RESEARCH ARTICLE

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In Vitro Anticancer Activities of *Senna singueana* Leaf Extracts against HeLa Cell Lines

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Abstract

Objective: The present demonstrated that SSL-CH extract selectively inhibits the growth of HeLa cell lines though activation of p^{53} and *Bax* genes medaied proapoptotic pathway signifying it is a potential alternative anticancer agent for cervical cancer. **Materials and Methods:** The selective cytotoxicity of diethyl ether, chloroform, ethyl acetate, and ethanolic leaf extracts of *S. singueana* were evaluated against cervical HeLa and normal mouse fibroblast L929 cell lines using MTT assay. At (IC_{50}) concentration 20 µg/ml) of effective chloroform leaf extract of *S. singueana* (SSL-CH) was chosen to evaluate its nuclear apoptotic changes by using Acridine Orange/Etidium Bromide dual staining. Subsequently, apoptotic induction potential of SSL-CH extract was assessed by RT-qPCR analysis of apoptotic genes p^{53} and *Bax*. **Results:** Treatment SSL-CH extract selectively inhibited the dose depended proflretion of HeLa cell lines with IC_{50} of 20 µg/ml. AO/EB dual staining showed SSL-CH extract induced late apoptosis of 49.42% in compared to the control. RT-PCR analyses demonstrated that SSL-CH extract profoundly upregulated the proapoptotic genes p^{53} to12.85 (***p≤0.01), *Bax* to 11.61 (**p≤0.01), *caspase-9* to 40.62 (***p≤0.01), and *caspase-3* to 11.61 folds (**p≤0.01) respectively and down regulated antiapoptotic genes *Bcl-2* to 0.9 (**p≤0.001) and survivin gene to 1 (***p≤0.001) at $2\times IC_{50}$ (40 µg/mL) in comparison to control, confirming activation of the intrinsic apoptotic pathway. **Conclusion:** These findings demonstrated that SSL-CH extract could serve as a potential alternative therapeutic agent for cervical cancer treatment with affordable and fewer side effects comparison with cisplatin.

Keywords: *Senna singueana*- cytotoxicity- cervical cancer- apoptosis intrinsic pathway- nuclear changes

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Introduction

Cervical cancer is the heterogeneous malignancy and the second leading cancer mortality in females with the high risk at the age of 35-45 in low income countries [1,2].WHO by 2019 recognized it is an important issue, and allocated substantial resources and funds to advance cervical cancer treatments [2]. In spit the effectiveness of chemotherapy regimens for treating cervical cancer, it has significant drawbacks, including high doses, high costs, non-specificity, and multidrug resistance of chemotherapeutic agents emphasizes for alternative therapeutics to enhance treatment efficacy and patient outcomes [3].To overcome these challenges medicinal plants and plant-derived anticancer agents, such as paclitaxel, vincristine, gemcitabine, 5-fluorouracil, carboplatin, and oxaliplatin are used alone or in combination to treat cervical cancer and other malignancies [4]. As a result, these issues motivated the researchers to search novel anticervical cancer drugs capable of providing more effective treatment options.

Senna singueana (*S. singueana*), belongs to the family Fabaceae is widely distributed in Ethiopia has been extensively used by traditional healers to treat ulcer, gonorrhea, malaria, typhoid, cancer and additionally, it can be used to alleviate constipation [5]. The phytochemical analysis of organic crude root bark extract of *S. singueana*, has shown the presence of alkaloids, phenolic compounds, flavanoids, tannins, glycosides [6], and it has antioxidant [7], antimicrobial [8], anticancer [9] and antiplasmodial [10]. Several in vitro anticancer studies reported that organic leaf and stem bark extracts from Senna species have exhibited promising anticancer activities against colon (HCT-116, HT-29), ovarian (OVCAR-8), glioblastoma (SF-295) [11], breast (MCF-7), prostate (C4-2WT, LNCaP), lung

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(SKLU-1) [12], A549), colorectal cancer (SW-620) [13], and leukemic cancer cells (JURKAT, K562) [14]. These reports have made this plants to be attractive candidates for anticervical cancer drug. Despite these promising findings, research on *S. singueana* is specifically remains limited. However till date, only a few studies have investigated the cytotoxic effects of leaf extracts of *S. singueana* on HeLa cell lines, with no valid investigation of the underlying molecular mechanisms.

Hence the present studies aimed to screening the selective in vitro cytotoxicity of various organic leaf extracts from *S. singueana* against HeLa cell lines and elucidate the underlying molecular mechanisms of apoptosis induction

Materials and Methods

Preparation of extracts

S. singueana leaves were collected from Axum city, Ethiopia in January, 2021 and authenticated in National Herbarium of Ethiopia by Mr. Melaku Wendaferash (voucher specimen number AM-002). The fresh leaves of *S. singueana* were dried in shade at room temperature for ten days and ground by electrical grinder into powder.

