International Journal of Chemistry Studies
www.chemistryjournal.in
ISSN: 2581-348X
Received: 27-10-2024, Accepted: 26-11-2024, Published: 11-12-2024
Volume 8, Issue 2, 2024, Page No. 59-64

Phytochemical screening and *in vitro* anti-breast cancer activity of *Securidaca longependunculata* root bark extracts on mcf-7 cells using mtt assay

L A Magashi
Department of Chemistry, Kaduna State College of Education Gidan Waya, North Western, Nigeria

Abstract
Breast cancer remains a significant public health concern, necessitating the exploration of novel therapeutic agents from natural sources. This study evaluates the *in vitro* anti-breast cancer activity of *Securidaca longependunculata* root bark extracts on MCF-7 breast cancer cells, utilizing the MTT assay to assess cell viability and investigates the phytochemical constituents in the plant. The root barks of *Securidaca longependunculata* was extracted, weight 100g with soxhlet extractor using six different solvents based on polarity guided method and the respective extracts were concentrated under reduced pressure. Percentage Yields and Physico-Chemical Evaluation of the Extracts from the various fractions were recorded, with highest yields from 70 % MeOH, Methyl acetate and MeOH fractions and 25.513, 25.504 and 5.766 % respectively. The extracts exhibited significant cytotoxic effects on MCF-7 cells, with an IC_{50} - 223.3 µg/ml and % Inhibition of 64.62 in Methyl acetate and IC_{50} - 242 µg/ml with % Inhibition of 61.22 in MeOH fractions compared to Oxaliplatin IC_{50} of 38.04µg/ml and % Inhibition of 74.96 µg/ml from their respective concentrations, 320 µg/ml and 100 µg/ml. Phytochemicals were screened to identify the active compounds responsible for the observed effects and results revealed the presence of various bioactive compounds, including flavonoids, alkaloids, Phenolics, Saponins and tannins. These findings reveals notable cytotoxicity which may support the development of novel therapeutic agents derived from this plant. This warrants further investigation into its bioactive constituents and the development of novel breast cancer therapeutic agents from the plant.

Keywords: *Securidaca longependunculata*, phytochemicals, MCF-7 Cells, anti-cancer and MTT assay

Introduction
Breast cancer is one of the leading causes of cancer-related morbidity and mortality among women globally, representing a significant public health challenge (Bray *et al*., 2018) [2]. Despite advancements in early detection and treatment modalities, the disease continues to pose a substantial burden due to its complex biology and the emergence of resistance to conventional therapies (Siegel *et al*., 2020) [11]. As a result, there is an urgent need for innovative therapeutic strategies that can enhance treatment efficacy while minimizing adverse effects. Natural products have gained attention in recent years as a promising source of novel anticancer agents, owing to their diverse chemical structures and biological activities (Newman & Cragg, 2016) [9]. *Securidaca longependunculata*, a medicinal plant traditionally used in various cultures for its therapeutic properties, has shown potential in treating a range of ailments (Farnsworth & Soejarto, 1991) [5]. However, its anticancer properties, particularly against breast cancer, remain underexplored. Plants have been a vital source of anti-cancer agents, and their potential in cancer treatment is well-documented (Cragg & Newman, 2013) [4]. This study aims to evaluate the *in vitro* anti-breast cancer activity of *Securidaca longependunculata* root bark extracts on MCF-7 breast cancer cells. By employing the MTT assay to assess cell viability, this research seeks to elucidate the cytotoxic effects of the extracts and identify the phytochemical constituents responsible for these effects. The findings from this study could pave the way for the development of novel therapeutic agents derived from this plant, contributing to the ongoing efforts to combat breast cancer effectively.

Materials and methods
Plant identification and collection
Securidaca longependunculata root bark was collected from southern part of kaduna state Nigeria, wild sources, authenticated at the biological sciences department Gombe State University, assigned a voucher No. FHJ-654 and voucher specimen was deposited. The plant material was air-dried under shade for several days (about two weeks) at room temperature on a clean surface. Later it was pulverized to powder using wooden pestle and mortar, and stored in brown paper envelopes until needed for use.

