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Antioxidant and antiproliferative activity of Portulaca Oleracea and Azanza Garckeana on cervical cancer

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Abstract

Background: Mortality and incidence rates of cervical cancer are on the rise in Sub-Saharan Africa. Current cervical cancer management strategies are not fully effective as they bring about major side effects and issues such as drug resistance. Indigenous plants have been used in ethnomedicine to treat conditions related to cancer.

Aim: The present study was aimed at evaluating the antioxidant and anti-proliferation potential of extracts derived from *Portulaca oleracea* and *Azanza garckeana* plants.

Methods: Phytochemicals were extracted from all plant parts of the two plants with methanol, ethanol, chloroform, distilled water, isopropanol and hexane. Qualitative phytochemical analysis was conducted. The total phenolic and flavonoid content were estimated, and antioxidant activity was determined using DPPH (2,2-diphenyl-1-picryl hydrazyl) and ABTS (2,2'-azino-bis (3-ethylbenzothiazoline-6- sulfonic acid) assays. The antiproliferative activity of extracts on HeLa cells was analysed with 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT).

Results: *P. oleracea* and *A. garckeana* stem and leaf methanolic extract had high total phenolic content between 14.25 and 16.39 TE mg/100 g and flavonoid content between 41.70 and 52.03 QE mg/100 g. Methanolic and ethanolic extracts displayed anti-proliferative activity towards HeLa cells used in study and the extracts affected cell viability in a dose dependent fashion ranging between 69 and 92% reduction in cell viability at 400 µg/ml extract concentration. The IC_{50} values of extracts ranged between 242.09 and 329.31 µg/mL and 22.78 µg/mL ± 10.37 for cisplatin.

Conclusions: *Portulaca oleracea* and *Azanza garckeana* demonstrate anti-proliferative and antioxidant activity, which indicate the potential of these plants in anticancer drug development.

1. Introduction

Cervical cancer in Botswana has been considered to be the most prevalent cancer in women between the ages of 15–45, with the incidence rates anticipated to be 374 women diagnosed with the disease per year[1].The cancer has also been associated to be the leading cause of high cancer mortality rates in middle and low income countries[2]. Cervical cancer is characterized by the development of tumours around the cervical region in the body. The initiation of cervical cancer has been attributed to factors such as radiation which leads to oxidative stress; the accumulation of reactive oxygen species (ROS) and the Human Papilloma virus(HPV) [3–5]. This accumulation of reactive oxygen species leads to DNA mutations which marks the onset of cervical cancer [3, 5]. Studies have demonstrated that certain natural compounds such as polyphenols, flavanols, carotenoids and steroids have antioxidant properties, making them chemo preventive and chemotherapeutic agents as they scavenge accumulated free radicals in the body [3–4, 6–8].

The *Portulaca oleracea* and *Azanza garckeana* medicinal plants commonly known as the pig weed and African chewing gum respectively are sources of these antioxidants. The *P. oleracea* shrub is characterized by small and round fleshy leaves and red juicy stems[9] whereas *A. garckeana* plant is characterized by creased and large green leaves and yellow flowers[10].

Key studies have indicated that phytochemicals such as phenols and flavonoids from *Portulaca oleracea* and *A. garckeana* can inhibit the proliferation of cervical cancer cells[6, 9, 11–12]and the phytochemicals can be used to develop anticancer drugs replacing conventional methods such as chemotherapy. There is a need to consider alternative cancer management strategies such as the use of phytochemicals derived from *P. oleracea* and *A. garckeana*. Therefore, the present study was conducted to determine the antioxidant and anti-proliferation potential of phytochemicals derived from *Portulaca oleracea* and *A. garckeana* in HeLa cells which are cervical cancer cells.

2. Materials and methods

2.1 Plant collection and extraction

Portulaca oleracea whole plant was collected from Palapye, Khurumela Ward (23°25'11"S 26°44'0"E) and *Azanza garckeana* leaves, stems and roots were carefully collected from Serowe (22° 26' 52"S 26° 44' 46"E). The identity of the plants was verified by the Herbarium Unit of the Botswana National Museum. The leaves, stems and roots of the two plants were separated ,washed and dried for seven days .Extraction was through maceration using a ratio of 20:200 for solvent to extract. Extracts were filtered with a funnel and Whatman filter paper of 0.45 µm to remove insoluble material. The remaining solvent was concentrated at 45°C using a rotary evaporator (Heidolph Laborato 4000 efficient, Bavaria, Germany) to acquire the crude extracts. Extraction yield of the different solvents was determined using the formula;

Extraction yield = $\frac{weight\ of\ the\ extract\ after\ evaporating\ solvent\ and\ drying}{dry\ weight\ of\ the\ sample}$ × 100 [Formula 1]

2.2 Qualitative and quantitative phytochemical analysis

Preliminary phytochemical screening was conducted to qualitatively detect the presence of phenols, flavonoids, saponins, tannins, terpenoids, steroids and cardiac glycosides as according to methods described by Gallo et al. [13–16]. Total phenolic content was determined following revisions of the Folin-Ciocalteu method by Singleton and Rossi[17]. Total flavonoid content of *P. oleracea* and *A. garckeana* extracts was determined following revisions of a protocol by Gallo et al.[16].

