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Phytochemical constituents of ethyl acetate fraction of both roots and leaves of *Sansevieria triafasciata* cultivated in Iraq and assessment of its anti-proliferative effect on breast cancer cell line

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ABSTRACT: Aims of study are identification, isolation, identification and determination of some type bioactive constituents presents in *Sanseveria trifasciata (S.trifasciata)* with assessment of the effectiveness of ethyl acetate fraction roots and leaves as antiproliferative agents against breast cancer (MDA-MB-231) cell lines. The roots and leaves of *S. trifasciata* were macerated in n-hexane for defatting, then the defatted components were extracted using a hot extraction technique with 85% aqueous ethanol, and the extracted parts were fractionated first with chloroform, ethyl acetate, and then n-butanol. A fraction of ethyl acetate is analyzed by analytical high performance liquid chromatography (HPLC) to determine its component. Assessment of its cytotoxic activity was performed using the MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) assay. for 24 hours, multiple concentrations of extracts are used to treat the breast cancer (MDA-MB-231) cell line. After 72 hours of treatment, media were removed and cell viability was calculated. The qualitative HPLC analysis showed that the ethyl acetate fraction of roots contains apigenin, quercetin, chlorogenic acid, luteolin, catechin, and caffeic acid but did not contain kaempferol. On the other hand, the ethyl acetate fraction of leaves contained apigenin, kaempferol, chlorogenic acid, luteolin, and catechin but does not contain quercetin. A cytotoxic study (MTT assay) showed that the IC50% for roots was 89.83 µg/ml and the IC50% for leaves was 77.6 µg/ml. Ethyl acetate extracts of *S. trifasciata* from both roots and leaves showed anti-breast cancer cell lines through inhibition of proliferation. However, leaves showed a more inhibitory effect at the lower concentration than roots.

Keywords: Cytotoxic effect; Ethyl acetate fraction; HPLC; MDA-MB-231 cell line; *S.trifasciata*.

1. INTRODUCTION

The evergreen perennial *Sansevieria trifasciata* forms dense stands distributed underground. Around a single growth point, a rosette of its stiff, vertically oriented leaves that have dark cross-banded coloring patterns emerges [1] . *Trifasciata* is a particular epithet that means "three bundles." It is a common houseplant with flowers and yellow striped leaves that have the ability to absorb benzene, formal aldehyde, toluene, xylene, and carbon dioxide. So, *S. trifasciata* is also called viper's bowstring hemp [2] . *S. trifasciata* is native to West Africa. Outside of its native range, *S. trifasciata* has spread to other parts of the world, including Asia, Australia, Europe, and the Americas[3] and also to Iraq as an ornamental plant, and now widely spread to tropical and subtropical regions[4] . Mature leaves usually range between 70 and 90 cm long and 5–6 cm wide, with a height reaching 2 m[4,5]. The plant is adapted to a range of conditions and can tolerate both drought and low light levels. These plants' latex can be used as an insect and snake repellant in Africa[6,7]. However, as the use of particular medicinal plants to treat specific conditions became more prevalent, the empirical framework gradually shifted towards a more evidence-based approach, relying on clarifying facts to guide the use of medicinal plants[8,9] . Studies on the *S. trifasciata* extract demonstrate that the plant contains the majority of biologically active phytochemicals, making it significant in traditional medicine[10], like alkaloids, flavonoids, tannins and cardiac glycosides . also, steroidal saponins are present in plant which are mild toxic to dogs and cats leading to gastrointestinal upset if consumed[11,12]. The medicinal uses of *S. trifasciata* are to cure diarrhea, coughing, abdominal pain, colic, hemorrhoids, irritation of the respiratory

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tract, asthma, hair growth, cough, foot wounds, leprosy, hypertension, rheumatism, enlargement of glands, nutritional deficiencies, and snake bite treatment, in addition to being used as an attractive plant. Many studies have shown that *S. trifasciata* has antibacterial activity[10,13],antioxidant[14], anti-alopecia[15], antiallergic and anti-anaphylactic activity[16–18] and anti -inflammatory activity[17,19].

Cancer currently kills more people than coronary heart disease and stroke combined, according to WHO projections for 2011. Breast cancer, one of the most common cancers among women worldwide[20]. Furthermore, novel synthetic chemotherapeutic medications currently being utilized in clinical settings have fallen short of expectations over the past ten years, despite the considerable cost of their research. Therefore, there is a constant demand for new, effective, and affordable anticancer drugs. Breast cancer frequently metastasizes to distant organs such as the bone, liver, lung, and brain and is an incurable illness. Early diagnosis of the illness can lead to a favorable prognosis and a high survival rate. Parallel to this, there is growing evidence that chemicals derived from plants have the ability to suppress several stages of carcinogenesis and related inflammatory processes. The use of therapeutic plants was initially instinctive, much like the way animals instinctively seek out natural remedies[21,22].

Eventually, extracts of the plant's material, like roots, leaves, and stems by ethyl acetate, can be considered as possible therapies for many types of cancer. The MDA-MB-231 cell line, which is an epithelial type of cancer cell line that is usually utilized in research, is used in the current work. Since it is considered invasive in vitro, it remains poor in metastasis, but on the other hand, in vivo, it shows benefits as a model of experimental metastasis[23]. So, the aim of the current study is to determine the main active components of the ethyl acetate fraction of both roots and leaves of *S. trifasciata* and evaluate the cytotoxic activity of both fractions against a human breast cancer cell line by MTT assay.

