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Original Research Article

In vitro cytotoxic activity of *Sansevieria trifasciata* against various cancer cell lines

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Abstract

Purpose: *To determine the in vitro anticancer properties of Sansevieria trifasciata extracts.*
Methods: *The cytotoxic activity of n-hexane, ethyl acetate and methanol leaf extracts at different concentrations was assessed against Vero, pancreatic (AsPC-1), lung (A549), esophageal squamous carcinoma (KYSE 150) and rhabdomyosarcoma (RD) cancer cell lines using MTT assay. The apoptosis-inducing potential of selected S. trifasciata leaf extract was determined using a caspase 3/7 assay kit.*
Results: *Ethyl acetate extract of S. trifasciata leaf showed significant anticancer activity against RD cells and methanol extract against KYSE cells ($p < 0.05$). Ethyl acetate and methanol extract possessed higher toxicity towards RD and KYSE 150 cell lines and exhibited relatively lower toxicity towards Vero normal cells. Ethyl acetate extract-treated RD cells and KYSE 150 cancer cells had more typical apoptotic morphologic features when compared to normal cells.*
Conclusion: *Sansevieria trifasciata possesses anticancer activity and provides new insights for future molecular work on investigating its utilization in cancer treatment.*

Keywords: *Sansevieria trifasciata, Cancer cell lines, Apoptosis, Chemotherapeutic potential, MTT assay*

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INTRODUCTION

Worldwide, cancer continues to be the second most common cause of death. The International Agency for Research on Cancer reported 19.3 million new cancer cases and almost 10.0 million deaths worldwide in 2020, according to Global Cancer Observatory (GLOBOCAN) data. Cancer cases are further expected to rise by over 50 %, with 32.2 million cases in 2040 [1]. Even with certain notable advancements in cancer treatment, adverse side effects and medication resistance continue to produce unsatisfactory therapeutic results. Thus, the need for other sources to address the problems stated is a global priority [2].

Cancer chemoprevention agents with natural phytochemical compounds are recognized as an emerging approach to prevent, delay, impede, or control malignancy, especially for advanced metastasized cancer. Many cultures throughout

the world still rely on indigenous medicinal plants to alleviate various health problems [3] and 60 to 75 % of FDA-approved anti-infectious and anticancer drugs are proven to be derived from natural products of plant origin [4]. *Sansevieria trifasciata, a* member of the family Asparagaceae, is used in the treatment of various diseases such as urinary diseases, diabetes mellitus, pharyngitis, earache, skin itch, and also possesses analgesic, antipyretic, antioxidants along with antibacterial properties [5].

Apoptosis, one of the primary types of cell death, is characterized by a variety of unique morphological and biochemical alterations, including chromatin condensation, membrane blebbing, cell shrinkage, and the formation of apoptotic bodies [6]. Because cancer can result from a lack of apoptotic regulation, apoptosis plays a crucial function in cancer treatment [7].
Despite the many therapeutic potentials that *S. trifasciata* has demonstrated, relatively few studies concerning the cytotoxic activity of *S. trifasciata* in contrast to various cancer cell lines have been reported. Therefore, this study investigates the potential anticancer activity of *S. trifasciata*.

EXPERIMENTAL

Preparation of plant extract

Fresh leaves of *S. trifasciata* were harvested from a local field close to the School of Medical and Life Sciences, Sunway University, Selangor, Malaysia and were authenticated by a taxonomist (Mohd Hafizi Adzmi Hanafi, reference no. KM0016/22, at the Institute of Bioscience, Universiti Putra Malaysia (UPM), Seri Kembangan, Malaysia. The leaves were dried and pulverized. Thereafter, approximately 200 g of the pulverized leaf was extracted sequentially in a Soxhlet apparatus with solvents of different polarities such as n-hexane, ethyl acetate and methanol. The amount of solvent used to wet the plant sample and fill the round bottom flask was set to a quantitative relation of approximately 1:2, in which two parts of solvent were utilized for one part of plant material. The selected solvent (100 mL) was transferred into the round bottom flask, followed by a small amount of boiling chips. The resulting extract was concentrated in a rotating device, filtered through a 0.22 µm filter, and stored at -20 °C in a dark glass bottle. After the extract was dissolved in DMSO, the final DMSO concentration was adjusted to less than 0.1 %. The leaf extraction or percentage yield (Y) was determined using Eq 1.

$$Y (\%) = (A/W)100 \quad \text{……………..} \quad (1)$$

where A is the actual yield and W is the weight of plant.

