J Herbmed Pharmacol. 2023; 12(3): 432-441.

http://www.herbmedpharmacol.com doi: 10.34172/jhp.2023.48

Journal of Herbmed Pharmacology

Dual antineoplastic and photodynamic effects of methanolic extract of *Tecoma stans* yellow flowers for cancer treatment

Asmaa Y Khattab[1], Nagwa E Awad[2], Doaa Abdel Fadeel[1*], Maha Fadel[1]

[1]Pharmaceutical Nano-Technology Unit, Department of Medical Applications of Laser, National Institute of Laser Enhanced Sciences (NILES), Cairo University, P.O. 12613, Giza, Egypt
[2]Pharmacognosy Dept., National Research Centre, Dokki, Giza, Egypt

ARTICLE INFO

Article Type:
Original Article

Article History:
Received: 1 March 2023
Accepted: 30 April 2023

Keywords:
Bignoniaceae
Photosensitizing agents
Anthocyanins
Coumarins
Phytochemicals

ABSTRACT

Introduction: *Tecoma stans* is a fast-growing plant from the family Bignoniaceae. Various parts of *T. stans* have been used in different biological applications, especially in cancer treatment. Photodynamic therapy (PDT) is a promising modality for cancer treatment that depends on the interaction between a photosensitizer, light, and oxygen. Searching for photosensitizers from plant origin is crucial to provide nontoxic photosensitizers with high economic value. This study aims to evaluate the anticancer and photodynamic activities of *T. stans* methanolic flower extract (TSFE).

Methods: The phytoconstituents of TSFE were analyzed by the UPLC/MS/MS technique. The cytotoxicity of TSFE was examined on the breast carcinoma (MCF-7) and lung carcinoma (A549) cell lines, in dark and after irradiation by blue light (400-450 nm).

Results: TSFE contained various phytochemical components with antineoplastic activity. Moreover, TSFE contained coumarins and anthocyanins that may act as photosensitizers. TSFE showed negligible cytotoxicity against MCF-7 cell lines at all tested concentrations in dark. A non-significant cell viability change was observed upon radiation ($P>0.05$). TSFE showed significant dark cytotoxicity on A549 cells, which improved significantly after light radiation ($P<0.05$).

Conclusion: TSFE is a promising anticancer and natural photosensitizer for PDT and this study may inspire further ethnobotanical investigations into promising new natural anti-cancers and photosensitizers.

Implication for health policy/practice/research/medical education:
Tecoma stans methanolic flower extract (TSFE) contained coumarins and anthocyanins that may act as photosensitizers. The TSFE showed cytotoxicity against lung cancer and breast cancer cell lines after being irradiated. Therefore, TSFE may be a promising natural photosensitizer to be used in photodynamic therapy of different cancers.

Please cite this paper as: Khattab AY, Awad NE, Fadeel DA, Fadel M. Dual antineoplastic and photodynamic effects of methanolic extract of *Tecoma stans* yellow flowers for cancer treatment. J Herbmed Pharmacol. 2023;12(3):432-441. doi: 10.34172/jhp.2023.48.

Introduction

Plants have been used for centuries as the main source of medication in folk medicine, especially in developing countries. They are considered cheaper and safer with fewer adverse reactions than many synthetic chemicals. Moreover, plants contain several phytochemical constituents which possess a wide range of biological activities (1).

Tecoma stans (L.) Juss. Ex Kunth (Bignoniaceae) is a fast-growing ornamental plant grown in Egypt and in many tropical and subtropical areas (2,3). It is widely used in folk medicine for several purposes. Previous pharmacological studies have revealed that *T. stans* has various biological activities such as antidiabetic, anticancer, antioxidant, antispasmodic, antimicrobial, and antifungal activities (4-7). The wide range of activities is attributed to a diversity of phytoconstituents isolated from different parts of the plant.

In the field of cancer treatment, searching for anti-cancer drugs that are derived from plants has attracted great attention. The anti-cancer plant-derived compounds may be direct cytotoxic compounds or photosensitive ones

***Corresponding author**: Doaa Abdel Fadeel,
Email: d_fadeel@cu.edu.eg, doaa.fadeel@niles.edu.eg

called photosensitizers. A photosensitizing (PS) agent can be excited with a specific wavelength of light in the presence of tissue oxygen. This photochemical interaction results in the generation of reactive oxygen species and free radicals that are cytotoxic to the cells. These cytotoxic species exert their cytotoxic effects only at the site where they are generated. Therefore, by exposing the site of action only to the specific light wavelength, a high degree of selectivity can be attained. This light-dependent modality of treatment is known as photodynamic therapy (PDT) (4,8), which is considered a promising choice for cancer therapy because of its low systemic toxicity and can be used repeatedly.

A significant challenge in the field of PDT is to get photosensitizers from natural plant extracts. Natural photosensitizers are preferred to synthetic ones owing to their high production yield, high biodegradability, high safety, efficacy, and low cost (9,10). It has been reported that herbal remedies that include a mixture of PS phytochemicals can be employed as alternatives to conventional PSs for PDT (11,12). The chemical structures of several phytochemicals contain chromophores that can absorb light at appropriate wavelengths effectively. Thus, these phytochemicals are effective PDT candidates but need to be investigated for their chemical characteristics, provenance from nature, and photo-pharmaceutical characteristics (12).

In this context, *T. stans* is one of the promising plants that may contain PS agents, which can be used in PDT, in addition to other cytotoxic phytoconstituents.

This study aims to determine whether a methanolic extract of *T. stans* flowers (TSFE) can be utilized to treat cancer both conventionally and using PDT. Flowers were selected due to their high content of many phytocomponents with various activities. We analyzed the phytochemical components of TSFE and investigated both the cytotoxicity and the photodynamic activities of the whole extract on breast cancer and lung cancer cell lines.

Materials and Methods

Plant collection extract preparation

Flowers of *T. stans* were collected from El merryland garden (Heliopolis, Cairo, Egypt) in August 2019. This plant material was identified and authenticated at the National Research Center, Egypt. Fresh flowers were collected, washed with distilled water, dried on a 3 mm PC sheet for one hour at room temperature, and cut into small pieces for further evaluation.

The small pieces of the fresh flowers (0.6 kg) were soaked in methanol and ground using an electric mixer then, transferred to a 5-L percolator and allowed to dropwise at a rate of 5 drops/min, methanol was added (500 mL) until complete exhaustion of the flower's marc. The collected methanol extract was evaporated under reduced pressure using a rotavapor (Heidolph, Germany) until complete dryness. The dried extract (138 gm equal to 23%w/w fresh flowers) was stored in a quick-fit flask in the dark and frozen for further phytochemical and biological analysis. The extract was prepared in dim laboratory light and kept in darkness.

