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Phytochemicals Screening and Photocytotoxicity of *Strobilanthes crispa* (L.) Blume (Acanthaceae) Leaf Extracts

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Strobilanthes crispa (L.) Blume (*S. crispa*) is a well-known folklore medicinal plant traditionally used in Malaysia and Indonesia for its diverse range of traditional applications and medicinal properties. While previous studies have established its effectiveness as an anticancer agent against various cancer types, its impact on skin cancer cells remains largely unexplored. In this study, ethanol, hexane, and ethyl acetate leaf extracts of *S. crispa* were screened for phytochemicals and analyzed for their photocytotoxicity against the A431 human skin squamous carcinoma cell line by the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenultetrazolium bromide (MTT) assay. In general, the qualitative analysis indicated the presence of terpenes, alkaloids, phenols, quinones, and chlorophylls in all three crude extracts, while steroids, flavonoids, and anthraquinones were not detected. Upon activation with photodynamic therapy (PDT) treatment, the cell viability decreased. Among the extracts, the hexane crude extract exhibited the highest photocytotoxic activity against the A431 cell line, with an IC_{50} value of 6.06 ± 0.21 µg/mL, followed by the ethanolic crude extract (IC_{50} = 8.83 ± 0.55 µg/mL) and the ethyl acetate crude extract (IC_{50} = 9.37 ± 0.71 µg/mL). However, when tested without PDT treatment, none of the three crude extracts exhibited significant activity against the A431 cell line, even at concentrations as high as 200 µg/mL after 24 hours of incubation in the dark. The results of the present study suggest that *S. crispa* could serve as a valuable source of photosensitizing agents for photodynamic therapy in cancer treatment.

Keywords: *Strobilanthes crispa*; phytochemical screening; PDT treatment; A431 cells; photocytotoxicity

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Strobilanthes crispa (L.) Blume (*S. crispa*), known locally as "Pecah kaca" [1-2], "yellow Strobilanthus" in English [3], and "黑面将军" in Chinese [4], is a folklore medicinal plant in Malaysia and Indonesia. It belongs to the genus *Strobilanthes* (Acanthaceae) and has a rich history of traditional use in the treatment of various ailments, such as breast and uterine cancers, diabetes mellitus, hypertension, gastrointestinal and kidney diseases [5-7], typhoid [8], jaundice, piles, high cholesterol, and ulcers [9-10]. The accepted scientific name for this plant is *S. crispa*, as listed in the Plant List and it was first published in 1826 [11]. According to Kew's taxonomic resource available at http://www.plantsoftheworldonline.org/, *S. crispa* is a native plant to regions spanning from Jawa to the Lesser Sunda Islands [12]. It is currently distributed in various parts of Madagascar and the Malay Archipelago [5, 13-14] (Brunei, Indonesia, East Malaysia, Papua New Guinea, and the Philippines [15]), at an altitude ranging from 50 to 1,200 m [8,16], mainly as a woody shrub [3]. In fact, recent pharmacological studies have shown that various extracts of *S. crispa* have a wide range of pharmacological activities based on its traditional uses. These activities include anti-hyperglycemic [2], antioxidant [17], antimicrobial [18], wound healing [19], anti-trypanosomal [20], anti-inflammatory [21], anti-obesity [22], anti-urolithiatic [23], anti-angiogenic [24] and vasorelaxant activity [25]. Additionally, previous studies have highlighted the potential of *S. crispa* as an anticancer agent, showing promising results in treating cervical [3], liver, lung [24], prostate, breast [26], colon [27], and nasopharyngeal [28] cancers. However, studies specifically targeting skin cancer cells have not been conducted.