Preliminary phytochemical analysis

Leaf extracts of *S. trifasciata* were subjected to preliminary identification of phytochemical constituents using standard procedures [8-9].

Cell culture

Pancreatic cancer cells (AsPC-1), lung cancer cells (A549), esophageal squamous carcinoma cells (KYSE 150), and rhabdomyosarcoma cancer cells (RD) were provided by Professor Dr. Poh Chit Laa of Sunway University's School of Medical and Life Sciences' Centre for Virus and Vaccine Research. American Type Culture Collection provided Vero cells. At 37 °C in a humidified atmosphere with 5 % CO_2, cells were cultured in DMEM (GIBCO, USA) supplemented with 10 % heat-inactivated FBS and 100 units/mL of pen/strep (100 µg/mL) as the antibiotic source.

Cell viability assay

S. trifasciata extracts were evaluated for their impact on viability of Vero, AsPC-1, A549, KYSE150, and RD cells. In 96-well plates, cells were cultivated at a density of 1 × 10^4 per well and incubated until 80 % adherence was achieved. Then cells were treated with different concentrations of *S. trifasciata* extract (62.5, 125, 250 and 500 µg/mL). The cells of control group were incubated with 100 µL of 2 % DMEM. After 24 h, 20 µL of MTT reagents (Macklin, Canada) were added in the dark at 37 °C under 5 % CO_2 for 4 h. Subsequently, 100 µL of 10 % sodium dodecyl sulfate/0.01 M hydrochloric acid was added to each well to solubilize the formazan crystals formed. Using a microplate reader, the absorbance was measured at 570 nm with a reference wavelength of 630 nm. (Infinite® F50 Robotic; Tecan, Männedorf, Switzerland).

Morphological analysis

The KYSE 150 and RD cells were treated with various concentrations of ethyl acetate extract (31.25 and 125 µg/mL) for 2 h in 96-well plates, using untreated cells as negative control. Following treatment, phosphate-buffered saline (PBS) was used to wash the cells, and the morphological changes of treated KYSE 150 and RD cells were observed under an inverted light microscope.

Apoptosis analysis by Caspase 3/7 assay

The KYSE 150 and RD cells were treated with various concentrations of ethyl acetate extract (15.625 and 31.25 µg/mL) and untreated cells as negative control. Utilizing 100 µL of Caspase-Glo® 3/7 reagent, which was added to each well and allowed to incubate for an hour at room temperature, apoptosis triggered by ethyl acetate extract was identified. The signals produced were read using a microplate reader.

Statistical analysis

All tests were conducted in duplicates in two independent experiments. Using GraphPad Prism version 8.0, the data were analyzed using ANOVA and Bonferroni post hoc test. Values were reported as mean ± standard deviation (SD). Statistical significance was assigned to values with ($p < 0.05$).

RESULTS

Percentage yield of the extraction of *S. trifasciata* leaf

The yield of the leaf extraction is displayed in Table 1.

Preliminary phytochemical classes analysis of extract

The results of phytochemical screening are summarized in Table 2.

In vitro cytotoxic activity of *S. trifasciata* leaf extract

Table 3 displays the findings of extract's cytotoxic activities. KYSE 150 cell line was the most sensitive to treatment of hexane extract (IC_{50}: 13.8 ± 0 µg/mL), while hexane extract exhibited weak or inactive cytotoxic activity against A549, RD and AsPC-1 cell lines.