Qualitative estimation of TSFE by ultra-performance liquid chromatography coupled to tandem high-definition mass spectrometry (UPLC-MS/MS)

Sample preparation

UPLC-MS/MS analysis was done, using AB Sciex TripleTOF 5600+, in the basic research department, proteomics unit, at 57357 Children's Cancer Hospital (Alsayeda Zeinab, Cairo, Egypt).

First, a reconstitution working solvent was prepared as a mixture of deionized water, methanol (purchased from Fisher Scientific, UK), and acetonitrile (purchased from Sigma-Aldrich, Germany) in ratios of 50: 25: 25, respectively. One milliliter of the prepared solvent was added to 50 mg of weighted TSFE and vortexed for 2 minutes followed by ultrasonication for 10 minutes and centrifugation for 10 minutes at 10 000 rpm. Then, 50 µL of the prepared extract solution was diluted with 1 ml of the reconstitution solvent. Finally, 10 µL of the extract solution (contained 1 µg/µL TSFE) was injected in positive mode and negative mode. 10 µL of the reconstitution solvent was injected as a blank sample.

Acquisition Method

Data processing

MS-DIAL 3.52, Database was used: Respect negative (1573 records) and positive (2737). Master View was used for feature (peaks) extraction from Total ion chromatogram (TIC) based on the following criteria: Features should have a Signal-to-Noise greater than 5 (non-targeted analysis). Feature's intensities of the sample-to-blank should be greater than 3.

Spectrophotometric analysis

UV-visible absorption spectra of a diluted methanolic TSFE were recorded in the wavelength range of 200-800 nm using a Rayleigh 2601 double-beam spectrophotometer (Beijing, China), using methanol as a reference blank.

Cytotoxicity assay

Cell culture

The cytotoxicity of TSFE was tested using two cell lines, Breast Adenocarcinoma cells (MCF-7) and Lung Cancer cells (A-549). Both cell lines were obtained from Nawah Scientific Inc., (Mokatam, Cairo, Egypt). Cells were cultured in DMEM media supplemented with 100 mg/mL of streptomycin, 100 units/mL of penicillin, and 10% of heat-inactivated fetal bovine serum (all obtained from Lonza, Belgium), and incubated in humidified 5% (v/v)

CO2 atmosphere at 37°C.

Dark and photo-cytotoxicity

Aliquots of 100 µL cell suspension (5×10^3 cells) were seeded in 96-well plates and incubated in complete media for 24 hours. Cells were treated with another aliquot of 100 µL media containing the flower extract at various concentrations (0.01 to 100 µg/L) and incubated for 72 hours. For dark cytotoxicity, the cell viability was assessed by sulforhodamine B (SRB) assay, immediately after the incubation period. The media was replaced with 150 µL of 10% trichloroacetic acid (TCA) to fix the cells and incubated at 4°C for 1 hour. The TCA solution was removed and the cells were washed 5 times with distilled water. Aliquots of 70 µL SRB solution (0.4% w/v) were added and incubated in a dark place at room temperature for 10 min. Plates were washed 3 times with 1% acetic acid and allowed to air-dry overnight. Then, 150 µL of tris(hydroxymethyl)aminomethane (10 mM) was added to dissolve the protein-bound SRB stain; the absorbance was measured at 540 nm using a BMG LABTECH®-FLUOstar Omega microplate reader (Ortenberg, Germany).

For photocytotoxicity determination, the cells were irradiated after the 72 hours incubation period. The irradiation was done using a light emitting diode (LED) light source fitted with a 450 nm band path filter (Photon scientific, Cairo, Egypt) at 90 mW/cm^2 for 15 minutes. Afterward, an SRB assay was conducted as described above.

Results

Identification of the compounds

Interpretation of UPLC/MS/MS was conducted using the database of 57357 Children's Cancer Hospital library (Alsayeda Zeinab, Cairo, Egypt). The spectra of the unknown components were compared to the spectra of the known authentic reference standards from the library. Eighty-seven and 44 compounds were identified from the positive acquisition mode (Figure 1) and the negative acquisition mode (Figure 2), respectively.

The structures of the identified compounds were confirmed based on the peak area, retention time, molecular formula, and molecular weight (Table 1). The results revealed that TSFE contained a diversity of phytochemical components such as coumarins (8%),

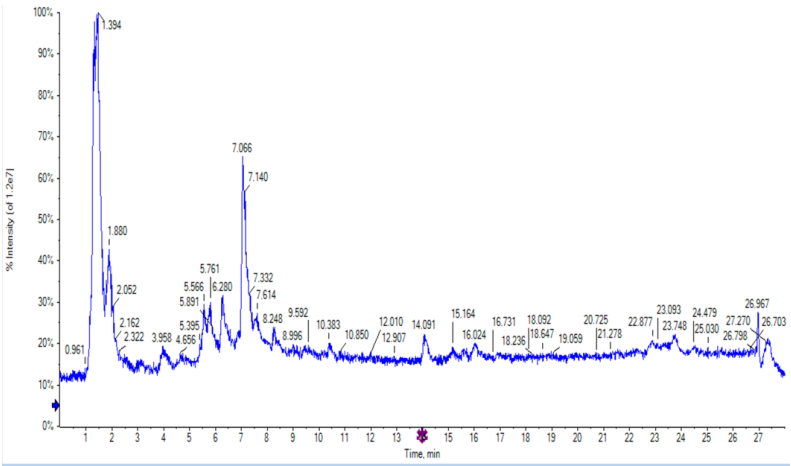

Figure 1. Total ion chromatogram in positive ionization mode of methanolic extract of *Tecoma stans* yellow flowers.

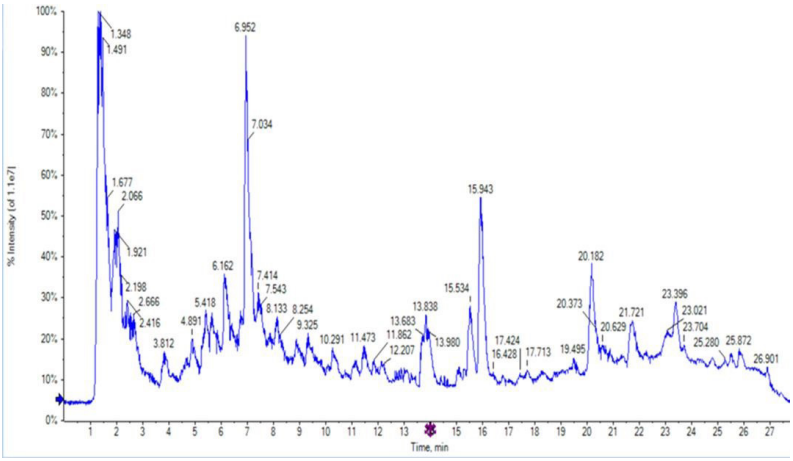

Figure 2. Total ion chromatogram in negative ionization mode of methanolic extract of *Tecoma stans* yellow flowers.