Photodynamic therapy (PDT) is a minimally invasive therapeutic approach that has gained clinical

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approval due to its selective cytotoxicity against cancer cells. For PDT to effectively target cancer cells, three crucial prerequisites must be fulfilled: the presence of a photosensitizing agent, an oxygen-enriched environment, and exposure to light with specific wavelengths and energies. It is of utmost importance that none of these elements harm the cells themselves [29]. The process of PDT involves the introduction of a photosensitizer (PS) that can be localized to the tumor site. Subsequently, the tumor is exposed to light of a specific wavelength, thereby activating the PS [30]. This activation triggers a series of photochemical reactions that generate reactive oxygen species (ROS) through electron or hydrogen transfer (Type I reaction) and singlet oxygen ($^1O_2$) via energy transfer (Type II reaction) [31]. The production of ROS leads to the oxidation of crucial cellular macromolecules, ultimately leading to the destruction of tumor cells [30]. In other words, PDT could initiate three primary cell death mechanisms: apoptosis, necrosis, and autophagy-associated cell death [29]. By leveraging these mechanisms, PDT provides a targeted therapeutic strategy against cancer cells while minimizing damage to healthy cells.

Most PSs used in cancer therapy are derived from tetrapyrrole backbones, some of which naturally occur in plants [32]. In a study by Villacorta et al. [33], it was discovered that ethanolic crude extracts of *Lumnitzera racemose* and *Albizia procera* exhibited cytotoxic effects on breast carcinoma (MCF-7) cells when exposed to light, indicating the potential of plant extracts as a source for identifying promising PSs. In line with our study, this study aimed to explore the effect of PDT utilizing *S. crispa* extracts on A431 human non-melanoma skin squamous cell carcinoma. This study pursued two specific objectives: (1) to obtain *S. crispa* leaf extracts and conduct a qualitative phytochemical screening, and (2) to assess the potential induction of the photocytotoxic effect of *S. crispa* on skin cancer cells through the application of external energy, specifically light.

MATERIALS AND METHODS

Chemicals and Materials

All chemicals used were of analytical grade. The chemicals included ethanol (EtOH; C_2H_5OH; Chem Soln; #CER0978-20230317), hexane (C_6H_{14}; Chemiz; #2208121), ethyl acetate (EA; $C_4H_8O_2$; Chemiz; #2111151), isopropyl alcohol (IPA; C_3H_8O; Thermo Fisher Scientific; #2240646), dichloromethane (DCM; CH_2Cl_2; Thermo Fisher Scientific; #2224582), chloroform (TCM; $CHCl_3$; Univar; #506415), sulphuric acid (H_2SO_4; Thermo Fisher Scientific; #RA-160513), acetic anhydride (CH_3CO; Merck; #K49771242808), Dragendorff's reagent, sodium hydroxide (NaOH; Chemsoln; #PTNN110711), ferric chloride ($FeCl_3$; Chemiz; #1811301), hydrochloric acid (HCl; R&M Chemicals; #PNKR110319), ammonium hydroxide (NH_4OH; R&M Chemicals; #PGNE111019), diethyl ether ($C_4H_{10}O$; HmbG Chemicals; #2K120049-06011), acetone (C_3H_6O; Chemiz; #2205011), and methanol (MeOH; CH_3OH; Chemsoln; #CER5624-20210915).

Besides, human skin squamous carcinoma (A431) cell line was purchased from American Type Culture Collection (ATCC, USA). The reagents used in bioassay involved 1X Dulbecco's phosphate buffered saline (DPBS; ATCC, USA; #80722201), Dulbecco's modified Eagle's medium (DMEM; Capricorn Scientific, Germany; #CP22-5390), fetal bovine serum (FBS; Capricorn Scientific, Germany; #CP21-4466), penicillin-streptomycin (Pen/Strep; Capricorn Scientific, Germany; #CP22-5084), trypsin-EDTA (Sigma-Aldrich, USA), hybrid-max dimethyl sulfoxide (DMSO; Sigma-Aldrich, USA; #RNBK7775), cisplatin (EDQM, France; #7.0), 3-(4,5-dimethylthiazol- 2-yl)-2,5-diphenultetrazolium bromide (MTT; Merck Millipore, USA; #3770285).