Ethyl acetate extract possessed significant cytotoxic activity against RD (IC_{50}: 19.09 ± 12.86 µg/mL), and KYSE 150 cells (IC_{50}: 20.22 ± 1.65 µg/mL), while insignificant against A549 and AsPC-1 cells. Lastly, KYSE 150 was the most sensitive to the treatment of methanol extract (IC50: 14.80 ± 0.49 µg/mL), while the extract exhibited weak or inactive cytotoxicity against RD, A549 and AsPC-1 cells.

Assessing the selectivity of *S. trifasciata* leaf extract using normal cell lines

The cytotoxicity of *S. trifasciata* leaf extract was determined in the cancer cell lines as well as the normal cell line. The findings are shown in Table 4. The ethyl acetate and methanol extract have higher toxicity towards RD and KYSE 150 cells and exhibit relatively lower toxicity towards Vero normal cells.

Therefore, RD cells and KYSE 150 cells were selected for further experiments to determine the presence of apoptosis by measuring Caspase 3/7 levels.

Apoptosis of RD and KYSE 150 cells

As determined by the MTT assay (Figure 1) and selectivity index (SI), ethyl acetate extract which exhibited noticeable cytotoxic activity and relatively higher selectivity against RD and KYSE 150 cancer cell lines was then employed in further investigations to determine the apoptotic activity.

Table 1: Weight of extraction, percentage of yield, color and solidity of *S. trifasciata* leaf extract

Extract	Weight of extract (g)	Yield (%)	Colour	Consistency
n-hexane	3.95	1.98	Dark green	Semi-solid
Ethyl acetate	7.63	3.82	Dark green	Semi-solid
Methanol	14.27	7.14	Dark brown	Semi-solid

Table 2: Phytochemical analysis testing of different extracts of *Sansevieria trifasciata*

Constituent of detection	Types of biochemical test	Results		
		n- hexane	Ethyl acetate	Methanol
Flavonoid	**Alkaline**	**+**	**-**	**-**
Alkaloid	**Wagner's reagent**	**+**	**-**	**-**
Phenolic	**Ferric chloride**	**+**	**+**	**-**
Terpenoid	**Salkowski**	**-**	**-**	**-**
Tannin	**Ferric chloride**	**+**	**+**	**-**
Glycoside	**Modified Borntrager (C-glycoside)**	**+**	**+**	**+**
Saponin	**Foam**	**-**	**-**	**+**

Note: –: not present, +: present in low concentration

Table 3: IC_{50} values (µg/mL) of *S. trifasciata* leaf extract against cancer and normal cell lines as determined by MTT assay

Extract	Cancer cell lines												Normal cell line		
	RD			KYSE 150			A549			AsPC-1			Vero		
	1	2	Mean ± SD (µg/mL)	1	2	Mean ± SD (µg/mL)	1	2	Mean ± SD (µg/mL)	1	2	Mean±SD (µg/mL)	1	2	Mean±SD (µg/mL)
Hexane	186.21	190.55	188.38±3.07	13.8	13.8	13.8±0.00	91.2	131.83	111.52±28.73	-	-	-	13.8	14.45	14.13±0.46
Ethyl acetate	10	28.18	19.09±12.86	19.05	21.38	20.22±1.65	69.18	93.33	81.26±17.08	83.18	109.62	96.4±18.7	144 .54	158.49	151.52±9.86
Methanol	32.36	33.11	32.74±0.53	15.14	14.45	14.80±0.49	91.2	102.33	96.77±7.87	52.48	52.48	52.48±0	436.52	489.78	463.15±37.66

Table 4: Selectivity index (SI) of *S. trifasciata* leaf extract calculated by the IC_{50} against Vero cells and cancer cell lines. Data were obtained from two independent experiments and presented as mean ± standard deviation (SD)