Table 1. UPLC/MS/MS of the phytochemical components of methanolic extract of *Tecoma stans* yellow flowers (TSFE)

Retention time	Parent ion m/z		Identified compound	MS/MS Fragments	Molecular formula	Chemical class	Ref.
	$[M-H]^-$	$[M+H]^+$					
1.12	117		Succinic acid	117, 73[M^--CO_2]	$C_4H_6O_4$	Phenolic acid	(13)
1.19	191		Quinic acid	191,173[M^--H_2O], 147[M^--CO_2], 111[M^- -2H_2O-CO_2], 93[M^- -3H_2O-CO_2], 85[M^-- 2CO_2-H_2O]	$C_7H_{12}O_6$	Phenolic acid	(14)
1.21	359		Rosmarinic acid	359, 315[M^--CO_2], 197[M^--Hexose], 222.9,161, 179[M^--H-caffeic acid], 147	$C_{18}H_{16}O_8$	Phenolic acid	(15)
1.32	133		Malic acid	133, 115[M^--H_2O], 89[M^--CO_2], 71[M^--CO_2, H_2O], 59[M^--CH_3, CO_2]	$C_4H_6O_5$	Phenolic acid	(13)
1.34	477		Isorhamnetin-3-O-glucoside	477, 409, 297, 135 341[477-2CO_2, H_2O-CH_3] 311[341-CH_3] 179[477-CH_3-2CO_2]	$C_{22}H_{22}O_{12}$	Flavonoids (Flavonols)	(16)
1.34	341		Sucrose	341, 179[M^- -Hexose],161[M^- -Hexose -H_2O], 149, 119	$C_{12}H_{22}O_{11}$	Sugar	(17)
1.35	269		Apigenin	268, 200, 180[M^--2CO_2], 88[M^--Hexose, H_2O], 89	$C_{15}H_{10}O_5$	Flavonoids (Flavones)	(14)
1.39	283		Acacetin	283, 103[M^--Hexose -H_2O], 75 [M^--Rhamnose - CO_2 - H_2O]	$C_{16}H_{12}O_5$	Flavonoids (Flavones)	
1.40		137	Sabinene	137, 122[M^+-CH_3], 119, 110, 109, 65	$C_{10}H_{16}$	Bicyclic Monoterpenes	
1.44	449		Okanin-4'-O-glucoside	449, 403, 306[M^--Rhamnose, 4H_2O, 2CO_2], 241, 240[M^--Rhamnose, H_2O, 3CH_3], 179, 143, 101	$C_{21}H_{22}O_{11}$	Flavonoids (Chalcones)	(18)
1.48	497		Dicaffeoylshikimic acid	335[M^- -caffoeyl],179,161,135	$C_{16}H_{16}O_8$	Phenolic acid	
1.50		137	γ-Terpinene	137, 119, 109, 91, 65	$C_{10}H_{16}$	Monoterpene	
1.53	445	447	Baicalein 7-O-glucuronide	447, 429[M^+-H_2O] 349[M^+-CO_2-3H_2O] 267[M^+-Hexose-H_2O], 171, 131, 130	$C_{21}H_{18}O_{11}$	Flavonoids	(19)
1.83		317	3 3'4' 5-tetrahydroxy-7-methoxyflavone (rhamnetin)	317, 263[M^+-3 H_2O], 203, 179, 173[M^+-Hexose-H_2O], 161, 137, 85	$C_{16}H_{12}O_7$	Flavonoids (Flavonols)	(20)
1.84		109	1,4-Benzoquinone	109, 94[M^+-CH_3] ,81,72,66,55, 51[81-CH_3] 54[72-H_2O]	$C_6H_4O_2$	Benzoquinones	
1.86		179	Daphnetin	179, 161 [M^+-H_2O], 133 [M^+-H-COOH], 133, 117[M^+-H_2O-CO_2], 105, 89, 71, 59	$C_9H_6O_4$	Coumarin	(21)
2.00		195	Ferulic acid	195, 179, 178[M^+-H_2O], 93[M^+-4H_2O-2CH_3]	$C_{10}H_{10}O_4$	Phenolic acid	
3.16		419	Kaempferol-3-o-alpha-L-arabinoside	419, 251[M^--pentose, 2 H_2O], 200, 149[M^--2CO_2, Rhamnose, 2 H_2O], 97[M^--4 CO_2-Rhamnose], 71	$C_{20}H_{18}O_{10}$	Flavonoids (Flavonols)	