Preparation of Plant Sample

S. crispa specimen was obtained from TKC Herbal Nursery, located in Seremban, Malaysia. The authentication of the plant specimen was conducted under the expertise of Dr. Mohd Firdaus Ismail, who is the botanist of the Institute of Bioscience at the University Putra Malaysia (UPM) in Serdang, Selangor, Malaysia. Following authentication, the voucher specimen was securely deposited at the herbarium within the Biodiversity Unit of the UPM. The assigned voucher number for the *S. crispa* specimen was SK3267/17. To proceed with further processing, the leaves were carefully detached from the plants, weighed, and washed thoroughly under running tap water to eliminate any impurities. Subsequently, the leaves were dried in an oven at 40 °C until a constant weight was achieved. Once the leaves were completely dried, they were finely ground into a powder using an electric blender, ensuring efficient extraction of the crude constituents.

Figure 1. Freshly harvested *S. crispa* leaves.

Preparation of Crude Extracts

The dried *S. crispa* leaves, with a total mass of 1398.33 g, were subjected to cold maceration with 80% ethanol to obtain the ethanolic crude extract, which weighed 103.73 g, as described in the previous study [35]. Subsequently, the dried ethanolic crude extract underwent partitioning with hexane and distilled water in a separating funnel. The resultant aqueous layer was subjected to an additional partition step with ethyl acetate (EA), while the hexane layer was collected and dried at 45°C using a rotary evaporator. In the second round of partitioning, the aqueous layer was discarded, and the EA layer was collected and dried at 45°C using a rotary evaporator. The dried hexane and EA crude extracts were then transferred to separate glass vials, and their respective weights were recorded.

Phytochemical Screening

Phytochemical screening is a widely recognized analytical method for the qualitative identification of specific phytochemicals in plant extracts [36]. In our investigation, we utilized three distinct crude extracts: ethanol, hexane, and EA. These samples were meticulously prepared by dissolving the respective crude extracts in their designated solvents. Isopropyl alcohol served as the dissolving solvent for most of our tests, with exceptions being made for Dragendorff's test and thin-layer chromatography, where dichloromethane (DCM) was used, as well as for the ferric chloride test, which required the use of distilled water. The concentrations of the ethanol, hexane, and EA crude extracts were standardized to 1 mg/mL.

The Salkowski test was employed to detect terpenes and steroids in the crude extracts. To test for terpenes, 1 mL of the extract was combined with 2 mL of chloroform and 3 mL of concentrated sulphuric acid, resulting in the expected formation of a reddish-brown coloration at the interface [37]. The test for steroids involved mixing 2 mL of chloroform, 10 drops of acetic anhydride, and 2 drops of concentrated sulphuric acid with 1 mL of the extract. A positive outcome is indicated by a change in color from red to blue and finally bluish [38]. In addition, alkaloids were assessed using Dragendorff's test, which involved adding a few drops of Dragendorff's reagent, a solution made by combining equal volumes of potassium iodide dissolved in water and bismuth subnitrate in glacial acetic acid, to 1 mL of the extract, resulting in the formation of a reddish-brown precipitate. Furthermore, flavonoids were examined using the alkaline reagent test, where 1 mL of 2M sodium hydroxide was added to 1 mL of the extract, leading to the formation of a yellow coloration. Phenols were detected using the ferric chloride test, wherein 2 mL of 5% ferric chloride was added to 1 mL of the extract, and a positive result is indicated by the formation of a dark green or bluish-black coloration. The quinone test was conducted using the concentrated hydrochloric acid test, wherein 1 mL of concentrated hydrochloric acid was added to 1 mL of the extract, resulting in the formation of a green coloration for a positive result. Other than that, anthraquinones were assessed using the ammonium hydroxide test, involving the addition of a drop of concentrated ammonium hydroxide to 1 mL of the extract, and the expected outcome is the formation of red coloration after 2 minutes [35]. Lastly, thin layer chromatography (TLC) was employed to detect the presence of chlorophylls. In this test, 3 drops of the crude extract were spotted to the origin on the TLC plate, which was subsequently developed using an appropriate solvent system. For the ethanol crude extract, the solvent system consisted of DCM and methanol in a volume ratio of 4:1. For the hexane crude extract, the solvent