All analyses were performed in triplicates. Total phenolic and flavonoid content were calculated using the following equation;

TPC (mg / g) = $\frac{TE\ from\ calibration\ curve \times volume\ \ of\ extract\ solution\ in\ mL}{weight\ of\ extract\ in\ g}$ [Formula 2]

TFC (mg/g) = $\frac{QE\ from\ calibration\ curve\ \ \times volume\ of\ the\ extract\ solution\ in\ mL}{Weight\ \ of\ extract\ in\ g}$ [Formula 3]

2.3 Evaluation of antioxidant activity using DPPH and ABTS assay

The antioxidant activity of *A. garckeana* and *P. oleracea* extracts was evaluated following a protocol by Gallo et al [17]. All analyses were performed in triplicates. The DPPH radical scavenging ability was calculated in terms of percentage inhibition as follows;

% inhibition = $\frac{Absorbance\ of\ control - Absorbance\ of\ test\ sample}{Absorbance\ of\ control}$ [Formula 4]

The Trolox equivalents were obtained through using the equation from the calibration curve (Y = mx + C) as follows;

Trolox equivalent = $\frac{(Absorbance\ of\ control - absorbance\ of\ test\ control) - C}{m} \times\ w$ [Formula 5]

Where w represents weight of dry sample and m is the gradient from the calibration curve.

2.4 Identification of bioactive compounds using GC-MS

Extracts from *P. oleracea* and *A. garckeana* were filtered with a 0.21 nm filter (Millipore Sigma) to obtain clean extracts. The GC-MS procedure was adapted from Samer and Hala et al. [18] with a few modifications.

2.5 Determination of the anti-proliferative activities of *P. oleracea* and *A. garckeana* extracts

2.5.1 Cell culture

HeLa cells were maintained in DMEM (Thermo Fisher Scientific, Grand Island, USA), 10% Foetal Bovine Serum (FBS, Thermo Fisher Scientific, Grand Island, USA), penicillin streptomycin(Pen-Strep) (Thermo Fisher Scientific, Grand Island, USA) and L-glutamine (L-Glut) (Thermo Fisher Scientific, Grand Island, USA) and incubated under 37°C and 5% CO_2 until confluent.

HeLa cells of passage 21 at a seeding density of 1×10^5 cells/mL were plated in T-75 flasks and incubated at 37°C for 24 hrs.

2.5.2 Determination of cell viability using MTT assay

An estimated density of 5000 cells /well was seeded in a 96-well plate (FalconTM, Thermo Fisher scientific, Grand Island, USA). The cells were incubated at 37°C, 5% CO2 for 24 hrs. The MTT viability assay was adapted from Gallo et al. [16].

The percentage cell viability was calculated as follows;

% cell viability = $\frac{Absorbance\ of\ treatments}{Absorbance\ of\ control} \times 100$ [Formula 5]

The analysis was done in triplicates followed by the determination of the minimum inhibitory concentration (IC_{50}).

2.6 Statistical analysis

Data was shown as mean ± standard deviation. For cell culture experiments the analysis was based on both standard deviation and standard error of the mean. Statistical comparisons were evaluated using one way analysis of variance (ANOVA) and Tukey Kramer test for multiple comparisons (SAS-JMP software version 16). Differences were presumed significant at $P < 0.05$.

3. Results

3.1 Qualitative phytochemical analysis

Different phytochemicals were detected in six different extracts of *Portulaca oleracea* and *Azanza garckeana* leaves, stems and roots (Tables 1 and 2). The results indicate more phytochemicals were detected in methanolic extracts when compared to the other extracts and fewer phytochemicals were detected in hexane extracts. This indicated that *P. oleracea* was constituted by mostly polar components. Phenols were detected in most leaf and stem extracts when compared to root extracts. Results show that phytochemicals were detected across various plant parts except for tannins in the leaves and cardiac glycosides in the roots in *Azanza garckeana* (Table 2). In the *Azanza garckeana* stem extracts, flavonoids were the most abundant when compared to other phytochemicals. In conclusion, the phytochemical composition of both *Azanza garckeana* and *Portulaca oleracea* was not very dissimilar.

Table 1
Phytochemicals detected in different extracts of different parts of *Portulaca oleracea*

Plant	Plant part	Phytochemical	Absence/Presence of phytochemical in extract					
Portulaca oleracea	Leaves		Methanol	Ethanol	Isopropanol	Distilled water	Chloroform	Hexane
		Phenols	+	+	+	+	+	-
		Saponins	+	+	+	+	-	-
		Flavonoids	+	+	+	+	-	-
		Tannins	+	+	-	-	-	+
		Terpenoids	+	-	+	-	-	-
		Steroids	+	+	+	-	-	-
		Cardiac glycosides	+	+	+	-	-	-
	Stems	Phenols	+	+	+	+	+	-
		Saponins	+	+	-	-	-	-
		Flavonoids	+	+	+	-	-	-
		Tannins	+	-	-	+	+	-
		Terpenoids	+	+	-	+	-	-
		Steroids	+	-	-	+	-	-
		Cardiac glycosides	+	+	+	+	-	-
	Roots	Phenols	+	+	+	+	-	-
		Saponins	+	+	-	+	-	-
		Flavonoids	+	+	+	-	+	-
		Tannins	+	+	-	+	+	-
		Terpenoids	+	+	-	-	-	-
		Steroids	+	+	-	-	+	+
		Cardiac glycosides	-	-	-	-	+	-

KEY ; **+** indicates presence of phytochemical. **–** indicates absence of phytochemical.

Table 2
Phytochemicals detected in different extracts of different parts of *Azanza garckeana*

Plant	Plant part	Phytochemical	Absence/Presence of phytochemical in extract					
Azanza garckeana	Leaves		Methanol	Ethanol	Isopropanol	Distilled water	Chloroform	Hexane
		Phenols	+	+	+	-	-	-
		Saponins	+	+	-	-	-	-
		Flavonoids	+	+	+	-	+	+
		Tannins	-	-	-	-	-	-
		Terpenoids	+	+	-	-	-	+
		Steroids	+	+	-	-	+	-
		Cardiac glycosides	-	-	+	-	+	-
	Stems	Phenols	+	+	+	-	+	-
		Saponins	+	+	+	-	-	-
		Flavonoids	+	+	+	+	+	-
		Tannins	+	+	-	-	-	-
		Terpenoids	+	-	-	+	-	-
		Steroids	+	+	-	+	+	-
		Cardiac glycosides	-	+	+	-	-	-
	Roots	Phenols	+	+	-	+	-	-
		Saponins	+	-	+	+	-	-
		Flavonoids	+	+	+	-	-	-
		Tannins	+	+	-	-	-	+
		Terpenoids	+	-	+	-	+	-
		Steroids	+	-	+	-	+	-
		Cardiac glycosides	-	-	-	-	-	-

KEY ; **+** indicates presence of phytochemical. **–** indicates absence of phytochemical.