Table 1. Continued

Retention time	Parent ion m/z		Identified compound	MS/MS Fragments	Molecular formula	Chemical class	Ref.
	$[M-H]^-$	$[M+H]^+$					
3.87		225	Methyl jasmonate	225, 207[M+-H_2O], 189[M^+-2H2O], 175, 163[M^+-CO_2, H_2O], 147, 91	$C_{13}H_{20}O_3$	Flavonoids	
4.10		193	Scopoletin	178[M^+ - CH_3] ,133[M^+ -CO-CH_3-OH]	$C_{10}H_8O_4$	Coumarin	(22)
4.6	463	465	Quercetin-4'-glucoside	463, 394[463-3H_2O, CH_3], 354.9,301, 286.9[M^+-CO_2-Pentose], 218.9[M^+-CO_2-Rhamnose-3H_2O], 190.9,112.9	$C_{21}H_{20}O_{12}$	Flavonoids (Flavonols)	
5.16		380	S-Lactoylglutathione	380, 218[M^+-Hexose] 336[M^+-CO_2], 327, 201, 183, 165, 137	$C_{13}H_{21}N_3O_8S$	Oligopeptides	
5.50	461		Kaempferol-3-Glcuronide	461, 392[M^--CH_3- 3 H_2O], 324, 285[M^--Pentose-CO_2], 256[M^--CH_3-2CO_2- H_2O]	$C_{21}H_{18}O_{12}$	Flavonoids (trihydroxyflavone)	
5.76	507		Syringetin-3-O-galactoside	507, 463[M^--CO_2], 394.9,354.9, 287[M^--2CO_2, Pentose], 258.9, 218.9	$C_{23}H_{24}O_{13}$	Flavonoid galactosides	(23)
6.46	384.86	387	1-O-b-D-glucopyranosyl sinapate (Hydroxycinnamic acid)	384, 385, 340 [M^--CO_2], 316, 248 [M^--H_2O -CO_2- 2CH_3], 180, 112[M^--Hexose, 2H_2O-CO_2-2CH_3]	$C_{17}H_{22}O_{10}$	Phenolic acid	(24)
6.51		181	Caffeic acid	181, 164, 163[M^+-H_2O], 147, 148[M^+-CH_3- H_2O], 68	$C_9H_8O_4$	Phenolic acid	(25)
6.72	625		Quercetin-3,4'-O-di-beta-glucoside	625,459, 431[M^--pentose+CO_2+H_2O, 417, 163[M^-- Hexose+2 Pentose-2 H_2O,161, 113[M^--Hexose- Rhamnose- Pentose- 4H_2O]	$C_{27}H_{30}O_{17}$	Flavonoids (Flavonols)	
7.29	447		Quercitrin	447, 402[M^--CO_2] 285[M^--Hexose] ,284, 255[M^--Hexose-2CH_3], 112.9	$C_{21}H_{20}O_{11}$	Flavonoids (Flavonols)	(20)
7.39		595	Cyanidin 3-O-rutinoside	595, 449[M^+-CO_2- 4H_2O- 2CH_3], 433[M^+-Hexose], 287[M^+-Hexose- rhamnose], 85	$C_{27}H_{31}O_{15}^+$	Anthocyanins	(26)
7.55	609		Delphinidin-3-O-(6''-O-alpha-rhamnopyranosyl-beta-glucopyranoside)	609, 565[M^--H_2O], 301[609-Rhamnose- Hexose]	$C_{27}H_{31}O_{16}^+$	Anthocyanin	(27)
7.95	431		Kaempferol-3-O-alpha-L-rhamnoside	431, 363, 295[M^--2CH_3- 2 CO_2-H_2O], 227[M^--3CO_2, 4H_2O], 180, 112	$C_{21}H_{20}O_{10}$	Flavonoid (Proanthocyanidins)	
8.26		146	Indole-3-carboxaldehyde	146, 118, 117, 91,65	$C_9H_7N_O$	Indoles Alkaloid	
8.70	609		Luteolin-7,3'-di-o-glucoside	609, 563[M^--CO_2], 489[M^--5H_2O-CH_3], 472, 471, 309[M^--Pentose- 3CO_2- 2H_2O], 112	$C_{27}H_{30}O_{16}$	Flavonoid glycoside	(20)
8.72	609		Rutin	609, 563, 564, 471, 472, 453, 301[M^- -rutinoside], 309, 248, 171, 112	$C_{27}H_{30}O_{16}$	Flavonoids (Flavonols)	(28)
8.92	151		Oxypurinol	151, 136[M^--CH_3], 92[M^--CH_3- CO_2]	$C_5H_4N_4O_2$	Pyrazoles alkaloid	
9.69		433	Apigenin-8-C-glucoside (Vitexin)	433, 389[M^+-CO_2], 357, 247, 191[M^+-Hexose-2H_2O-CO_2], 133, 89[133-CO_2]	$C_{21}H_{20}O_{10}$	Flavone glycoside	(29)

Table 1. Continued

Retention time	Parent ion m/z		Identified compound	MS/MS Fragments	Molecular formula	Chemical class	Ref.
	$[M-H]^-$	$[M+H]^+$					
10.69	177		Aesculetin	177, 133[M^- -CO_2], 89[M^- -$2CO_2$] 149,105	$C_9H_6O_4$	Coumarin	(21)
11.34	339		Esculin	339, 295[M^--CO_2], 221, 189[M^-- Pentose-H_2O], 149[M^--Pentose-CO_2, 59	$C_{15}H_{16}O_9$	Coumarin	
13.37		355	Chlorogenic acid	267[M^+-CO_2-$3H_2O$], 201, 181[M^+-caffeic acid], 193[M^+-caffeoyl acid], 103[M^+-Rhamnose-$2CO_2$-H_2O], 73	$C_{16}H_{18}O_9$	Phenolic acid	(30)
15.03	447		Luteolin 8-C-glucoside	447, 379, 311[M^--$2CO_2$, $2CH_3$, H_2O], 279[M^--Pentose, $2H_2O$], 278	$C_{21}H_{19}O_{11}^-$	Flavonoids	(31)
15.16	285	287	Luteolin	287, 269[M^+ -H_2O], 218[M+-$3H_2O$, CH_3], 150, 112	$C_{15}H_{10}O_6$	Flavonoids (Flavones)	(20)
15.8	115		Maleic acid	115, 97[M^--H_2O]	$C_4H_4O_4$	Phenolic acid	
16.41		271	Genistein	271, 253[M^+-H_2O], 271, 227[M^+-CO_2], 213, 195, 171, 109	$C_{15}H_{10}O_5$	Flavonoids (Isoflavones)	(32)
16.47		135	Cinnamyl alcohol	135, 117[M^+-H_2O], 91, 77, 75, 73	$C_9H_{10}O$	Phenolic alcohol	(24)
16.90		303	Quercetin	303, 286, 285, 273[M^- -CO],255[M^- -CO-H_2O]243, 207, 151, 133, 123	$C_{15}H_{10}O_7$	Flavonoids (Flavonols)	(20)
20.37		593	Acacetin-7-O-neohesperidoside	593, 513[M+-CO2-2H2O], 512, 496, 495[M+-3H2O-, CO2], 327[M+-Hexose-CO2, 4CH3], 258, 133, 89	$C_{28}H_{32}O_{14}$	Flavonoid glycoside	
21.19	593		Kaempferol 7-neohesperidoside	593, 525, 457, 389[M^--3 CO_2-$4H_2O$], 321[M--Hexose- CO_2- 2 H_2O-CH_3], 248, 253, 238[M^- -Pentose-Rhamnose-CO_2- CH_3- H_2O], 112	$C_{27}H_{30}O_{15}$	Flavonoids (Flavonols)	
21.70	306		Glutathione	306, 170[M^--$2CO_2$-CH_3- H_2O], 102[238-$2CO_2$, CH_3, H_2O], 239[M^--$2H_2O$, $2CH_3$]	$C_{10}H_{17}N_3O_6S$	Oligopeptides	
22.25	415		Diadzein-8-C-glucoside (Puerarin)	415, 347, 279[M^--$2CO_2$-$2CH_3$- H_2O], 112[347-Pentose-2 CO_2- CH_3].	$C_{21}H_{20}O_9$	Flavonoids (Isoflavones)	(33)
25.99	623	625	Isorhamnetin 3-rutinoside (anthocyanine)	625, 608, 607[M^+-H_2O], 369, 282, 296[M^+-$3CO_2$- CH_3- $2H_2O$-Rhamnose], 222, 221[M^+-Hexose-Pentose- $2H_2O$-CO_2- $2CH_3$, 231, 147	$C_{28}H_{32}O_{16}$	Flavonoids (Flavonols)	(16)
26.28	299		3,5,7-Trihydroxy-4'-methoxyflavone	299, 284, 281[M^--H_2O], 253, 137[M^--Hexose]	$C_{16}H_{12}O_6$	O-methylated flavone	

flavonoids (49%), alkaloids (4%), anthocyanins (4%), phenolics (21%), oligopeptides (4%), terpenes (4%), benzoquinone (4%), and sugars (2%) (Figure 3).