system included hexane, diethyl ether, and acetone in volume ratios of 6:3:2. For the EA crude extract, the solvent system was composed of DCM and methanol in a volume ratio of 19:1. The presence of green spots on the TLC plate indicates the presence of chlorophyll [38].

Cell Culture

The A431 human skin squamous carcinoma cell line obtained from the American Type Culture Collection (ATCC) was employed in this assay, utilizing passages ranging from 57th to 60th. The cells were cultivated in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% fetal bovine serum (FBS) and 1% of 10,000 U/mL Penicillin-Streptomycin (Pen/Strep). The cultures were maintained in a humidified incubator at 37°C with 5% CO_2. Subculturing was performed when the cells reached a confluence of 70% to 80%.

Cell Viability 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenultetrazolium bromide (MTT) Assay

The ethanol, hexane, and EA crude extracts of *S. crispa* were prepared as stock solutions at a concentration of 5.0 mg/mL in 0.1% dimethyl sulfoxide (DMSO). Prior to the assay, these stock solutions were further diluted in an FBS-free culture medium to achieve a range of concentrations (3.125, 6.25, 12.5, 25.0, 50.0, 100.0, 200.0 µg/mL). As a positive control, cisplatin was prepared in 0.1% DMSO and diluted in FBS-free culture medium to concentrations ranging from 0.625 to 40 µg/mL. The negative control consisted of FBS-free culture medium only.

A431 cells were seeded in 96-well plates at a density of 2.5×10^4 cells/well and incubated for 48 hours until they reached 80% to 90% confluency. Following this, the cells were washed with 1X Dulbecco's phosphate-buffered saline (DPBS) and treated with 100 µL of the respective treatment solutions. After a 2-hour incubation period with the treatment, PDT treatment was performed for 10 minutes using a specific wavelength of 660 nm and a light dose of 17.04 J/cm^2. Subsequently, the cells were incubated for an additional 24 hours in a CO_2 incubator in the dark. Another set of cells treated with the same solutions, but not exposed to PDT, served as the light control for the photo-independent cytotoxicity assay.

After 24 hours of incubation, cell viability was assessed using the colorimetric MTT assay. A total of 20 µL of 5 mg/mL MTT solution in 1X DPBS was added into each well, followed by incubation at 37°C under 5% CO_2 for 4 hours. Following incubation, the contents of each well were discarded, and 100 µL of 99.9% analytical grade DMSO was added, followed by another incubation at 37°C with 5% CO_2 for 20 minutes. The absorbance at 570 nm was measured using a microplate reader (Model Infinite 200 Pro, Tecan, Switzerland). The entire experiment was repeated to obtain two sets of three biological replicates (n = 6). The percentage of cell viability was calculated using the following formula:

$$\text{Percentage of cell viability, \%} = 100\% - \left[\text{Absorbance}\left(\frac{\text{Blank} - \text{Treatment}}{\text{Blank}}\right) \times 100\%\right]$$

Statistical Analysis

Analysis of MTT assay data will be performed using GraphPad Prism software. The IC_{50} values and statistical significance of the obtained data will be determined.

RESULTS AND DISCUSSION

Phytochemical Screening

In this study, phytochemical screening was conducted on the crude extracts of *S. crispa* leaves, specifically the ethanol, hexane, and EA crude extracts, to identify the possible phytochemical groups present. The outcomes of various phytochemical tests conducted on each crude extract are presented in **Table 1**. The screening results indicated the presence of terpenes, alkaloids, phenols, quinones, and chlorophylls in all tested crude extracts. However, the tests for steroids, flavonoids, and anthraquinones yielded negative results, suggesting the absence of these compounds in *S. crispa* leaves.