3.2 Quantitative phytochemical analysis

3.2.1 Total phenolic and flavonoid content of different extracts from *P. oleracea* and *A. garckeana* leaves, stems and roots

Total phenolic content (TPC) was determined from the Trolox standard curve ($y = 0.001x + 0.0897$, $R^2 = 0.9508$) and the quercetin standard curve ($y = 0.0009x-0.0086$, $R^2 = 0.9967$) was used to determine the total flavonoid content (TFC). The findings indicated that the total phenolic content of *P. oleracea* leaf and stem methanolic extracts was significantly

greater, at 16.25 ± 0.48 TE mg/100 g and 16.39 ± 0.51 TE mg/100 g, respectively (Fig. 1). The methanolic and ethanolic extracts of *A. garckeana* leaves came next, with corresponding concentrations of 14.00 ± 1.00 TE mg/100 g and 12.21 ± 0.06 TE mg/100 g (Fig. 1).

Results showed that *A. garckeana* leaf methanolic and ethanolic extracts had significantly high TFC of 52.03 ± 2.44 QE mg/100 g and 55.06 ± 0.43 QE mg/100 g when compared to other extracts (Fig. 1). *P. oleracea* stem hexane extracts displayed significantly high TFC of 16.14 ± 0.55 QE mg/ 100 g despite the low polarity of this solvent. Extracts of *P. oleracea* and *A. garckeana* leaves displayed significantly high TFC when compared to the root extracts of these plants across all extraction solvents. Most extracts showed statistical difference from each other except stem and root extracts across chloroform and hexane. Furthermore, the data show that while root extracts had a considerably lower TPC across all solvents, leaf extracts had a higher TPC across all solvents when compared to stem and root extracts

3.3 Antioxidant activities of the extracts

3.3.1 DPPH and ABTS radical scavenging activities of different extracts from *P. oleracea* and *A. garckeana* leaves, stems and roots

The antioxidant concentration from DPPH and ABTS assays was estimated from the Trolox standard curve ;y = 0.0007x + 0.1159, R^2 = 0.9608 and y = 0.0008x + 0.0895, R^2 = 0.9851 respectively. Differences in means were statistically significant at a P-value of $P < 0.05$ hence the use of different mean separation letters as seen in Fig. 2. *P. oleracea* root methanolic extracts displayed the highest concentration of antioxidants and highest radical scavenging activity from both DPPH and ABTS assays at 673.71 ± 23.23 Mmol/g ,706.25 ± 13.25 TE Mmol/g and 87.69% ± 2.43 respectively followed by *P. oleracea* stem methanolic extracts 705.00 ± 4.42 TE Mmol/g. On the other hand, the *P. oleracea* root hexane extracts were found to have the lowest antioxidant concentration of 57.50 ± 9.72 TE Mmol/g and therefore the lowest radical scavenging activity of 20.25% ± 1.16 when compared to other extracts.

Methanol, ethanol and chloroform extracts were found to contain a significantly high amount of antioxidants and therefore exhibiting significantly high radical scavenging activity (Fig. 2) when compared to distilled water and hexane whereas hexane extracts were found to contain a significantly low concentration of antioxidants and therefore displayed significantly reduced radical scavenging activity.

3.4 Identification of bioactive compounds in different extracts of *P. oleracea* and *A. garckeana*

Most compounds identified from Table 3–4 were based on probable compounds which had reported bioactivity whereas some compounds were identified based on the percentage probability. Various compounds were identified by GC-MS which may explain the antioxidant and antiproliferative activity of the extracts as the compounds had anticancer, antitumor and antioxidant activity (Table 3–4). These compounds may be responsible for the antioxidant activity of the extracts observed during DPPH and ABTS assays. Furthermore, the compounds have diverse biological activity from anti-microbial, anti-viral, anti-diabetic, antifungal and neuroprotective.

Table 3
Different bioactive compounds identified from *P. oleracea* extracts

Name of compound	Molecular weight (g/mol)	Formula of compound	Biological activity [Reference]	Extract identified from
6-((1,3-	608.55	C29	Anticancer[19, 20]	
dihydroxypropan-2-		H28N4O11		Stem chloroform
yl)amino)-2,10-				
dihydroxy-12-				
((2*R*,3*R*,4*S*,5*S*,6*R*)-				
3,4,5-trihydroxy-6-				
(hydroxymethyl)tetrah				
ydro-2*H*-pyran-2-yl)-				
12,13-dihydro-5*H*-				
indolo[2,3-				
a]pyrrolo[3,4-				
c]carbazole-5,7(6*H*)-				
dione				
7,9-Di-tert-butyl-1-	276.37	C17 H24O3	-Antioxidant[21]	
oxaspiro (4,5)deca-6,9-			-Antimicrobial	Stem chloroform
diene-2,8-dione			[21]	
l-Propyl14-methyl- pentadecanoate	298.50	C19H38O2	Antioxidant [22]	Stem chloroform
Benzonitrile,4-(6- butyl-2-naphthyl)-	285.38	C21H19N	-Antitumor [23] -Antioxidant [23]	Stem chloroform
Cyclononasiloxane, octadecamethyl-	667.39	C18H54O9 Si9	-Antioxidant [24] -Antimicrobial [2 4]	Leaf chloroform Root methanolic Leaf methanolic Stem methanolic Leaf ethanolic Stem ethanolic