According to Ho and Chun [15], about25 grams of leaf powder (1:10 w/v) was extracted over 12hrs using 250 ml of diethyl ether, chloroform, ethyl acetate, and ethanol by using Soxhlet apparatus. The percentage yield was calculated.

Percentageyield=(Extract weight of the plant (g))/(Dry weight of the plant (g))×100

Qualitative phytochemical screening

The qualitative phytochemicalanalysis was carried out using modified phytochemical screening methods described by [16].

In vitro cytotoxicity assay

The cytotoxic effect of *S. singueana* leaf extract against HeLa and normal mouse fibroblast L929 cell lines were measured by MTT (3-(4, 5-dimethylthiazol-2-yl)-2, 5 diphenyltetrazolium bromide) assay [17]. Cells were seeded with a density 1×10^4 cell/well in a 96 well plates and treated with 25, 50,100, 250 and 500µg/ml of extracts and 2.5, 5, 10, 20 and 40 µg/ml cisplatin in triplicates except to wells with basal control, where nutrient medium (DMEM) was added and incubated for 24 hours in 5% CO2 incubator at 37°C. After the treatment, 20 µl of MTT (5mg/ml) solution was added and allowed further 4 hours of incubation in dark 5% CO_2 incubator at 37°C. The formazans was solubilized with 100µl of 10% Sodium Dodecyl Sulfide (SDS) and measured using micro ELISA plate-readerat 570 nm. The cytotoxicity was determined using following formula.

Cytotoxicity (%)=100- (OD of test)/(OD of control)×100

IC_{50} was calculated using cell proliferation curve. To determine the selective cytotoxicity of the plant extracts tested, selective index was calculated according to the given formula.

SelectiveIndex(SI)=(IC_{50} of normal cell lines)/(IC_{50} of cancer cell lines)×100

Cellular Morphological analysis

To determine the apoptotic cellular morphological changes induced by chloroform leaf extracts of *S. singueana* (SSL-CH) on HeLa and L929 cell lines, 5×10^5 cell/well were seeded into 12 well plates. After 24 hours of treatment with SSL-CH extract at IC_{50} (20 µg/ml), $2\times IC_{50}$(40 µg/ml) and $4\times IC_{50}$ (80 µg/ml), the cellular morphological changes of apoptosis were observed using an inverted phase contrast microscope at 1000X [18].

Acridine Orange/Ethidium Bromide (AO/EtBr) dual staining assay

AO/EtBr dual staining assay was used to determine the apoptotic induction potential of SSL-CH extract using fluorescent microscopy. HeLa cells with density of 5×105 cells/well were seeded in 12 well plates with DMEM and treated with SSL-CH extract at IC_{50} (20 µg/ml)for 24 hours in 5% CO_2 incubator. After treatment, cells were stained using 1µl of AO/EtBr solution (1µg/ml each PBS) [19] and observed under inverted fluorescent phase contrast microscope 1000X.AO/EtBr staining distinguishes between viable cells, early apoptotic, late apoptotic or necrotic cell. Viable cells showed green, early apoptotic cells shown bright green, orange color were seen in late apoptotic and red necrotic cells [20].

RT-q PCR analysis

The m-RNA expression of apoptosis-related genes *p^{53}, Bax, survivin, Bcl-2*, *caspases- 3* and *9* were evaluated by RT-qPCR. HeLa cell lines with 5×10^5 cells density were seeded in 12 well plates then, treated with IC_{50} (20µg/ml) and $2\times IC_{50}$ (40µg/ml) SSL-CH extract and incubated for 24 hours in 5% CO_2 incubator [21, 22]. TRIzol reagent (Ambion, USA) was used to extract the total RNA from treated and untreated HeLa cell line by following the manufacturer's protocol. The concentration and purity of RNA was measured using Nanodrop 2000 spectroscopy (Thermo scientific). The cDNA synthesis was carried out using cDNA synthesis kit PrimeScriptTM (TAKARA BIO INC, Japan) based on the manufacturer's instructions. The *mRNA* gene expression of target genes were investigated by Quantitative real-time PCR (RT-qPCR) with GoTaq® qPCR Master Mix (Promega, USA) and Glyceraldehyde-3- phosphate dehydrogenase (GAPDH) was used as an internal control. The gene expression levels of *p^{53}, Bax, Bcl-2 survivin*, *caspase 9* and 3 were determined by cyclic threshold method (ct method) using ($^{-\Delta\Delta ct}$) formula.

Statistical analysis

The results from three distinct trials are presented as mean ± standard deviation (SD). Statistical differences between test and control groups were analyzed using one-way ANOVA and Tukey's test, with statistical significance $p\leq0.05$, $p\leq0.01$, and $p\leq0.001$.