Extract	Cancer cell lines											
	RD			KYSE 150			A549			AsPC-1		
	1	2	Mean ± SD	1	2	Mean ± SD	1	2	Mean± SD	1	2	Mean± SD
Hexane	1.16	1.16	1.16± 0.004	1	1	1± 0	0.58	0.54	0.56± 0.03	-	-	-
Ethyl acetate	2.2	1.52	1.86± 0.48	1.72	1.65	1.69± 0.05	1.20	1.12	1.16± 0.06	1.15	1.08	1.11± 0.05
Methanol	1.75	1.74	1.74± 0.01	2.24	2.28	2.26± 0.03	1.35	1.31	1.33± 0.02	1.53	1.53	1.53± 0

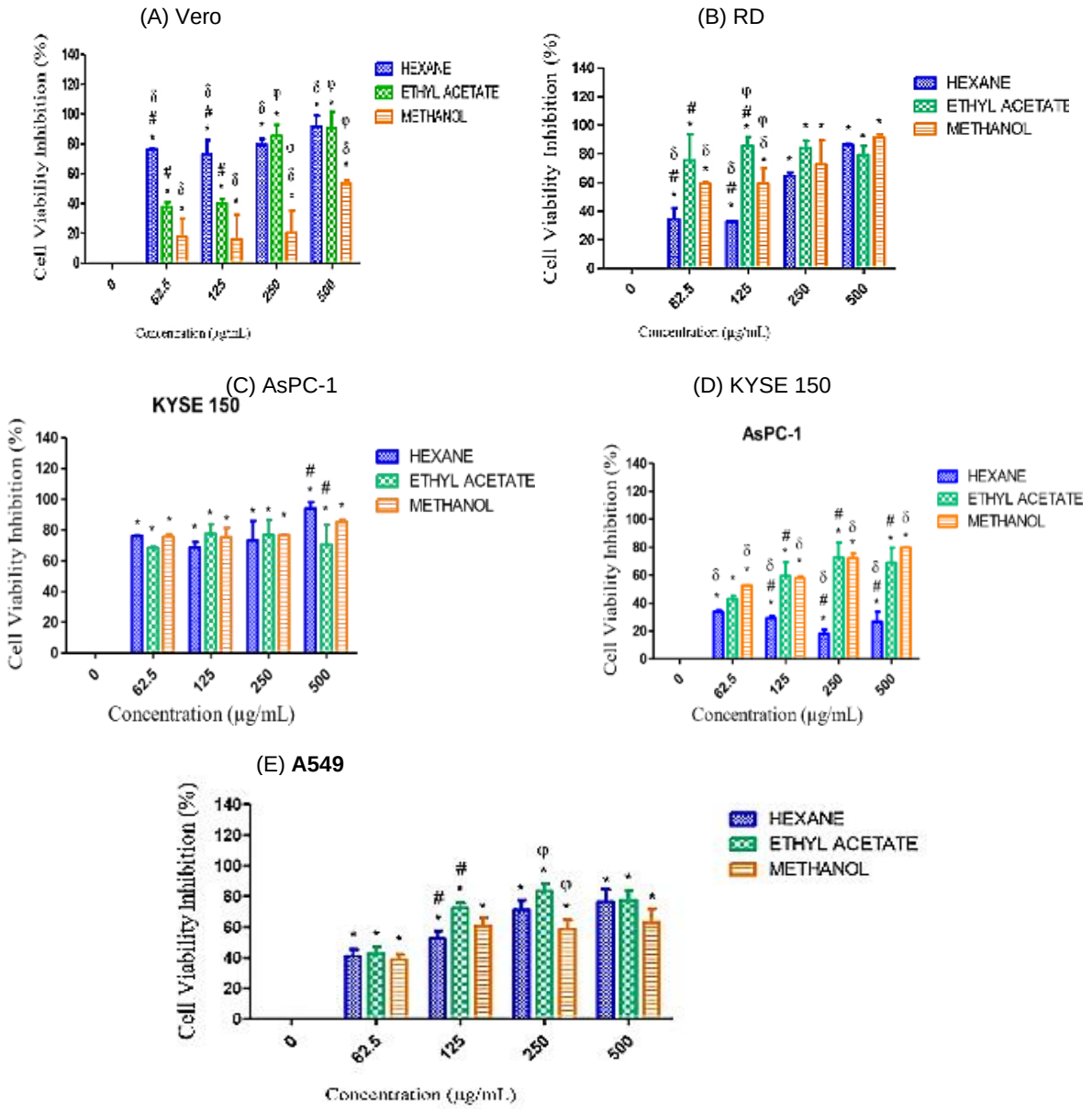

Figure 1: Percentage of (A) Vero, (B) RD, (C) KYSE 150, (D) AsPC-1 and (E) A549 cells viability inhibition after treating with different concentrations of hexane, ethyl acetate and methanol extract of *S. trifasciata* against as evaluated by MTT assay. ***Note:*** $^{*}P < 0.05$ versus concentration at 0 µg/mL (Control group); $^{\#}P < 0.05$ between hexane and ethyl acetate extract; $^{\delta}P < 0.05$, between hexane and methanol extract; $^{\phi}P < 0.05$ between ethyl acetate and methanol extract

Morphological changes induced by ethyl acetate extract of *S. trifasciata* leaf in KYSE 150 and RD cells

The morphological changes induced are shown in Figure 2. Two hours of treatment of KYSE 150 and RD cells with 31.25 µg/mL and 125 µg/mL (concentrations causing 50% cell viability inhibition) reduced cell number and cell membrane blebbing due to loss of cellular viability.