Spectrophotometric analysis

The UV-VIS spectrum of the diluted methanolic TSFE (Figure 4) showed a wide absorption spectrum in the UVA and visible regions with characteristic peaks at the range of 400-450 nm.

Cytotoxicity assay

As illustrated in Figure 5, TSFE showed negligible cytotoxicity against McF-7 cell lines at all the tested concentrations in dark. Upon radiation at 450 nm, the cell viability was 84% at the highest tested concentration (100 µg/mL), but the decrease was statistically non-significant ($P>0.05$). On the other hand, the extract showed significant dark and photo-cytotoxicity on A549 cell lines at higher concentrations (Figure 6). At a concentration of 100 µg/mL, the cell viability in dark was 78%, which significantly decreased to 64% after irradiation ($P<0.05$). These results suggest that TSFE is a promising natural photosensitizer for PDT.

Discussion

In this study, we analyzed the phytochemical components of TSFE by UPLC/MS. Each of the identified compounds has a wide range of biological activities as reported by several previous studies, as summarized in Supplementary file 1 (Table S1). Therefore, TSFE can be considered a rich source of natural bioactive constituents that may exert different biological activities.

TSFE has previously been studied for different biological activities. Ha et al isolated five components from TSFE and proved their activities as alpha-glucosidase inhibitors (5). Sugavanam et al reported CNS depressant activity of the TSFE (34). Gonçalves et al identified the phytoconstituents of several classes of TSFE and studied their antibacterial activity (35). Here, we focused on the antineoplastic activity of TSFE, either due to the presence of cytotoxic components, PS agents, or both. For this purpose, the cytotoxicity of TSFE was evaluated on two

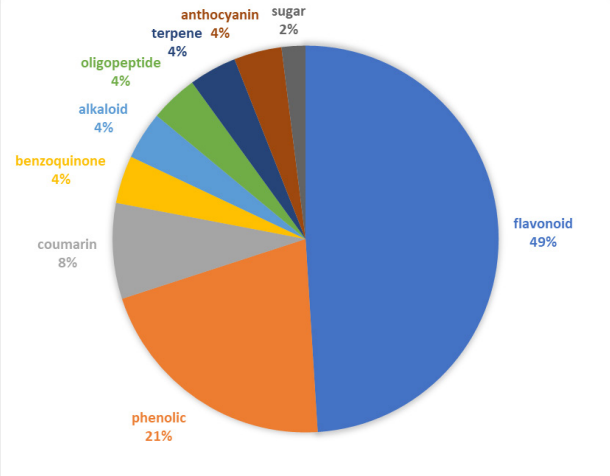

Figure 3. Pie chart showing different phytochemical classes identified via UPLC/MS/MS of crude methanolic extract of *Tecoma stans* yellow flowers.

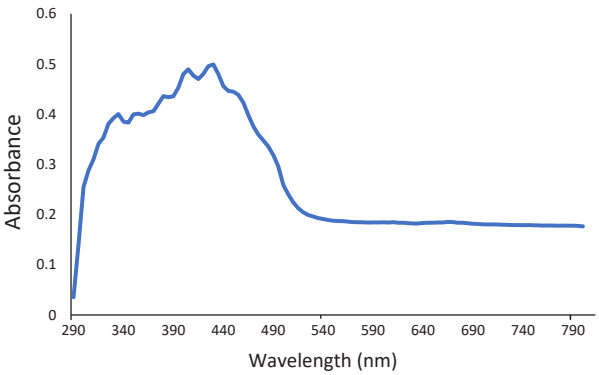

Figure 4. A UV-Visible spectrum of *Tecoma stans* flower extract.

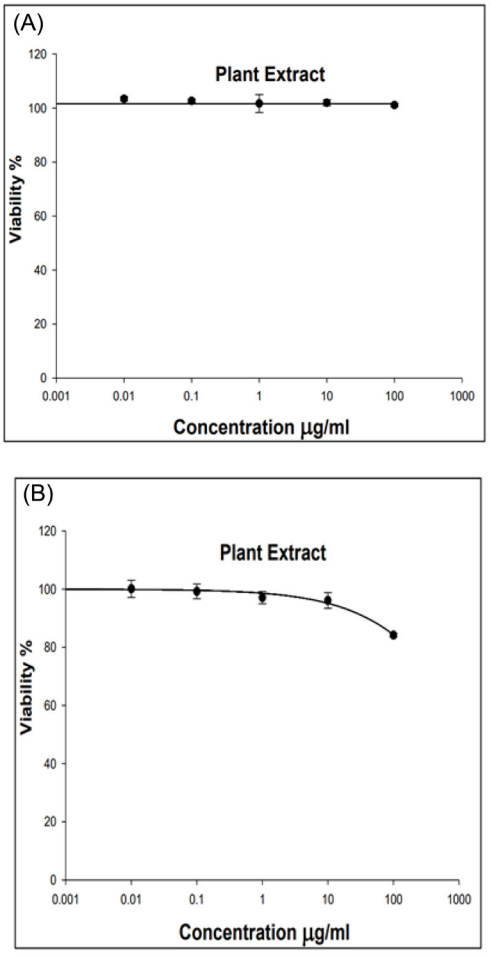

Figure 5. In vitro cell viability of breast cancer cell lines (McF-7) when treated with different concentrations of *Tecoma stans* methanolic flower extract (TSFE) in the dark (A) and after light irradiation (B).

