JOTCS A
Journal of the Turkish Chemical Society
Section: A
Chemistry
TURKISH CHEMICAL SOCIETY

Phytochemical Screening, Antioxidant and Anticancer Activities of *Euphorbia hyssopifolia* L. against MDA-MB-231 Breast Cancer Cell Line

Asseel Azaat*[1], Georget Babojian[1], Nizar Issa[1]

[1]Damascus University, College of Sciences, Damascus, Syria.

Abstract: *Euphorbia hyssopifolia* L. is an annual herb producing toxic latex. To our knowledge, this study is the first one that focuses on accurately identifying the bioactive compounds in *E. hyssopifolia* and evaluating its antioxidant and anti-breast cancer activities. Phytochemical qualitative screening tests were performed. Total phenolic contents (TPC) and total flavonoid contents (TFC) were determined by Folin Ciocalteu and Aluminum chloride methods, respectively. Bioactive compounds in *E. hyssopifolia* latex were identified by using GC-MS analysis. Antioxidant activity was evaluated using DPPH and ABTS assays, and anticancer activity of latex against MDA-MB-231 breast cancer cell line was studied using flow cytometry methods. Results revealed the presence of flavonoids, tannins, alkaloids, diterpenes, steroids, and cardiac glycosides in the plant, whereas saponins were absent. Latex outperformed methanolic 70% extract in terms of TPC and TFC (39.52 ± 0.36 mg GAE/g E and 28.66 ± 0.10 mg RE/g E, respectively). GC-MS analysis of *E. hyssopifolia* latex resulted in the identification of 26 compounds, of which triterpenoids constitute 67.0172%, followed by lupeol (23.7089%) and betulin (14.0098%). According to the reference studies, most of the compounds found in latex have many biological activities. Latex outperformed all extracts and ascorbic acid in terms of antioxidant activity (IC_{50}= 0.029 mg/mL for DPPH, IC_{50}= 0.001 mg/mL for ABTS). Flow cytometry methods revealed that *E. hyssopifolia* latex induced cell cycle arrest at G1 phase (61%) and apoptosis (21.93%) of MDA-MB-231 cells after treating with latex at 10 µg/mL for 24 hours. However, more studies should be performed to explore bioactive compounds in *E. hyssopifolia* and determine the underlying mechanism of its latex anti-breast cancer effects.

Keywords: *Euphorbia hyssopifolia*, apoptosis, *Euphorbiaceae*, necrosis, anticancer.

Submitted: November 10, 2021. **Accepted:** February 03, 2022.

Cite this: Azaat A, Babojian G, Issa N. Phytochemical Screening, Antioxidant and Anticancer Activities of Euphorbia hyssopifolia L. against MDA-MB-231 Breast Cancer Cell Line. JOTCSA. 2022;9(1):295–310.

DOI: https://doi.org/10.18596/jotcsa.1021449.

***Corresponding author. E-mail:** Asseel7777@hotmail.com

INTRODUCTION

The genus *Euphorbia* belongs to *Euphorbiaceae*, the sixth largest family among flowering plants. It includes about 2100 species, and it is the most diverse genus of spermatophytes on the Earth. Species of *Euphorbia* are widely represented in the Mediterranean Basin, the Middle East, South Africa, and Southern USA (1-3).

Members of this genus are characterized by the production of a milky irritant latex produced by laticiferous vessels. Latex is a complex mixture of proteins, starch, sugars, alkaloids, tannins, oils, resins, and gums. It serves as a defense material and prevents herbivorous insects from feeding (4).

There are 45 species and 6 varieties of *Euphorbia* in Syria, except for the species studied in this research, most of which are herbaceous (5).

Euphorbia hyssopifolia L. is an annual herb commonly found in the tropical and sub-tropical regions of Africa and America, and is often found growing along road sides and in fields, erect or suberect, 25.89 ± 2.75 cm height, usually glabrous. Leaves opposite with serrated margin and obovate shape. Cyathia axillary and terminal, single or in small cymes, glands 4, yellow-green, appendages white or pale pink. Capsule triangular, ovoid, seeds are blackish, each side has 3 or 4 transverse furrows, caruncle being absent (6,7).

E. hyssopifolia was registered for the first time in the flora of Syria in 2018. It produces latex which is a health hazard to humans and livestock. Direct contact of latex with the eye may cause blindness. It is known to contain phenolic compounds which are inhibitory to seed germination and seedling growth as well as bacteria. This latex has also diuretic and purgative effects. It was used to treat respiratory infections and to induce bronchial relaxation in asthma. The juice is said to remove warts and the leaves can be boiled with *Phyllanthus niruri*to make tea for the treatment of gonorrhea (8-10).

Phytochemical screening of the plant revealed the presence of alkaloids, flavonoids, carbohydrates, vitamin A, reducing sugars, saponins, glycosides, and steroidal aglycone. It also contains mono and sesquiterpenes, triterpenes, and steroids (11,12).

