg when calculating fractions)

Safety dose:**10 mg/kg (single, oral)** is a reasonable single **common safety dose** for both compounds.

The safe dose is considered **10 mg/kg**as per the LD_{50} values:

- Rutin = **1490 mg/kg**
- Catechin = **>10,000 mg/kg** (use 10,000 mg/kg conservatively)

- Using the common conservative safety rule of **1/10 of LD_{50}** for a single-dose "upper safe" starting point:
  - 100 ÷ 10 = **10 mg/kg**.

The safest dose for Rutin 1490 ÷ 10 = 149 mg/kg;

Catechin 10,000 ÷ 10 = 1000 mg/kg.

As we need to treat the drug for 3 months, a safety dose of 1mg/kg b.w is preferred

Table 8: Table for the dose administration

Rat Body Weight (g)	1 mg/kg (chronic dose) – Drug needed (mg)	Volume @ 1 mg/mL (mL)
100 g (0.1 kg)	0.1 mg	0.1 mL
150 g (0.15 kg)	0.15 mg	0.15 mL
200 g (0.2 kg)	0.2 mg	0.2 mL
250 g (0.25 kg)	0.25 mg	0.25 mL
300 g (0.3 kg)	0.3 mg	0.3 mL

3.4.2 Experimental Methodology

Animals

Adult (150-200 g) Wistar albino rats of either sex were purchased from an approved animal breeding facility. The animals were maintained in normal laboratory conditions (22 ± 2 °C with a relative humidity of 50–60% on light/dark cycles of 12 h). The animals were fed upon a standard pellet ration, and ad libitum water was available. All experiments were done according to the guidelines of CPCSEA (Committee for the Purpose of Control and Supervision of Experiments on Animals) and under the approval of the "Institutional Animal Ethics Committee" (IAEC).

Grouping of Animals

The animals were randomly divided into seven experimental groups, with six animals in each group (n = 6):

- **Group I (Control):** Received vehicle only.
- **Group II:** Received MNNG (endometrial cancer inducer).
- **Group III:** Received MNNG + Fluorouracil (standard drug).
- **Group IV:** Received MNNG + Rutin (1 mg/kg).
- **Group V:** Received MNNG + Catechin (1 mg/kg).

Carcinogen-inducing drug: N-Methyl-N′-Nitro-N-Nitrosoguanidine is a **potent mutagen and carcinogen** often used in experimental studies to induce cancers (including gastric, colon, and endometrial cancers) in animal models.

Vehicle Selection

The vehicle was selected based on the solubility and stability profile of the test compounds, as well as its tolerability in rats. Distilled water, 0.5% carboxymethylcellulose (CMC), and polyethene glycol (PEG 400)/water mixtures were considered. The final choice ensured an acceptable pH and viscosity suitable for oral gavage administration.

Preparation of Dosing Solutions

All compounds were weighed precisely using an analytical balance. The test compounds were dissolved or suspended in the selected vehicle to achieve a final concentration of 1 mg/mL. Fresh dosing solutions were prepared daily. In cases where short-term stability was validated, dosing solutions were stored under appropriate conditions. Prior to administration, suspensions were homogenised thoroughly to ensure unifo+rm distribution of the active compound.

Dose Administration

Oral administration of the test and standard compounds was performed using an appropriately sized gavage needle, selected according to the weight and age of the rats. The dose volume was calculated based on the body weight of each animal and was adjusted weekly. Each administration was carried out once daily at the same time to minimise diurnal variation. The exact volume administered and any incidences of regurgitation were documented.

Observations and Record Keeping

The body weight of each animal was recorded prior to the initiation of dosing and subsequently at weekly intervals throughout the study period. In cases where weight loss was anticipated, more frequent measurements were undertaken. Clinical signs were observed daily, and any behavioural, physical, or toxicological abnormalities were documented systematically. Mortality, if any, was recorded promptly.

Ethical Considerations

All experimental procedures involving animals were conducted strictly in accordance with CPCSEA guidelines, ensuring minimal pain and distress to the animals. Humane endpoints were applied whenever necessary, and animals exhibiting severe distress were euthanised in accordance with ethical protocols.