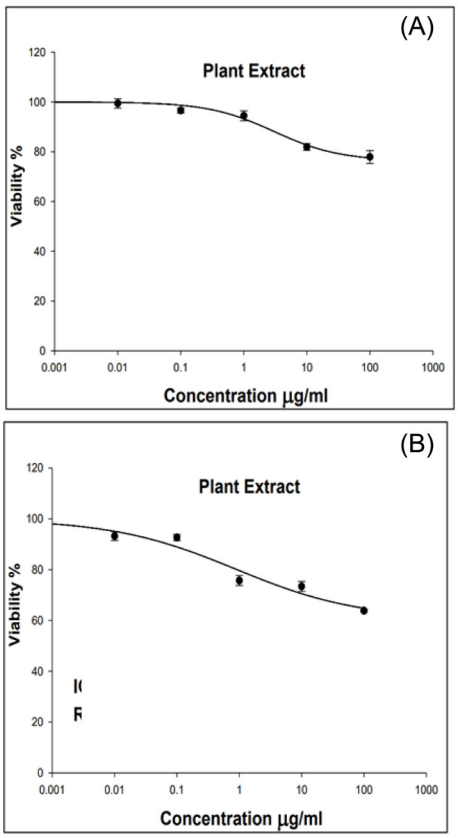

Figure 6. *In vitro* cell viability of lung cancer (A-549) cell lines when treated with different concentrations of *Tecoma stans* methanolic flower extract (TSFE) in the dark (A) and after light irradiation (B).

different cancer cell lines. The assessment of cytotoxicity was carried out in dark and after blue light irradiation to assess the photodynamic activity of TSFE. The results showed significant cytotoxicity of TSFE against the A-549 lung cancer cell line. These are consistent with those obtained by Robinson et al who reported high cytotoxicity of methanolic TSFE on A-549 cells (36). This high cytotoxicity may be attributed to the presence of phytoconstituents that exert antineoplastic activities, as revealed by UPLC/MS results (Table 1). Examples of identified constituents with proposed antineoplastic activity are apigenin (37), rosmarinic acid (38), esculin (39), genistein (40), luteolin (41), kaempferol (42), and isorhamnetin (43).

Moreover, our results revealed that the cytotoxicity was enhanced significantly after light radiation. The UV-visible spectrum of TSFE (Figure 4) showed a strong absorption peak in the visible region, especially in 400-450 nm, suggesting that TSFE could be excited by visible blue light, thus it might be a promising photosensitizer (44). The high photo-cytotoxicity may be due to the presence of PS phytoconstituents such as coumarins (as esculin and daphnetin) and anthocyanins (revealed from the results of UPLC/MS). These compounds can generate reactive oxygen species upon excitation by light.

Coumarins are organic dyes that have shown good PS properties in the treatment of many skin conditions such as psoriasis and vitiligo (12,45,46). In addition, anthocyanins derivatives, contained in TSFE, show photochemical and photophysical properties as they can absorb light in the blue wavelength (400-450 nm), which makes them potential PS in PDT of cancer (47).

A few previous studies have addressed the PDT activity of TSFE. Mamone et al reported that TSFE was a good photosensitizer in the photodynamic inhibition of the *Staphylococcus epidermis* (44). On the other hand, Tariq et al have studied the *T. stans* branch and leaves extract on the rhabdomyosarcoma cell line. They reported marked cytotoxicity of the tested extract, which was significantly enhanced upon the combination with a photosensitizer and silver nanoparticles due to the synergistic mechanism between anticancer and photodynamic activity (4).

Our results showed that the cytotoxicity against breast cancer cell McF-7 was not significant in dark, but it increased non-significantly by applying the light that enhance the poor activity.

The fruit extract of *T. stans* was reported to be highly cytotoxic against the HEPG-2 cell line but exhibited lower cytotoxicity against the MCF-7 cancer cell line (2). However, Thirumal et al reported a good antiproliferative effect of ethanolic TSFE on MCF-7 cells with IC50 of 70 µg/mL (48). Moreover, the ethanolic bark extract of *T. stans* was able to induce apoptosis in human breast cancer cell lines at all concentrations (49). These studies used different parts of the plant and different types of extract that may contain different phytoconstituents with various concentrations. In this study, MCF-7 cells might be resistant to the components of TSFE as they were reported to develop resistance to many chemotherapeutic drugs (50). Slight enhancement of the cytotoxicity on MCF-7 after light radiation proves that the TSFE is a promising PS in PDT, but further investigation is needed.

Conclusion

The current investigation revealed that the methanolic extract of *T. stans* flowers contained a great number of bioactive constituents responsible for many biological activities. The phytochemicals identified through UPLC-MS analysis confirmed the presence of both antineoplastic phytoconstituents and PS compounds. This makes the flower extract of *T. stans* a promising anticancer drug and promising PS in PDT. This study could be a promising base for further investigations in other biological and medical aspects.

Authors' contribution

MF and NEA were responsible for conceptualizing and supervising the whole work. AYK and DAF were responsible for carrying out the experimental work and preparing the initial draft of the manuscript. All authors

shared equally in revising the manuscript

Conflict of interests

The authors have no conflict of interest to declare.

Ethical considerations

All authors have inspected the ethical issues of plagiarism, misconduct, data fabrication, falsification, double publication, or redundancy related to the manuscript.

Funding/Support

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Supplementary file 1

Supplementary file 1 contains Table S1.