Extraction of the plant materials
The plant samples were extracted from root bark of *Securidaca longependunculata*, weight (100 g) with 250 ml Petroleum ether using Sexhlet extractor. The extraction was carried out continuously for 8 hours. The procedure was repeated using; methyl acetate, acetone, chloroform, methanol and 70 % Methanol/water sequentially in order of polarity. 250 ml of each solvent with their respective extracts were collected and concentrated under reduced pressure (using the rotary evaporator), the residual solvents were evaporated in an Oven at 105 ^{o} C, dried and weighed. The extracts were stored in a desiccator.

Identification of phytochemical constituents
The phytochemical components of Cassia sieberiana were determined using the methods of (Harborne, 1973; Safowara, 1993 & Ciulei, 1994) [3, 6] with some minor modification.

Test for alkaloids

Dragendoff's test: To a 0.5 ml test sample, 0.2 ml of HCl was added. To the mixture, 2-3 drops of Dragendoff's reagent was added and the appearance of orange or red precipitate and turbid solution indicates the presence of alkaloids.

Wagners's test: A fraction of extract was treated with 3-5 drops of Wagner's reagent and observed for the formation of reddish-brown precipitate (or colouration) indicates the presence of alkaloids.

Test for carbohydrates

Molisch's test: Few drops of Molisch's reagent (α- napthol dissolved in alcohol) was added to 0.5 ml of sample and mixed with 0.2 ml of sulphuric acid (added along the sides of the test tube) and observed for the appearance of a purple colour ring for positive test.

Test for tannins

Braymer's test: To a 0.5 ml of plant extract, 2 ml water was added and mixed. The mixture was heated on water bath for 10 minutes. The mixture was filtered and ferric chloride was added to the filtrate and observed for dark green solution which indicates the presence of tannin.

Test for terpenoids

Salkowki's test: In a test tube with 0.2 ml of chloroform, 0.5 ml of plant extract was added. To the mixture, concentrated sulphuric acid was added carefully to form a layer. Presence of reddish brown colour at the interface would show would show the presence of Terpenoids.

Test for Glycosides

Borntrager's test: To 0.5 ml of filtered hydrolysate, 0.3 ml of Chloroform is added and shaken, Chloroform layer is separated and 10 % Ammonia solution is added to it. Pink colour indicates presence of glycosides. 0.2 ml of sample was mixed with 0.2 ml of chloroform. 0.2 ml of acetic acid was added to this solution and the mixture was cooled on ice. Sulphuric acid was added carefully and the colour change from violet to blue to green indicates the presence of steroidal nucleus (A glycone portion of glycoside).

Test for steroids:

Salkowski tests: Chloroform solution of the extract when shaken with concentrated sulphuric acid and on standing yields red colour solution indicates the presence of steroids.

Test for saponins

Frothing test: 2 drops of olive will be added to the frothing solution and shaken vigorously. Frothing indicates the presence of Saponins.

Test for Saponins (Foam test: To 0.2 ml of extract was added 0.6 ml of water in a test tube. The mixture was shaken vigorously and observed for the formation of persistent foam that confirms the presence of Saponins.

Test for phenols

FeCl3 test: To 0.5 ml of various solvent extracts of sample, 0.4 ml of distilled water followed by a few drops of 10 % aqueous ferric chloride solution was added. Formation of blue or green colour indicated the presence of phenols.

Test for cardiac glycosides

Keller-Killani test: To a 5 ml of various solvent extracts were mixed with 2 ml (10µL) of glacial acetic acid containing one drop of ferric chloride ($FeCl_3$) solution, followed by the addition of 1 ml (40 µL) concentrated sulphuric acid. Brown ring was formed at the interface which indicated the presence of deoxysugar of cardenoloides. A violet ring may appear beneath the brown ring, while in the acetic acid layer, a greenish ring may also form just gradually throughout the layer indicates the presence of cardiac glycosides.

Salkowski test: Sample mass of 0.5 g of the extract was dissolved in chloroform (2.0 cm3). Three drops of conc. H2SO4 was carefully been added to form a lower layer. The change in the reaction was observed and recorded.

