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In- vitro and in-silico studies of anticancer activity for chemical compounds of *Tecoma stans* extract against human breast cancer (MCF-7)

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ABSTRACT

The anti-cancer characteristics of *Tecoma stans* leaf and root extract are described in this work. Initially, methanol was used to extract the crude extract from the dried leaves and roots of *Tecoma stans*. After that, the extract was examined using FTIR, UV-Visible, and HPLC spectroscopy. The extract was then tested using an in-silico method for molecular docking and compared to Paclitaxel (controller of MCF-7) for its antioxidant and anti-cancer activities on the MCF-7 human breast cancer cell line. *Tecoma stans* roots and leaf extract included chemical substances that showed different effects on the 6QGK and 4AGC proteins, which are known to affect the survival of tumor cells. *Tecoma stans* leaf and root extracts were shown to have promise anti-cancer action, with the root extract having somewhat greater potential.

Key words: *Medicinal plant, UV-visible, FTIR, HPLC, DPPH assay, Anti-cancer activity, Molecular Docking.*

Introduction

Medicinal plants play a vital role in promoting a healthy lifestyle and enhancing well-being. Studies have determined the bioactive components of certain plants, including flavonoids and phenols, through UV-visible and FTIR spectroscopy. HPLC analysis revealed the presence of approximately twenty-three compounds in the leaves, stems, and fruits of medicinal plants (Senthamilselvi, Kesavan, and Sulochana, 2011;Solomon, Muruganantham, and Senthamilselvi, 2015 and Huang *et al.,* 2006). The ethyl acetate extract of leaves and flowers exhibited significant antioxidant activity in a dose-dependent manner, as determined through in-vitro antioxidant activity and cytotoxicity analysis (Atmani *et al*., 2009; Gonzalez-Angulo, Morales-Vasquez, and Hortobagyi, 2007; Tong *et al.* 2018). The IC-50 values for DPPH scavenging assays were also determined.

Materials and Methods

Collection of plant materials

The Poondi Thanjavur district in Tamil Nadu, India, yielded 3 kilogram of *Tecoma stans* leaves and roots. The plant components were ground into a fine powder, allowed to dry under shade for three weeks, and then extracted using methanol and Soxhlet extraction method. For later usage, the semisolid crude extract that was produced was refrigerated. Several techniques were used to analyze the phytochemicals in the methanol extract. A UV-visible spectrophotometer (JASCO, V-630) and an FTIR-4000 system from JASCO were used to characterize the extract.

Peak values from the FTIR and UV-visible investigations were noted. The following were the LC-MS/HPLC settings for the analysis: 5% B for the first three minutes, a progressive rise to 20% B for the next three, a further increase to 40% B for the next four minutes, and finally a final climb to 50% B for the final five minutes. A Thermo Electron LTQ-Orbitrap XL mass spectrometer equipped with a nano-electrospray ion source was used to conduct the MS study. Data-dependent automatic switching between MS and MS/MS acquisition modes was used in the MS analysis.

In-vitro antioxidant activity was determined by DPPH Method

The MS analysis involved a steep increase from 5% B to 95% B over a period of 63 minutes; followed by a gradual increase from 20% to 40% B. Thermo Electron LTQ-Orbitrap XL mass spectrometer was used, with a Nano electrospray ion source, and operated under X Caliber 2.1 version software in positive ionization mode. The analysis employed data-dependent automatic switching between MS and MS/MS acquisition model, ensuring accurate results.

Radical scavenging activity (%) = 100- [A_0-A_S/ A_0] X 100

Where A_0 is the absorbance of the control and as is the absorbance of the samples at 517 nm

In-vitro anticancer activity was determined by the following methods.

In-vitro anti-cancer activity

The National Centre for Cell Science (NCCS) in Pune provided the MCF-7 human breast cancer cell line for this study. The cells were cultured in Eagles Minimum Essential Medium (EMEM) supplemented with 10% fetal bovine serum (FBS), penicillin, and streptomycin in a 5% CO_2 humidified atmosphere at 37°C (Mitra *et al.,* 2016). The cytotoxic assay was performed using the MTT reduction assay. The cell suspension was diluted with a medium containing 5% FBS to achieve a final density of 1X105 cells/mL. 96-well plates were seeded with 10,000 cells/well and incubated at 37 °C, 5% CO_2, 95% air, and 100% relative humidity for cell attachment (Lacaille-Dubois *et al.,* 2000; Sankar, Maheswari, *et al.*, 2014). Aliquots of leaf and root extracts were dispensed into the designated wells, and the MTT metabolic product was dissolved in 100µl of DMSO. The IC-50 concentration was determined graphically, and the medium without samples was used as a control. The impact of the samples on MCF-7 antiproliferation was quantified as % cytotoxicity using specific formulas (Masoud and Pagès, 2017).