These findings contrast with the results of previous studies conducted by Manaf and Daud [39], Fardiyah et al. [40], and Gul et al. [41], who identified alkaloids, tannins, flavonoids, saponins, terpenoids, and steroids in alcoholic plant extracts. Besides, a recent study by Chen et al. [10] suggested that *S. crispa* contains a variety of phytochemicals, including terpenoids, flavonoids, phenolic compounds, sulfur-containing compounds, steroids, chlorophylls, benzofuran, fatty acids, and other simple metabolites. The variations in results may be attributed to various factors. For instance, the location of plant cultivation can influence the chemical composition and bioactivity of the plant. Factors such as soil conditions, climate, and growing conditions can affect the presence and concentration of specific compounds in the plant [42]. Additionally, as our samples were extracts rather than pure isolated compounds, the phytochemical groups may not have been present in detectable amounts, resulting in negative screening results. Therefore, further isolation and characterization of compounds are necessary to verify and identify the exact phytochemical constituents present in *S. crispa* leaves.

Table 1. Observations of different phytochemical tests.

Test	Observations*		
	Ethanolic crude	Hexane crude	EA crude
(A) Terpenes	+	++	+++
(B) Steroids	ND	ND	ND
(C) Alkaloids	+	++	+++
(D) Flavonoids	ND	ND	ND
(E) Phenols	++	+	+++
(F) Quinones	+	++	+
(G) Anthraquinones	ND	ND	ND
(H) Chlorophylls	+	+++	+++

* "+++": high concentration; "++": medium concentration; "+": low concentration; "ND": non-detectable.

Photocytotoxic Effect of *S. crispa* Crude Extracts in A431 Cells

The field of cancer chemotherapy has sparked significant interest in natural products, either in their original form as found in nature or chemically modified form [43]. These compounds have garnered attention as potential cancer therapeutics [43], and numerous researchers around the world have focused on screening bioactive compounds derived from plant remedies that exhibit anticancer properties [44]. In fact, many clinically approved anticancer drugs are derived from plant compounds [45]. Despite this, limited research has been conducted on the potential of plant-derived PS compounds [33], such as those present in *S. crispa*, particularly regarding their anti-cancer effects on the A431 skin cancer cell line. To address this gap, our study employed the MTT assay, a widely used *in vitro* cell proliferation assay, to evaluate the potential of *S. crispa* as a photosensitizing anticancer drug. The MTT assay colorimetrically measures the activity of mitochondrial dehydrogenase, a key indicator of cell viability [46]. The assay involves the reduction of MTT reagent to formazan within living cells and the resulting formazan is quantified by measuring its absorbance at 570 nm using a plate reading spectrophotometer [47]. It is assumed that the amount of formazan is directly proportional to the number of viable cells [48]. This cost-effective, reliable, and convenient method is commonly employed to assess the preliminary anticancer potential of natural products and extracts [46].