Results

The percentage yield

The percentage yields of the extracts ranged from 3.3 to 9.9 g per 25 g of *S. singueana* leaves. It was found that ethanolic leaf extract of *S. singueana* has shown highest percentage yield of 9.9g followed by ethyl acetate 7.4 g, chloroform 5.7g and the lowest diethyl ether 3.3g. Our findings are consistent with those of Gebrelibanos [23], who reported that *S. singueana* leaf extract has shown the highest percentage yield in methanol extract (15.6 g/100 g) through maceration.

Phytochemical screening

As presented in Table 1, phytochemical assessment of leaf extracts of *S. singueana* showed that diethyl ether extracts had moderate presence of alkaloids, phenolics, flavonoids, and tannins, but chloroform extracts was identified with significant levels of flavonoids, alkaloids, tannins, and phenolics. Ethyl acetate extracts were abundant in tannins, flavonoids, and phenolics, while ethanolic extracts were moderate in alkaloids, phenolics, flavonoids and tannins, despite no saponins or glycosides observed. These findings confirm *S. singueana* potential as a source of novel drugs against cancer [24].

Our results are coincides with Mbugua et al. [25], reported methanolic and dichloromethane (1:1) and aqueous leaf extracts of S.didymobotrya were shown the presence of flavonoids, phenolic compounds, tannins, terpenoids, glycosides, and saponin absence of but not alkaloids.

In vitro cytotoxicity and Selective Cytotoxic index (SI)

The in vitro anti cancer activities of leaf extracts (SSL-DE, SSL-CH and SSL-EA) of *S. singueana* haves shown a dose-dependent selective cytotoxicity against HeLa cell lines with cytotoxicity at 25-500µg/mL treated for 24hours ranging from 11.06 % to 97.47 % (Figure1 and Table 2). On the other hand SSL-ET extract has shown less cytotoxic effects on HeLa cell lines. The cytotoxicity of standard cisplatin which has shown significant cytotoxicity ranging from 23.15 % to 87.56%at concentration range of

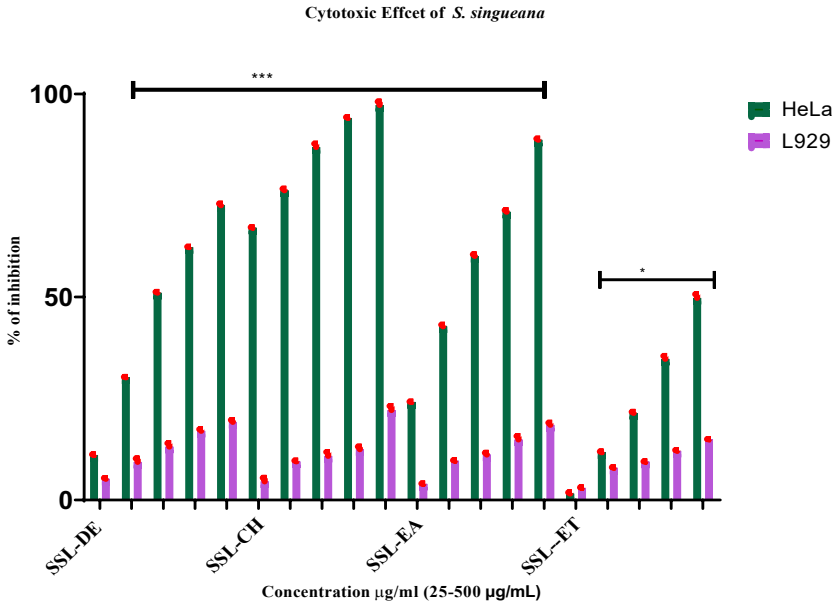

Figure 1. Cytotoxiceffect of Leaf Extracts of *S. singueana* at 25-500 µg/ml on HeLa and L929 Cell Lines after 24 hours of Treatment

Table 1. Phytochemical Screening of Leaf Extracts of *S. singueana*

	Senna singueana			
Phytochemicals	Diethyl ether	Chloroform	Ethyl acetate	Ethanol
Alkaloids	++	+++	++	++
Phenolic compounds	++	+++	+++	++
Flavonoids	++	+++	+++	++
Tannin	++	+++	+++	++
Glycosides	-	++	++	-
Terpenoids	-	++	+	+
Saponin	+	-	-	+
Steroids	+	-	++	-
Anthraquinones	+	+	++	++

"-"Absence; "+"Minor presence; "++" Moderate presence; "+++"Maximum presence of secondary metabolites.