2. RESULTS and DISCUSSION

2.1. Phytochemical Analysis

The many forms of secondary metabolites present are listed in Table 1 below, where (+, -) denotes the existence or lack of respective phytochemical ingredients**.**

Table 1. List of the secondary metabolites in leaves and roots

Tested component	Type of test	Results for leaves	Results for roots
Phenolic compounds	Gelatin test	+	+
	10%lead acetate	+	+
	5%ferric chloride	+	+
Tannins	Braymer's test	-	-
Flavonoids	shinoda	+	+
	Alkaline reagent test	+	+
	Lead acetate test	+	+
Alkaloids	Mayer's reagent	-	-
	Dragendorff	-	-
	Wagner	-	-
Phytosterols	Liebermann -burchard's	-	-
Coumarin	Florescence response test	-	-
Saponin	Foam test	-	-
Cardiac glycoside	Baljit test	-	-
	Keller -kiliani	-	-

2.2. Qualitative analysis by HPLC

Compounds were qualitatively identified by comparing retention time of standards as in Figures 1-7 with ethyl acetate fractions (roots and leaves) where shown below as in Figures 8 and 9:

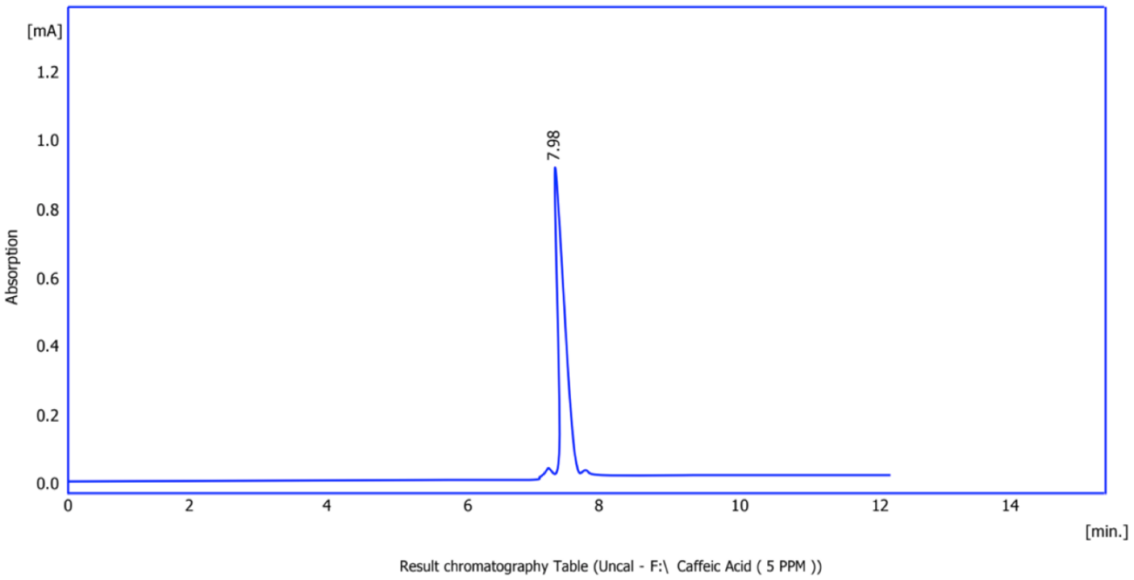

Figure 1. HPLC chromatogram of caffeic acid standard

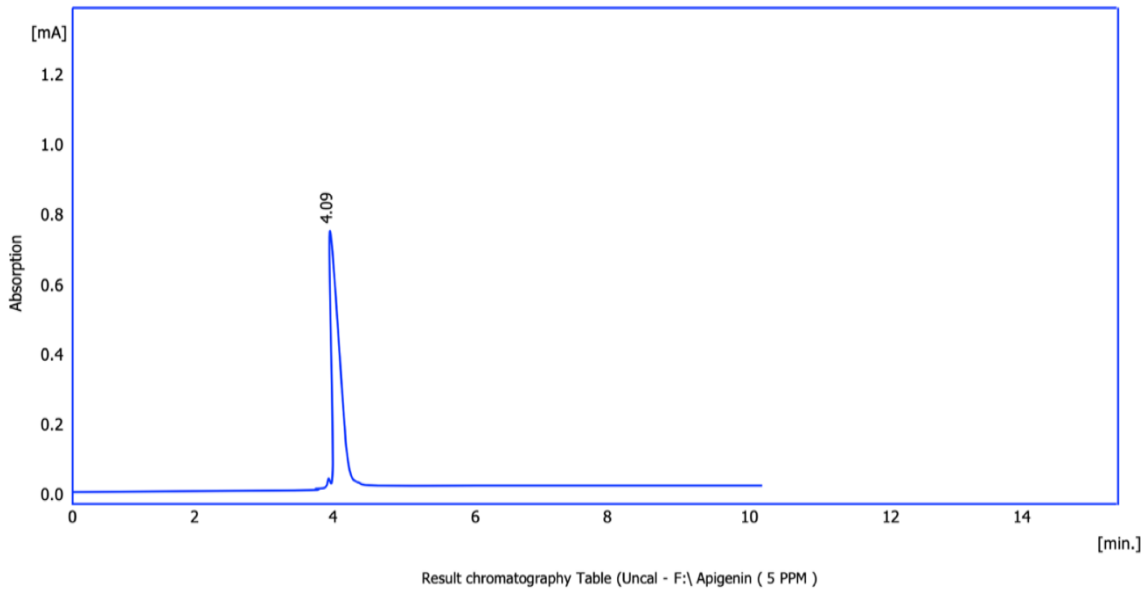