Name of compound	Molecular weight (g/mol)	Formula of compound	Biological activity [Reference]	Extract identified from
Colchicine,N-	563.59	C31H33N	Anti-inflammatory[25]	Stem chloroform
desacetyl-N-[4-		O9		
hydroxy-3,5-				
dimethoxycinnamoyl]-				
Cholestan-26-oic acid, 3,7,12-trihydroxy-, (3α,5β,7α,12α)-	450.65	C27H46O5	-Antioxidant [26],Antimicrobial[26]	Stem chloroform Leaf chloroform Root methanolic Stem methanolic Leaf ethanolic
Octasiloxane, 1,1,3,3,5,5,7,7,9,9,11,11,13,13,15,15-hexadecamethyl-	579.30	C16H50O7Si 8	Anticancer [27]	Stem chloroform Root methanolic Leaf ethanolic Stem methanolic Stem ethanolic
Molybdenum,tetrakis[μ- (acetato-O:O')] di-, (Mo-Mo)	428.10	C18H12Mo 2O8	Antioxidant [28] Anticancer[28]	Stem chloroform
3 Beta-chloro-5alpha- cholestane-5,6beta-diol6- acetate	481.10	C29H49Cl O3	Antioxidant [29]	Stem chlorofom
3-O-Methyl-D-glucose	194.18	C7H14O6	Antioxidant [30]	Root methanolic Stem ethanolic
Nordextromethorphan	257.37	C17H23N O	-Neuroprotective [31]	Root methanolic

Name of compound	Molecular weight (g/mol)	Formula of compound	Biological activity [Reference]	Extract identified from
			-Anti-inflammatory [31]	Leaf ethanolic
Heptasiloxane, 1,1,3,3,5,5,7,7,9,9,11,11, 13,13-tetradecamethyl	505.09	C14H44O6 Si7	-Antioxidant [32] -Antibacterial [32, 33]	Root methanolic Leaf ethanolic Stem methanolic
(6*R*)-5,6,7,8- Tetrahydrobiopterin	241.25	$C_9H_{15}N_5O_3$	Antioxidant[34, 35]	Leaf ethanolic
4-Ethyl-2*E*-(hydroxyimino)- 5-nitro-3*E*-hexenamide	215.21	C8H13N3 O4	-Anticancer [36] -Anti-HIV [36] -Anti-bacterial [36]	Leaf methanolic
Cyclooctasiloxane, hexadecamethyl-	593.23	C16H48O8 Si8	-Antioxidant [38] -Antimicrobial [37]	Leaf methanolic Stem methanolic
10-Acetoxy-1,4a-dimethyl-9- oxo-1,2,3,4,4a,9- hexahydrophenanthrene-1- carboxylic acid, methyl ester	342.38	C20H22O5	Anticancer [39]	Leaf methanolic
Benzenepropanoic acid, 3,5- bis(1,1-dimethylethyl)-4- hydroxy-, methyl ester	292.41	C18H28O3	-Antimicrobial [40] -Antioxidant [40]	Leaf methanolic
1-Methyl-10,18-bisnorabieta- 8,11,13-triene	256.42	C19H28	Anticancer, Antidiabetic, Antimicrobial, Anti-inflammatory [41]	Leaf methanolic Stem ethanolic
Cyclopropa[3',4'] benz[1',2':4,5]azuleno[1,8a- d]-1,3-dioxole-5b,7,7a-triol, 3a,5a,6,7,8,8a,8b,11- octahydro-10- (hydroxymethyl	490.58	C27H38O8	Antioxidant, Anti-tumor, Anti- inflammatory and Anti- diabetic [42]	Leaf methanolic
Podofilox	414.40	C22H22O8	Anticancer [43, 44]	Leaf methanolic
Salmeterol	415.56	C25H37N O4	Anti- inflammato ry [45]	Stem methanolic

Name of compound	Molecular weight (g/mol)	Formula of compound	Biological activity [Reference]	Extract identified from
3*H*-Furan-2-one, 3- cyclohexylidene-5-(4- hexanoyloxyphenyl)-	386.44	C22H26O6	Anti- cancer, Anti-inflammato ry, Anti-viral [46]	Stem methanolic
Cyclohexasiloxane, dodecamethyl-	444.92	C12H36O6Si6	Antioxidant, Antimicrobial [39]	Stem methanolic
Cycloheptasiloxane, tetradecamethyl-	519.08	C14H42O7Si7	Antioxidant [39]	Stem methanolic
Acetamide, 2-(1,2,3,4- tetrahydro-3,3-dimethylisoquinolin-1- ylideno)-*N*-(2-chlorophenyl)-	326.82	C19H19ClN2O	Anti- inflammatory, Anti-neoplastic, Antioxidant [47]	Stem ethanolic
Aminoglutethimide	232.28	C13H16N2O2	Anti-cancer [48]	Stem ethanolic

Table 4
Different bioactive compounds identified from *A. garckeana* extracts

Name of compound	Molecular weight (g/mol)	Formula of compound	Biological activity [Reference]	Extract identified from
3-Methylmannoside	194.18	C7H14O6	-Anti-inflammatory [49]	Leaf ethanolic
			-Anti-diabetic [49]	
			-Anti-viral [49]	
			-Anti-bacterial [49]	
3*H*-Furan-2-one,3-cyclohexylidene-5-(4-hexanoyloxyphenyl)-	386.44	C22H26O6	-Anticancer [31]	Leaf ethanolic
			-Anti-inflammatory [31]	
			-Anti-Viral [31]	
Cyclononasiloxane, octadecamethyl-	667.39	C18H54O9Si9	-Antioxidant [24]	
			-	Leaf ethanolic
			Antimicrobial	Leaf methanolic Root methanolic Stem methanolic
			[24]	
Octasiloxane, 1,1,3,3,5,5,7,7,9,9,11,11,13,13,15,15-hexadecamethyl-	579.30	C16H50O7Si8	Anticancer [27]	Leaf ethanolic Leaf methanolic

