



ORIGINAL RESEARCH ARTICLE

Bioactive principles of *Steganotaenia araliacea* extracts and their anti-proliferation activities against cervical cancer cells

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Abstract

Cervical cancer prevalence and death cases in sub-Sahara Africa are high. Despite advances in medicine, cancer treatment remains difficult because the disease is highly complex, current chemotherapy lacks selectivity, side effects are often severe and overall treatment costs are high. There is urgent need for alternative anticancer drugs. Traditional medicine, which makes extensive use of natural remedies, is regarded as reliable, however, it is still insufficiently studied and the mechanisms underlying the potent activity of specific metabolites have not been scientifically explained. In this study, we evaluated bioactive principles of *Steganotaenia araliacea* (anti-cancer remedy in Kenya) on anti-proliferation activity of Cervical Cancer Cells (CCCs) using MTT assays. We hypothesized that organic extracts of *S. araliacea* stem bark contains compounds that inhibit sporadic growth cervical cancer cells. Further, all crude extracts of *S. araliacea* recorded some antioxidant activity against normal cells. The sample was sequentially extracted using hexane, dichloromethane, ethyl acetate and 50:50 dichloromethane-methanol, followed by anti-proliferation evaluations against CCCs. Qualitative analysis indicated presence of saponins, tannins, flavonoids, phenols, alkaloids and steroids. The most active crude at all concentrations (50:50 dichloromethane-methanol extract) was subjected to column chromatographic separations. Three pure compounds (α -amyrin, β -sitosterol and stigmasterol) characterized by GC-MS, FTIR and NMR were isolated from this extract and anti-proliferation activity evaluated against CCCs. β -sitosterol had significantly ($p < 0.05$) higher anti-proliferation activity against CCCs similar to that of Tamoxifen (anti-cancer drug). This study lays a solid background in identification of lead compounds towards development of a novel cervical cancer drugs. The reported extracts can also be used as herbal remedies for cervical cancer among the local communities.

Key words: Anticancer, Cervical cancer, *Steganotaenia araliacea*, anti-proliferation, Terpenoids

1.0 Introduction

Cancer arises from the transformation of normal cells into tumor cells in a multi-stage process that generally progresses from a pre-cancerous lesion to a malignant tumor. Cancer is one of the leading causes of death, accounting for nearly 10 million deaths in 2020 globally (Ferlay et al., 2020). The five most common cancers in Kenya are in the order breast > cervical > prostate > esophageal > colorectal (Ng'ang'a et al., 2018). Most cancer patients frequently face significant delays in diagnosis due to shortcomings in healthcare systems, including a lack of oncologists and many patients are diagnosed at advanced stages, severely limiting their chances of survival (Gatobu et al., 2025). Cervical cancer develops at the periphery of the cervix and the uterus. The major risk factor is the infectious Human Papilloma Virus (HPV), which is sexually transmitted (Kimani et al., 2012). The most common treatments of cancer are surgery, chemotherapy, and radiation. However, these alternatives have serious drawbacks such as being non-selective, nerve problems, skin and nail changes, blood clots, damage to nearby tissues, drug reactions, damage to other organs and loss of immunity among many others (Aguko et al., 2024). With these challenges, there is need of attempting to understand which complementary therapies, including traditional medicines, if they are safe and effective to combine with conventional cancer treatments (Makau-Barasa et al., 2017).

Secondary metabolites from traditionally used medicinal plants can be targeted for the discovery of lead compounds towards drug development and cosmetic applications (Goma and Ezeala, 2017). *Steganotaenia araliacea*, is a small tree whose aqueous extracts has been used by Kitui locals to relief general body pains in cancer patients (Omolo et al., 2014). The *S. araliacea* roots have been reported to treat snakebites and painful chest conditions (Mard et al., 2011). It has also been reported to contain some antioxidant and antibacterial activity (Ojerinde, 2013; Hamambulu, 2021). Anticancer and antidiuretic activities of *S. araliacea* crude extracts have also been reported (Agunu et al., 2005). Different parts of *S. araliacea* plant have been used as remedies for different ailments without giving any chemical composition. We herein report active organic extracts and pure compounds from *S. araliacea* stem bark against Cervical Cancer Cells (CCCs) for the first time. We specifically evaluated i) sequential extracts (hexane, dichloromethane, ethyl acetate and 50:50 mixture of dichloromethane-methanol) of *S. araliacea* crude extracts against CCCs and ii) the anti-proliferation activity of isolated and characterized pure compounds against CCCs.

2.0 Materials and methods

2.1 Plant materials

S. araliacea plant in its natural habitat in Kaliku, Kitui County was identified by a plant taxonomist from KEFRI. The stem bark of the plant was harvested with a sterile stainless-steel knife and kept in airtight container for transportation to KEFRI Natural Products Laboratory. Aerial parts of the plant were also collected, taken to the Department of East Africa Herbarium, in the National Museum of Kenya (NMK) where the plant was authenticated by a taxonomist, deposited in their herbarium and assigned a voucher specimen number (NMK/BOT/2022/SA-007). The collected



samples (*S. araliacea* stem barks) were air dried, powdered, weighed and stored in air tight containers awaiting extraction.