Few studies have demonstrated the toxic effects of *E. hyssopifolia*. One study revealed the genotoxic effect of its ethanolic extracts on HepG2 cells. Another study pointed out that its aqueous extract has toxic effects on the normal structure and functions of the liver and the heart of albino rats (12,13).

Breast cancer is one of the most common cancers that affects about 12% of women in the world. Despite advances in chemotherapy, breast carcinoma treatment is still a great challenge in the clinical therapy (14). Therefore, breast cancer should be at the forefront of medicinal plant research.

Up today, no study focused on accurately identifying the bioactive compounds in *E. hyssopifolia* and evaluating its antioxidant and anti-breast cancer activities.

This study aims to perform qualitative screening (flavonoids, tannins, alkaloids, saponins, diterpenes, and steroids), quantitative screening (total phenolic and flavonoid contents), identification of bioactive compounds in *E. hyssopifolia* using GC-MS analysis, and study its antioxidant and anticancer activities on MDA-MB-231 breast cancer cell line.

MATERIALS AND METHODS

Chemicals and Reagents

All solvents used in this study were of analytical grade and purchased from Merck. All other chemicals, including standard compounds (gallic acid and rutin), reagents and cell culture chemicals were purchased from Sigma-Aldrich.

Plant Materials

E. hyssopifolia was collected from Al-Bramka Zone (33°30'37.1"N, 36°16'56.7"E) in Damascus Governorate in September 2019 and 2020. The plants were identified by Prof. Babojian, Botany Department, Sciences College, Damascus University, Damascus, Syria.

Latex Collection

Crude white milky latex was obtained through cutting and squeezing the stems of the fresh plants, then it was dried in an oven at 45 °C up to constant weight (15). Dried latex was stored in a freezer at -20 °C until use.

It should be noted that dried latex was re-dissolved in DMSO when total phenolic and flavonoid contents were determined, and when DPPH and ABTS assays were performed.

It is also useful to point out that latex used in flow cytometry methods to study its anticancer activity on breast cancer cells was not dried and was kept wet in freezer at -20 °C until use.

Preparation of the Extracts

Different extracts (aqueous, methanolic 70%, ethyl acetate, and *n*-hexane extracts) were prepared using maceration method; 40 g of powdered aerial parts were separately extracted in 400 mL of the organic solvents by maceration (3 times for 72 hours; re-extracted every 24 hours) at room temperature as mentioned in (16). After filtration, the solvents were removed using a rotary evaporator under reduced pressure and kept in a freezer at -20 °C.

The ethyl acetate and *n*-hexane extracts were re-dissolved in DMSO when total phenolic and flavonoid contents methods, and DPPH and ABTS assays were performed, whereas aqueous and methanolic 70% extracts were re-dissolved in their solvents.

Preparation of Latex Extract for GC-MS Analysis

Methanol (99.9%, 5 mL) was added to 500 µL of latex and kept in the shaker for 24 hours at room temperature. The sample was centrifuged at 3000 rpm for 10 min and filtered. The supernatant was concentrated to volume of 100 µL and kept in the freezer at -20 °C until use.

Phytochemical Qualitative Screening Tests

Test of flavonoids

a) Shinoda test: 2 to 3 mL of the extract and small pieces of metallic magnesium were added into a small porcelain lid, followed by careful dropwise addition of concentrated HCl. Appearance of purple color indicates the presence of flavonoids (17).

b) Alkaline Test: Method of (18) was applied with some modifications. 2 to 3 mL of the extract and few drops of 5% NaOH were added into a test tube. The appearance of intense yellow color that became colorless on addition of few drops of dilute HCl indicates the presence of flavonoids.

Test of tannins

Few drops of 5% ferric chloride reagent were added to the extract. Formation of an intense green or black color indicates the presence of tannins (19).

Test of alkaloids

a) Wagner's Test: 1 mL of each extract was mixed with equal volumes of Wagner's reagent (Iodine in potassium iodide). Formation of reddish brown precipitate indicates the presence of alkaloids.

b) Hager's test: To 2 mL of each extract, few drops of Hager's (Saturated picric acid solution) reagent were added. Presence of alkaloids was indicated by formation of a bright yellow-colored precipitate.

c) Mayer's Reagent: 1 mL of each extract was mixed with few drops of Mayer's reagent (Potassium Mercuric Iodide Solution). Formation of light yellow precipitate indicates the presence of alkaloids (20).

Test of saponins

Approximately 200 mg of each extract was shaken with 5 mL of distilled water in a test tube and heated on water bath to boil. Presence of saponins was indicated by formation of strong and stable foam (21).

Test of diterpenes

3 mL of each extract was mixed with 3 mL of 10% copper acetate solution (note that the concentration is specific to the research). Presence of diterpenes was indicated by formation of green color (22).