The powdered dried leaves of *S. crispa* were subjected to a cold maceration process to obtain an ethanolic crude extract with a final yield of 7.42% (103.73 g). Subsequently, the crude extract was further fractionated using hexane (a non-polar solvent), EA (a moderately polar solvent), and distilled water (a highly polar solvent), yielding recoveries of 1.25% (17.53 g), 0.91% (12.76 g), and 4.73% (66.18 g), respectively. To evaluate the photocytotoxicity of these extracts, the percentage of cell viability was employed as a measure of their effects. **Figure 2** illustrates that ethanol, hexane, and EA crude extracts at concentrations up to 200 µg/mL had no observable impact on the A431 cell line after incubation in the dark for 24 hours without PDT treatment. However, upon photo-activation at a wavelength of 660 nm and a light dose of 17.04 J/cm^2 for 10 minutes, the cell viability drastically decreased from 117.99 ± 13.67% to 12.44 ± 1.60% for the ethanolic crude extract, from 117.87 ± 7.96% to 8.48 ± 1.30% for the hexane crude extract, and from 154.85 ± 16.99% to 23.07 ± 3.76% for the EA crude extract, as depicted in **Figure 2**. These findings indicate that the crude extracts exhibited cytotoxic effects on the A431 cell line only when exposed to 660 nm light, and that extract-based PDT reduced cell viability in a concentration-dependent manner. Importantly, the cell viability observed in A431 cells subjected to light irradiation alone (light control) was 79.42 ± 8.21%, suggesting no significant cell death. Taken together, the results of this preliminary study highlight a notable difference between cells treated with PDT and those without PDT, suggesting the presence of potential photo-sensitizing anticancer bioactive compounds in the crude extracts of *S. crispa*.