2.2 Extraction

A 500 g sample of plant material was subjected to sequential extraction using organic solvents of increasing polarity, including n-Hexane, dichloromethane (DCM), ethyl acetate, and a 1:1 mixture of dichloromethane-methanol. Each extraction was performed for 48 hours to extract phytochemicals. The solvents were subsequently removed by distillation, and the 1:1 dichloromethane-methanol extract, which exhibited significant anti-proliferative activity against cervical cancer cell lines, was concentrated and dried under reduced pressure, yielding 9.6 g of a yellow-brown residue. The resulting crude extract was further analyzed for its bioactive components.

2.3 Qualitative phytochemical screening

Basic phytochemical screening analysis for presence of active compounds like saponins, tannins, flavonoids, phenols, alkaloids, steroids and anthraquinones was carried out on sequentially extracted stem extract using standard procedure (Shaikh and Patil, 2020).

2.4 Column chromatography

A separating column of 60 cm by 6cm in length and internal diameter respectively was packed with Kieselgel silica gel 60G (70-230 mesh) in hexane. 9.6g of dichloromethane–methanol crude extract which showed highest anti-proliferation activity on cervical cancer cell lines was packed on top of the stationary phase and the column run using hexane and ethyl acetate by gradient elution technique. TLC was used to monitor the eluates. A total of 130 fractions were collected and fractions with similar TLC profiles were pooled together and solvent distilled off under reduced pressure. This afforded two fractions **A** and **B**. Fraction **A** yielded a single spot when subjected to TLC using different solvent systems and it showed to be a homogenous compound **R1** as a white amorphous solid, soluble in dichloromethane, acetone and ethyl acetate. Further, its melting point was 178°C. Fraction **B** yielded two spots on the TLC and it was purified using a smaller column containing sephadex LH-20 where methanol was used for elution. These afforded two distinct compounds **R2** and **R3** as single spots in TLC that were homogeneous compounds. Compound **R2** was a white powder, soluble in dichloromethane, ethyl acetate and ethanol. Its melting point was 137°C. Compound **R3** was a white waxy powder coupled with a characteristic odor, soluble in ethanol, dimethyl ether and DMSO with a melting point of 162°C. **R1**, **R2** and **R3** were further subjected to IR, GC-MS and NMR to ascertain their chemical structures.

2.5 GC - MS conditions

One microliter (1µl) of the isolates was injected into the GC using an auto sampler. The column used for separations was DB-5MS (30 m length, 0.25 mm internal diameter, and 0.25 µm in

thickness - Agilent Technologies, JW Scientific Products, USA). Ultrapure helium was used as the carrier gas at a flow rate of 1.0 mL/min. Injection temperature was set to 280° C. Split Injection mode was used at a ratio of 5:1. The oven temperature program was set as follows: 50° (1min); 15° C/min to 120° C (1min); 3° C/min to 280° C (4 minutes). Total run time was 64.00 minutes. The samples were analysed in full scan mode in the range 50 – 600 m/z. Solvent cut – time was set at 3.5 minutes. Ion source temperature was set at 200 ° C and interface temperature was set at 250° C. Mass spectra of eluted compounds were matched against the NIST-17 Library for identification.

2.6 Spectroscopic characterization

Different spectroscopic methods were used to elucidate structures of the isolated compounds. The spectroscopic techniques used were infra-red spectrum and GC-MS. Infra-red was recorded using Agilent 5500 compact FTIR spectrometer and GC-MS was recorded at high resolution on a mass spectrometer (Agilent 5977C GC/MSD) at the National Phytotherapeutics Research Centre – Kenyatta University and data given in m/z values. NMR analyses were performed on a Bruker AVIII (500 MHz) spectrophotometer with CDCl₃ as the solvent and TMS as the internal standard. JEOL M Station JMS-700 mass spectrometer was used to obtain the mass spectral data.

2.7 Antioxidant activity

The DPPH radical scavenging activity of the compounds was determined using a standard method (Kumawat et al., 2012) with Vitamin C (ascorbic acid) as the positive control. 0.1 mM DPPH solution in methanol was prepared and mixed with 1 mL of hexane, dichloromethane, ethyl acetate and methanol-dichloromethane extracts (at 6.25, 12.5, 25, 50, 100µgmL⁻¹ concentrations) with 2 mL of DPPH solution. The mixtures were incubated in the dark at room temperature for 30 minutes. Further, the absorbance was measured at 517 nm using a UV-Vis spectrophotometer and the percentage of DPPH radical scavenging activity was calculated using:

$$\%Inhibition = \frac{A_{control} - A_{sample}}{A_{control}} \times 100$$

Where:

A_{control} – Absorbance of DPPH without sample

A_{sample} – Absorbance of DPPH with antioxidant sample

2.8 Cell Culture and treatment

The cervical cancer cell lines used was HeLa (HPV-18 positive) and supplemented with 10% Fetal Bovine Serum (FBS) (for cell growth), 1% Penicillin-Streptomycin (100 U/mL penicillin, 100 µg/mL streptomycin) (to prevent contamination) and 1% L-Glutamine to enhance cell metabolism. Further, they were incubated at 37°C in a humidified incubator with 5% CO₂. The cells were harvested using 0.25% Trypsin-EDTA, re-suspend in fresh medium and counted using an

automated cell counter. The cell density was determined using trypan blue exclusion assay (Patel et al., 2009). The cells were seeded independently in 96 well plate at 2×10^4 cells per well at a volume of 100 μ l and incubated at 5% CO₂ humidified incubator at 37°C for 24 hours. The treatment was done by adding 50 μ l of crude extracts and isolated compounds of *S. araliacea* at 7 concentrations namely 1000, 333.33, 111.11, 37.04, 12.35, 4.12, 1.37 μ g mL⁻¹ and the last row of the 96 well plates were left untreated. They were then incubated in 5% CO₂ humidified environment at 37°C for 48 h after which the MTT viability assay was carried out.

2.9 Viability MTT test

This is a colorimetric assay based on enzymatic activity of mitochondria succinate dehydrogenase enzymes in living cells to reduce the yellow water-soluble substrate 3 - (4,5-dimethyl thiazol2-yl) - 2, 5-diphenyl tetrazolium bromide (MTT) into formazan. A method by (Mosmann, 1983) was adopted whereby the viability test was done by the tetrazolium 3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyltetra-zolium bromide (MTT) dye reduction assay. After 48 hours of incubation, the culture medium in plates was discarded and wells washed with phosphate buffered saline (PBS). A volume of 10 μ l of PBS solution containing 10 μ g/mL of MTT was pipetted into each well and the plates incubated for 3 hours. A volume of 10 μ L of Pure DMSO was added and incubated for 30 minutes. The plates were mildly shaken at room temperature and the Optical density (OD) determined at 560 nm using a microplate reader spectrophotometer.

2.10 Determination of anti-proliferation activity

The proliferation of the cells after treatment was calculated using a formula developed by (Awasure, 2012; Patel, 2009).

$$\text{Proliferation rate} = \frac{At - Ab}{Ac - Ab}$$

Where At, Ab and Ac represents absorbance of test compound, absorbance of blank and absorbance of negative control (cell plus media), respectively.

2.11 Data analysis and presentation

Anti-proliferation data were rank transformed, subjected Analysis of Variance (ANOVA) and the means separated by Student Newman-Keuls' post-hoc analysis test using SPSS version 20 software. Qualitative data generated from phytochemical screening of the plant extract were also tabulated. Interpretations of mass spectrum in GC-MS were concluded by using database of National Institute Standard and Technology (NIST).

3.0 Results

3.1 Qualitative phytochemical screening

The qualitative phytochemical analyses of hexane, dichloromethane, ethyl acetate and DCM - methanol sequential extracts of *S. araliacea* indicated presence of tannins, saponins, flavonoids, steroids, phenols, steroids and alkaloids in all the extracts as shown in Table 1. However, anthraquinones were absent in all the sequential extracts of *S. araliacea* stem bark.

Table 1: Phytochemicals screening of S. araliacea stem bark extracts

Phytochemical	Hexane	DCM	EtOAc	DCM-MeOH
Saponins	+	+	+	+
Tannins	+	+	+	+
Flavonoids	+	+	+	+
Phenols	+	+	+	+
Alkaloids	+	+	+	+
Steroids	+	+	+	+
Anthraquinones	-	-	-	-

DCM: dichloromethane; EtOAc: ethyl acetate; DCM-MeOH: 50:50 mixture of dichloromethane and methanol; (+) = positive result; (-) = negative result

3.2 Radical scavenging activity

The results of radical scavenging activity of hexane, dichloromethane, ethyl acetate and dichloromethane - methanol extracts of *S. araliacea* against normal cells are summarized in Table 2. Generally, all the crude extracts of *S. araliacea* recorded some radical scavenging activity against normal cells as indicated by mean values > 6.4% (Table 2). The dichloromethane-methanol extract showed significantly ($p < 0.05$) higher mean radical scavenging activity than hexane, dichloromethane or ethyl acetate extracts (Table 2). For instance, at $12.5 \mu\text{g mL}^{-1}$, the mean radical scavenging activity against normal cells of hexane, dichloromethane, ethyl acetate and dichloromethane - methanol extracts were 21.2%, 40.1%, 23.4% and 48.1% respectively (Table 2).

The mean radical scavenging activity of *S. araliacea* dichloromethane - methanol extract against normal cells was significantly ($p < 0.05$) lower than that of ascorbic acid (positive control) – a known antioxidant in most concentrations (Table 2). It was noted that at $12.5 \mu\text{g mL}^{-1}$, *S. araliacea* dichloromethane - methanol extract (48.1%) had significantly higher ($p < 0.05$) mean radical scavenging activity against normal cells than ascorbic acid which had 38.8% (Table 2). The mean radical scavenging of the extracts of *S. araliacea* (hexane, dichloromethane, ethyl acetate and methanol) and ascorbic acid significantly ($p < 0.05$) increased with increase in their concentrations (Table 2).