Name of compound	Molecular weight (g/mol)	Formula of compound	Biological activity [Reference]	Extract identified from
				Root methanolic Stem methanolic
Xanthylium,9-[2-(ethoxycarbonyl)phenyl]-3,6-bis(ethylamino)-2,7-dimethyl-, chloride	507.06	C28H30ClN2O3	Antioxidant [50]	Leaf ethanolic
3-O-Methyl-D-glucose	194.18	$C_7H_{14}O_6$	Antioxidant [31]	Leaf methanol Stem methanol
3(2*H*)-Benzofuranone,6-methoxy 2-[(3-methoxyphenyl) methylene]-, (*E*)-	289.29	$C_{17}H_{14}O_4$	Anti-tumor[51]	Leaf methanol
7-Chloro-*N*-{3- [(diethylamino)methyl]-4-[(trimethylsilyl)oxy] phenyl}- *N*-(trimethylsilyl)quinolin-4-amine	500.22	$C_{26}H_{38}ClN_3O_2Si_2$	Anti-inflammatory [52]	Leaf methanol
Cyclohexasiloxane, dodecamethyl-	444.92	C12H36O6Si6	Antioxidant Antimicrobial [39]	Root methanol
Cyclooctasiloxane, hexadecamethyl-	593.23	C16H48O8Si8	Antioxidant, Antitumor, Antifungal, Antibacterial [53–54]	Root methanol
Benzoic acid, 4-amino-2-hydroxy-, tris(trimethylsilyl) deriv.	631.09	$C_{16}H_{31}NO_3Si_3$	Anti-inflammatory, Anti-fungal [55, 56]	Root methanol Stem methanol
Tetracosamethyl- cyclododecasiloxane	889.84	C24H72O12Si12	Antioxidant, Anti- bacterial [57–58]	Root methanol

Name of compound	Molecular weight (g/mol)	Formula of compound	Biological activity [Reference]	Extract identified from
4-Pyrimidinecarboxylic acid, 2,6-*bis*[(tert-butyldimethylsilyl) oxy]-, tert-butyldimethylsilyl ester	498.88	C23H46N2O4Si3	Antioxidant [59]	Stem methanol
1-Methyl-10,18-bisnorabieta- 8,11,13-triene	256.43	C19H28	Antioxidant, Anti- microbial, Anti-inflammatory [60]	Stem methanol
2(1H)-Pyrimidinone, 5-chloro-4,6-diphenyl-	282.72	$C_{16}H_{11}ClN_2O$	Cytotoxic , Anti-inflammatory [61–62]	Root methanol
10-Acetoxy-1,4a-dimethyl-9- oxo-1,2,3,4,4a,9-hexahydrophenanthrene-1- carboxylic acid, methyl ester	342.38	$C_{20}H_{22}O_5$	Anticancer [40]	Leaf methanol
Cholestan-26-oicacid, 3,7,12-trihydroxy-, (3α,5β,7α,12α)-	450.65	C27H46O5	Antioxidant [29, 30]	Leaf ethanolic

3.5 Antiproliferative activity of *P. oleracea* and *A. garckeana* extracts

3.5.1 Effect of *P. oleracea* and *A. garckeana* extracts on HeLa cell morphology

HeLa cells were treated at different concentrations of extracts and cisplatin ;12.5, 25, 50, 100, 200 and 400 µg/mL and exposed to the different concentrations of the extracts for 24 hrs. HeLa cells shrunk and detached from the cell culture surface (Fig. 3) after 24 hr exposure to extracts. Moreover, the cells lost their fibrous polygonal shape and were seen floating in media (Fig. 3) and lost their compactness as they no longer covered a large portion of the cell culture surface. After exposure to extracts for 24 hrs cell death was seen as indicated by small aggregates of dead cells (Fig. 3).

3.6 Effects of *A. garckeana* and *P. oleracea* extracts on the viability of HeLa cells

MTT assay was used to determine the viability of HeLa cells after treatment with *A. garckeana* leaves methanolic extracts, *A. garckeana* leaf ethanolic extracts, *P. oleracea* leaves methanolic extracts and *P. oleracea* stem methanolic extracts to study the antiproliferative effects of the extracts. The anticancer drug cisplatin was used as a positive control. The results were found to be statistically significant at a p-value of $P < 0.05$. Cell viability was affected in a dose-dependent manner (Fig. 4). *A. garckeana* leaf ethanolic extracts greatly reduced cell viability significantly by

92.00% from 97.00–5.00% at 400 µg/ml extract concentration and *P. oleracea* stem methanolic extracts slightly reduced cell viability significantly by 69.00% from 99.00–30.00% at 400 µg/ml extract concentration (Fig. 4).

Cisplatin significantly reduced cell viability from 95.00–12.00%, a confirmation of its cytotoxic effect on cells as an anticancer drug.

HeLa cells showed a significantly lower cell viability after exposure to a high concentration of 400 µg/mL of the four extracts. Cisplatin as a positive control caused a significant decrease in cell viability across all concentrations.

The IC_{50} values of the four used extracts and cisplatin were determined (Table 5). Results were found to be statistically significant at $P < 0.05$. The IC_{50} value indicates the potency of an extract or drug and level of its cytotoxic activity. This value is the concentration required to reduce proliferation of cells by 50.00%. Therefore, the lower the IC_{50} value, the greater the cytotoxic effect of the extract.