% Cytotoxicity = 100 - [$Abs_{(sample)}$ / $Abs_{(control)}$] X 100.

% Cell Viability = [$Abs_{(sample)}$ / $Abs_{(control)}$] X 100.

In-silico docking studies

The protein data bank (PDB) and Pubchem were used to obtain protein structures and ligands for an interaction study (Linkuvien et al., 2018). The Swiss Dock server was used to identify clusters and potential interactions. The ligands were prepared using a ligand preparation pipeline available in POAP, which utilizes Open Babel for ligand preparation and optimization. The 3D structural coordinates were converted through a weighted rotor search, generating 50 conformers for each ligand. The best structure with the lowest energy was selected, and the ligands were converted into PDBQT format using the prepare ligand pipeline. The POAP virtual screening pipeline was employed for docking multiple ligands with multiple proteins (Sankar *et al*., 2014). The input for the virtual screening process included receptor files with grid and docking parameters, as well as the prepared receptor structure. The top hit compounds and complex files containing the protein were obtained. A 2D plot was generated using Discovery Studio software to visualize the ligand structures. The Parallelized Open Babel and Auto dock Pipeline (POAP) was developed to automate and optimize the ligand database and perform virtual screening with minimal user intervention (Yuriev, Holien, and Ramsland, 2015).

Statistical analysis

To assure accuracy, the trials were run three times. Using a linear regression technique, the MS-Windows-based Graphpad Instat (version 3) program was used to graphically predict the quantity of extract needed to suppress free radicals concentration by 50% IC-50. Graphical/mean ± standard deviation findings were displayed (Heryanto Heryanto *et al., 2024)*.

Results and Discussion

The dried *Tecoma stans* leaves and roots were extracted with methanol to produce a crude extract. UV-visible spectroscopy was used to characterize

the extract. The leaf extract showed spectral bands at 205, 288, and 320 nm, while the roots extract showed peaks at 208 and 289 nm, indicating the presence of flavonoids (Linkuvien *et al.*, 2018).

The leaf extract exhibited spectral bands at approximately 205, 288, and 320 nm, as depicted in

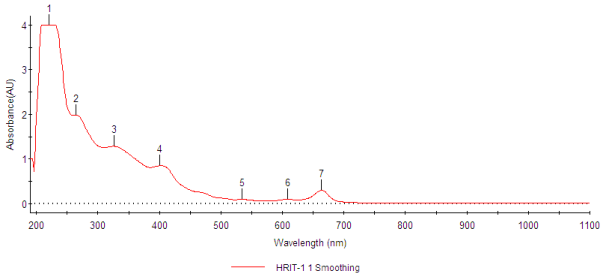

Fig. 1. UV spectrum of leaves extract

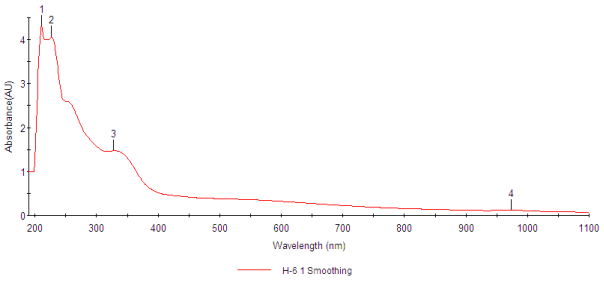

Fig. 2. UV spectrum of roots extract

Fig. 1, whereas the roots extract displayed peaks at 208 and 289 nm in Fig. 2. Both figures illustrated that the spectral lines corresponded to the presence of flavonoids, which typically fall within the range of 210–290 nm.

The FTIR spectrum of the leaves' crude extract was illustrated in Fig. 3, showing characteristic peaks at 3406 cm^{-1}, 2929 cm^{-1}, 1710 cm^{-1}, 1516 cm^{-1}, 1394 cm^{-1}, 1383 cm^{-1}, 1263 cm^{-1}, 1159 cm^{-1}, and 880 cm^{-1} corresponding to -O-H, -C-H, -C=O, -C-C, -O-H bending, -C-H, -C=C, -C-O, and -C-H stretching vibrations, respectively, associated with aromatic