References

1. Ibrahim AM, Ahmed AK, Hammam OA, Abdel-Ghaffar F. Immunotoxical, neurotoxical, histopathological and immunohistopathological alterations of *Nerium oleander* and *Tecoma stans* methanolic extract on *Biomphalaria alexandrina* snails. Acta Trop. 2022;230:106405. doi: 10.1016/j.actatropica.2022.106405.
2. Marzouk MS, Gamal-Eldeen AM, Mohamed MA, El-Sayed MM. Antioxidant and anti-proliferative active constituents of *Tecoma stans* against tumor cell lines. Nat Prod Commun. 2006;1(9):735-43. doi: 10.1177/1934578x0600100908.
3. Elsayed HE, Kamel RA, Ibrahim RR, Abdel-Razek AS, Shaaban MA, Frese M, et al. Cytotoxicity, antimicrobial, and in silico studies of secondary metabolites from *Aspergillus* sp. isolated from *Tecoma stans* (L.) Juss. ex Kunth leaves. Front Chem. 2021;9:760083. doi: 10.3389/fchem.2021.760083.
4. Tariq H, Rafi M, Amirzada MI, Muhammad SA, Yameen MA, Mannan A, et al. Photodynamic cytotoxic and antibacterial evaluation of *Tecoma stans* and *Narcissus tazetta* mediated silver nanoparticles. Arab J Chem. 2022;15(3):103652. doi: 10.1016/j.arabjc.2021.103652.
5. Ha KN, Nguyen TV, Mai DT, Tran NM, Nguyen NH, Vo GV, et al. Alpha-glucosidase inhibitors from *Nervilia concolor*, *Tecoma stans*, and *Bouea macrophylla*. Saudi J Biol Sci. 2022;29(2):1029-42. doi: 10.1016/j.sjbs.2021.09.070.
6. Anand M, Basavaraju R. A review on phytochemistry and pharmacological uses of *Tecoma stans* (L.) Juss. ex Kunth. J Ethnopharmacol. 2021;265:113270. doi: 10.1016/j.jep.2020.113270.
7. Khattab A, Awad NE, Fadeel DA, Fadel M. Reviewing the reported pharmacognostic and pharmacological investigations on *Tecoma stans* Juss. ex Kunth. J Herbmed Pharmacol. 2023;12(1):25-40. doi: 10.34172/jhp.2023.03.
8. Mamone L, Di Venosa G, Sáenz D, Batlle A, Casas A. Methods for the detection of reactive oxygen species employed in the identification of plant photosensitizers. Methods. 2016;109:73-80. doi: 10.1016/j.ymeth.2016.05.020.
9. Singh S, Maurya IC, Sharma S, Srivastava P, Bahadur L. Effect of dye extraction solvent on the photovoltaic performance of *Tecoma stans*-sensitized solar cells. J Electron Mater. 2020;49(7):4355-63. doi: 10.1007/s11664-020-08154-2.
10. Bruschi ML, da Silva JB, Rosseto HC. Photodynamic therapy of psoriasis using photosensitizers of vegetable origin. Curr Pharm Des. 2019;25(20):2279-91. doi: 10.2174/1381612825666190618122024.
11. Senapathy GJ, George BP, Abrahamse H. Exploring the role of phytochemicals as potent natural photosensitizers in photodynamic therapy. Anticancer Agents Med Chem. 2020;20(15):1831-44. doi: 10.2174/1871520620666200703192127.
12. Sulaiman C, George BP, Balachandran I, Abrahamse H. Photoactive herbal compounds: a green approach to photodynamic therapy. Molecules. 2022;27(16):5084. doi: 10.3390/molecules27165084.
13. Jaitz L, Mueller B, Koellensperger G, Huber D, Oburger E, Puschenreiter M, et al. LC-MS analysis of low molecular weight organic acids derived from root exudation. Anal Bioanal Chem. 2011;400(8):2587-96. doi: 10.1007/s00216-010-4090-0.
14. Bouhafsoun A, Yilmaz MA, Boukeloua A, Temel H, Harche MK. Simultaneous quantification of phenolic acids and flavonoids in *Chamaerops humilis* L. using LC–ESI-MS/MS. Food Sci Technol. 2018;38:242-7. doi: 10.1590/fst.19917.
15. Sundaram RS, Ramanathan M, Rajesh R, Satheesh B, Saravanan D. LC-MS quantification of rosmarinic acid and ursolic acid in the *Ocimum sanctum* Linn. leaf extract (Holy basil, Tulsi). J Liq Chromatogr Relat Technol. 2012;35(5):634-50. doi: 10.1080/10826076.2011.606583.
16. Diep T, Pook C, Yoo M. Phenolic and anthocyanin compounds and antioxidant activity of tamarillo (*Solanum betaceum* Cav.). Antioxidants (Basel). 2020;9(2):169. doi: 10.3390/antiox9020169.
17. Li S, Lin Z, Jiang H, Tong L, Wang H, Chen S. Rapid identification and assignation of the active ingredients in Fufang Banbianlian Injection using HPLC-DAD-ESI-IT-TOF-MS. J Chromatogr Sci. 2016;54(7):1225-37. doi: 10.1093/chromsci/bmw055.
18. Peng A, Lin L, Zhao M, Sun B. Classification of edible chrysanthemums based on phenolic profiles and mechanisms underlying the protective effects of characteristic phenolics on oxidatively damaged erythrocyte. Food Res Int. 2019;123:64-74. doi: 10.1016/j.foodres.2019.04.046.
19. Tu Y, Zhou L, Li L, Wang L, Gao S, Hu M. Development and validation of an LC-MS/MS method for the quantification of flavonoid glucuronides (wogonoside, baicalin, and apigenin-glucuronide) in the bile and blood samples: application to a portal vein infusion study. Anal Biochem. 2020;601:113723. doi: 10.1016/j.ab.2020.113723.
20. Dos Santos Szewczyk K, Pietrzak W, Klimek K, Grzywa-Celińska A, Celiński R, Gogacz M. LC-ESI-MS/MS identification of biologically active phenolics in different extracts of *Alchemilla acutiloba* Opiz. Molecules. 2022;27(3):621. doi: 10.3390/molecules27030621.
21. Soares MS, da Silva DF, Amaral JC, da Silva MM, Forim MR, Rodrigues-Filho E, et al. Rapid differentiation of graft *Citrus sinensis* with and without *Xylella fastidiosa* infection by mass spectrometry. Rapid Commun Mass Spectrom. 2020;34 Suppl 3:e8745. doi: 10.1002/rcm.8745.
22. Zeng Y, Li S, Wang X, Gong T, Sun X, Zhang Z. Validated LC-MS/MS method for the determination of scopoletin in rat plasma and its application to pharmacokinetic studies. Molecules. 2015;20(10):18988-9001. doi: 10.3390/molecules201018988.
23. Grace MH, Xiong J, Esposito D, Ehlenfeldt M, Lila MA. Simultaneous LC-MS quantification of anthocyanins and