Table 5
Half maximal inhibitory concentrations (IC50) of *A. garckeana* and *P. oleracea* extracts and cisplatin on HeLa cells after cell viability assay

Sample	Extract	IC50 (µg/mL)
A. garckeana leaves	Methanolic	176.44 ± 25.49 (D)
A. garckeana leaves	Ethanolic	190.47 ± 46.10 (C)
P. oleracea leaves	Methanolic	329.31 ± 54.69 (A)
P. oleracea stems	Methanolic	242.09 ± 23.60 (B)
Cisplatin	Anti-cancer drug(cisplatin)	22.78 ± 10.37 (E)

4. Discussion

Medicinal plants have been widely reported to provide therapeutic effect due to the presence of phytochemicals which demonstrate pharmacological effects in many ways [10, 63]. This study assessed and contrasted the phytochemical properties, antioxidant and cytotoxic effect of *A. garckeana* and *P. oleracea* extracts. Results from qualitative phytochemical screening (Tables 1 and 2) show that more phytochemicals were detected in methanol extracts and less phytochemicals were detected in hexane extracts implying effect of polarity on extraction of compounds. This is in support of a study which reported that methanol is the most effective solvent during extraction because of its ability to impede polyphenol oxidase to oxidize phenolics [17].The variation of the TPC content from results and of other studies may be attributed to differences in processing, extraction techniques and the age and growth stage of plant as pointed out by studies [17–18, 64]. The current study revealed that leaf extracts had a significantly higher total phenolic and flavonoid content in both *P. oleracea* and *A. garckeana*. All these results show a relationship between solvent polarity, part of plant used to extract and extraction of phytochemicals as reported in other studies [65–66]. It is paramount for both the ABTS and DPPH assays to be used when evaluating antioxidant activity of extracts as a greater comprehensive evaluation of antioxidant activity of the extracts is provided since the assays measure different components of antioxidant activity [13, 67].

In the current study, high antioxidant activity as indicated in results presented from Fig. 3 was observed in most methanol extracts and GC-MS analysis as presented from Table 3–4 indicated the presence of many antioxidant compounds in methanolic extracts of *both P. oleracea* and *A. garckeana*. Furthermore, GC-MS results indicated from Table 3–4 in the current study indicate the presence of compounds which have antioxidant activity. The high

antioxidant activity seen in some of the extracts from both *P. oleracea* and *A. garckeana* extracts may be due to the antioxidant compounds present in extracts which participate in hydrogen electron transfer (HAT), single electron transfer (SET) and chelation of transition metals reactions [68].

Some of the compounds that were detected from extracts with high antioxidant activity include cholestan-26-oic acid, 3,7,12-trihydroxy-, (3α,5β,7α,12α)- and 3-O-methyl-D-glucose. These compounds display antioxidant action through the presence of hydroxyl groups which modulate this activity [69].These findings correlate with findings of another study which highlighted that *P. oleracea* varieties display a significantly high antioxidant activity because polar solvents are highly effective in extracting antioxidants [17]. Within this study, hexane extracts displayed low antioxidant activity despite high total flavonoid composition. This may be attributed to the structure of the flavonoids without hydroxyl groups which leads to reduced antioxidant activity as reported previously reported [17].

Cytotoxicity investigations of these extracts on HeLa cells showed that the extracts affect cell morphology as indicated from Fig. 3 .The cell viability assay results correlated with findings from a previous study which indicated that *P. oleracea* extracts bring about antiproliferative effects in a dose dependent fashion [70].The antiproliferative activity displayed by extracts from *A. garckeana* and *P. oleracea* may be due to the presence of anticancer and antioxidant compounds such as 10- acetoxy-1,4a-dimethyl-9-oxo-1,2,3,4,4a,9-hexahydrophenanthrene-1-carboxylic acid, methyl ester as reported in GC-MS results from current study (Table 3–4).Studies have reported that these compounds exert their antiproliferative effect by inducing apoptosis, causing DNA damage, increasing oxidative stress in HeLa cells which leads to oxidative damage, interfering with cell cycle progression and interacting with pathways such as MAPK and NF-kB pathways[22, 66, 67, 70].

5. Conclusion

This study showed that *P. oleracea* and *A. garckeana* different extracts possess antioxidant and antiproliferative properties and have bioactive phyto-compounds which may be responsible for the antioxidant and antiproliferative effect. Therefore, the support the traditional use of traditional use of the plant extracts in cervical cancer management.

Declarations

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Author contributions

O.M. . did data, collection, investigation, data analysis and wrote the original draft. N.T.G. critically revised the article, did data analysis, and interpretation. M. K. did methodology, and critical revision of the article. K.M. did conceptualization, methodology, and critical revision of the article. G.G. did conceptualization, methodology, and critical revising of the article. All authors reviewed and approved the final version of the article.

Conflict of interest

No potential conflict of interest reported by authors.

Data Availability

Data is available upon request from the authors.

Ethics approval and consent to participate: The collection of the plants used in the study complies with local or national guidelines with no need for further affirmation.

Consent for publication: The authors declare that this paper does not contain any studies with human participants or animals.

Competing interests: The authors declare no competing interests.

Clinical study: This study was not a clinical and therefore has no registry, trial registration number or data of registration.