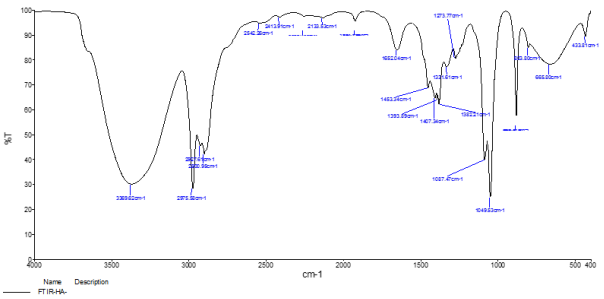

Fig. 3. FTIR spectrum of leaves extract

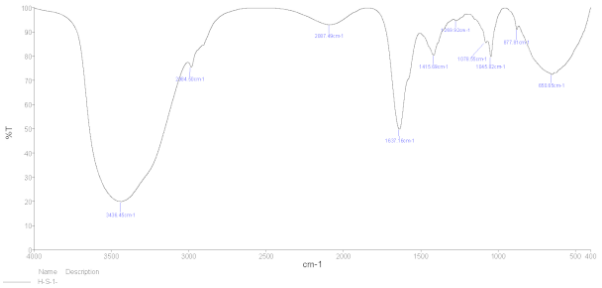

Fig. 4. UV-Visible spectrum of roots extract

groups. Similarly, the FTIR spectrum of the roots' crude extract was displayed in (Fig. 4), revealing characteristic peaks at 3378 cm^{-1}, 2975 cm^{-1}, 2130 cm^{-1}, 1451 cm^{-1}, 1230 cm^{-1}, and 803 cm^{-1} related to -C-H, -C a"C, C-C, -C-N, and C-H vibrations attributed to aromatic groups (Veeravelan *et al.*, 2024; Rauf *et al.* 2024; Rauf *et al.* 2022; Valentini, Nappi, and Nitti, 2002).

HPLC chromatogram comparison of methanol extract of *Tecoma stans* root

The HPLC method was utilized for the detection of

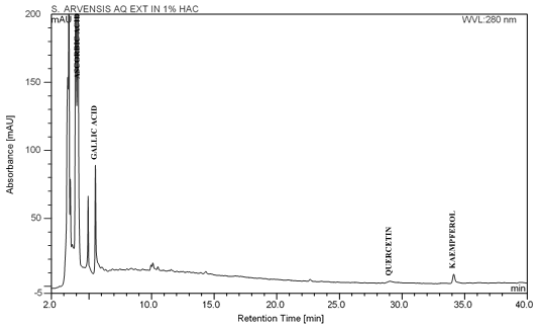

Fig. 5. HPLC chromatogram comparison of methanol extract of Tecoma stans root

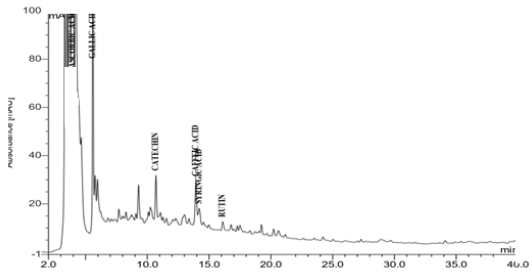

Fig. 6. HPLC chromatogram comparison of methanol extract of Tecoma stans leaf

flavonoid compounds in the analysis of extracts from leaves and roots. The analysis revealed the presence of ten flavonoid compounds, including 1-

Table 1. Flavonoid compounds of *Tecoma stans* root extract

Peak#	Retention. time	Area	Height	Area %	Height %	Compounds Identified
1	4.092	14720743	268439	76.762	77.433	Ascorbic acid
2	5.698	9311807	113050	22.048	21.296	Gallic acid
3	29.054	606	128	0.145	0.219	Quercetin
4	34.25	1025	301	1.045	1.052	Kaempferol

Table 2. Flavonoid compounds of *Tecoma stans* leaf

Peak#	Ret. Time	Area	Height	Area %	Height %	Compounds identified by literature*
1	3.576	5646975	204074	18.819	15.79	Quercetin
2	4.391	14458315	651188	48.184	50.385	Gallic Acid
3	5.228	14458315	384109	27.78	29.72	Apigenin
4	7.235	14458315	39956	3.908	3.092	Resorcinol
5	9.364	369061	12170	1.23	0.942	Caffeic Acid
6	16.942	137	14	0	0.001	Cyanidin -3-O-glucoside

Ascorbic acid; 2-Gallic Acid, 3-Quercetin, 4-Kaempferol, 5-Ascorbic acid, 6-Gallic Acid, 7-Catechin, 8-Caffeic acid, 9-Syringic acid, and 10-Rutin (shown in Table 1 & 2).