Table 2. *In vitro* Selective Cytotoxic Effects of Organic Leaf Extracts of *S. singueana* after 24 hours of Incubation

Extracts /Standard	Concentration (µg/ml)	% Cytotoxicity		IC_{50} (µg/ml)		Selective Index (SI)
		HeLa Cells	L929	HeLa Cells	L929	
SSL-DE	25	11.06 ±017	5.21±0.22	64.32	>500	
	50	30.21±0.13	9.37±0.85			7.81
	100	51.06±0.19	13.17±0.81			
	250	62.28±0.12	17.02±0.41			
	500	72.58±0.43	19.25±0.47			
	25	67.09±0.10	4.6±0.87		>500	
SSL-CH	50	76.29±0.85	9.25±0.22	18.1		27.62
	100	86.92±0.19	10.85±0.98			
	250	94.09±0.84	12.5±0.67			
	500	97.30±0.10	22.17±0.98			
SSL-EA	25	24.14±0.31	3.98±0.1			
	50	42.79±0.38	9.65±0.14	50.24	>500	9.95
	100	60.09±0.42	11.19±0.45			
	250	70.98±0.40	14.9±0.86			
	500	85.74±0.26	18.5±0.52			
	25	1.52±0.34	2.98±0.14	>500	>500	1
SSL-ET	50	11.73±0.29	7.96±0.12			
	100	21.44±0.64	9.48±0.03			
	250	34.77±0.39	12.1±0.04			
	500	49.79±0.48	14.95±0.20			
	2.5	23.15±38	ND			ND
Cisplatin	5	38.74±29	ND	9.53	ND	
	10	54.16±0.43	ND			
	20	73.06±0.19	ND			
	40	87.56±0.76	ND			

ND, not determined

2.5 to 40 µg/ml for 24 hours.

As shown in Table 2 and Figure1, the treatment with SSL-DE, SSL-CH and SSL-EA extracts have shown the highest Cytotoxic effect, inhibiting the growth of HeLa cell lines, with percentage inhibitions of 72.58%, 97.30% and 85.74%, respectively with significance level of (***p≤0.001) as compared with the control. On the other hand the extract SSL-ET has shown less selective cytotoxic effects on HeLa cell lines with percentage inhibitions of 49.79% with significance level of (*p≤0.01) in contrast to the control. The IC_{50} values for DE, CH, EA and ET extracts and cisplatin against HeLa and L929 cell lines were found to be 64.32 and >500 µg/ml, 18.1 and >500 µg/ml, 50.24 and >500 µg/ml, 500 and >500 and 9.53µg/ml, respectively. The cytotoxic activity of *S. singueana* leaf extracts on HeLa cell lines was observed in the following order:

SSL-CH >SSL-EA > SSL-DE > SSL-ET

Additionally, the SI values of SSL-DE, SSL-CH, SSL-EA, and SSL-ET extracts on HeLa cell lines compared with L929 cell lines were 7.81, 27.62, 9.9 and 1, respectively (Table 2). Our results have shown that SSL-CH had a cytotoxic concentration (CC_{50}) of >500 µg/ml against L929 cell lines, with an IC_{50} of 20 µg/ml against HeLa cell lines, resulting in a selective index of 27.62 (Table2).Based on the IC_{50} value of 20 µg/ml, SSL-CH extract was selected to evaluate the apoptotic induction potential of SSL-CH extract in HeLa cell lines by studying pro and anti apoptotic gene expression using real-time qPCR

These findings are in line with Onyegene-Okerenta et al. [13], who reported that ethyl acetate leaf extract

Table 3. Apoptotic Induction Effect of SSL-CH at IC_{50} on HeLa Cells

	Number of Apoptotic cells /100			
Test	Live cells (Green)	Early Apoptotic (Green &yellow)	Late Apoptotic (Orange & light red)	Necrosis (Red)
Control	90.43±0.15	5.57± 0.78	3.35 ± 0.25	0.9± 0.19
SSL-CH	8.45 ±066	33.32 ±0.57		8.82±0.3
(IC_{50} 20 µg)			49.42±0.12	