Test for flavonoids

Lead acetate test: To 0.5 ml of extract, few drops of lead acetate solution was added to it and observed for yellow coloured precipitate indicates the presence of flavonoids.

Ferric chloride test: To 0.5 ml of extract, few drops of 10 % ferric chloride solution were then added. A green-blue or violet colouration indicated the presence of a phenolic hydroxyl group which indicates the presence of flavonoids.

Test for quinones

HCl test: To 1 ml of the extract 5 ml of HCl was added and observed for the presence of yellow colour precipitate indicates the presence of Quinones.

Test for anthraquinones

To a 1ml of benzene, 0.6 g of the powder sample was added in a conical flask and soaked for 10 minutes and then filtered. Further 1ml of 10 % ammonia solution was added to the filtrate and shaken vigorously for 30 seconds and pink, violet, or red color indicated the presence of Anthraquinones in the ammonia (lower) phase.

Test for coumarins

To a test tube 0.5 ml of the moistened various extracts was added. The mouth of the tube was covered with filter paper treated with 1N NaOH solution. Test tube was placed for few minutes in boiling water and then the filter paper was removed and examined under the UV light for yellow fluorescence indicated the presence of Coumarins.

Anticancer inhibition assay (Cytotoxicity studies) for MCF-7 cell line

Preparation of test solutions

For cytotoxicity studies, 32 mg/mL sample stocks were prepared in DMSO. Serial two fold dilutions from 320 µg/ml to 10 µg/ml were prepared which is then used for treatment.

Cell lines and culture medium

All the cell lines were procured from American Type Culture Collection (ATCC) Bangalore, India. Stock cells was cultured in DMEM supplemented with 10% inactivated

Fetal Bovine Serum (FBS), penicillin (100 IU/ml), streptomycin (100 µg/ml) in a humidified atmosphere of 5 % CO2 at 37ºC until confluent. The cell was dissociated with cell dissociating solution (0.2 % trypsin, 0.02 % EDTA, 0.05 % glucose in PBS). The viability of the cells are checked and centrifuged. Further, 100 µl of the diluted cell suspension was seeded in a 96 well plate at the confluency of 80 % at a start and incubated for 24 hrs at 37ºC, 5 % CO_2 incubator.

Procedure: The monolayer cell culture was trypsinized and the cell count was adjusted to 1.0 x 105 cells/ml using respective media containing 10 % FBS. To each well of the 96 well microtiter plate, 100 µl of the diluted cell suspension was added with confluency of 80 % at a start. After 24 h, when a partial monolayer was formed, the supernatant was flicked off, washed the monolayer once with medium and 100 µl of different test concentrations of *Securidaca longependunculata* root bark extracts were added on to the partial monolayer in microtiter plates. The plates were then incubated at 37ºC for 24hrs in 5% CO_2 atmosphere. After incubation the test solutions in the wells were discarded and 100 µl of MTT in the medium (5 mg/10 ml of MTT in PBS) was added to each well. The plates were incubated for 4 h at 37º C in 5% CO_2 atmosphere. The supernatant was removed and 100 µl of DMSO was added and the plates were gently shaken to solubilize the formed formazan. The absorbance was measured using a microplate reader at a wavelength of 590 nm. The percentage growth inhibition was calculated using the following formula and concentration of test drug needed to inhibit cell growth by 50 % (IC_{50}) values is generated from the dose-response curves for each cell line.

Calculating inhibition

% Inhibition = 100 – (OD of sample/OD of Control) x 100.