HPLC chromatogram comparison of methanol extract of *Tecama stans* leaves

Four flavonoid chemicals have been identified by study of the root extract: 1-Ascorbic acid, 2-Gallic Acid, 3-Quercetin, and 4-Kaempferol. Table 2 further indicates that the extract also includes 5-Ascorbic acid, 6-Gallic Acid, 7-Catechin, 8-Caffeic Acid, 9-Syringic Acid, and 10-Rutin.*Tecoma stans* roots' flavonoid components are shown in Fig. 6.

In-vitro antioxidant activity of Tecoma stans leaf and roots extract

The investigation of the antioxidant activity of *Tecoma stans* leaves and roots extract through a DPPH assay (Yuriev, Holien, and Ramsland, 2015) .

DPPH radical scavenging activity

A popular technique for assessing the antioxidant capabilities of plant extracts is the DPPH assay. Fig-

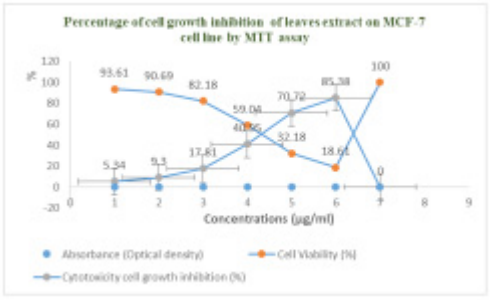

Fig. 8 leaves extract on MCF-7 cell line by MTT assay

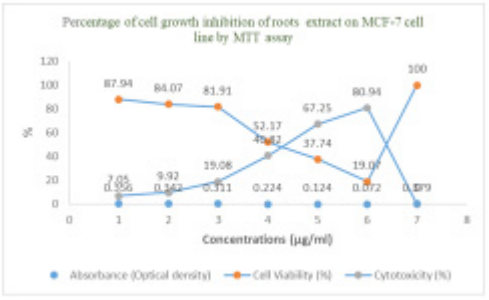

Fig. 9 of cell growth inhibition of roots extract on MCF-7 cell line by MTT assay

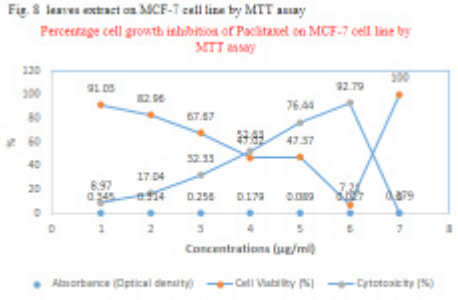

Fig. 10 Paclitaxel on MCF-7 cell line by MTT assay

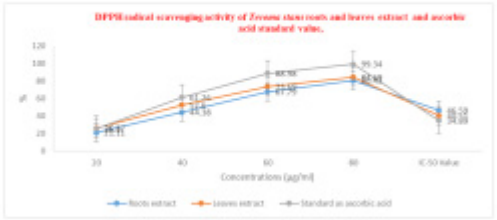

Fig. 7 Comparison of DPPH radical scavenging activity of *Tecoma stans* plants extract

ure 7 illustrates the comparison between ascorbic acid and *Tecoma stans* leaves' ability to scavenge DPPH radicals in this study (Ichsan Rauf *et al.*, 2024). The ascorbic acid, leaf extract, and root extract had IC-50 values of 34.89 ìg/ml, 40.51 µg/ml, and 46.59 µg/ml, respectively. The plant extract inhibited DPPH activity in a dose-dependent manner. The content of L-ascorbic acid directly affects its capacity to scavenge DPPH radicals. Out of the leaf and roots extracts, the floral extract showed the most promise and was almost as active as the standard.

In-vitro anticancer activity of *Tecoma stans* leaf and roots extract

Tecoma stans leaf and roots extracts were tested on the MCF-7 cell line, a cell line used in *In vitro* breast cancer research due to its unique characteristics resembling the mammary epithelium. The extracts showed potential inhibitory effects on cell growth, with the lowest observed growth inhibition being 6.39% at 12.5 µg/ml for the *Tecoma stans* leaf extract, and the highest at 81.38% at 400 µg/ml for the *Tecoma stans* root extract. Similarly, the *Tecoma stans* extract showed significant anticancer activity, comparable to the standard Paclitaxel. The IC-50 values of the leaves extract, roots extract, and standard Paclitaxel were 205.35, 196.61, and 147.42 ìg/ml, respectively. The results suggest that *Tecoma stans* extracts can potentially inhibit cell growth and improve the effectiveness of Paclitaxel in treating breast cancer (Tong *et al.* 2018).