The onset of apoptosis was observed which was characterized by shrinkage of cells and nucleus, followed by detachment of cells from the surrounding tissue. The cells became convoluted and formed extensions called budding. The formation of apoptotic bodies with the cellular organelles and fragments of nuclei closely packed inside the cells was also recognized in Figure 3 and Figure 4.

DISCUSSION

Cancer is a devastating disease that has become one of the world's most pressing health concerns [10]. Several clinically approved cancer treatments are available [11]. However, the of conventional chemotherapeutic agents is often

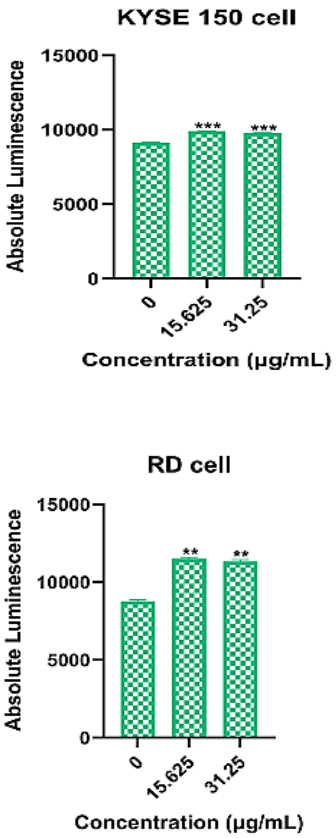

Figure 2: Apoptotic (Caspase 3/7) activity in (A) KYSE 150 (B) RD cells treated with 15.625 and 31.25 µg/mL of ethyl acetate extract for 2 h. *P < 0.05 versus control.

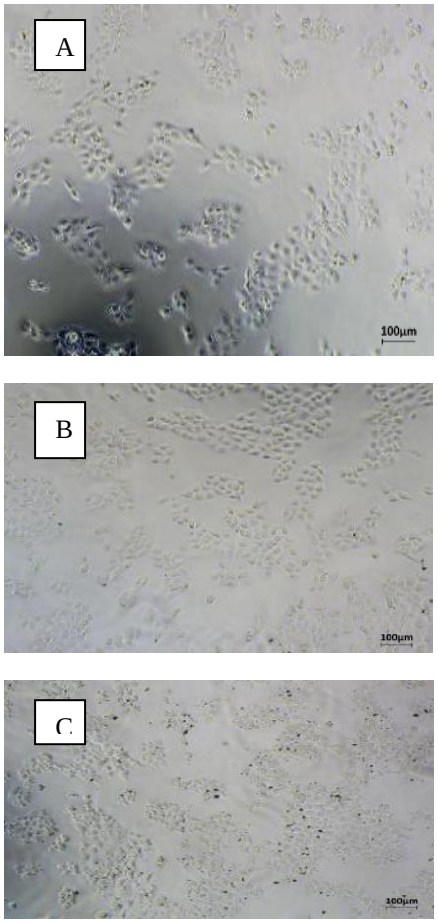

Figure 3: Changes in morphology caused by *S. trifasciata* ethyl acetate extract in (A) KYSE 150 cells, (B) at 31.25 µg/mL and (C) at 125 µg/mL after 2 h treatment compared with untreated control. Magnification: 10X