Standard and control

Standard – Oxaliplatin Control – Water

Results and discussions

Table 1 presents the physico-chemical evaluation of *Securidaca longependunculata* root bark extracts which provides essential insights into their potential as therapeutic agents. The high yield percentages, extraction solvents, color, texture and the presence of various bioactive compounds indicate that these extracts may possess significant pharmacological properties. Understanding the physico-chemical characteristics of the extracts is vital for optimizing extraction methods, standardizing formulations, and guiding future research aimed at developing effective breast cancer therapies. This study underscores the importance of solvent polarity in extracting bioactive compounds, as evidenced by the higher yields obtained from the MeOH, 70 % MeOH and Methyl acetate fractions of 7.208 %, 31.891 % and 31.880 % with crude extract recovered 5.766 g, 25.513 g and 25.504 g respectively in (Table 1). This highlights the need for careful selection of extraction method and solvent to maximize the yield of therapeutic compounds from natural sources. This can be seen in (Table 2), secondary metabolites or phytochemicals presents in the plant resulted from different polarity of the extracting solvents and the extraction method. Phytochemicals screening results showed the presence of Saponins, Quinones, Phenolic, Steroids, Tannins, Flavonoids, Terpenoids, Anthraquinones, Cardiac glycosides, Alkaloids, Carbohydrates, Glycosides and Coumarins, qualitatively from the extracts of Petroleum ether, Methyl acetate, Acetone, Chloroform, MeOH and 70 % MeOH respectively. Phytochemical analysis was conducted to identify the bioactive components of the extracts, providing insights into their potential mechanisms of action. Previous studies indicate that flavonoids and tannins possess anti-cancer properties by promoting apoptosis and inhibiting tumor growth (Khan & Khan, 2018; Sahu & Mohanty, 2019) [7, 10]. Hence, the presence of Flavonoids, Alkaloids, Phenolic acids, Saponins and Tannins are supporting the observed cytotoxic effects in MCF-7 cells (Plate 1) as reported (Mokhber-Dezfuli & Zare, 2016) [13]. These compounds are well-documented for their antioxidant, anti-inflammatory, and anticancer properties (Khan & Khan, 2018) [7]. The presence of flavonoids and phenolics, in particular, has been associated with the inhibition of cancer cell proliferation and induction of apoptosis (Sahu & Mohanty, 2019) [10]. The findings suggest that the observed cytotoxic effects may be attributed to a synergistic action of these compounds, warranting further investigation into their individual and collective roles in mediating anti-cancer activity. The results of this study provide compelling evidence for the cytotoxic effects of *Securidaca longependunculata* root bark extracts on MCF-7 breast cancer cells.

The MTT assay results indicate that both Methyl acetate and MeOH fractions exhibit significant cytotoxicity, with IC_{50} values of 223.3 µg/ml and 242 µg/ml, respectively (Figure 2 &3). These values suggest that while the extracts are effective, they are less potent than *Oxaliplatin* (Figure 1), a standard chemotherapeutic agent, which has an IC_{50} of 38.04 µg/ml (Kumar & Singh, 2015) [8]. The percentage inhibition rates of 64.62 % and 61.22 % for the Methyl acetate and MeOH fractions, respectively (Table 3), further highlight the potential of these extracts as anti-cancer agents (Bhandari & Bhandari, 2016) [1]. The cytotoxic effects of *Securidaca longependunculata* root bark extracts on MCF-7 cells suggest potential anti-breast cancer properties. Phytochemical analysis identified bioactive compounds, which may contribute to the observed cytotoxicity. These findings are consistent with previous studies on related plant species.

Table 1: Percentage Yields and Physico-Chemical Evaluation of the Extracts for *Securidaca longependunculata* (root bark) [B]

S/NO.	Extraction Solvents	Recovery (g)	% Yields	Colour	Texture
1.	Petroleum ether	1.124	1.405	Yellow	Oily
2.	Methyl acetate	25.504	31.880	Dark brown	Solid
3.	Acetone	1.226	1.533	Brown	Solid
4.	Chloroform	0.160	0.200	Yellow	Oily
5.	Methanol	5.766	7.208	Brown	Solid
6.	70%MeOH	25.513	31.891	Black	Solid

%YIELDS = Recovery (yield obtained)/Theoretical yields × 100

Table 2: Phytochemical screening for *Securidaca longependuncula* (root bark) [B]