In-silico docking analysis

The current investigation explores the role of B-cell lymphoma 2 (Bcl-2) and Vascular endothelial growth factor receptor 2 (VEGFR2) proteins in promoting tumor cell survival. The study tested these proteins against various compounds derived from *Tecoma stans* leaves and roots, such as 3,5-O-Dicaffeoylquinic acid, Isorhamnetin-3-O-rutinoside, 1-O-Caffeoyl-5-O-feruloylquinic acid, Quercetin-7-O-rhamnoside, Quercetin-3-O-glucoside, Chrysoeriol, 2',6-Dihydroxyflavone, 6-Ethoxy-3(4'-hydroxyphenyl)-4-methyl coumarin, 4-Hydroxy- 2', 3, 4', 6'-tetramethoxychalcone, and Maltotriose. The bio-computational analysis assessed the activation of gene expression using the scoring function. The highest energy score for 3,5-O-Dicaffeoylquinic acid (-8.0) and the lowest energy score for Maltotriose (-5.3), indicating potential binding interactions and subsequent expression of Bcl-2. The probable binding interaction of the ligand (VEGFR2) suggests the initiation of apoptosis. Isorhamnetin-3-O-rutinoside (-8.3) exhibited the highest apoptotic activity among the 10 compounds. The expression of VEGFR2 was

Table 3. Docking results against 6QGK and 4AGC

S. No.	Name of the compound	GOLD Score (kcal/mol)	
		6QGK	4AGC
1	Syringic acid	-28.04	-37.73
2	Gallic acid	-23.18	-29.63
3	Quercetin	-32.6	-52.3
4	Rutin	-33.33	-23.68
5	Kaempferol	-33.8	-53.05
6	Ascorbic acid	-26.47	-30.13
7	Caffeic acid	-26.09	-36.12
8	Catechin	-36.57	-47.78

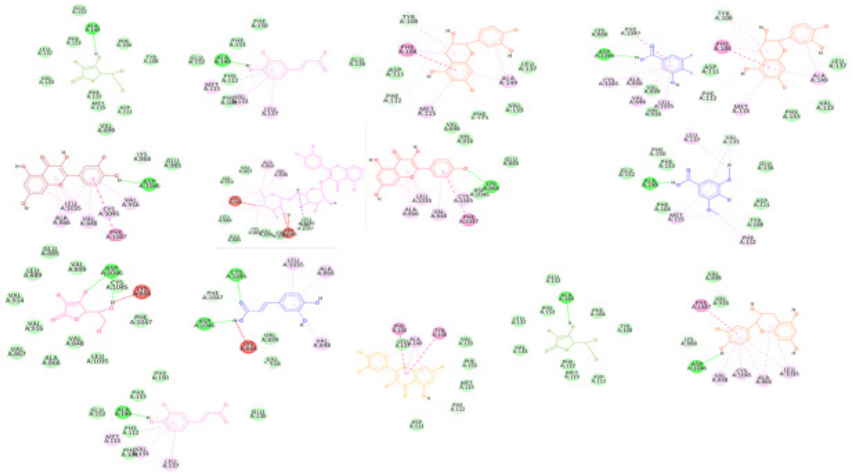

Fig. 11. Docking results against 6QGK and 4AGC

inhibited by the 10 compounds, as confirmed by docking studies. The study highlights the importance of energy and binding in promoting gene expression and promoting tumor cell survival.

In silico docking analysis of *Tecoma stans* leaves and root extract

Among the 8 compounds, the highest binding energy of gallic acid (-23.18) was observed in 6QGK / BCL2 while rutin(-23.68) was observed in 4AGC/ VEGFR2

Conclusion

The results of the present research demonstrate that *Tecoma stans'* leaves and roots are a rich source of natural antioxidants that may be helpful in the treatment of oxidative stress-related illnesses like cancer and arthritis. The MCF-7 cell line was used to validate the anticancer characteristics, and in silico research revealed that the cancer genes Bcl-2 and VEGFR2 were suppressed. The findings of this study validate the anti-tumor properties of the *Tecoma stans* leaf and root extract. For the therapeutic benefit to be confirmed, clinical trials are required. The antioxidant qualities of *Tecoma stans'* leaves and roots have never been scientifically verified before, and this study supports the plant's traditional use in South India. The roots extract exhibits the highest potential for action among the extracts.

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Conflict of Interest: None
Funding: Self
Ethical Clearance: Not required

Declaration of Competing Interest

The authors affirm that they possess no recognized conflicting financial interests or personal associations that may have seemed to impact the research presented in this manuscript.

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