Table 2: Mean percentage radical scavenging activity of *S. araliacea* crude extracts at varying concentrations against normal cells

Extracts	Radical Scavenging Activity (%)				
	6.25µgmL ⁻¹	12.5 µgmL ⁻¹	25.0 µgmL ⁻¹	50.0 µgmL ⁻¹	100.0 µgmL ⁻¹
n-Hexane	6.4±0.0 ^{Aa}	21.2±0.0 ^{Ba}	39.1±0.0 ^{Cb}	50.2±0.0 ^{Db}	57.4±0.0 ^{Eb}
DCM	23.6±0.0 ^{Ac}	40.1±0.0 ^{Bc}	44.2±0.0 ^{Cc}	54.2±0.0 ^{Dc}	61.4±0.0 ^{Ec}
EtOAc	15.4±0.0 ^{Ab}	23.4±0.1 ^{Bb}	31.2±0.0 ^{Ca}	36.2±0.0 ^{Da}	40.4±0.0 ^{Ea}
DCM-MeOH	26.3±0.0 ^{Ad}	48.1±0.0 ^{Bd}	58.8±0.0 ^{Cd}	74.8±0.0 ^{Dd}	80.5±0.0 ^{Ed}
Ascorbic acid [#]	29.1±0.1 ^{Ae}	38.8±0.0 ^{Bc}	93.1±0.0 ^{Ce}	96.7±0.0 ^{De}	98.5±0.1 ^{De}

DCM:dichloromethane; EtOAc: ethyl acetate; MeOH: methanol; DCM-MeOH:50-50 mixture of dichloromethane and methanol #known anti-oxidant (positive control); Means followed by the same capital and small letters in a row and column, respectively are not significantly different ($p>0.05$, ANOVA, SNK post-hoc analyses)

3.3 Anti-proliferation/inhibition activity of cervical cancer cells lines on treatment with *S. araliacea* extracts

The anti-proliferation activity of cervical cancer cells lines on treatment with *S. araliacea* organic extracts at varying concentrations was evaluated and the results summarized in Figure 1. It was noted, the entire stem bark organic extracts of *S. araliacea* significantly ($p<0.05$) inhibited proliferation rate of cervical cancer cells. At lower *S. araliacea* extract concentrations, the mean cancer cell inhibition significantly ($p<0.05$) increased with increase in concentration (Figure 1). At lower concentrations, the mean cancer cell inhibition on treatment with *S. araliacea* extract was concentration dependent. An optimal concentration for all the extracts (dichloromethane-methanol, dichloromethane, Ethyl acetate and n-Hexane) was 111.11µg/mL. The order of anti-proliferation activity against cervical cancer cells was dichloromethane-methanol > dichloromethane > ethyl acetate > n-Hexane (Figure 1).

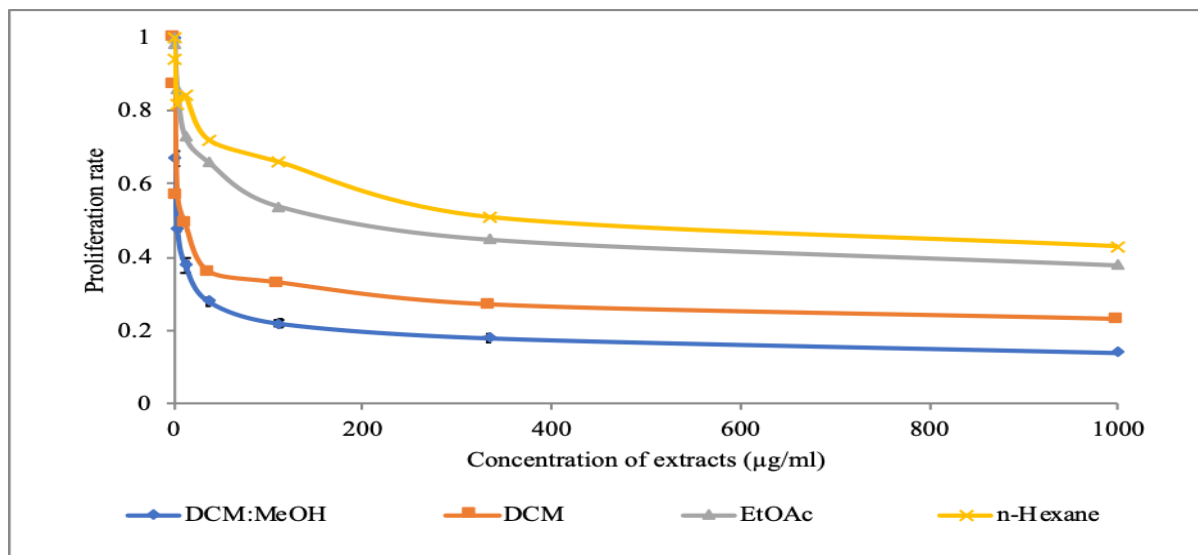


Figure 1: Anti-proliferation Activity of Cervical Cancer Cells Lines on treatment with *S. araliacea* extracts at various concentrations DCM:MeOH; EtOAc: ethyl acetate; MeOH: methanol: DCM:MeOH:50-50 mixture of dichloromethane and methanol