S/no	Phytochemicals	Type of test	Pet. ether	Methyl acetate	Acetone	Chloroform	MeOH	70%MeOH
1	Saponins	a. Frothing test	-	-	+	+	+	+
		b. Foam test	+	+	+	+	+	+
2	Quinones	Conc. HCl	+	+	+	+	+	+
3	Phenolic	FeCl3 test	+	+	+	+	+	+
4	Steroids	a. Salkowski's test	+	+	+	+	+	+
		b. Lieberman Burchardt		+			+	+
5	Tannins	Braymer's test	+	+	+	+	+	+
6	Flavonoids	a. Lead acetate	+	+	+	+	+	+
		b. Iron (III) chloride	+	+	+	+	+	+
7	Terpenoids	Salkowki's test	-	+	-	+	+	+
8	Anthraquinone	10% Ammonia solution	-	-	-	-	-	-
9	Cardiac glycosides	a. Keller-Killani test	-	-	-	-	-	-
		b. Salkowski's test		-			-	-
10	Alkaloids	a. Dragendoff's test	-	+	-	-	+	+
		b. Wagner's test	-	+	-	-	+	+
11	carbohydrates	Molisch's test		-			-	-
12	Glycosides	Borntrager's test		-			-	-
13	Coumarins	NaOH		-			+	+

Table 3: MTT Assay of MCF-7 cell line for *Securidaca longependuncula*

Compound name	Conc. µg/ml	OD at 590nm	% Inhibition	IC_{50} µg/mL
Control	0	0.661	0.00	
Oxaliplatin	3.125	0.530	5.54	
	6.25	0.504	10.23	
	12.5	0.444	20.95	38.04
	25	0.325	42.11	
	50	0.246	56.16	
	100	0.141	74.96	
MA B	10	0.644	2.63	
	20	0.610	7.77	
	40	0.555	16.10	**223.3**
	80	0.471	28.79	
	160	0.374	43.45	
	320	0.234	64.62	
70%MeOH B	10	0.637	3.66	
	20	0.617	6.79	
	40	0.564	14.67	IC_{50} was not calculated
	80	0.511	22.77	due to lesser inhibition
	160	0.468	29.24	
	320	0.428	35.24	
MeOH B	10	0.630	4.75	
	20	0.606	8.38	
	40	0.562	15.01	242
	80	0.477	27.83	
	160	0.383	42.09	
	320	0.257	61.22	

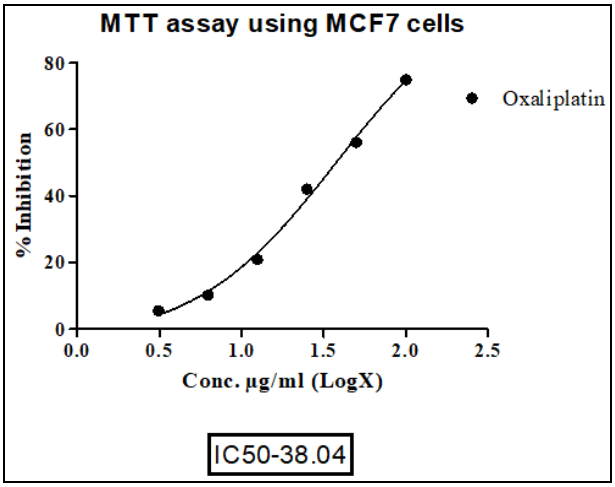


Fig 1: MTT assay for Oxaliplatin (Standard)