Test of steroids

Salkowski's test*:* 2 mL of extract were mixed with 2 mL of chloroform and 2 mL concentrated sulfuric acid was added carefully. Formation of red color in the chloroform layer indicates the presence of steroids (23).

Test of cardiac glycosides

Extract (2 mL) was treated with 2 mL glacial acetic acid and few drops of $FeCl_3$. A brown color ring indicates the presence of positive test (24).

Phytochemical Quantitative Screening Methods

TPC of the extracts and latex was determined by Folin Ciocalteu method (26). 1 mL of the sample was combined with 4 mL of 2% Na_2CO_3 and 4.8 mL of the sample solvent (water for aqueous extract, methanol 70% for methanolic 70% extract and DMSO for ethyl acetate and *n*-hexane extracts and latex). 0.2 mL of 2 M Folin-Ciocalteu reagent (Sigma-Aldrich) was added to the mixture and mixed thoroughly. After incubation for 60 min in the dark, absorbance at 760 nm was measured by UV-visible spectrophotometer (Optizen, Mecasys- Korea). Sample solvent was used as a blank. Total phenolic content was determined as milligrams of gallic acid equivalents per gram of sample (GAE mg/gE) using a standard calibration curve between 0 to 300 ppm (Figure 1). Total phenolic contents of samples were determined in triplicate. Data were expressed as mean ± standard deviation (SD).

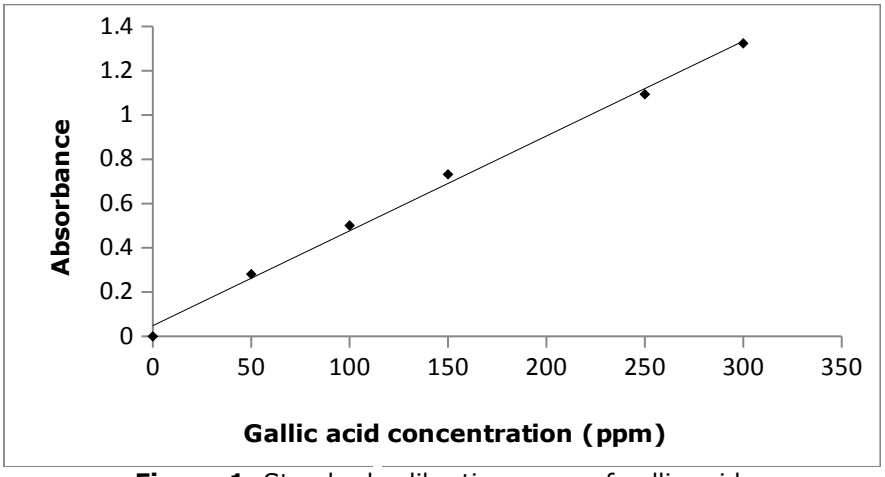


Figure 1: Standard calibration curve of gallic acid.

TFC

Aluminum chloride colorimetric method was used for determination of flavonoids with some modifications (26). Briefly, 0.75 mL of sample was mixed with the same volume of the sample solvent (as we did in TPC determination), 1.5 mL of 2% aluminum chloride, and 6 mL of 5% potassium acetate were added. After incubation for 40 min in the dark, the absorbance of the reaction mixture was measured at 415 nm spectrophotometrically. Sample solvent was used as a blank. Total flavonoid content was determined as milligrams of rutin equivalents per gram of sample (RE mg/gE) using a standard calibration curve between 0 to 150 ppm (Figure 2). Total flavonoid contents of samples were determined in triplicate. Data were expressed as mean ± standard deviation (SD).

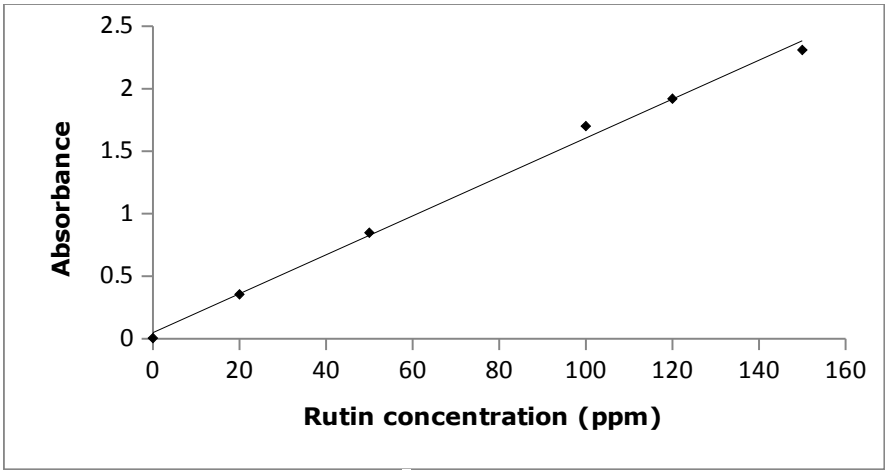


Figure 2: Standard calibration curve of rutin.