3.4 Anti-proliferation activity of normal cell lines on treatment with *S. araliacea* extracts

The anti-proliferation activity of normal cell lines on treatment with *S. araliacea* organic extracts at varying concentrations was evaluated and the results summarized in Table 3. Generally, there was a significant ($p < 0.05$) reduction in proliferation rate of normal cells on treatment with either dichloromethane-methanol, dichloromethane, EtOAc or n-Hexane extracts as compared to untreated cells (Table 3). For instance, the mean percentage anti-proliferation activity of 1.37 and 4.12 µg/mL of *S. araliacea* dichloromethane-methanol extract was 10 and 15, respectively (Table 3). The highest cell viability was observed in untreated normal cells and lowest at 333.33 µg/mL which was not significantly different ($p > 0.05$) from that of 1000 µg/mL for each extract (Table 3). The mean anti-proliferation activity of normal cells on treatment with the most active anticancer dichloromethane-methanol extract was not significantly ($p > 0.05$) different from that of Tamoxifen (positive control – a known anticancer drug).

Table 3: Proliferation Activity of Normal Cell Lines on treatment with *S. araliacea* extracts at various concentrations

Concentration (µg/ML)	n-Hexane	DCM	EtOAc	DCM-MeOH	ϕControl
0.00	1.00±0.23 ^h	1.00±0.01 ^e	1.00±0.03 ^f	1.00±0.02 ^f	1.00±0.02 ^d
1.37	0.94±0.42 ^g	0.91±0.00 ^d	0.90±0.01 ^e	0.90±0.01 ^e	0.93±0.00 ^c
4.12	0.90±0.00 ^f	0.84±0.01 ^c	0.85±0.02 ^d	0.85±0.03 ^d	0.91±0.01 ^c
12.35	0.84±0.02 ^e	0.83±0.00 ^{bc}	0.80±0.02 ^c	0.82±0.01 ^{bcd}	0.84±0.02 ^b
37.04	0.82±0.03 ^{cde}	0.80±0.03 ^b	0.79±0.04 ^c	0.81±0.02 ^{bc}	0.82±0.00 ^{ab}
111.11	0.80±0.01 ^{bcd}	0.80±0.02 ^b	0.75±0.01 ^b	0.80±0.00 ^{ab}	0.82±0.01 ^{ab}
333.33	0.77±0.00 ^b	0.78±0.00 ^{ab}	0.71±0.12 ^a	0.79±0.02 ^a	0.81±0.03 ^a
1000.00	0.73±0.02 ^a	0.77±0.01 ^a	0.69±0.01 ^a	0.78±0.00 ^a	0.80±0.02 ^a

DCM: dichloromethane; EtOAc: ethyl acetate; MeOH: methanol; DCM-MeOH: 50-50 mixture of dichloromethane and methanol; ϕTamoxifen - reference drug (positive control); Mean values followed by the same superscript

3.5 Spectroscopic characterization

3.5.1 GC-MS fragmentation

HR-MS spectrum of compound **R1** gave an [EI (+)]: m/z ratio for this compound as 426.0 while the m/z ratio calculated for molecular ion, $C_{30}H_{50}O$ [M^+] was 425.8 which corresponded to (3S,4aR,6aR,6bS,8aR,11R,12S,12aR,14aR,14bR)-4,4,6a,6b,8a,11,12,14b-octamethyl-2,3,4a,5,6,7,8,9,10,11,12,12a,14,14a-tetradecahydro-1H-picen-3-ol, the IUPAC name of α -amyrin. HR-MS spectrum of compound **R2** gave an [EI (+)]: m/z ratio for this compound as 414.0 while the m/z ratio calculated for molecular ion, $C_{29}H_{50}O$ [M^+] was 414.71 which corresponded to 17-(5-Ethyl-6-methylheptan-2-yl)-10,13-dimethyl-2,3,4,7,8,9,11,12,14,15,16,17-dodecahydro-1H-cyclopenta[a]phenanthren-3-ol, the IUPAC name of β -sitosterol. HR-MS spectrum of compound **R3** gave an [EI (+)]: m/z ratio for this compound as 412.0 while the m/z ratio calculated for molecular ion, $C_{29}H_{48}O$ [M^+] was 412 which corresponded to (22 E)-stigmasta-5,22-dien-3 β -ol, the IUPAC name of stigmasterol.