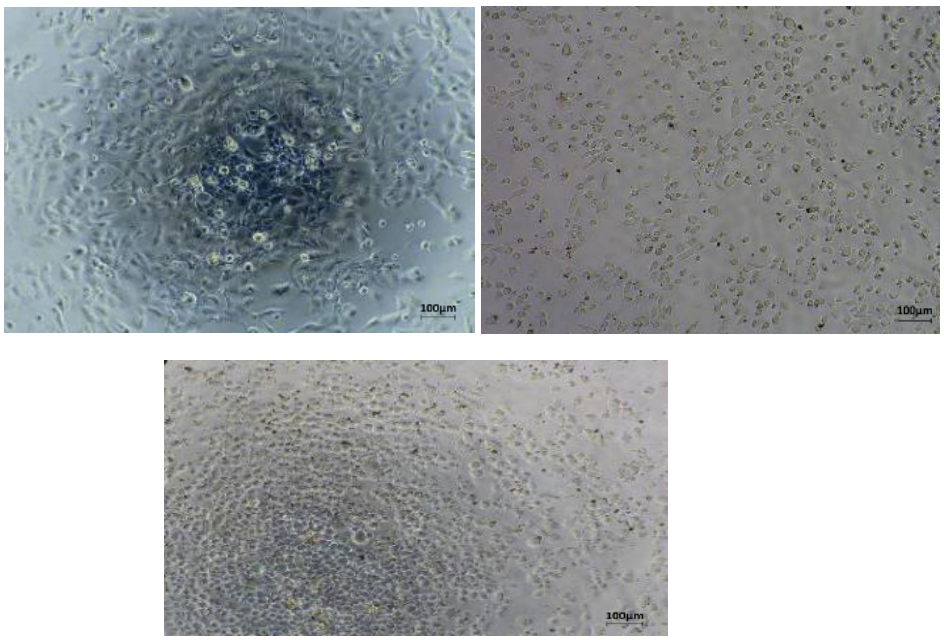

Figure 4: Changes in morphology brought upon by the *S. trifasciata* ethyl acetate extract in RD cells at (B) 31.25 µg/mL and (C) 125 µg/mL after 2 h treatment compared with untreated controls (A). Magnification: 10x

accompanied by harmful side effects and chemo-resistance. Hence, it is crucial to seek a novel anticancer agent with high efficacy and low toxicity.

Sansevieria trifasciata is important in ethnomedicine for the treatment of several ailments [12]. Its anti-inflammatory activities have been demonstrated [13]. However, relatively few studies concerning the cytotoxic activity of *S. trifasciata* leaf extract against different cancer cell lines have been reported. The phytochemical screening of these extracts indicated the presence of several phytochemical classes that are known to possess strong anti-cancer properties and combat various diseases through specific modes of action [14].

Based on the result obtained, the hexane and methanol extract from the leaves of *S. trifasciata* exhibited a noticeable cytotoxic effect on KYSE 150 cancer cell lines while ethyl acetate extract displayed a higher cytotoxic effect on RD cells and KYSE 150 cancer cell lines. The low cytotoxicity of the plant extract against A549 and AsPC-1 cancer cells may be due to the resistance of the cancer cell lines to the type of secondary metabolites present.