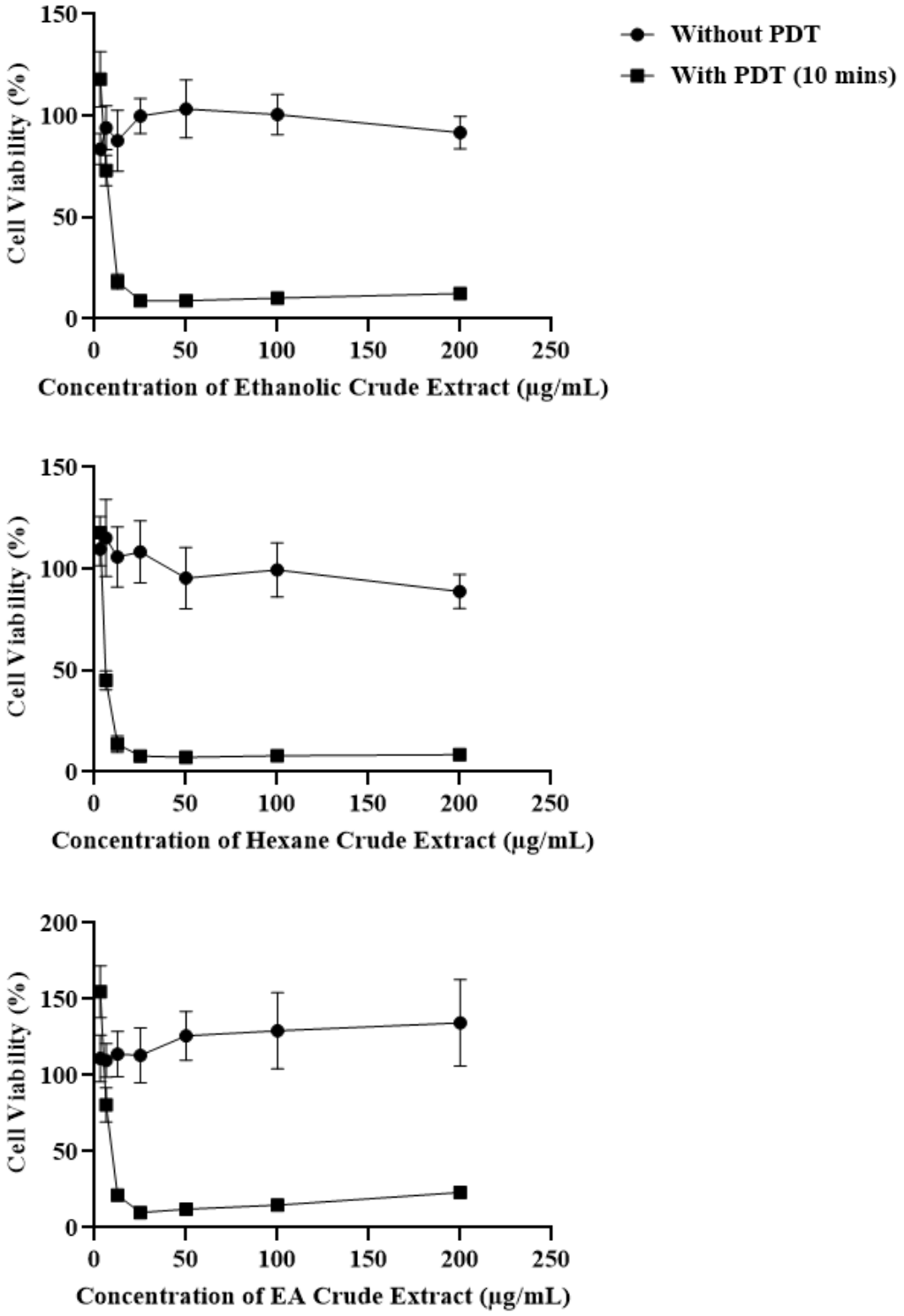

Figure 2. Photo-independent cytotoxicity (extracts without PDT) and photo-dependent cytotoxicity (extracts with PDT) at different concentrations of ethanol, hexane, and EA crude extracts of *S. crispa* on the A431 cell line.

Table 2. Half maximal inhibitory concentration (IC_{50}) of different crude extracts on A431 cells treated with and without PDT.

Crude Extract	PDT Treatment	IC_{50} of crude extract (μg/mL)
Ethanol	Without PDT	NA
	With PDT (10 mins)	8.83 ± 0.55 [a]
Hexane	Without PDT	NA
	With PDT (10 mins)	6.06 ± 0.21 [b]
EA	Without PDT	NA
	With PDT (10 mins)	9.37 ± 0.71 [a]
Cisplatin	Without PDT	27.83 ± 1.76

Note: NA: No activity, *Each data was expressed as the mean ± standard deviation with n = 6. In the last column, values followed by the same letter (e.g., 'a') are considered significantly similar at $p \leq 0.05$ as determined by the one-way ANOVA and post-hoc Tukey HSD test. Conversely, values with different letters (e.g., 'a' and 'b') indicate significant differences.

For the three crude extracts, no noticeable effect was observed in the A431 cell line without PDT treatment, even at concentrations as high as 200 μg/mL, as depicted in **Figure 2**. These results are consistent with the findings of Rahmat et al. [49], who reported similar non-toxic effects of ethanolic crude extracts of *S. crispa* on various cancer cell lines, including HepG2 liver cancer, MCF-7 hormone-dependent breast cancer, MDA-MB-231 non-hormone-dependent breast cancer, and Caco-2 colon cancer, at concentrations up to 100 μg/mL. Likewise, Ghasemzadeh et al. [3] demonstrated high IC_{50} values of ethanolic crude extract of *S. crispa* from different locations against the HeLa human cervical cancer cell line. Extracts of leaves from Kelantan, Malaysia exhibited higher IC_{50} of 182.5 μg/mL compared to those of extracts from Selangor, Malaysia (IC_{50} = 266.4 μg/mL) and Penang, Malaysia (IC_{50} = 331.5 μg/mL). Additionally, Koh et al. [28] reported high IC_{50} values for both EA (IC_{50} = 119.00 ± 48.10 μg/mL) and hexane (IC_{50} = 123.50 ± 37.50 μg/mL) crude extracts of *S. crispa* against the CNE-1 human nasopharyngeal cancer cell line. These observations indicate that the crude extracts of *S. crispa* are less effective as stand-alone anticancer agent, as indicated by their high IC_{50} values exceeding 20 μg/mL [50]. However, as demonstrated in **Figure 2**, when the A431 cell line treated with the extracts was subjected to PDT for 10 minutes, a significant reduction in IC_{50} values was observed compared to the control group. The IC_{50} values decreased from over 200 μg/mL to 8.83 ± 0.55 (22-fold lower), 6.06 ± 0.21 (33-fold lower), and 9.37 ± 0.71 μg/mL (21-fold lower) for the ethanol, hexane, and EA crude extracts, respectively. These values were lower than that of the positive control cisplatin (IC_{50} = 27.83 ± 1.76 μg/mL). These findings are consistent with the study conducted by Tan et al. [34], where HepG2 cells treated with *S. crispa* did not exhibit significant dark toxicity at the tested concentrations. However, when the cells were treated with the methanolic extract of *S. crispa* and subjected to PDT, cell viability was effectively reduced, with an IC_{50} value of 8.51 ± 0.70 μg/mL.