3.5.2 FT-IR spectroscopic analysis

The IR spectrum of compound **R1** showed a characteristic intense absorption broad band attributed to O-H stretching ($3450 - 3600\text{cm}^{-1}$), intense $-\text{CH}_3$ stretches and vibrations (2937cm^{-1}), intense $-\text{CH}_3$ stretches and vibrations (2921cm^{-1}), asymmetric ethylenic double bond (1739cm^{-1}) and $\text{C}=\text{C}$ vibrations (1463cm^{-1}). The IR spectrum of compound **R2** showed characteristic absorptions peaks at 3440.38 , 2946.82 , 1596.70 , 1400.38 , 1219.52 and 1067.33cm^{-1} attributed to O-H stretching, C-H stretch of CH_3 , $\text{C}=\text{C}$ stretch, cyclic methylene group vibrations, C-O stretch and C-OH vibration of secondary alcohols, respectively. The IR spectrum compound **R3** showed a characteristic intense absorption broad band attributed to O-H stretching vibrations ($3450 - 3600\text{cm}^{-1}$), intense SP^2 C-H stretching at (3063cm^{-1}), symmetric and asymmetric stretching

vibrations 2921cm^{-1} and 2855.1cm^{-1} respectively. Unsymmetrical ethylene double bond (1639cm^{-1}) and C=C vibrations (1453cm^{-1}).

3.5.3 ^1H NMR spectra Analysis

Compound **R1** ^1H NMR analysis exhibited characteristic peaks at 5.15 ppm (1H, H-12) and 4.52 ppm (1H, m, H-3) and other signals corresponding to eight methyl groups (H-23 to H-30) at 0.85 ppm (1H, d, H-30), 0.96 ppm (3H, s, H-26), 0.88 ppm (3H, s, H-23), 1.00 ppm (3H, s, H-25), 0.83 ppm (3H, d, H-29), 1.05 ppm (3H, s, H-27), 0.79 ppm (3H, s, H-28), and 0.82 ppm (3H, s, H-24). These spectral characteristics aligned with established features of triterpenoid amyrins. The presence of a doublet at H-18 suggests a single proton at H-19, which couples with H-18, leading to the observed splitting pattern. This implied that the remaining position on C-19 is occupied by a methyl group (CH_3), forming a vicinal dimethyl arrangement at H-19 and H-20. Collectively, these findings supported the identification of the isolated compound as α -amyrin. The ^1H -NMR data aligns with that of α -amyrin isolated from *Hoya multiflora* (Ebajo et al., 2015).

The ^1H NMR spectrum of **R2** exhibited a deshielded singlet at 5.30 ppm corresponding to the olefinic proton (H-6) in the steroid nucleus. Multiplets observed in the range of 3.50–3.60 ppm is attributed to the C-3 hydroxyl methine proton. The aliphatic region displays multiple signals between 0.68–1.20 ppm, corresponding to methyl protons at C-18, C-19, C-21, C-26, and C-27. The presence of a characteristic doublet at 0.83 ppm ($J \approx 6.5$ Hz) indicated the presence of the ethyl group at C-24, distinguishing β -sitosterol from stigmasterol. These spectral features confirmed the sterol framework and the structural integrity of β -sitosterol (Pierre and Moses, 2020). Compound R2 had a characteristic singlet at 5.35 ppm corresponding to the olefinic proton (H-6) within the steroid nucleus. The presence of two additional vinylic protons at 5.10 and 5.00 ppm indicated the C-22 and C-23 olefinic protons hence distinguishing stigmasterol from β -sitosterol. A multiplet at 3.52 ppm is assigned to the C-3 hydroxyl methine proton. The aliphatic region (0.68–1.20 ppm) displayed multiple signals from methyl groups at C-18, C-19, C-21, C-26, and C-27. These spectral features are consistent with the structural identity of stigmasterol isolated from *Commiphora africana* resin (Wairagu et al., 2022).

3.6 Anti-proliferation/Inhibition activity of cervical cancer cells lines on treatment with pure Isolates of *S. araliacea* dichloromethane-methanol extract

The anti-proliferation activity of cervical cancer cells lines on treatment with pure isolates of *S. araliacea* dichloromethane-methanol extract at varying concentrations was evaluated and the results summarized in Figure 2. Generally, all the pure isolates from *S. araliacea* dichloromethane-methanol extract significantly ($p < 0.05$) inhibited proliferation rate of cervical cancer cells as compared to untreated cervical cancer cells (Table 4). There was an observed high mean proliferation rate on untreated cervical cancer cells. In each concentration of the isolates evaluated, the mean inhibition activity β -sitosterol to cervical cancer cells was significantly ($p < 0.05$) higher than that of α -amyrin and stigmasterol (Figure 2). The order of anti-proliferation

activity of the isolates against cervical cancer cells was β -sitosterol > α -amyirin > stigmasterol (Figure 2).