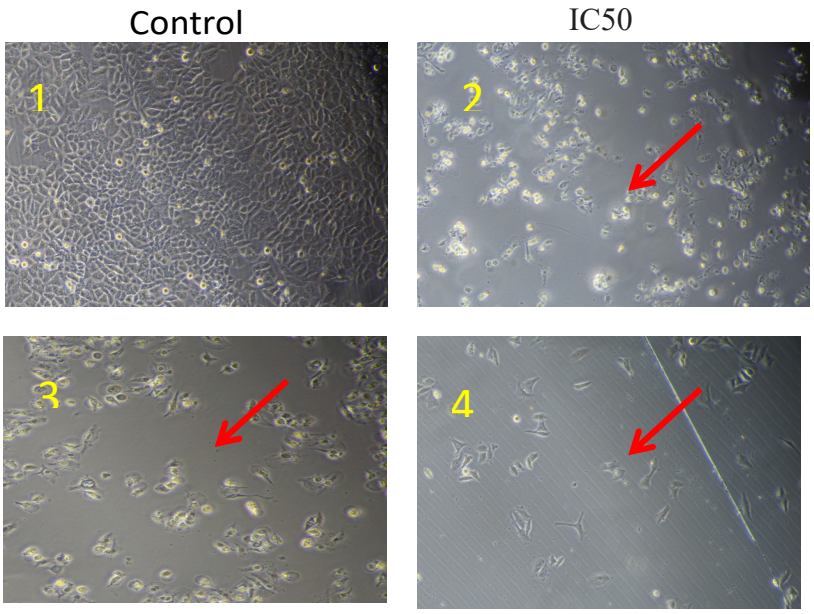

Figure 2 (a). Morphological Changes of HeLa Cell Lines Observed under Phase Contrast Microscope (1000X) (1) Control (2,3 and 4) HeLa cell lines treated with chloroform leaf extract of S. singueana (SSL-CH) at IC_{50} (20µg/mL), 2 fold IC_{50} (40µg/mL), and four fold IC_{50} (80µg/mL), respectively for 24 hours. Morphological changes are indicated in red arrows.

of S. alata had shown strong anti proliferation activity against breast cancer (MCF-7), colon cancer (HCT-116, HT-29),and prostate cancer(C4-2WT) with lowest IC_{50} values of 5.9, 11.86, 4.97 and 9.48 µg/mL, respectively. Our results also coincide with [26] reported that N-hexane, dichloromethane and chloroform leaf extracts of S. alata have shown potential selective cytotoxic effects against breast MCF-7 cell lines with lowest IC_{50} values of 0.013, 47.11 and 57.61 µg/mL respectively. In the same way ethanolic leaf extract *S.singueana* have significantly reduced cell population of HeLa and prostate PC-3 cancer cell lines with lowest IC_{50} values of 42.1 and 9.9 µg/mL, respectively [9].

Morphological changes

SSL-CH extract treated HeLa cell lines at IC_{50} (20 µg), 2× IC_{50} (40 µg), and 4× IC_{50} (80 µg) for 24 hours displayed substantial morphological shift confirming apoptosis, in contrast to L929 cell lines which demonstrated only slight changes. As shown in Figure 2a and 2b significant

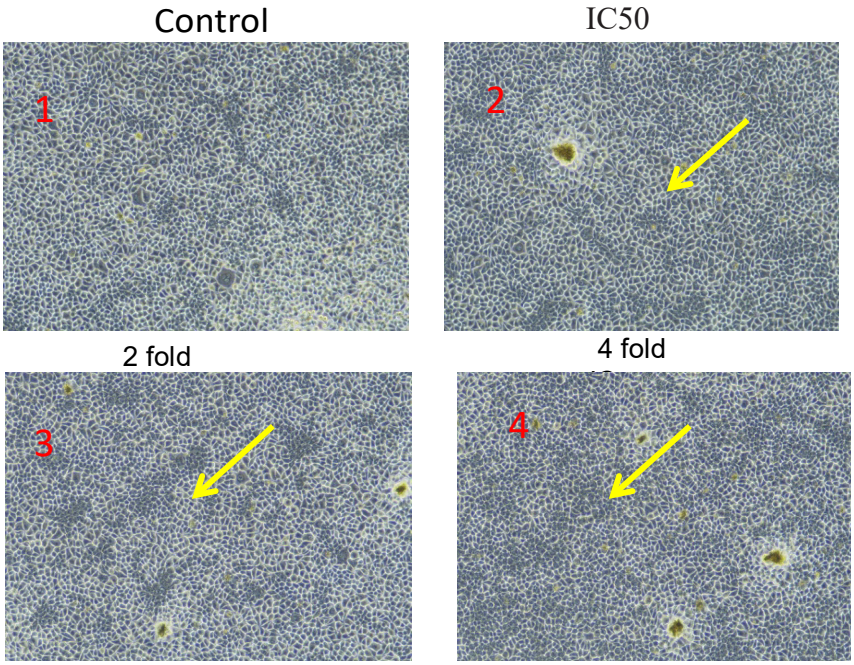

Figure 2 (b). Morphological Changes of Mouse Fibroblast Cell Lines (L929) Observed under Phase Contrast Microscope (1000X) (1) Control (2,3and 4) L929 cell lines treated with chloroform leaf extract of *S. singueana* (SSL-CH) at IC 50 (20µg/mL), 2 fold IC 50 (40µg/mL), and four fold IC_{50} (80µg/mL) respectively for 24 hours. Morphological changes are indicated in yellow arrows.

Table 4. Modulation Effect of SSL-CH at IC_{50} on Proapoptotic and Antiapoptotic Gene Profile on HeLa Cell Lines

S.No	Gene	Control	*S. singueana* chloroform leaf extract (SSL-CH)			
			Fold of up regulation		Fold of down regulation	
			IC_{50}	2 fold IC_{50}	IC_{50}	2 fold IC 50
1	*Bax*	1.07	6.86	12.68	-	-
2	*P53*	1.08	5.74	13.93	-	-
3	*Bcl-2*	1.06	-	-	0.32	0.16
4	*Survivin*	1.12	-	-	0.36	0.12
5	*Capases-9*	1.75	28.05	42.37	-	-
6	*Capases-3*	1.75	14.77	28.59	-	-

changes comprised detachment, loss of adherence, and round shape, along with apoptotic characteristics such as cell shrinkage and abnormal membrane bulges illustrate the distinct effects of SSL-CH on HeLa cells relative to the control.