Figure 2. HPLC chromatogram of apigenin standard

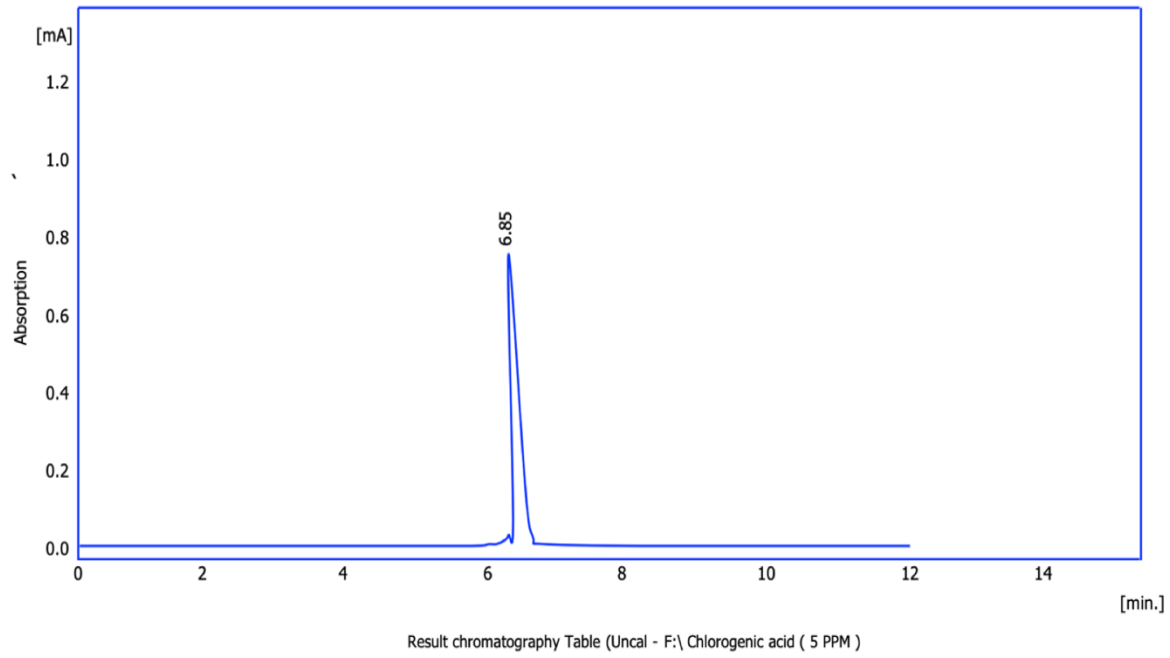

Figure 3. HPLC chromatogram of chlorogenic acid standard

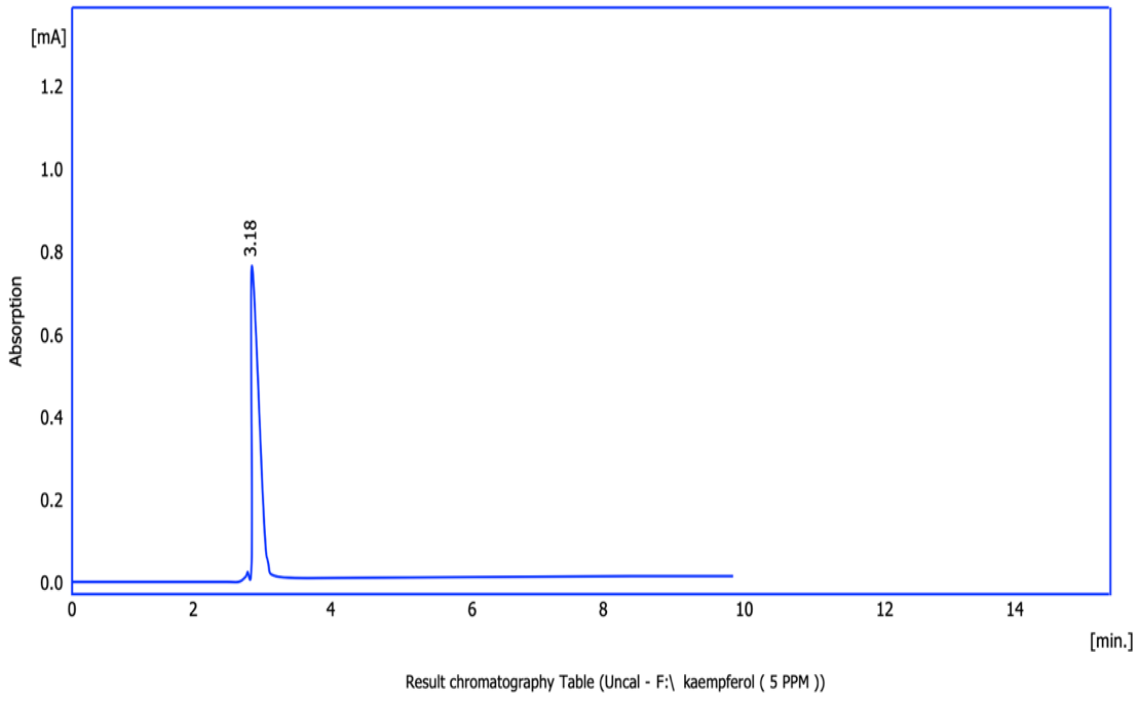

Figure 4. HPLC chromatogram of kaempferol standard

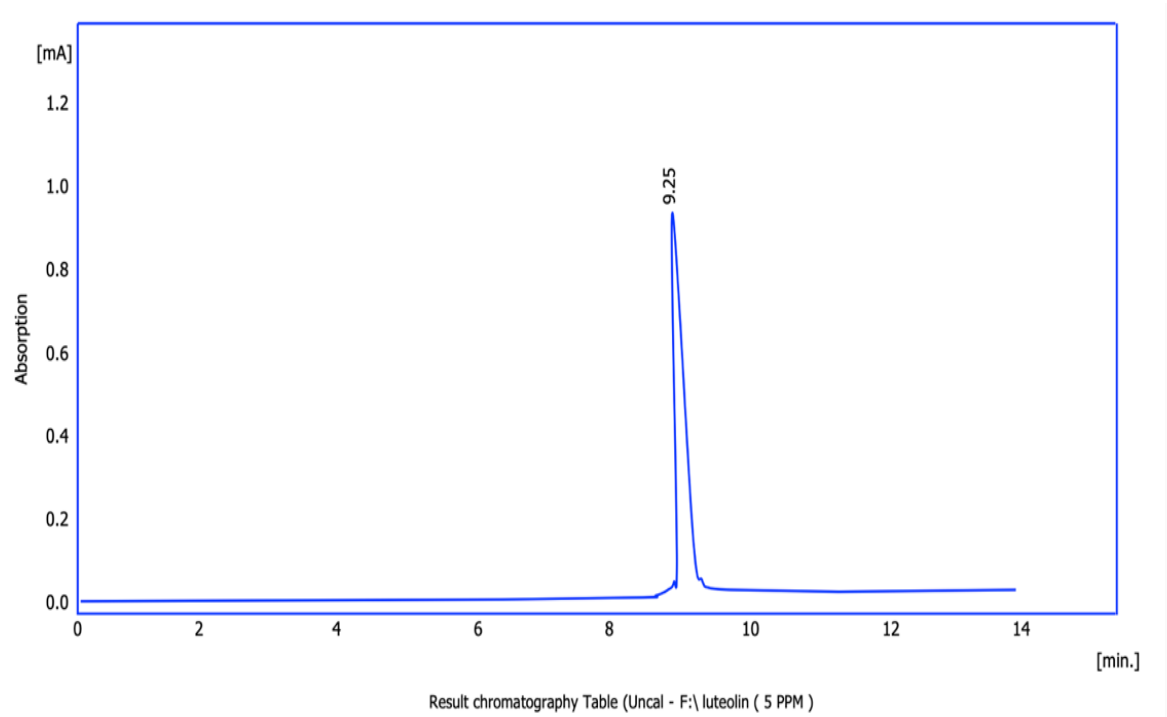

Figure 5. HPLC chromatogram of luteolin standard

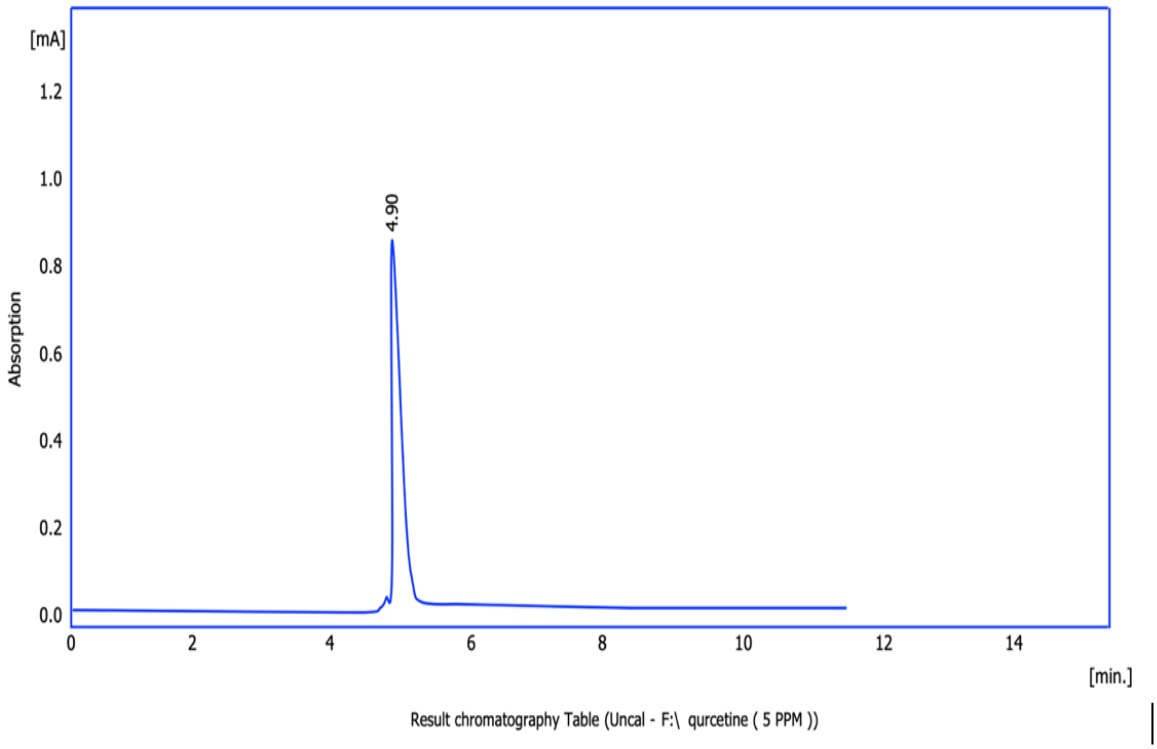

Figure 6. HPLC chromatogram of quercetin standard

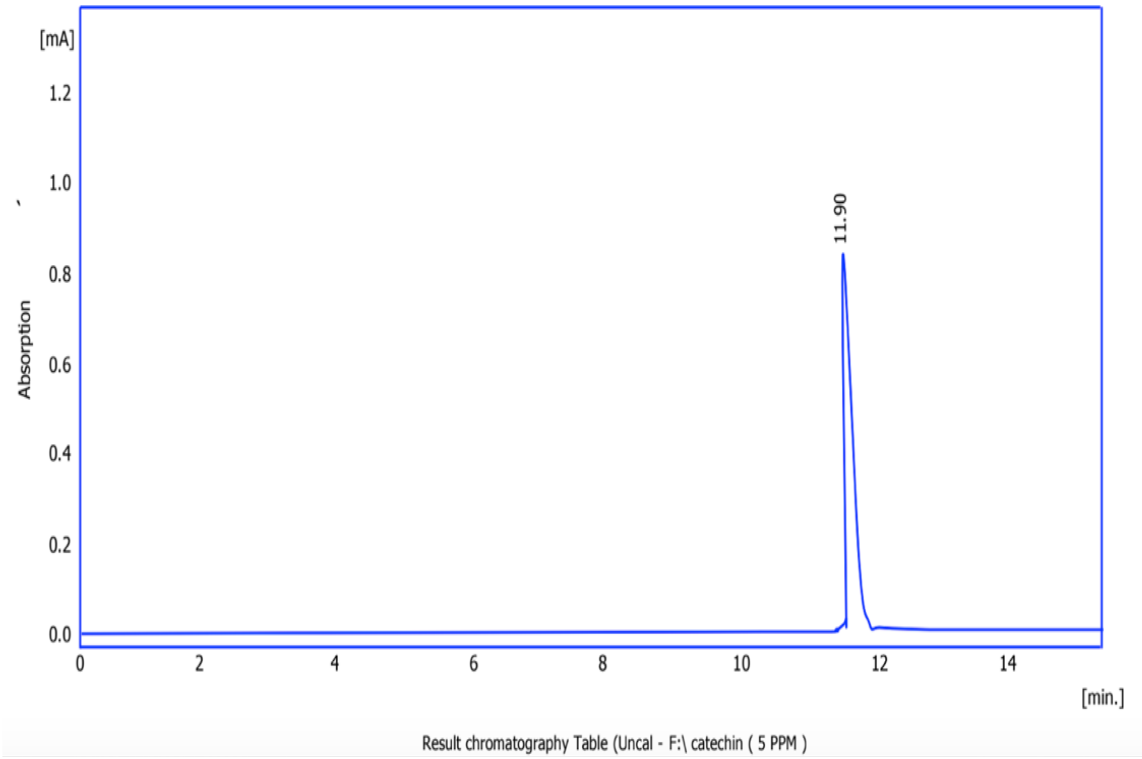

Figure 7. HPLC chromatogram of catechin standard