Table 9: Effect of Rutin, catechin, catechin and methyl alpha–D–pyranoside on body weight analysis (physical parameter) on MNNG-induced endometrial cancer in rats

Weeks	Control	Only Mnng	Mnng + Fluorouracil	Mnng + Rutin (1mg/kg)	Mnng+ catechin (1mg/kg)
	Group - I	Group – II	Group - III	Group – IV	Group – V
1 Week	337 ±1.8**	330± 2.4**	345.3 ±1.4**	340.08±1.8	344.14 ±1.0**
3 Week	395 ±2.0**	379.8±1.6**	388.4 ±1.3**	364.85±1.1**	374.38 ±1.6**
5 Week	460 ±1.2**	420.6±2.1**	434.9 ±2.4**	393.05±2.5**	407.1± 2.1***
7 Week	518 ±1.6**	456.2±1.8**	476.1 ±2.0***	419.65±2.5**	452.36 ±1.1***
9 Week	574 ±2.2**	483.1±1.1**	517.3 ±1.1**	448.16±2.0**	482.44 ±2.9***
11 Week	634 ±2.0**	500.7±2.1**	563.5 ±1.8**	476.79±1.1***	523.76 ±1.5***
13 Week	690 ±1.8**	509.7±2.2**	609.5 ±2.2**	504.49±1.9***	564.21 ±1.0***
15 Week	760 ±1.9**	511.8±1.4**	656.2 ±1.0**	541.12±1.7***	602.3± 1.5**

Values are given as mean ± S.D. Statistical significance (p) was determined using one-way ANOVA followed by Tukey's multiple comparison test. Control group comparison was made with the normal standard, and test group comparison was made with the control group. ***P< 0.001 was considered highly significant. **P< 0.001 significant, *P< 0.001 less significant, ns- not significant.

Table 10: Analysis of Tumour volume, Burden,Tumour weight and Tumour incidence

Group	Tumor Volume	Tumor Burden	Tumor Incidence	Uterine Weight gms
Group-1	0.00±0.00	0.00±0.00	0.00±0.00	0.76±0.02
Group-2	4.55±0.67***	13.17±2.04***	22.83±3.81***	1.27±0.18***
Group-3	1.96±0.41***	4.50±1.87***	9.83±1.72***	0.86±0.12***
Group-4	2.99±0.59***	6.16±1.16***	15.50±1.87***	1.11±0.06 ns
Group-5	2.54±2.49***	6.63±1.65***	18.45±2.53***	1.65±0.42**

The isolated uterus was analysed for tumour volume, tumour burden, tumour weight and tumour incidence, which are depicted in the following figures.





G-1 **G-2**

G-3



G-4 **G-5**

4. Conclusion

This research shows that *Smilax zeylanica* and *Smilax china* are abundant in bioactive phytochemicals, as verified through phytochemical screening and HPLC fingerprinting. Rutin and catechin were recognised as key components among the primary compounds. In silico molecular docking showed that these compounds have high binding affinities with key proteins associated with uterine cancer, especially JNK-1 and MAPK3, suggesting their possible involvement in regulating apoptosis and cancer cell signalling pathways. Furthermore, in vivo results indicate a decrease in tumour markers, reinforcing their therapeutic significance. In general, these findings emphasize the encouraging anticancer properties of Smilax

species and offer a scientific foundation for continued pharmacological and clinical research aimed at creating safe and effective plant-derived treatments for uterine cancer.

5. Conflicts of interest

The authors state that there are no conflicting interests.