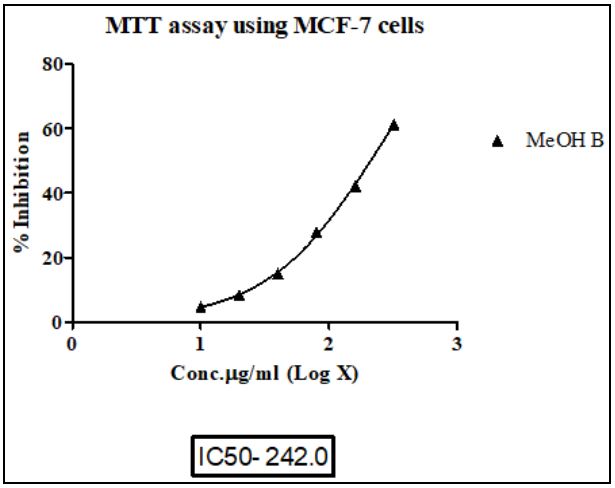


Fig 2: MTT assay for MeOH B

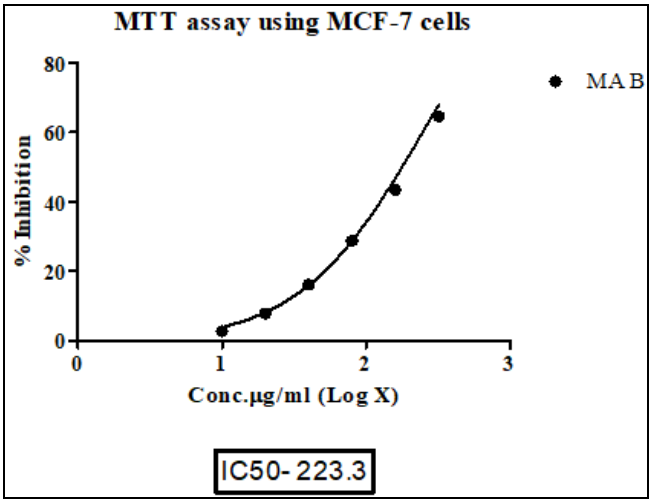


Fig 3: MTT assay for MA B

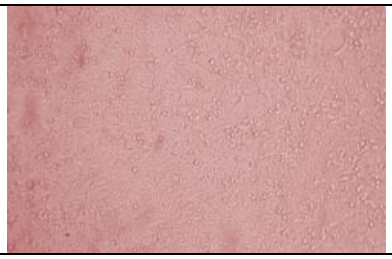
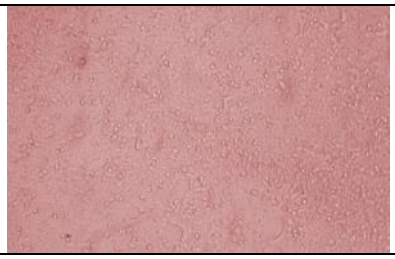
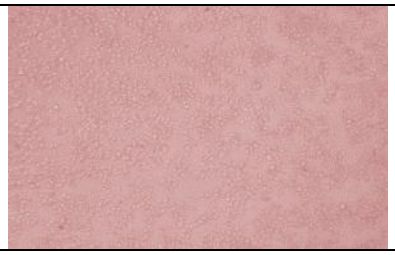
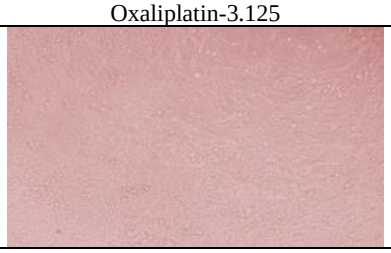
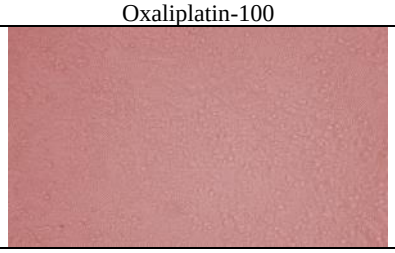
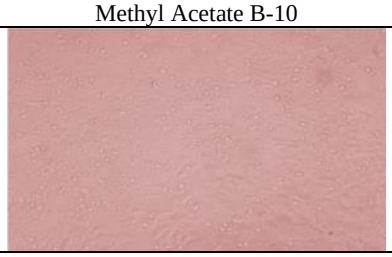
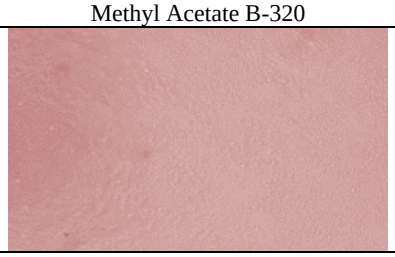
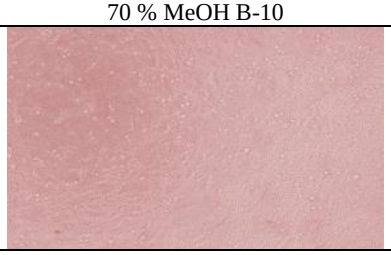
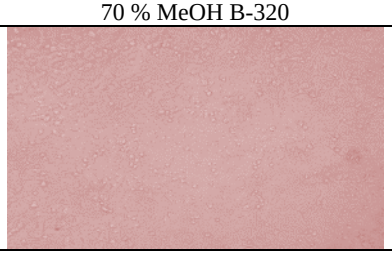
	