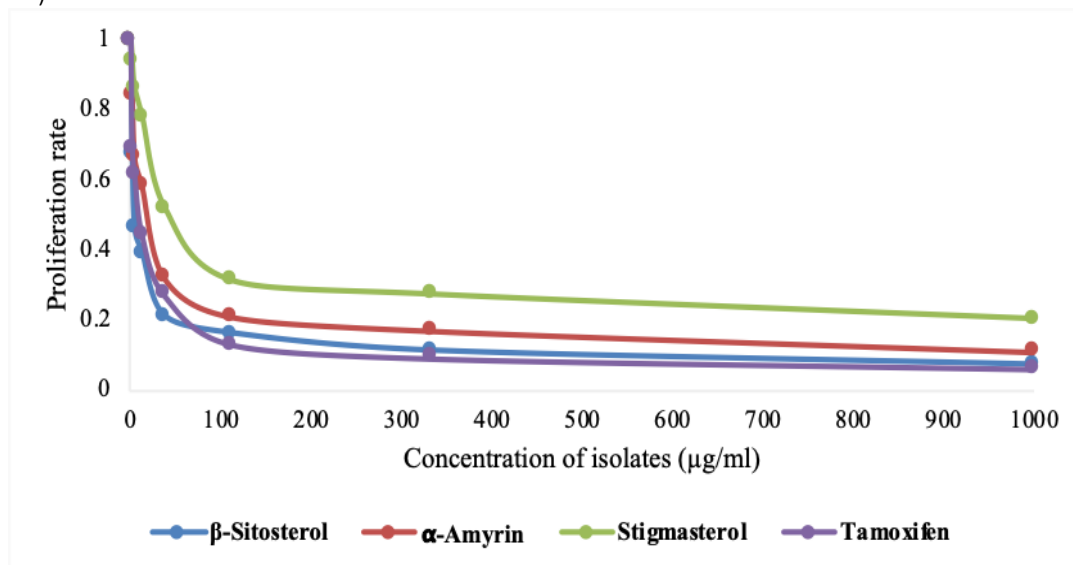


Figure 2: Anti-proliferation Activity of Cervical Cancer Cells Lines on treatment with pure isolates

3.7 Anti-proliferation activity of normal cell lines on treatment with *S. araliacea* isolates

The anti-proliferation activity of normal cell lines on treatment with pure isolates of *S. araliacea* dichloromethane-methanol extract at varying concentrations in water was evaluated and the results summarized in Table 4. There was a significant ($p < 0.05$) reduction in proliferation rate of normal cells on treatment with either β -sitosterol, α -amyirin, stigmasterol or Tamoxifen as compared to untreated cells (Table 4). The pure isolates demonstrated a dose-dependent reduction in proliferation of normal cells (Table 4). For instance, the mean percentage anti-proliferation activity of 1.37 and 4.12 $\mu\text{g/mL}$ of β -sitosterol was 6 and 7, respectively (Table 4).

On treatment with β -sitosterol, the highest cell viability was observed in untreated normal cells and lowest at 12.35 $\mu\text{g/mL}$ which was not significantly ($p > 0.05$) different from that at 37.04 $\mu\text{g/mL}$ (Table 4). The mean anti-proliferation activity of normal cells on treatment with the most active anticancer isolate (β -sitosterol) was not significantly ($p > 0.05$) different from that of Tamoxifen (positive control – a known anticancer drug) (Table 4). For instance, at 4.12 $\mu\text{g/mL}$ of β -sitosterol and Tamoxifen, their mean anti-proliferation activity was 9%.

Table 4: Anti-proliferation Activity of Normal Cells Lines on treatment with pure isolates of *S. araliacea* at various concentrations

Concentration (µg/mL)	α-Amyrin	β-sitosterol	Stigmasterol	ϕControl
0.00	1.00±0.01 ^f	1.00±0.02 ^c	1.00±0.02 ^f	1.00±0.02 ^d
1.37	0.97±0.02 ^e	0.94±0.03 ^b	0.94±0.01 ^e	0.93±0.00 ^c
4.12	0.95±0.03 ^{de}	0.93±0.01 ^b	0.91±0.00 ^{de}	0.91±0.01 ^c
12.35	0.94±0.02 ^d	0.93±0.03 ^b	0.88±0.02 ^d	0.84±0.02 ^b
37.04	0.78±0.01 ^c	0.92±0.02 ^{ab}	0.82±0.01 ^c	0.82±0.00 ^{ab}
111.11	0.76±0.01 ^c	0.91±0.03 ^a	0.77±0.02 ^b	0.82±0.01 ^{ab}
333.33	0.70 ±0.02 ^b	0.91±0.02 ^a	0.72±0.03 ^b	0.81±0.03 ^a
1000.00	0.64±0.01 ^a	0.89±0.01 ^a	0.69±0.01 ^a	0.80±0.02 ^a

ϕTamoxifen - reference drug (positive control); Mean values followed by the same superscript letter are not significantly different ($p>0.05$; one-way ANOVA, SNK post-hoc analyses)