Gas Chromatography-Mass Spectroscopy (GC-MS) Analysis of Latex

Methanolic extract of latex was subjected to GC-MS for the identification of bioactive volatile compounds. GC-MS analysis of the samples was carried out using Agilent series A7890 with nonpolar HB-5 MS column (30 m, 0.25 mm, 0.25 µm). Helium was used as the carrier gas and the temperature programming was set with initial oven temperature at 80 °C, increased at 8 °C/min to 200 °C and held for 1 minute, and the final temperature of the oven was 300 °C and held for 20 minutes. 1 µL sample was injected with splitless mode, ionization potential 70 eV, and a scan range of 50 to 550 amu. The total running time for a sample was 52 minutes. The chemical components of the extract were identified by comparing the retention times of chromatographic peaks with NIST Library to relative retention indices.

Antioxidant Activity Assays

DPPH (2,2-diphenyl-1-picrylhydrazyl) assay

The free radical scavenging activities of extracts and latex on the DPPH radical were measured using the method described by (27). 0.3 mL of tested samples at different concentrations was added to 3 mL of DPPH solution (45 µg/100 mL of ethanol). The sample solvent (water for aqueous extract, methanol 70% for methanolic 70% extract, and DMSO for ethyl acetate and *n*-hexane extracts and latex) was used as a blank. After the mixture was shaken and left at room temperature for 30 min, the absorbance was measured at 517 nm with a spectrophotometer. The results were compared to ascorbic acid which was prepared as standard with different concentrations from 0 to 0.1 mg/mL (Figure 3). The antioxidant activity of the tested samples was calculated by determining the decrease in absorbance at different concentrations by using the following equation:

$$\text{DPPH scavenging effect (\%)} = \left[\frac{(A_1 - A_2)}{A_1}\right] \times 100$$

Where:

- A_1 = the absorbance of the control reaction.
- A_2 = the absorbance in the presence of the sample.

The IC_{50} value, defined as the amount of antioxidant necessary to decrease the initial DPPH concentration by 50%, was calculated from the results and used for comparison.

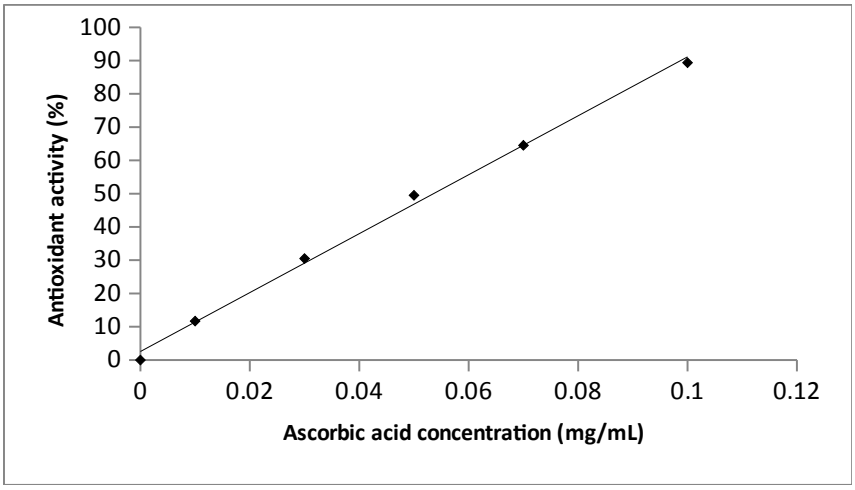


Figure 3: Standard calibration curve for antioxidant activity of ascorbic acid using DPPH assay.

ABTS (2,2'–Azino – bis (3-ethylbenzoline-6-sulfonic acid)) assay

The free radical scavenging activities of extracts and latex on the ABTS radical were measured using the method described by (28). The radical cation was prepared by mixing 7 mM ABTS stock solution with 2.45 mM potassium persulfate (1/1, v) and leaving the mixture for 4-16 h until the reaction was complete and the absorbance was stable. The $ABTS^+$ solution was diluted with ethanol to an absorbance of 0.700 ± 0.05 at 734 nm for measurements. The photometric assay was performed by adding 0.9 mL of $ABTS^+$ solution to 0.1 mL of tested samples and mixing for 45 sec, measurements were taken at 734 nm after 15 minutes. The results were compared to ascorbic acid which was prepared as standard with different concentrations from 0 to 0.015 mg/mL (Figure 4). The sample solvent (as we did in DPPH assay) was used as a blank. The antioxidant activity of the tested samples was calculated by determining the decrease in absorbance at different concentrations by using the following equation:

$$\text{ABTS scavenging effect (\%)} = \left[\frac{(A_1 - A_2)}{A_1}\right] \times 100$$

Where:
- A_1 = the absorbance of the control reaction.
- A_2 = the absorbance in the presence of the sample.