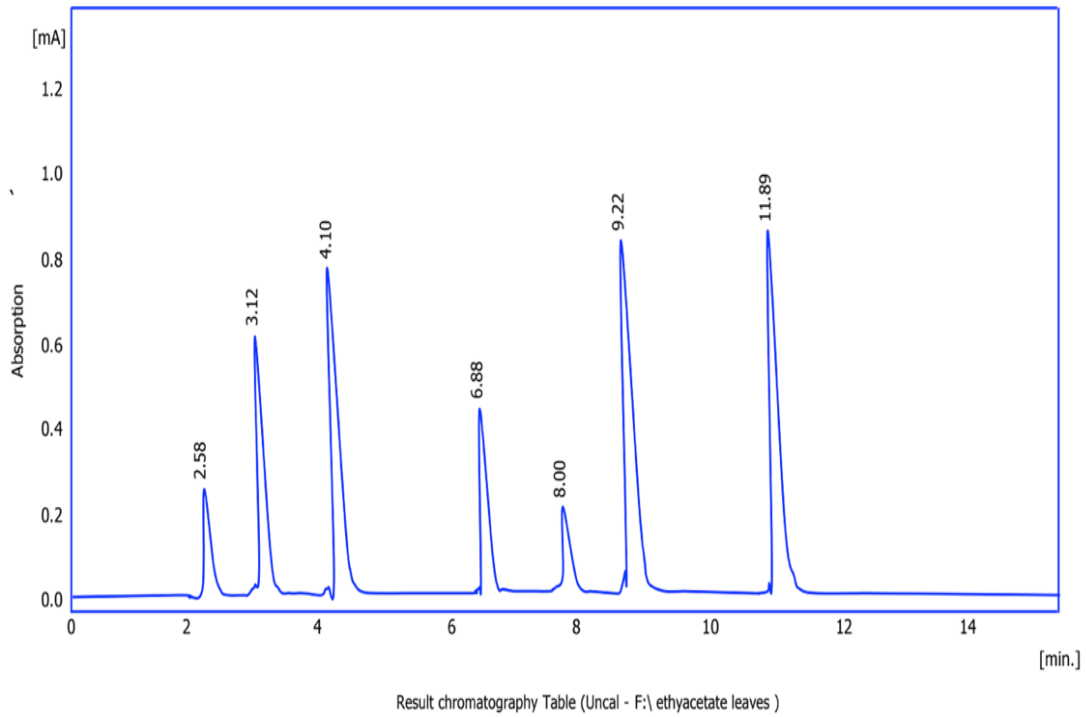

Figure 8. HPLC chromatogram of ethyl acetate leaves

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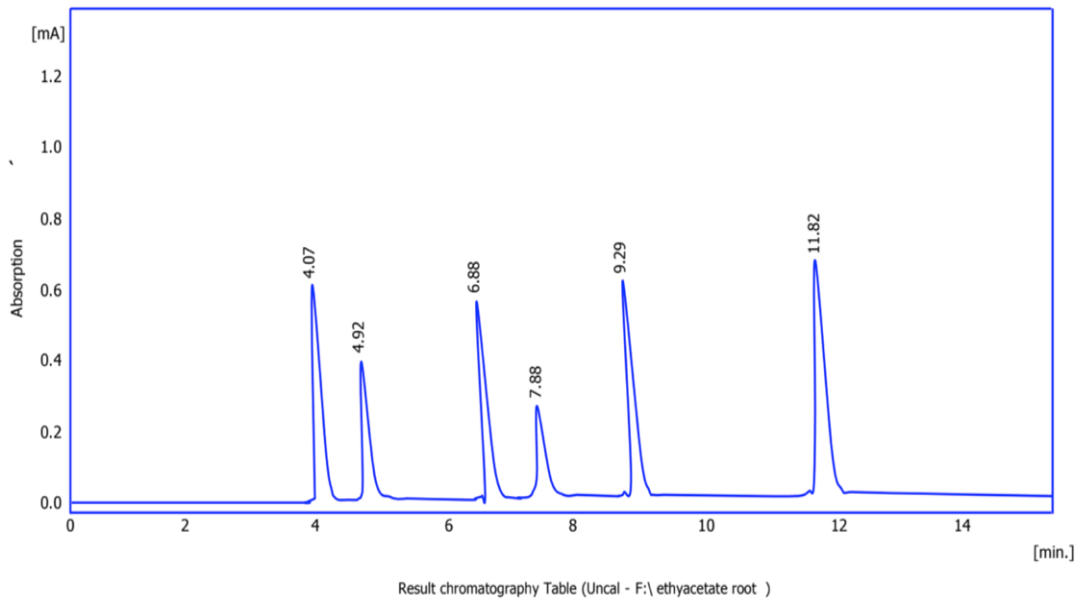

Figure 9. HPLC chromatogram of ethyl acetate roots

So, ethyl acetate fraction of roots show containing: Apigenin, quercetin, chlorogenic acid, luteolin, catechin and caffeic acid but not contain kaempferol. On the other hand, ethyl acetate fraction of leaves show containing: apigenin, kaempferol, chlorogenic acid, luteolin and catechin but not contain quercetin.