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Figures

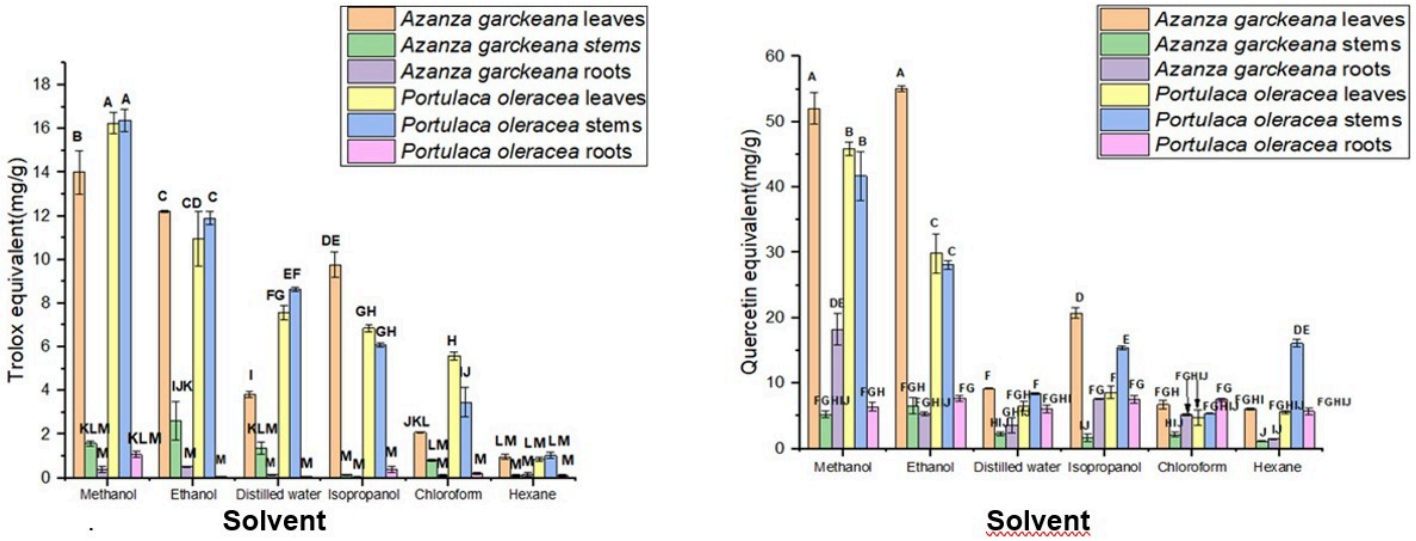

Figure 1

a) Total phenolic content and b) total flavonoid content expressed in TE and QE of leaves, stems and root extracts of six different solvents in both *P. oleracea* and *A. garckeana* (P-value < 0.05,considered statistically significant based on Tukey Kramer t-test).

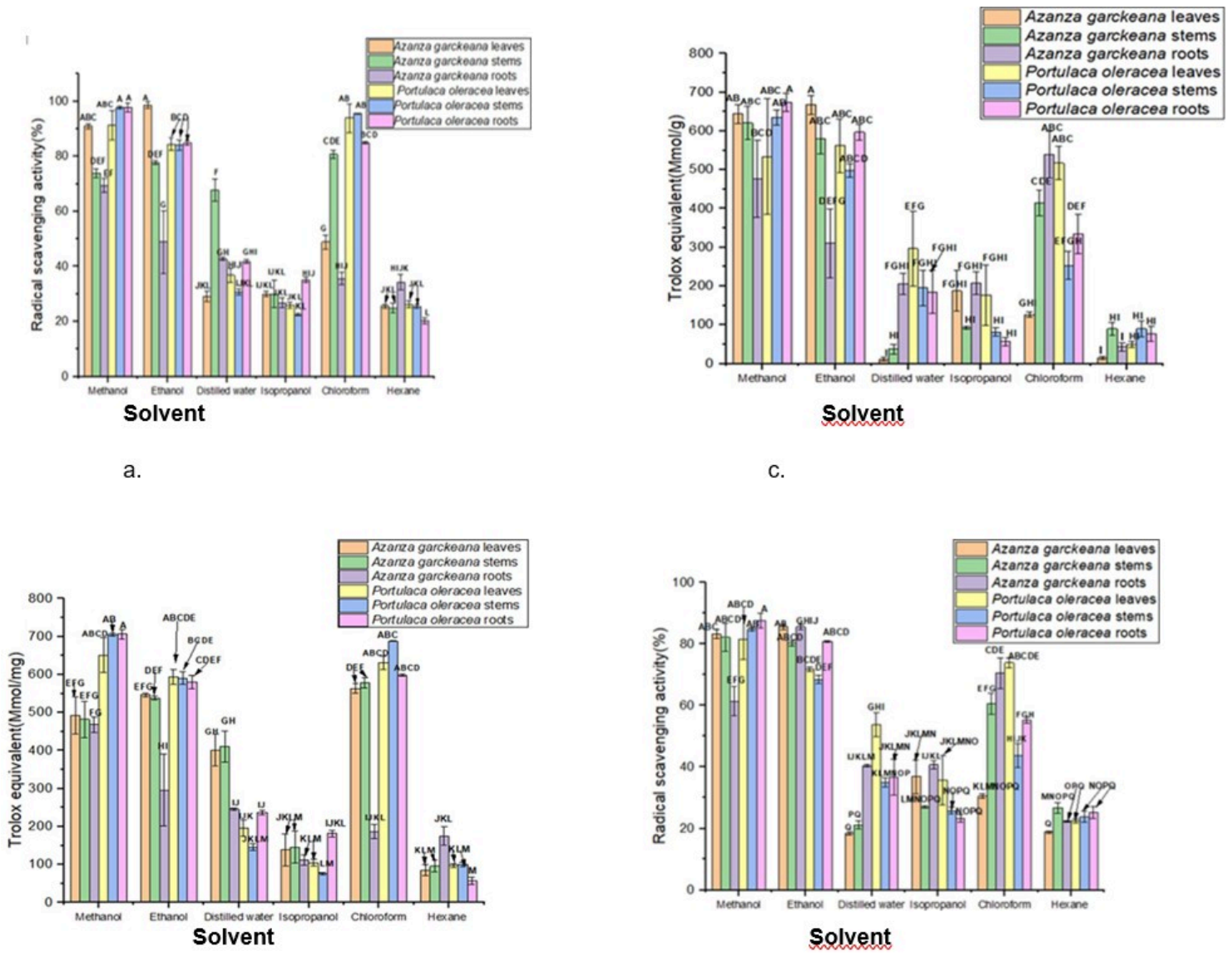

Figure 2

a) Antioxidant concentration expressed as Trolox equivalents (b.DPPH and c.ABTS) and radical scavenging activity (%) (a.DPPH and d.ABTS) of different extracts of leaves, stems and roots in *P. oleracea*and *A. garckeana*.