Our results are consistent with [26] reported that hexane, dichloromethane and chloroform extracts of S. alata have shown potent morphological changes on breast (MCF-7) cancer cell lines.

Effect of SSL-CH on induction of apoptosis by Acridine orange / Ethidium bromide (AO/EB) dual staining

As shown in Table 3 after 24 hours of treatment with SSL-CH extract at IC_{50} (20 µg/mL) it substantially decreased the viable HeLa cell from 90.43% to 8.45%. Early apoptotic, late apoptotic, and necrotic cells were significantly increased from 5.57 % to 33.32 % (***p≤0.01), 3.35% to 49.42%(***p≤0.001) and 0.9 % to 8.82% (*p≤0.05), respectively as in (Figure 4). As shown in Figure 3(A), phase contrast microscopy revealed that control HeLa cells are characterized by a regular monolayer appearance (indicated by a red circle with a yellow arrow). In contrast, Figure 3(B) showed, the cells become round and disconnected each other after treatment (indicated by a yellow circle with a red arrow).

Basal control HeLa cells were consistently green as shown in Figure 3 (C). whereas IC_{50} (20 µg/ml) of SSL-CH treated HeLa cells showed green, green mixed with orange, orange with light red, and red due to nuclear changes such as chromatin condensation and DNA fragmentation as in Figure 3(D).

Our results are agreed with Castro et al. [27] stated, the ethanolic root extract of Senna velutina induced 32.4% apoptosis in melanoma B16F10Nex-2 cell lines by causing extreme chromatin condensation and DNA fragmentation.

Effect of SSL-CH on m-RNA expression of pro apoptotic and anti apoptotic gene expression by Real time qPCR

As shown in Table 4 and Figure 5, *RT-qPCR m-RNA* gene expression studies revealed that the pro-apoptotic gene *Bax* in SSL-CH extract treated HeLa cells significantly (**p≤0.01) increased the m-RNA expression by 5.79 and 11.61 folds at IC50 (20 µg/mL) and 2 × IC_{50} (40 µg/mL), respectively in comparison to control. Additionally, anti-apoptotic gene *Bcl-2 m-RNA* gene expression revealed that SSL-CH extract treated

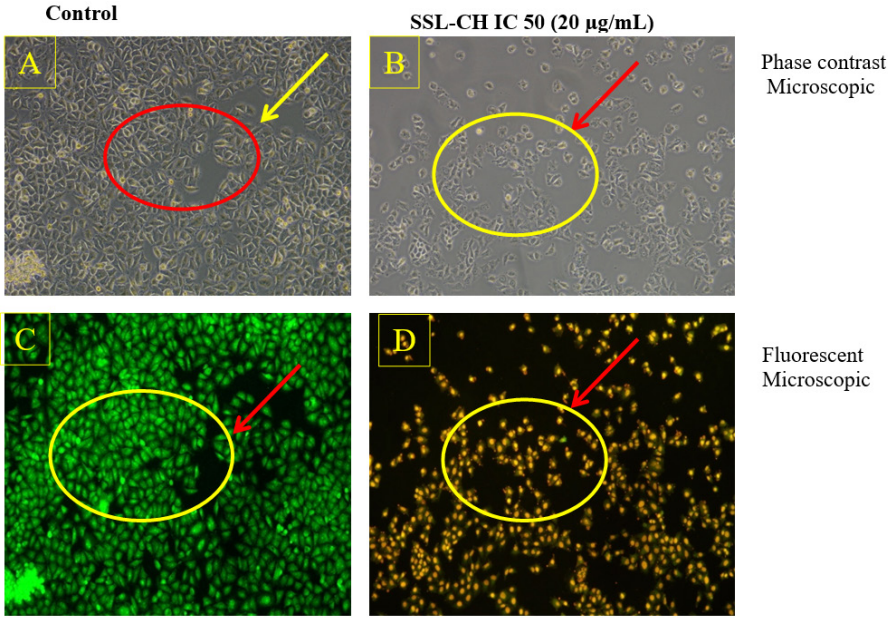

Figure 3. Nuclear Changes of HeLa Cell Lines Observed Untreated (A) Treated (B) under phase contrast microscope (1000X) and Untreated (C) Treated (D) observed under fluorescent microscopy using Acridine orange/Ethidium bromide dual staining with chloroform leaf extract of *S. singueana* (SSL-CH) at IC50 (20µg/mL) for 24 hours.