The IC_{50} value was calculated from the results and used for comparison.

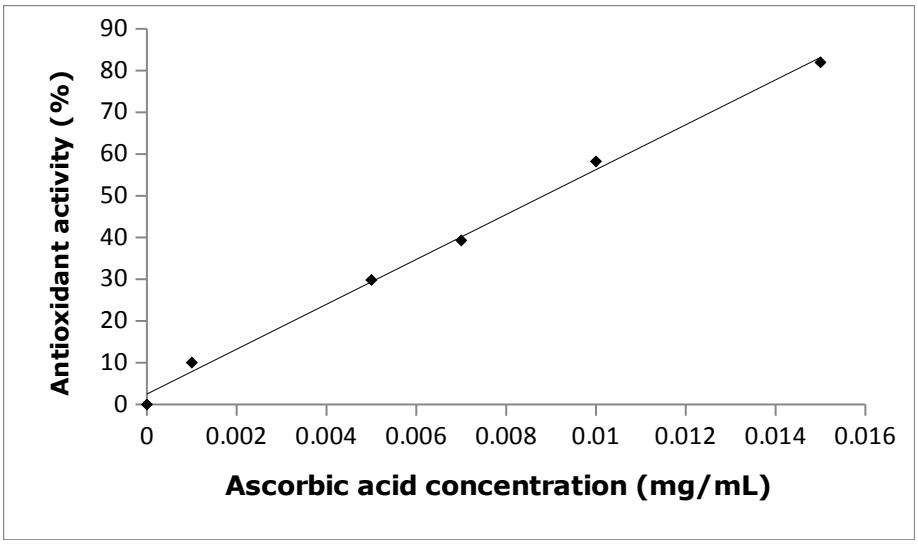


Figure 4: Standard calibration curve for antioxidant activity of ascorbic acid using ABTS assay.

Anticancer Activity of *E. hyssopifolia* Latex against MDA-MB-231 Breast Cancer Cell Line

This part of the research was performed in Atomic Energy Commission of Syria, Biotechnology Department.

Cell culture

MDA-MB-231 cells were purchased from ATCC. The cells were seeded in a six-well culture plate and grown in a humidified incubator (95%) at 37 °C with 5% CO_2. The cell culture medium was RPMI 1640 supplemented with 10% FBS and 1% penicillin/streptomycin. All cell culture chemicals were purchased from Sigma-Aldrich.

Cell cycle analysis by flow cytometry

Cell cycle analysis was performed by PI-based measurements of cell DNA content using flow cytometry. Cells were treated with various concentrations of latex (dissolved in DMSO) for 24 hours, followed by collection of both attached and detached cells. The pellet was rinsed twice with cold PBS and cells were fixed in 70% ice-cold ethanol overnight at 20 °C. Fixed cells were then washed twice with PBS, and DNA was stained with PI (Sigma-Aldrich) staining solution (20 µL of cell suspension were added to 2 mL of staining solution) and incubated in the dark for 5 minutes. Flow cytometry analysis was carried out using BD FACSCalibur Flow Cytometer.

Annexin V/PI apoptosis assay

Cells were cultured ($1x10^6$ cells/mL) overnight in 25 cm^2 cell culture flasks. Then, cells were treated with various concentrations of latex (dissolved in DMSO) for 24 hours. After treatment, both adherent and detached cells were collected and rinsed twice with cold PBS. The cell pellet was resuspended in 1 mL of annexin-binding buffer and incubated with 5 µL of Annexin V-FITC and 5 µL of PI for 15 minutes. The cells were analyzed by flow cytometry and data were analyzed using CellQuest Program. Data sets were expressed as mean ± standard deviation (SD).

Statistical Analysis

Experiments were performed in triplicate. Data were analyzed by SPSS software (version 22) using one-way ANOVA, LSD test. $P<0.05$ was considered statistically significant.

RESULTS AND DISCUSSION

Phytochemical Qualitative Screening of Secondary Metabolites

The results of phytochemical screening of *E. hyssopifolia* extracts showed that the plant contains flavonoids, tannins, alkaloids, diterpenes, steroids, and cardiac glycosides, whereas saponins were absent. Methanolic 70% extract was the richest in secondary metabolites, especially of flavonoids and tannins. On the other hand, alkaloids were found with a moderate presence in the aqueous extract. It is also noted that ethyl acetate extract was the poorest in secondary metabolites; it contains cardiac glycosides only. However, our results are similar to the study (11) which revealed that leaves of the plant contain flavonoids, tannins, alkaloids, and other secondary metabolites, except for the presence of saponins; the study indicated that they were present in the plant leaves, while they were absent in our extracts. This may be due to the difference in the environment of the plant.