Control	Control
	
Oxaliplatin-3.125	Oxaliplatin-100
	
Methyl Acetate B-10	Methyl Acetate B-320
	
70 % MeOH B-10	70 % MeOH B-320
	
MeOH B-10	MeOH B-320

Plate 1: Cytotoxicity of MCF-7 cell line for *Securidaca longependunculata* [B]

Conclusion

This study demonstrates that *Securidaca longependunculata* root bark extracts possess significant *in vitro* anti-breast cancer activity against MCF-7 cells, indicating their potential as a source of novel therapeutic agents. The identification of various bioactive compounds within the extracts provides a foundation for further exploration into their mechanisms of action and therapeutic applications. While the extract shows promise, their efficacy relative to the established chemotherapeutic agents suggests that they may be best utilized in combination therapies or as adjuncts to existing treatments.

Acknowledgment

Author would like to thank the Tertiary Education Trust Fund (Tetfund) – Nigeria for their financial support under Institution based Research intervention (IBR) 2024, in conducting this Research work.

References

1. Bhandari P, Bhandari S. Phytochemicals: A potential source of anticancer agents. J Cancer Res Ther,2016:12(1):1-5. doi: 10.4103/0973-1482.155080.
2. Bray F, Ferlay J, Soerjomataram I, Siegel RL, Torre LA, Jemal A. Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA Cancer J Clin,2018:68(6):394-424. doi: 10.3322/caac.21492.
3. Ciulei I. Methodology for the analysis of vegetable drugs: Chemical industries Branch, Division of Industrial Operations, UNIDO, Romania, 1994:24:26 67.
4. Cragg GM, Newman DJ. Plants as a source of anti-cancer agents. J Ethnopharmacol,2013:100(1-2):72-79. doi: 10.1016/j.jep.2005.04.001.
5. Farnsworth NR, Soejarto DD. Global importance of medicinal plants. In: Medicinal Plants: Their Role in Health and Medicine. World Health Organization, 1991, 1-12.
6. Harborne JB. Phytochemical Methods: A Guide to Modern Techniques of Plant Analysis. London: Chapman and Hall Ltd, 1973, 279.
7. Khan MI, Khan MI. Anticancer potential of natural products: A review. J Cancer Res Clin Oncol,2018:144(1):1-12. doi: 10.1007/s00432-017-2468-3.
8. Kumar S, Singh A. Phytochemicals as potential anticancer agents: A review. J Pharmacogn Phytochem,2015:4(4):1-7.
9. Newman DJ, Cragg GM. Natural products as sources of new drugs over the last 25 years. J Nat Prod,2016:79(3):629-661. doi: 10.1021/acs.jnatprod.5b01154.
10. Sahu R, Mohanty S. Phytochemical screening and evaluation of anticancer activity of *Securidaca longependunculata*. Int J Pharm Sci Res,2019:10(3):1234-1240. doi: 10.13040/IJPSR.0975-8232.10(3).1234-40.
11. Siegel RL, Miller KD, Jemal A. Cancer statistics, 2020. CA Cancer J Clin,2020:70(1):7-30. doi: 10.3322/caac.21590.
12. Sofowora A. Medicinal Plants and Traditional Medicines in Africa,2nd ed. Ibadan-Nigeria: Spectrum Books, 1993, 289.
13. Mokhber-Dezfuli N, Zare M. Phytochemical and pharmacological properties of *Securidaca longependunculata*: A review. J Med Plants Res,2016:10(8):133-139. doi: 10.5897/JMPR2015.5693.