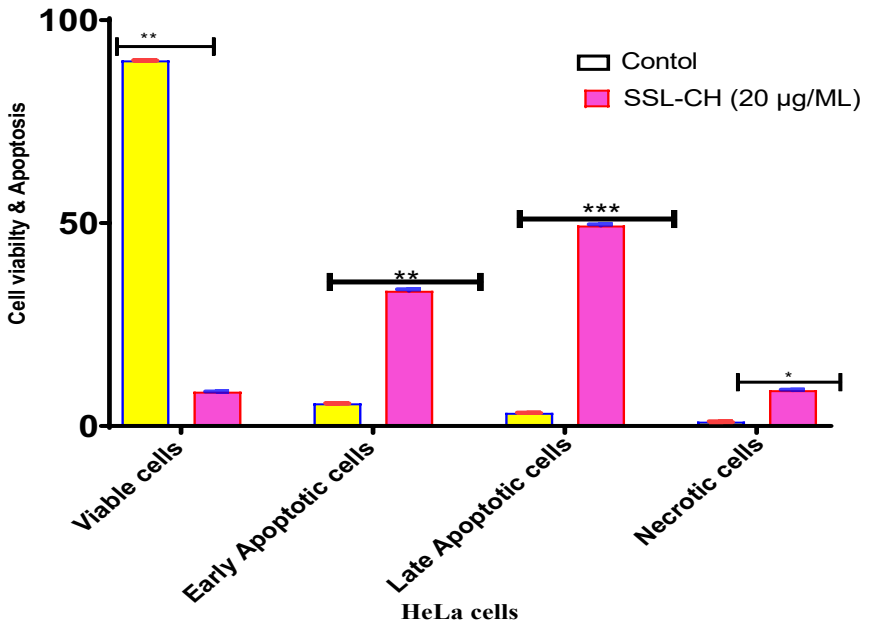

Figure 4. Apoptotic Effect of Chloroform Leaf Extract of *S. singueana* (SSL-CH) at IC_{50} (20µg/mL) against HeLa Cell Lines Treated for 24 hours. Acridine orange/ Ethidium bromide dual staining, has indicated the percentage of viable cells, early apoptotic cells, late apoptotic cells and necrotic cells.

HeLa cells was significantly (**p≤0.001) down regulated by 0.74 and 0.9 folds at IC_{50} (20µg/mL) and 2× IC_{50} (40µg/mL), respectively as compared to the control.

The SSL-CH extract significantly (***p≤0.001) elevated *p^{53} mRNA* expression in HeLa cells by 4.66 and 12.85 at IC_{50} (20 µg/mL) and 2× IC_{50} (40µg/mL), respectively whilst survivin expression significantly (***p≤0.001) reduced by 0.76 and 1 folds at IC_{50} (20 µg/mL) and 2× IC_{50} (40µg/mL) respectively compared to the control, indicating a significant inhibition of this protein, which normally inhibits apoptosis and promotes cell survival (Table 4, Figure 5).

The RT qPCR results demonstrated that SSL-CH extract significantly unregulated the levels of proapoptotic genes such as *p^{53}* and *Bax* while down regulating the levels of antiapoptotic genes *Bcl-2* and *survivin*. These findings have shown that SSL CH extract induced apoptosis in HeLa cell lines more efficiently.

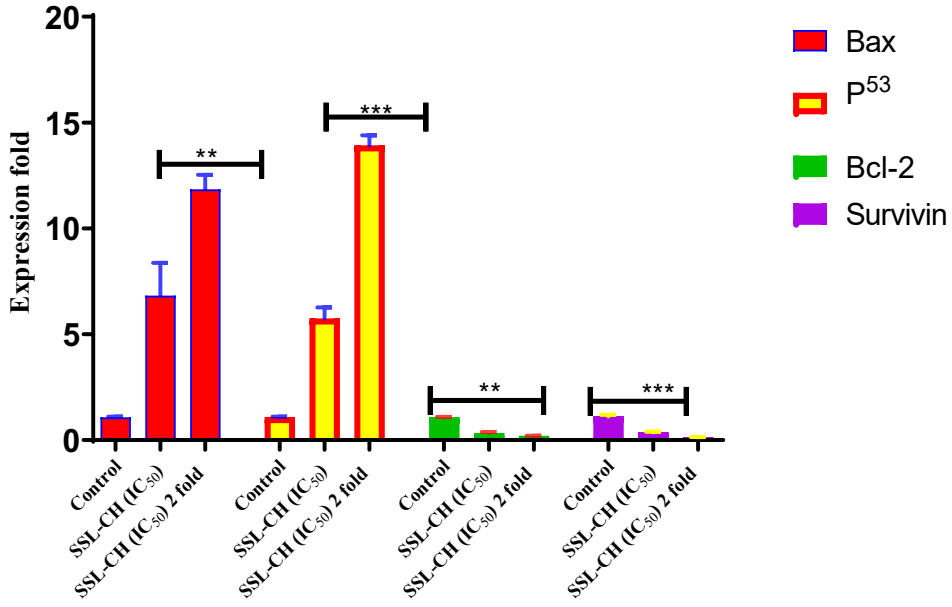

Figure 5. Apoptotic effect of *S. singueana* Chloroform Leaf Extract (SSL-CH) at IC_{50} (20µg/mL) and 2 fold IC_{50} (40µg/mL) on m-RNA gene expression analysis of proapototic (Bax & P^{53}) and anti apoptotic (Bcl-2 & Survivin) in HeLa cell lines incubated for 24 hrs and recorded using quantitative real time qPCR method.