It has been reported that the selectivity index (SI) > 1.0 is favorable as it indicates that treatment with extract has greater efficacy against cancer cells with low toxicity against normal cells [15], which would be a safer anticancer drug candidate for further testing *in vivo* models. Merely utilizing malignant cell lines to assess the anticancer potential of plant extract without identifying SI is a subpar predictor for subsequent investigations. The ideal anticancer medication should be able to eradicate cancer cells without endangering healthy, normal cells. Because traditional chemotherapeutic medicines lack selectivity and are extremely toxic to both cancer and healthy normal tissues, their usage is frequently followed by chemo-resistance and unpleasant side effects [2,16]. In the result obtained, the SI values of hexane extract on A549 and AsPC-1 cells were less than one, which can be toxic to Vero normal cells and cannot be used as an herbal drug. In contrast, ethyl acetate and methanol extract of *S. trifasciata* had comparatively higher SI values on RD and KYSE 150 cancer cell lines, which are presumably non-toxic and bioactive. Certain hazardous components are present in the extracts with comparatively low SI (< 1) data. Comparative analysis of MTT assay results suggested that the cytotoxic effect of ethyl acetate and methanol extract of S. *trifasciata* can be related to secondary metabolites such as polyphenols, flavonoids and alkaloids which were important for anticancer activity, especially saponin [12]. However, the ethyl acetate extract was selected to determine the apoptotic activity against RD and KYSE 150 cancer cells as it contains various phytochemicals as compared to methanol extract.

Known as "programmed cell death," apoptosis is a widely used process for tissue remodeling, cell replacement, and the removal of damaged cells. Anticancer drugs cause apoptosis, which decreases the sensitivity of treatment when it is disrupted [11]. Apoptosis is one of the key underlying mechanisms for cytotoxic antitumor agents, whereas studies have found that some natural compounds present in plants can induce apoptotic pathways that are blocked in cancer cells [17]. Ethyl acetate extract of *S. trifasciata* at 15.625 and 31.25 µg/mL was selected for cell death detection based on the results of the MTT assay for caspase 3/7. In this study, the apoptotic activity in KYSE 150 cells induced by ethyl acetate extract was the highest at 15.625 µg/mL as compared to 31.25 µg/mL, and this trend was similar to that of RD cell activity.

Under a light microscope, the morphological alterations of RD and KYSE 150 cells were examined to ascertain if the growth inhibitory action of *S. trifasciata* plant extract was connected to the induction of apoptosis. The American National Cancer Institute's (NCI) protocol states that a crude plant extract's upper limit for its IC_{50} is 30 µg/mL [19]. Nevertheless, it was discovered that after being exposed to 125 µg/mL and ethyl acetate extract (31.25) for two hours, the cells began to generate apoptotic bodies. Typical apoptotic morphological observation included cell rounding, membrane blebbing, and formation of several apoptotic bodies were observed, compared to homogeneous nuclear chromatin in the control cells [11]. Taken together, this finding revealed that ethyl acetate extract of *S. trifasciata* induces apoptosis in RD and KYSE 150 cells. Therefore, the beneficial role of *S. trifasciata* leaf extract in preventing apoptotic cell death is proposed. Future mechanistic research is necessary to determine whether the lethal impact of *S. trifasciata* extract on cancer cells is primarily caused by apoptosis induction or cell cycle arrest.

CONCLUSION

Ethyl acetate extract of *S. trifasciata* leaf shows potent anticancer activity against RD cells while ethyl acetate and methanol extract have the highest potency against KYSE 150 cells. The

present investigation provides evidence that *S. trifasciata* leaf extract may be an effective anti-cancer drug and justifies its use in ethnomedical settings.

DECLARATIONS

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Ethical approval

None provided.

Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Conflict of Interest

No conflict of interest associated with this work.

Contribution of Authors

We declare that this work was done by the authors named in this article and all liabilities pertaining to claims relating to the content of this article will be borne by the authors. Sheryar Afzal and V Appalaraju Study concept and design, manuscript handling and manuscript writing. Chan Zelynn performed experiments, data collection, and analysis, a major contribution to manuscript writing. Yuan Seng Wu supervised and designed the project, analysis and critical revision of the manuscript. All authors read and approved the final draft of the manuscript for publication.

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