4.0 Discussion

Spectroscopic identification of phytoconstituents in *S. araliacea* helps to predict the potential pharmacological activity (Emran et al., 2015). Crude extracts and fractions have broad-spectrum activity due to presence of different classes of active compounds as observed in *S. araliacea*. The high radical scavenging activity of *S. araliacea* dichloromethane-methanol extract is as a result of the presence of mid-polar and polar compounds such as tannins, flavonoids and terpenoids. This activity indicated that they can effectively counteract oxidative stress while also engaging multiple anticancer mechanisms such as disrupting their redox balance and apoptosis, processes known to inhibit cancer development and progression. This high activity was similar to the one observed by (Ramzi and Hosseininaveh, 2010) where radical scavenging against normal cells of methanolic extract of *Acalypha fruticosa* was observed to increase with concentration. High anti-proliferation activity of *S. araliacea* dichloromethane-methanol extract against cervical cancer cells was also observed which was attributed to the presence of mid-polar and polar compounds in the extract. Similarly, (Anal et al., 2014) reported dichloromethane-methanol extract of *Potentilla fulgens* having significant activity against human lung cancer (A-549) cell line. An oleanane-type triterpenoid saponin isolated from the stem bark extract of *Manilkara zapota* demonstrated concentration-dependent inhibition of five cancer cell lines—human leukemia (HL-60), human colon cancer (HT-29), human breast cancer (MCF-7), human skin cancer (A431), and human lung cancer (A549) (Awasare et al., 2012).

These findings highlight the significant potential of *S. araliacea* secondary metabolites as promising leads for anticancer drug development.

The *S. araliacea* dichloromethane-methanol extract had the highest anti-proliferation activity against cervical cancer cells and the polar isolates in the extract were responsible for this strong bioactivity and acceptable toxicity limits at higher concentrations. These observations

necessitated the isolation and characterization of compounds present in dichloromethane-methanol extract.

GC-MS fragmentation pattern and FTIR analyses of compound **R1**, isolated from the dichloromethane-methanol extract of *S. araliacea* had a unique fragmentation and characteristics peaks similar to that of α -amyrin isolated from the leaves of *Celastrus hindsii* (Viet et al., 2021). The IR spectrum of compound **R1** showed absorption bands characteristic of triterpenes similar to that of α -amyrin isolated from the leaves of *Orthosiphon stamineus* (Hossain and Ismail, 2013). Fragmentation pattern and FTIR analyses of compound **R2** isolated from the dichloromethane-methanol extract of *S. araliacea* showed similar fragmentation and absorption peaks comparable β -sitosterol isolated from *Commiphora africana* resin (Wairagu et al., 2022).

Fragmentation pattern from GC-MS and FTIR analyses of compound **R3** isolated from the dichloromethane-methanol extract of *S. araliacea* had similar profile and absorption peaks to that of stigmasterol isolated from the roots of *Combretum hypopolinum* (Muhammad et al., 2021). ^1H NMR analysis of compound **R1** was compared with literature data (Ebajo et al., 2015), confirming its identity. Alpha - amyrin, is a pentacyclic triterpenoid of the ursane type, and it features a double bond between C-12 and C-13 and a hydroxyl group at the 3- β position. It is classified as both a pentacyclic triterpenoid and a secondary alcohol. ^1H -NMR analysis confirmed that stigmasterol is a mixture of β -sitosterol and stigmasterol, with stigmasterol being the major component. The key structural difference between the two is the presence of a $\text{C}_{22} = \text{C}_{23}$ double bond in stigmasterol versus a single bond in β -sitosterol (Pierre et al., 2015).

The mean cancer cell inhibition on treatment with all the pure isolates of dichloromethane-methanol extract of *S. araliacea* (β -sitosterol, α -amyrin and stigmasterol) and Tamoxifen (positive control) were concentration dependent. An optimal concentration for all the isolates were 111.11 $\mu\text{g/mL}$. The high anti-proliferation activity of β -sitosterol against cervical cancer cells was also observed by (Muhammad et al., 2021) in anti-proliferative effects against the most prevalent cancers in women (breast (MCF-7), colon (HCT116) and cervical (HeLa)) in a dose-dependent manner. (Hossain and Ismail, 2013) also reported that β -sitosterol induced significant dose-dependent growth inhibition and suppressed the antigens in human colon cancer cells.

5.0 Conclusions

This study investigated the secondary metabolites of *S. araliacea* and their potential anti-cancer activity on cervical cancer cells. The α -amyrin, β -sitosterol and stigmasterol were isolated from the bark of the plant. The MTT cell viability study of α -amyrin, β -sitosterol and stigmasterol showed β -sitosterol to be the most active of the three compounds against the cervical cancer cell lines. Collectively, these constituents contribute to the overall cytotoxicity and antioxidant properties of the plant, although mechanism of action for the cytotoxic effect of *S. araliacea*



extracts remains unclear and the anti-proliferation effect has not been reported. Detailed studies are therefore needed, to establish the relative contribution of this pathway to cytotoxicity. Our results suggest that *S. araliacea* would be a potential candidate for the development of novel therapeutic agents to induce cell death in human cervical cancer cells. Phytosterols compounds were identified in the dichloromethane-methanol extracts of the bark by GC-MS analysis. This study reveals that the plant is rich in sterols, classes of compounds that are known to be biologically active and this validates its ethno-medicinal use.

6.0 Acknowledgements

6.1 Data availability

All data collected, analyzed and summarized in tables and figures are provided in this article.

6.2 Ethics approvals and consent to participate

The national regulations were followed in the collection and use of the plant materials. No special permit was required for the use of this plant species for use in experiments.

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6.4 Competing Interests

All the authors declare no competing interests.

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