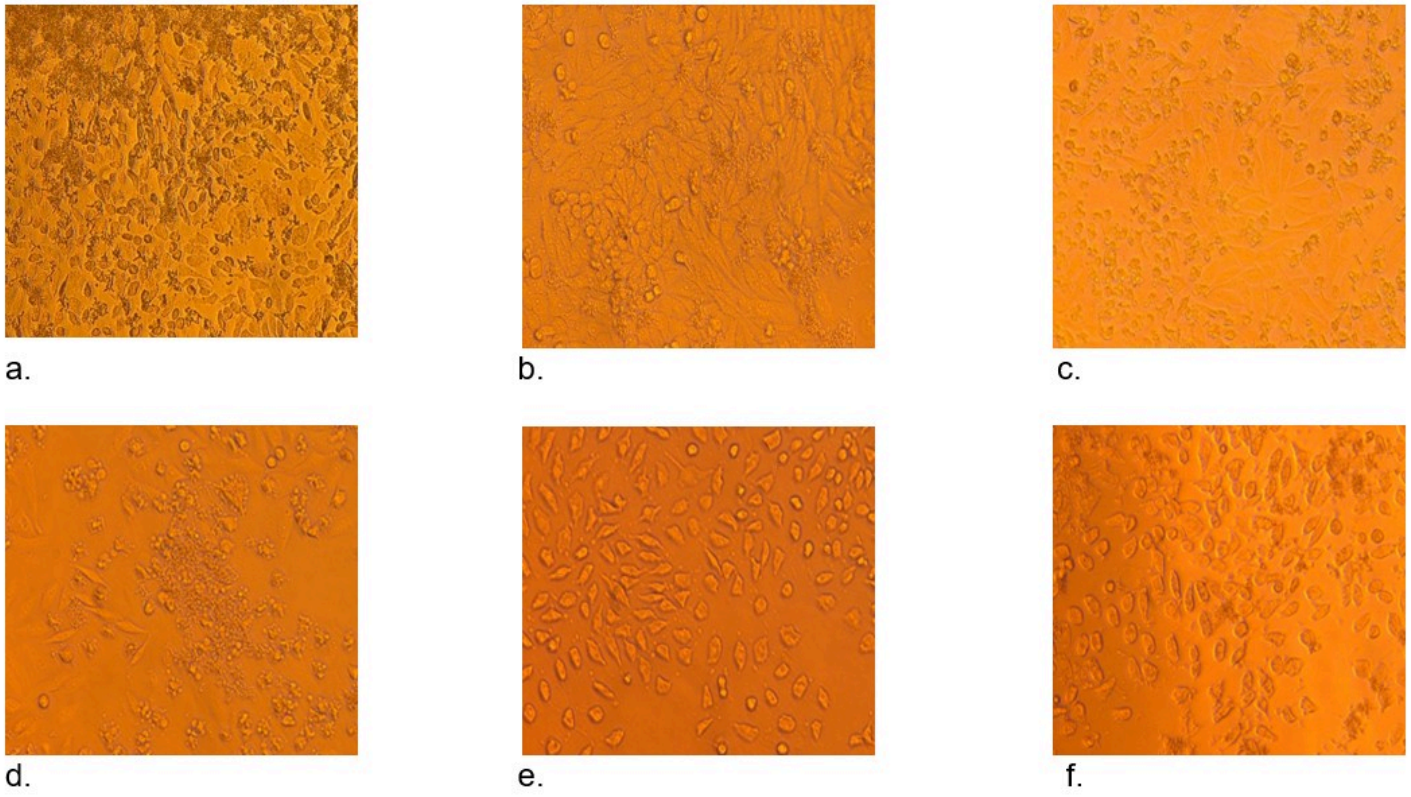

Figure 3

Morphology of HeLa cells (P20) after 24 hr incubation with *P. oleracea* and *A. garckeana* leaves methanolic extract (×40) at different concentrations (a and b; concentration of *P. oleracea*leaf methanol extract at 100 µg/ml and 400 µg/mL respectively, c and d ; concentration of *A. garckeana* leaf methanol extract at 100 µg/ml and 400 µg/mL respectively and e and f; concentration of cisplatin at 100 µg/ml and 400 µg/mL T respectively) captured under light inverted microscope (Olympus IX 83, Japan, Tokyo).

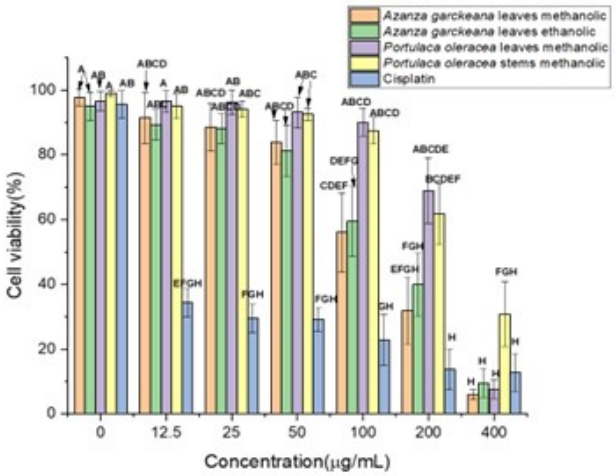

Figure 4

Cell viability of HeLa cells (P20, n=3) determined by MTT assay after 24 hr exposure of *A. garckeana* and *P. oleracea* extracts at different concentrations ($P < 0.05$ considered statistically significant based on Tukey Kramer t-test).