As summarized in **Table 1**, the phytochemical screening of the tested crude extracts of *S. crispa* revealed the presence of chlorophylls. According to Pucci et al. [51], chlorophyll derivatives have been recognized for their unique optical properties, are readily available in high purity from natural sources, exhibit low dark toxicity, and are rapidly eliminated from the body, making them promising PS candidates for PDT. Previous studies have demonstrated the photosensitizing potential of various chlorophyll derivatives when exposed to light in different cancer cell lines. For instance, Foscan (Temoporfin), a chlorin-based PS, has been successfully utilized for PDT in the treatment of advanced squamous cell carcinoma of the head and neck [52]. Similarly, Kamarulzaman et al. [53] observed the photocytotoxic effect of eight derivatives of pheophorbide-a and pheophorbide-b, isolated from *Piper penangense*, against the human promyelocytic leukemia cell line (HL60). Furthermore, Ahn et al. [54] and Liu et al. [55] investigated the impact of pheophorbide-a-based PDT on murine oral squamous cancer AT-84 cells and human prostate cancer PC-3 cells *in vitro*, respectively, using a wavelength of approximately 660 nm. Both studies demonstrated the induction of apoptotic cell death in the cancer cells following pheophorbide-a-based PDT treatment. Hence, the observed photo-cytotoxicity of *S. crispa* crude extracts may be attributed to the presence of chlorophylls, which are known for their photosensitizing properties when exposed to light. Therefore, further research efforts should focus on isolating and characterizing the compounds present in *S. crispa* extracts to determine the exact PSs responsible for their anticancer properties.

CONCLUSION

The ethanol, hexane, and EA crude extracts of *S. crispa* leaves were successfully obtained through cold maceration extraction and solvent-solvent partitioning methods. The objectives of this study were to evaluate the phytochemical composition and photocytotoxicity

of these extracts against the A431 human skin squamous carcinoma cell line using the MTT assay. Qualitative analysis revealed the presence of terpenes, alkaloids, phenols, quinones, and chlorophylls in all three extracts, while no steroids, flavonoids, and anthraquinones were detected. Upon activation with PDT treatment, a significant decrease in cell viability was observed, suggesting the presence of potential photosensitizing bioactive compounds in the crude extracts of *S. crispa*. Among the extracts, the hexane crude extract exhibited the highest photocytotoxic activity against the A431 cell line with an IC_{50} value of 6.06 ± 0.21 µg/mL, followed by the ethanolic crude extract (IC_{50} = 8.83 ± 0.55 µg/mL) and the EA crude extract (IC_{50} = 9.37 ± 0.71 µg/mL). However, in the absence of PDT treatment, none of the crude extracts showed significant toxicity against the A431 cell line, even at concentrations as high as 200 µg/mL after 24 hours of dark incubation. These findings are noteworthy, as prior research has shown the photosensitizing potential of various chlorophyll derivatives in different cancer cell lines, suggesting a potential correlation between chlorophyll presence in the extracts and their activity. This contributes to the growing body of knowledge on the potential application of *S. crispa* extracts as promising candidates for PDT in human non-melanoma skin squamous cell carcinoma treatment.

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CONFLICTS OF INTEREST

The authors declare that they have no conflict of interest regarding the publication of this manuscript.

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