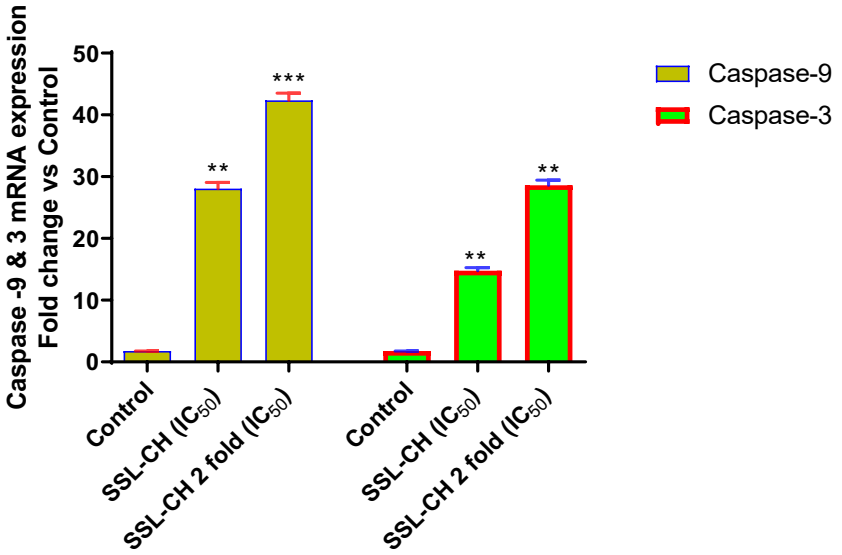

Figure 6. Apoptotic Effect of Chloroform Leaf Extract of *S. singueana* (SSL-CH) at IC_{50} (20µg/mL) and 2 fold (40µg/mL) on m-RNA gene expression analysis of caspase 9 & 3 in HeLa cell lines incubated for 24 hrs and recorded using quantitative real time qPCR methods.

Effect of SSL-CH extract on the modulation of caspase-9 and 3 gene expression by Real time qPCR

As shown in Figure 6 *caspase-9* gene expression increased steadily in comparison to the control. The RT-qPCR analysis revealed enhanced levels of caspase -9 (26.3folds at IC_{50} (20 µg/mL) of SSL-CH (**p≤0.01) and (40.62 folds) at 2x IC_{50} (40 µg/mL) (***p≤0.001). In the same way, there was a significant up regulation of caspase-3 mRNA expression, with increases of 13.02-foldat IC_{50} (20 µg/mL) (**p≤0.01) and 26.84-fold at 2x IC_{50} (40µg/mL) (**p≤0.01) in comparison to control.

Our findings are agreed with Castro et al. [27] stated, ethanolic leaf, extract of Senna velutina induced cell death in melanoma cancer cell lines B16F10-Nex2 by activating caspase -3 intrinsic apoptotic pathways. Overall, SSL-CH extract is an effective inducer of apoptosis via the intrinsic pathway, making it a promising candidate for further investigation as a potent anti cervical cancer agent.

Discussion

This current study showed chloroform leaf extract of *S. singueana* (SSL-CH) was abundant in high number of alkaloids, phenolic compounds, flavonoids, tannins, and glycosides which have concentration-dependent selective cytotoxic effects against HeLa cells by induction of apoptosis. SSL-CH is a potent pro-apoptotic agent, inducing prominent apoptotic morphological changes at 2× IC_{50} as well as strong late apoptosis at 4× IC_{50} as evidenced by AO/EB dual staining of the total cells (49.42%, $P < 0.01$). Furthermore, the extract increased the expression of p^{53} and *Bax*, by decreasing the expression of Bcl-2 and Survivin, and activating caspase-9 and caspase-3 through the intrinsic apoptotic mechanisms. SSL-CH extract could be a potential candidature for development of novel anticervical cancer agent.

Author Contribution Statement

Zenebe Teka, Berihu Tekluu and Tekleweyni Tadege were conducting experimental part in Department of Biology and Chemistry and acquisition of all the data. Neelima P, Kamalakararao K and Naveen Kumar A.D were involved in collection of review of literature, writing manuscript and editing. Krishna Chaithanya K performed stastically analysis and finally organizing manuscript according to the submission guidelines of Journal. All authors have read reviewed and verified final version of the manuscript.

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Data availability statement

All data that support the finding of this study are included within the article.

Conflict of interest

The authors have no conflict of interest.

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