2.3. Cytotoxicity study

The cytotoxic study of ethyl acetate fraction obtained from roots and leaves show cytotoxic effect against breast cancer cell lines .The results showed : both ethyl acetate fractions(roots and leaves) can show antiproliferation activity and breast cancer cell lines growth was also slowed in a concentration-dependent manner; the highest inhibition of cell done by roots occurred at concentration = 200µg/ml produced 71.089% inhibition as in figure (10), 200µg/ml for leaves produced highest inhibition 82.658% as in figure (11).

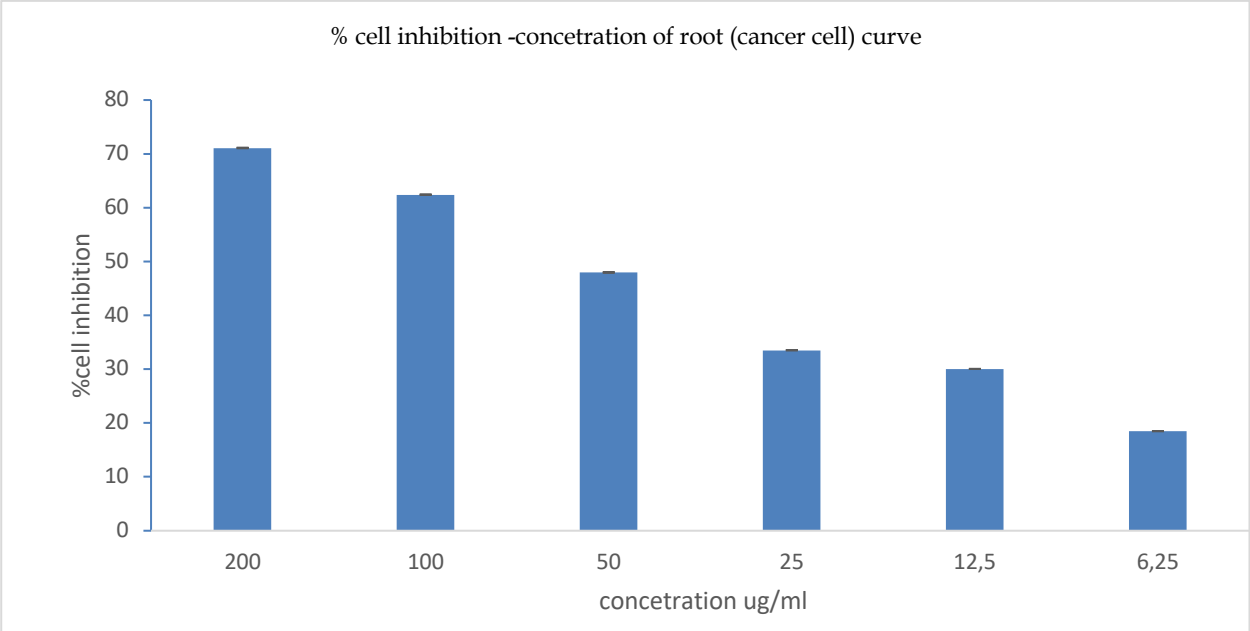

Figure 10. cytotoxic effect of Sanseveria t. roots at (MDA-MB-231) breast cancer cell. IC 50%=IC50=89.83 µg/ml

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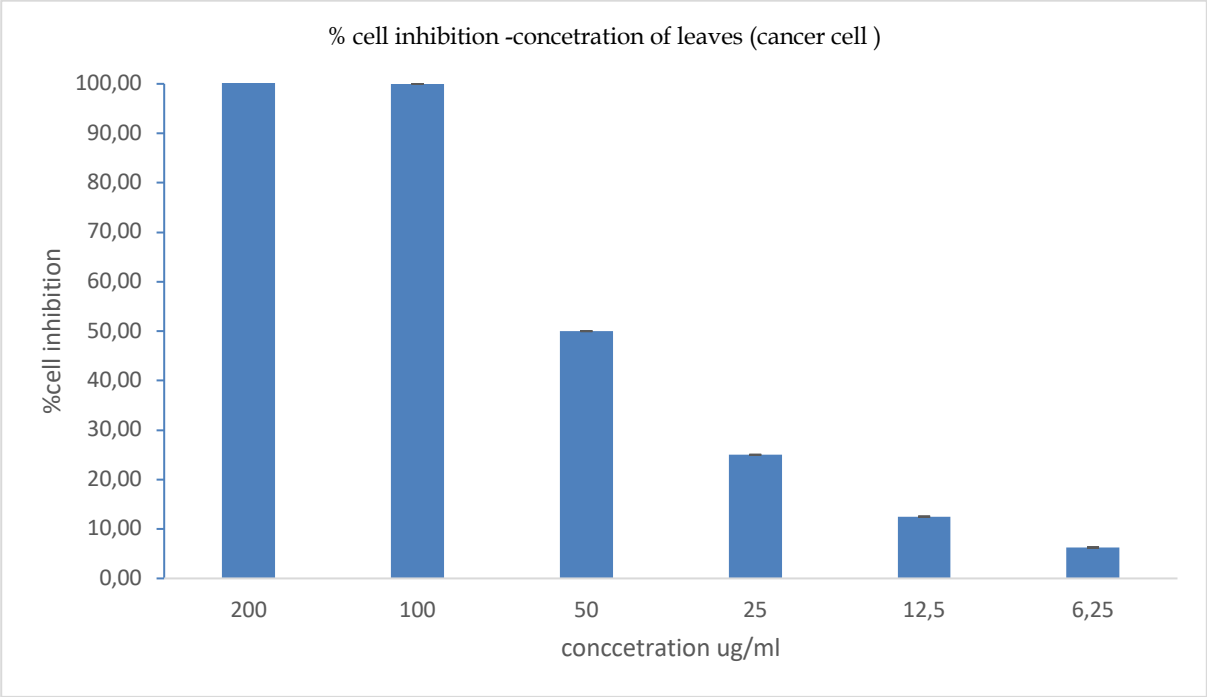