non-anthocyanin phenolics from blueberries with widely divergent profiles and biological activities. Food Chem. 2019;277:336-46. doi: 10.1016/j.foodchem.2018.10.101.
24. Mateos R, Baeza G, Sarriá B, Bravo L. Improved LC-MSn characterization of hydroxycinnamic acid derivatives and flavonols in different commercial mate (*Ilex paraguariensis*) brands. Quantification of polyphenols, methylxanthines, and antioxidant activity. Food Chem. 2018;241:232-41. doi: 10.1016/j.foodchem.2017.08.085.
25. Lin Y, Xu W, Huang M, Xu W, Li H, Ye M, et al. Qualitative and quantitative analysis of phenolic acids, flavonoids and iridoid glycosides in Yinhua Kanggan tablet by UPLC-QqQ-MS/MS. Molecules. 2015;20(7):12209-28. doi: 10.3390/molecules200712209.
26. Yang C, Wang Q, Yang S, Yang Q, Wei Y. An LC-MS/MS method for quantitation of cyanidin-3-O-glucoside in rat plasma: application to a comparative pharmacokinetic study in normal and streptozotocin-induced diabetic rats. Biomed Chromatogr. 2018;32(2):e4042. doi: 10.1002/bmc.4042.
27. Goszcz K, Deakin SJ, Duthie GG, Stewart D, Megson IL. Bioavailable concentrations of delphinidin and its metabolite, gallic acid, induce antioxidant protection associated with increased intracellular glutathione in cultured endothelial cells. Oxid Med Cell Longev. 2017;2017:9260701. doi: 10.1155/2017/9260701.
28. Brito A, Ramirez JE, Areche C, Sepúlveda B, Simirgiotis MJ. HPLC-UV-MS profiles of phenolic compounds and antioxidant activity of fruits from three citrus species consumed in Northern Chile. Molecules. 2014;19(11):17400-21. doi: 10.3390/molecules191117400.
29. Hassan WHB, Abdelaziz S, Al Yousef HM. Chemical composition and biological activities of the aqueous fraction of *Parkinsonea aculeata* L. growing in Saudi Arabia. Arab J Chem. 2019;12(3):377-87. doi: 10.1016/j.arabjc.2018.08.003.
30. Zălaru C, Crișan CC, Călinescu I, Moldovan Z, Țârcomnicu I, Litescu SC, et al. Polyphenols in *Coreopsis tinctoria* Nutt. fruits and the plant extracts antioxidant capacity evaluation. Cent Eur J Chem. 2014;12(8):858-67. doi: 10.2478/s11532-014-0539-x.
31. Soltana H, De Rosso M, Lazreg H, Vedova AD, Hammami M, Flamini R. LC-QTOF characterization of non-anthocyanic flavonoids in four Tunisian fig varieties. J Mass Spectrom. 2018;53(9):817-23. doi: 10.1002/jms.4209.
32. Tosun F, Erdem CK, Eroğlu Y. Determination of genistein in the Turkish *Genista* L. species by LC-MS. Pharmazie. 2003;58(8):549-50.
33. Zhang Y, Xu Q, Zhang X, Chen J, Liang X, Kettrup A. High-performance liquid chromatography-tandem mass spectrometry for identification of isoflavones and description of the biotransformation of kudzu root. Anal Bioanal Chem. 2005;383(5):787-96. doi: 10.1007/s00216-005-0068-8.
34. Sugavanam K, Velayutham S, Ganesan A. CNS depressent activity of different extracts of *Tecoma stans* flowers. Asian J Tradit Med. 2012;7(1):39-43.
35. Gonçalves TPR, Parreira AG, dos Santos Zanuncio VS, de Souza Farias K, da Silva DB, dos Santos Lima LA. Antibacterial and antioxidant properties of flowers from *Tecoma stans* (L.) Juss. ex Kunth (Bignoniaceae). S Afr J Bot. 2022;144:156-65. doi: 10.1016/j.sajb.2021.08.028.
36. Robinson JP, Suriya K, Subbaiya R, Ponmurugan P. Antioxidant and cytotoxic activity of *Tecoma stans* against lung cancer cell line (A549). Braz J Pharm Sci. 2017;53(3):e00204. doi: 10.1590/s2175-97902017000300204.
37. Zhou X, Wang F, Zhou R, Song X, Xie M. Apigenin: a current review on its beneficial biological activities. J Food Biochem. 2017;41(4):e12376. doi: 10.1111/jfbc.12376.
38. Han YH, Kee JY, Hong SH. Rosmarinic acid activates AMPK to inhibit metastasis of colorectal cancer. Front Pharmacol. 2018;9:68. doi: 10.3389/fphar.2018.00068.
39. Mokdad-Bzeouich I, Kovacic H, Ghedira K, Chebil L, Ghoul M, Chekir-Ghedira L, et al. Esculin and its oligomer fractions inhibit adhesion and migration of U87 glioblastoma cells and in vitro angiogenesis. Tumour Biol. 2016;37(3):3657-64. doi: 10.1007/s13277-015-4209-1.
40. Sharifi-Rad J, Quispe C, Imran M, Rauf A, Nadeem M, Gondal TA, et al. Genistein: an integrative overview of its mode of action, pharmacological properties, and health benefits. Oxid Med Cell Longev. 2021;2021:3268136. doi: 10.1155/2021/3268136.
41. Naso LG, Lezama L, Valcarcel M, Salado C, Villacé P, Kortazar D, et al. Bovine serum albumin binding, antioxidant and anticancer properties of an oxidovanadium(IV) complex with luteolin. J Inorg Biochem. 2016;157:80-93. doi: 10.1016/j.jinorgbio.2016.01.021.
42. Imran M, Salehi B, Sharifi-Rad J, Aslam Gondal T, Saeed F, Imran A, et al. Kaempferol: a key emphasis to its anticancer potential. Molecules. 2019;24(12):2227. doi: 10.3390/molecules24122277.
43. Luan Y, Luan Y, Zhao Y, Xiong F, Li Y, Liu L, et al. Isorhamnetin in Tsoong blocks Hsp70 expression to promote apoptosis of colon cancer cells. Saudi J Biol Sci. 2019;26(5):1011-22. doi: 10.1016/j.sjbs.2019.04.002.
44. Mamone L, Di Venosa G, Gándara L, Sáenz D, Vallecorsa P, Schickinger S, et al. Photodynamic inactivation of gram-positive bacteria employing natural resources. J Photochem Photobiol B. 2014;133:80-9. doi: 10.1016/j.jphotobiol.2014.03.003.
45. Bryantseva N, Sokolova I, Tsyrenzhapova A, Selivanov N, Khilya V, Garazd Y. Fluorescent characteristics of coumarin photosensitizers. J Appl Spectrosc. 2008;75(5):700-5.
46. Kasperkiewicz K, Erkiert-Polguj A, Budzisz E. Sunscreening and photosensitizing properties of coumarins and their derivatives. Lett Drug Des Discov. 2016;13(5):465-74.
47. Oliveira H, Correia P, Pereira AR, Araújo P, Mateus N, de Freitas V, et al. Exploring the applications of the photoprotective properties of anthocyanins in biological systems. Int J Mol Sci. 2020;21(20):7464. doi: 10.3390/ijms21207464.
48. Thirumal M, Kishore G, Srimanthula S. Anti-proliferative activity of various parts of *Tecoma stans* (L.) against human breast cancer cells in vitro. Res J Pharm Biol Chem Sci. 2013;4(2):305-13.
49. Anburaj G, Marimuthu M, Rajasudha V, Manikandan R. In vitro anti-cancer activity *Tecoma stans* against human breast cancer yellow elder (*Tecoma stans*). J Pharmacogn Phytochem. 2016;5(5):331-4.
50. Devarajan E, Chen J, Multani AS, Pathak S, Sahin AA, Mehta K. Human breast cancer MCF-7 cell line contains inherently drug-resistant subclones with distinct genotypic and phenotypic features. Int J Oncol. 2002;20(5):913-20.