Another study (12) indicated the presence of flavonoids and steroids in the ethanolic extract of the plant, which were present also in our methanolic 70% extract (Table 1).

Extraction Yield, Determination of Total Phenolic and Flavonoid Contents

No studies were found focused on the detection of diterpenes and cardiac glycosides in *E. hyssopifolia*. However, *Euphorbiaceae* (in all its genera) is known to contain diterpenes (29).

Results showed that maximum yield was obtained for aqueous extract (13.66% ± 3.37), followed by methanolic 70% extract (10.63% ± 0.12), ethyl acetate extract (2.80 ± 0.50), *n*-hexane extract (1.50% ± 0.26), and latex (0.05% ± 0.01). It is clear that yield of aqueous extract and methanolic 70% extract are significantly higher than other samples, and the yield of latex was the lowest (Table 2).

As for TPC and TFC, the extracts took the same order that they took in the results of the extraction yield; aqueous extract was the richest of these compounds (42.19 ± 0.70 GAE mg/g E for TPC, 35.71 ± 0.21 RE mg/g E for TFC), followed by methanolic 70% extract, ethyl acetate extract, and *n*-hexane extract. The surprising result is that the latex took the second order after the aqueous extract and outperformed significantly the methanolic 70% extract. TPC and TFC of latex were 39.52 ± 0.36 GAE mg/g and 28.66 ± 0.10 RE mg/g E respectively, even though it had the lowest yield (0.05% ± 0.01) (Table 2).

Table 1: Phytochemical screening of *E. hyssopifolia* extracts.

Extract	Flavonoids		Tannins	Alkaloids			Saponins	Diterpenes	Steroids	Cardiac glycosides
	NaOH	Shinoda		Wagner	Hager	Mayer				
Aqueous	++	++	+	++	++	++	-	+	-	-
MeOH 70%	+++	+++	+++	-	+	+	-	+	++	+
Ethyl acetate	-	-	-	-	-	-	-	-	-	++
n-hexane	-	-	-	+	+	+	-	-	-	+

—: absent, +: present, ++: moderately present, +++:abundantly present.

Table 2: Extraction yield, total phenolic (TPC) and flavonoid (TFC) contents of *E. hyssopifolia.*

Sample (extract/latex)	Yield extraction/Latex (%)	TPC (GAE mg/g E)	TFC (RE mg/g E)
Aqueous extract	13.66 ± 3.37	42.19 ± 0.70	35.71 ± 0.21
Methanolic 70% extract	10.63± 0.12	30.88 ± 0.21	17.95 ± 0.17
Ethyl acetate extract	2.80 ± 0.50	0.72 ± 0.05	0.31 ± 0.04
n-hexane extract	1.50 ± 0.26	0.45 ± 0.07	0.11 ± 0.02
Latex	0.05 ± 0.01	39.52 ± 0.36	28.66 ± 0.10

Each value is represented as mean ± SD (n = 3).

To our knowledge, there is no study determining the phenolic and flavonoid contents of *E. hyssopifolia so far*. By comparing our results with those of other *Euphorbia* species, differences were observed. One of these studies determined extraction yield, TPC and TFC in aqueous, methanolic, and ethyl acetate extracts of aerial parts of three *Euphorbia* species namely *E. hirta* L., *E. heterophylla* L. and *E. convolvuloides* Hochst. ex Benth.

Results showed that the yield of aqueous and methanolic extracts was higher significantly than that of ethyl acetate extracts. The values closest to ours are those of *E. convolvuloides*; yield extractions of aqueous, methanolic, and ethyl acetate extracts were 12.25%, 15.83%, and 3.29%, respectively.

The study also revealed that methanolic extracts of the three *Euphorbia* species possessed the highest TFC and TPC except the aqueous extract of *E. heterophylla* yielded the highest TPC (141.90 ± 3.34 mg GAE/g). TPC and TFC of ethyl acetate extracts were differed greatly between the three species; TPC values ranged from 29.61 ± 0.44 to 31.41 ± 0.53 mg GAE/g, whereas TFC values ranged from 3.44 ± 0.46 to 22.36 ± 0.32 mg RE/g (30).

Another study performed in Pakistan showed that ethanolic 50% extract of *E. dracunculoides* had the highest TPC and TFC; 17.35 ± 0.62 mg GAE/g and 7.57 ± 0.42 mg RE/g respectively, whereas *n*-hexane extract had the lowest values; 8.21 ± 0.49 mg GAE/g for TPC and 4.18 ± 0.25 mg RE/g for TFC (31).

Reflecting on our results and those of previous studies, it is clearly noted that extracts with high polarity (aqueous and alcoholic extracts) have higher yield extraction, TPC and TFC compared to extracts with lower polarity.

This is due to the fact that phenolic compounds constitute the largest share of secondary metabolites in plants (32), and they are generally highly soluble in alcohols, except for gallic, cinnamic, and coumaric acids prefer water, dichloromethane, and acetone, respectively (33). However, TPC and TFC in plants varies according to the species, environmental factors, and extraction methods.