Figure 11. Cytotoxic effect of *Sanseveria t.* leaves at (MDA-MB-231) breast cancer cell. IC 50%= IC50=77.6µg/ml

IC50% calculated and resulted as follows: IC50=89.83µg/ml for roots and IC50%=77.6µg/ml for leaves. so, leaves showed more inhibitory effect with lower concentration than roots. This may be related to the presence of kaempferol in leaves rather than roots. we were the first who reported the antiproliferative tumor activity of *S. trifasciata* in Iraq. A study was done in 2022 by Somashekara R etl using the same type of cell line (MDA-MB-231) breast cancer cell but using another species of *Sanseveria* which is *Sanseveria zeylanica* with the same conditions found that the IC50% of methanolic extract of *S. zeylanica* leaves = 1167.78 µg/ml [24]. So, our species is more effective than *zeylanica* by more than ten times. In addition, flavonoids and phenolic compounds were detected in both roots and leaves that are more likely to be responsible for antioxidant activity which can be done through metal binding activity, stimulation of antioxidant enzymes, the capture of reactive oxygen species, minimizing of oxidative damage caused by nitric oxide and inhibition of oxidases leading to synergetic effect.

3. CONCLUSION

Ethyl acetyl fractions of both roots and leaves of *S. trifasciata* have antiproliferative activity and may have encouraging anticancer potential for the breast cancer, but leaves show more action than roots. the species type *trifsciata* is recommended to be a good material for more studies leading to possible drug development for cytotoxic potentiation and specification.

4. MATERIALS AND METHODS

4.1. Plant materials Collection

The plant of *S. trifasciata* (roots and leaves) cultivated in Iraq was collected from a nursery in Babil city in August 2021. prof. Dr. Sukaena Abass performed the identification and authenticated of the plant done in the Biology Department -College of Sciences - University of Baghdad. Thoroughly washed plant as whole, divided into roots and leaves, both were shade-dried separately and converted into fine powder by using mechanical grinder to prepare for further extraction purposes.

4.2. Methods

About (250 gm) of each dried (roots and leaves) were weighted and macerated separately by using n-hexane (450 ml) for three consecutive days, to remove chlorophyll, waxes, the solvent must be replacing each day resulting in defatted plant powder. By using Soxhlet apparatus for hot extraction method, the defatted plant powder was packed in a thimble, were 750 ml of 85% of aqueous ethanolic solvent poured in the rounded bottom flask and onto the thimble, start heating by using heating mental for about 83 hours.

Filtrate the ethanolic extract and then evaporated by rotary evaporator under reduced pressure[25]. Then fractionate the resultant firstly with chloroform ,ethyl acetate and then with n-butanol according to increasing polarity in a separatory funnel to extract the active constituents according to the polarities basis starting with chloroform, ethyl acetate, and n-butanol[26,27]. After drying with rotary evaporator, weighted the result for more analysis [28].

4.2.1. Preliminary qualitative and phytochemical analysis

Depending on standard procedures, done on ethyl acetate fractions to identify the chemical constituents present in them[29–31] as summarized in Table 2.

Table 2. Chemical tests that used in the identification of the active constituents in ethyl acetate fractions.

Test	Procedure	Observation (indicated positive result)
Detection of glycosides		
Keller- Kilian's test	1ml of extract +1.5 ml glacial acetic acid +1 drop of 5%ferric chloride +conc.H2SO4(along with side of test tube)	Reddish brown layer formed
Baljit's test	2ml of the extract +drop of Baljit's reagent	Yellow-orange color
Flavonoids Detection tests		
Alkaline reagent test	I ml of extract+2ml of A 2%NaOH solution+ few drops of conc. HCL	An intense yellow color, becomes colorless on addition of diluted acid
Lead acetate test	Few drops of 10 % lead acetate solution were added to 1ml of extract	A yellow precipitate
Shinoda test	few drops of conc. HCL with fragments of magnesium ribbon were added to the extract that dissolved in 5ml alcohol	A pink to crimson colored Solution
Detection of phenolic compounds		
Ferric chloride test	few drops of 5 %ferric chloride solution were added to the extract	Dark green-bluish black color
Lead acetate test	3 ml of10%lead acetate solution were added to the extract which is dissolved in 5 ml of distilled water	A white precipitate
Gelatin test	1 %gelatin Solution and 10 % NaCl were added to the extract of plant that dissolved in 5 ml distilled water	White precipitate
Tests of alkaloids		
Dragendorff's test	Few ml of filtrate +1-2ml of Dragendorff reagent	Reddish Brown precipitate due to double salt formation
Mayer's test	Few ml of filtrate +1-2ml of Mayer's reagent (along the side of the tube)	A creamy precipitate due to double salt formation.
Wagner's test	Few ml of filtrate +1-2 ml of wagner's reagent (along the side of the tube)	A brown- reddish precipitate
Detection of saponin		
Foam test	1ml of the extract diluted with distilled water and shaken in graduated cylinder for 15 min.	Developed foam persist for more than 15 min.
Detection of tannins		
Braymer's test	1ml of filtrate+3ml distilled water +3drops 10%ferric chloride solution.	Blue green color
Detection of coumarins		
Fluorescence response test	Apply sample of each extract on silica gel chromatography plate, dry under UV light observation and then spray with 1%potassium hydroxide reagent	Blue green Fluorescence