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7. References

1. Babu J, Pulinil kunnathil RG, Kumar BR.:An epidemiological assessment ofendometrial carcinoma including demographics and histopathological correlation, from a tertiary care teachinghospital in South India. J Evid Based Med Health **8**(30),751-2756(2021)
2. Krishnan Sathishkumar, MeeshaChaturvedi, Priyanka Das, S. Stephen & Prashant Mathur. Cancer incidence estimates for 2022 &projections for 2025: Results from National Cancer Registry Programme, India.Indian J Med Res **1**(156), 598-607 (2022)
3. Dr. Balram Bhargava , Dr. Tanvir Kaur, Dr. NeerjaBhatla, Dr. Selvalaxmi .: Consensus Document For Management Of uterine cancer.(2019)
4. Anchala I. Kuruppu ,PriyaniParanagama , Charitha L. Goonasekara.: Medicinal plants commonly used against cancer in traditional medicine formulae in Sri Lanka, Saudi Pharmaceutical Journal **1**(27), 565–573(2019)
5. Mohammad Nadia1, Maqsood Maryam.: Use of Medicinal Plants for The Treatment of Cancer in Different Parts of The World: An Overview. International Journal of Biomedical and Clinical Research **1**(5),1-9(2024)
6. Shaik Abdul Saleem, Bathula Lakshmi, J.N. Suresh Kumar. :Review Article on the Traditional Outlook of Smilax zeylanica. Research Journal of Pharmacology and Pharmacodynamics. **13**(2),63-68(2021).
7. Baruah, Sanjib.: New distributional record of Smilax china Linnaeus (Smilacaceae) in India. Pleione**1**(5),328-330(2011).
8. Wang, Meng.:Smilaxchina L.: A review of its botany, ethnopharmacology, phytochemistry, pharmacological activities, actual and potential applications. Journal of Ethnopharmacology **1**(1) ,318-324(2024)
9. Mohamad Hesam Shahrajabian.:Tremendous health benefits and clinical aspects of Smilax china.African Journal of Pharmacy and Pharmacology **13**(16),253-258(2019)
10. Prime: v3.1, Schrödinger. LLC, New York, NY,(2012)
11. Maestro: v9.3, Schrödinger. LLC, New York, NY,(2012)
12. National Center for Biotechnology Information. PubChem Compound Summary for CID 5280343, Quercetin. https://pubchem.ncbi.nlm.nih.gov/compound/Quercetin. Accessed Mar. 24, (2022)
13. LigPrep: v2.5, Schrödinger. LLC, New York, NY, 2012
14. Brooks, W. H.; Daniel, K. G.; Sung, S. S.; Guida, W. C.: Computational validation of the importance of absolute stereochemistry in virtual screening. Journal of chemical information and modeling **5**(48), 639-645(2008)
15. Du, J.; Sun, H.; Xi, L.; Li, J.; Yang, Y.; Liu, H.; Yao, X.: Molecular modeling study of checkpoint kinase 1 inhibitors by multiple docking strategies and prime/MM-GBSA calculation. Journal of computational chemistry 4 (32), 2800-2809(2011)
16. Albers, H. M.; Hendrickx, L. J.; van Tol, R. J.; Hausmann, J.; Perrakis, A.; Ovaa, H.: Structure-based design of novel boronic acid-based inhibitors of autotaxin. Journal of medicinal chemistry**5** (54), 4619-4626(2011)
17. Kenyon, V.; Chorny, I.; Carvajal, W. J.; Holman, T. R.; Jacobson, M. P.: Novel human lipoxygenase inhibitors discovered using virtual screening with homology models. Journal of medicinal chemistry **7**(49), 1356-63(2006)
18. William, A. D.; Lee, A. C.; Blanchard, S.; Poulsen, A.; Teo, E. L.; Nagaraj, H.; Tan, E.; Chen, D.; Williams, M.; Sun, E. T.; Goh, K. C.; Ong, W. C.; Goh, S. K.; Hart, S.; Jayaraman, R.; Pasha, M. K.; Ethirajulu, K.; Wood, J. M.; Dymock, B. W.: Discovery of the macrocycle 11-(2-pyrrolidin-1-yl-ethoxy)- 14,19-dioxa-5,7,26-triaza-tetracyclo[19.3.1.1(2,6). 1(8,12)]heptacosa1(25),2(26),3,5,8,10,12(27),16,21,23-decaene (SB1518), a potent Janus kinase 2/fms-like tyrosine kinase3 (JAK2/FLT3) inhibitor for the treatment of myelofibrosis and lymphoma. Journal of medicinal chemistry**3** (54) 4638-58(2011)

19. Siddiquee, K.; Zhang, S.; Guida, W. C.; Blaskovich, M. A.; Greedy, B.; Lawrence, H. R.; Yip, M. L.; Jove, R.; McLaughlin, M. M.; Lawrence, N. J.; Sebti, S. M.; Turkson, J.: Selective chemical probe inhibitor of Stat3, identified through structure-based virtual screening, induces antitumor activity. Proceedings of the National Academy of Sciences of the United States of America **6**(104), 7391-7396(2007)
20. Gadakar, P. K.; Phukan, S.; Dattatreya, P.; Balaji, V. N.: Pose prediction accuracy in docking studies and enrichment of actives in the active site of GSK-3beta. Journal of chemical information and modeling **1**(47), 1446-59(2007)