E. hyssopifolia latex contained a relatively high TPC (39.52 ± 0.36 mg GAE/g E) and TFC (28.66 ± 0.10 RE mg/g E) compared to other *Euphorbia* species. For reference, a study has shown that TPC and TFC of methanolic extract of *E. tirucalli* latex were 10.50 ± 1.20 mg GAE/g E and 4.30 ± 0.50 mg catechin equivalent/g E respectively (34).

GC-MS Analysis of *E. hyssopifolia* Latex

The present study has identified 26 compounds in *E. hyssopifolia* latex, distributed as follows: triterpenoids (67.017%), phenolic compounds (11.281%), fatty acids and their derivatives (4.187%), steroids (2.647%), sesquiterpenes (0.778%), and other compounds (14.0895%) (Figure 5, Table 3).

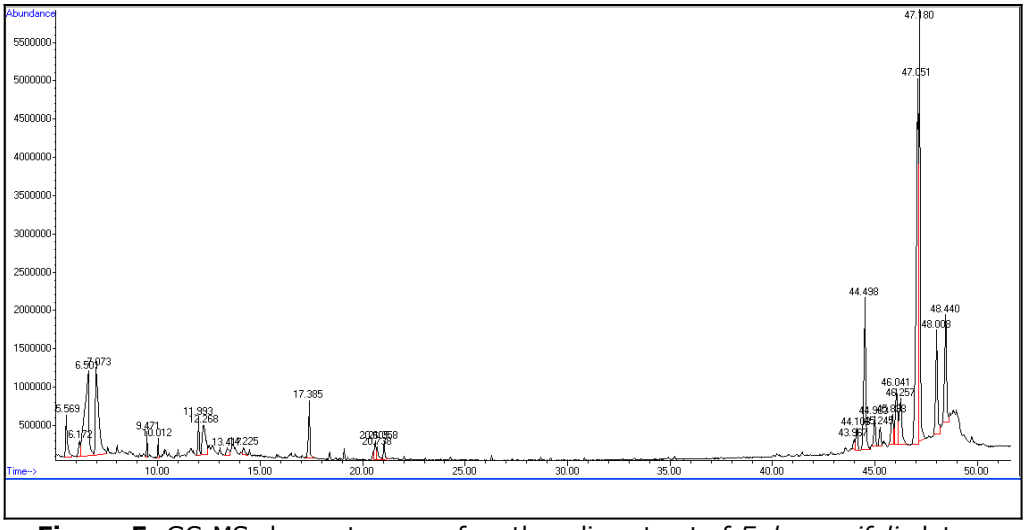


Figure 5: GC-MS chromatogram of methanolic extract of *E. hyssopifolia* latex.

Most of the compounds found in latex have many biological activities. Here, we highlight the major compounds. Lupeol, a pentacyclic triterpenoid, is the most abundant compound in latex (23.7089%). It has antioxidant, antimicrobial, anti-inflammatory, anti-tumor, and anti-cancer activities (35,36). Betulin is the second major compound in latex (found at 14.0098%). It is also a pentacyclic triterpenoid and has anti-tumor and anti-HPV (Herpes simplex virus) activities (37,38). Lanosterol is a triterpenoid found in latex at 7.6304%, and it has antioxidant activity (39).

Table 3: Screening of the compounds identified in methanolic extract of *E. hyssopifolia* latex.

No.	Compound nature	Area %	RT	Compound name
1	Pentacyclic triterpenoid	1.9872	5.5667	2,3-Dihydro-3,5-dihydroxy-6-methyl-4-pyran-4-one
2	Carboxylic acid	0.8068	6.1713	Benzoic acid
3	Organic compound	9.9094	6.4978	4-Hydroxydihydro-2(3H)-furanone
4	Phenolic compound	8.3532	7.0746	Pyrocatechol
5	Sesquiterpene	0.4061	9.4719	β-Caryophyllen
6	Sesquiterpene	0.3718	10.0139	α-Caryophyllene
7	Organic compound	1.1950	11.9942	1-Chlorocyclohexene
8	Phenolic compound	2.9273	12.2652	p-Hydroxybenzoic acid
9	Organic compound	0.6092	13.4187	5-Methyl-3-hexyn-2-ol
10	Organic compound	0.4465	14.2247	7-Methyl-Z-tetradecen-1-ol acetate
11	Saturated fatty acid	2.1633	17.3864	Palmitic acid
12	Monounsaturated fatty acid	0.7225	20.6036	cis-Vaccenic acid
13	Monounsaturated fatty acid	0.6664	20.7356	Oleic acid
14	Saturated fatty acid	0.6354	21.0553	Stearic acid
15	Steroid	0.4331	43.9649	Ergosta-7,22-dien-3.beta.-ol
16	Organic compound	1.1226	44.1108	Garcinielliptone FC
17	Tetracyclic triterpenoid	7.6304	44.4999	Lanosterol
18	Sterol	1.3322	44.9794	Obtusifoliol
19	Steroid	0.8822	45.2435	Ergosta-5,7-dien-3.beta.-ol
20	Triterpenoid of the sterol class	1.9876	45.841	Cycloartenol
21	Pentacyclic triterpenol	3.4377	46.0425	α-Amyrin
22	triterpenoid of the sterol class	3.3992	46.2580	Cycloartenol acetate
23	Pentacyclic triterpenoid	23.7089	47.0501	Lupeol
24	Pentacyclic triterpenoid	14.0098	47.1821	Betulin
25	Pentacyclic triterpenol	5.1167	48.0091	β-Amyrin acetate
26	Triterpene alcohol	5.7397	48.4398	cycloartenylferulate