Test	Procedure	Observation (indicated positive result)
Detection of phytosterols		
Libermann-Burchard test	Small portion of each plant extract was dissolved inn5ml of chloroform, chloroform layer then dried using anhydrous sodium sulfate and later mixed with 10 drops of acetic anhydride and 2drops of conc. H2SO4 (along the side of the tube)	Color change (the bluish green solution resulted due to the presence of steroidal nucleus as oxidation occur in steps)
Salkowski's test	Few ml of each fraction Filtrate was dissolved in 2 ml of chloroform followed by the careful addition of few drops of conc.H2SO4 (Shaken well and allowed to stand)	Red brown color in the lower layer due to oxidation reaction

4.2.2. High Performance Thin Layer Chromatography

By using standards (apigenin, kaempferol, quercetin, luteolin, chlorogenic acid, caffeic acid, catechin) with samples of ethyl acetate roots and leaves fractions in which dissolve in absolute methanol. 95% acetonitrile + 0.01% trifluoracetic acid (solvent A) and 5% acetonitrile + 0.01% trifluoracetic acid (solvent B) have been employed as the mobile phase [32], proceeding at a rate of 1 ml/min. the gradient program was as follows:

10% solvent, (A) +90% solvent (B)- 0 to 5 minutes.
25% solvent, (A) +75% solvent (B) - 5 to 7 minutes.
40% solvent, (A) +60% solvent (B) - 7 to 13 minutes.
50% solvent, (A) +50% solvent (B) -13 to 33 minutes.
70% solvent, (A) +30% solvent (B) - 33to 45 minutes.

And then returning to initial condition prior to injection of second substances, by using, UV- visible detector at 278 nm[33]. The retention time of the compound was compared to the retention period of standard to carry out the recognition of phenolic components[34, 35]. HPLC system equipped with C18-ODS column (250*4.6 mm*5 µl), Flow rate: 1ml/min. Detected at UV-visible detector at 278 nm, temperature: 40°C

4.2.3. Instruments

Instruments used in study described in table (3):

Table 3. Instruments used in the current study

No.	Item	company	Country
1	Cell culture plates	Santa Cruz biotechnology	USA
2	Co, incubator	Cypress diagnostics	Belgium
3	Laminar flow hood	K and K scientific supplier	Korea
4	Micropipette	Cypress diagnostics	Belgium
5	Microtiter reader	Gennex Lab	USA

4.2.4. Reagents and Chemicals

Table 4 illustrated the reagents and chemicals used in the current study

Table 4. Chemicals and reagents.

No	Item	Company	Country
1	Trypsin/EDTA	Capricon	Germany
2	DMSO	Sntacruz biotechnology	USA
3	RPMI 1640	Capricon	Germany
4	MTT Stain	Bio-world	USA

5	Fetal Serum Bovine	Capricon	Germany
6	Incubator	Cypress diagnostics	Belgium

1-EDTA (ethylene diamine tetra acetic acid):

When introduced into trypsin solutions, it operates as a metal chelator and increases activity. As EDTA is included, both calcium and magnesium ions get eliminated from the cell surface, and trypsin can hydrolyze particular peptide bonds. Cell-to-cell adhesion is primarily broken down by adding EDTA to trypsin.

2- Dimethyl sulfoxide's (DMSO's):

Impact on the phospholipids in membranes and their physical characteristics is thought to be the cause of the substance's cytotoxicity. A solvent with amphipathic characteristics, DMSO binds with the plasma membrane, forming pores, weakening membrane selectivity, and elevating cell permeability.

3- Cell culture uses a growth media called; RPMI 1640, also referred to as: RPMI medium.

4- The MTT assay (Molecular Targeted Therapies):

Used to determine metabolic activity of cells as a marker of survival of cell, proliferation, and cytotoxicity; as solution darken, more viable (metabolically active) cells are present.

5-Fetal bovine serum (FBS):

Academic and industrial researchers routinely augment basal growth media in cell culture applications with this byproduct of harvesting cattle for the meatpacking sector[36, 37].

4.2.5. Maintenance of cell cultures

RPMI-1640 has been used to maintain cancer cell with10% fetal bovine serum, 100 units/ml penicillin, and 100ug/ml streptomycin. Twice weekly reseeding and trypsin-EDTA incubation at 37 °C were used to passage the cells[38,39].

4.2.6. Cytotoxicity assay

MTT experiment was done with, plates of -96-well to measure the cytotoxicity that implanted with cell line of 1*104) cell/well. Additionally, the MDA-MB-231 cell lines were treated with various concentration after 24 hours. After 72 hours of treatment, the medium was removed, to determine the vitality of the cells. adding 28 µL of MTT solution with a concentration of (2 mg/mL), and then incubate for 1.5 hours and 37°C. Any crystals that sticked on walls can be removed after the MMT solution were removed through addition 130 µL DMSO -(Dimethyl Sulphoxide)-then another fifteen-minute incubating at 37°C with shaking. In triplicates, the assay's absorbance was measured on a microplate reader at a wavelength of 570 nm [40]. The dose- response curve of the cell line was used for estimating the amount of the test fractions expected to inhibit cell growth by 50% (IC50) values, as described by the formula below [24, 37].

% Cell viability = (Absorbance of treated cell/Absorbance of non-treated cell) × 100

% Cytotoxicity=100-Cell viability[37].

4.3. Statistical Analysis

The collected information was statistically analyzed using an unpaired t-test in SPSS, and the values were offered as the mean ±SD of three replicate measurements [36].

Author contributions: Concept – E.K.; Design – R.A., E.K.; Supervision – E.K.; Resources – R.A.; Materials – R.A.; Data Collection and/or Processing – R.A.; Analysis and/or Interpretation – R.A., E.K.; Literature Search – R.A., E.K.; Writing – R.A.; Critical Reviews – E.K.

Conflict of interest statement: "The authors declared no conflict of interest" in the manuscript.

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