Pyrocatechol was the most abundant phenolic compound (found at 8.3532%), and it was not previously recorded in other *Euphorbia* species. A study revealed that it has antioxidant and anti-breast cancer activity (40,41). GC-MS analysis showed the presence of another phenolic compound found at (2.9273%), which is p-hydroxybenzoic acid. A study has shown that it has antioxidant activity (42). Cycloartenol and its derivatives are triterpenoids present often in *Euphorbia* species, and they were found in *E. hyssopolia* latex. Cycloartenol was found at 1.9876%). It has antioxidant, anti-inflammatory, and anti-tumor activities (43,44). It should be noted that "Cycloartenyl ferulate" (found at 5.7397%) was not previously recorded in other *Euphorbia* species, and it has antioxidative, anti-allergic, anti-inflammatory, and anti-cancer activities (45). GC-MS analysis also showed the presence of other triterpenes commonly found in *Euphorbia* species, such as, amyrins and their derivatives. α-amyrin (a pentacyclic triterpene) was found at 3.4377%. It has antioxidant, analgesic, and anti-inflammatory activities (46,47). β-amyrin acetate was found at 5.1167%. It has antioxidant, anticonvulsant, anti-inflammatory, and antinociceptive activities (48). Garcinielliptone FC, a derivative of benzophenone, was found in *E. hyssopifolia* latex at 1.1226%. It was not previously recorded in other *Euphorbia* species. It has antioxidant, anti-leishmaniasis and anti-colorectal cancer activities (49-51).

Antioxidant Activity Using DPPH and ABTS Assays

It is known that a decrease in IC_{50} value indicates an increase in the antioxidant activity of the sample. The results of DPPH and ABTS assays appear to be compatible, as both assays indicate that latex significantly outperformed all extracts and ascorbic acid; IC_{50} values of it were 0.029 mg/mL and 0.001 mg/mL in DPPH and ABTS assays, respectively, noting that IC_{50} of ascorbic acid was 0.053 mg/mL and 0.008 mg/mL in DPPH and ABTS assays, respectively. The results of both assays also converge in that methanolic 70% extract came in the second order after latex, noting that its IC_{50} was equal to that of ascorbic acid in ABTS assay (0.008 mg/mL), whereas *n*-hexane extract was the least capable of scavenging free radicals (Table 4).

Table 4: Antioxidant activity of extracts and latex of *E. hyssopifolia.*

Sample (extract/latex)	IC_{50} using DPPH Assay (mg/mL)	IC_{50} using ABTS Assay (mg/mL)
aqueous extract	0.313	0.018
methanolic 70% extract	0.080	0.008
ethyl acetate extract	0.193	0.022
n-hexane extract	2.078	0.081
latex	0.029	0.001
ascorbic acid	0.053	0.008

On the other hand, DPPH and ABTS assays differed in the order of ethyl acetate and aqueous extracts in terms of their ability to scavenge free radicals. IC_{50} of ethyl acetate extract was lower in DPPH assay (0.193 mg/mL), that is, it was better than the aqueous extract which had IC_{50} of 0.313 mg/mL, while the order of the two extracts was reversed in ABTS assay; IC_{50} values were 0.018 mg/mL and 0.022 mg/mL for the aqueous extract and ethyl acetate extract, respectively (Table 4).

Latex's great ability to scavenge free radicals is due to its phenolic and flavonoid contents which are relatively high as previously mentioned. TPC and TFC were 39.52 ± 0.36 GAE mg/g and 28.66 ± 0.10 RE mg/g E respectively (Table 2). It is also due to its content of phenolic and non-phenolic antioxidants that appeared as a result of GC-MS analysis, which are: pyrocatechol, p-hydroxybenzoic acid (phenolic compounds), lupeol, lanosterol, cycloartenol, cycloartenyl ferulate, α-amyrin, β-amyrin acetate, and garcinielliptone FC (non-phenolic compounds). The high effectiveness of methanolic 70% extract in scavenging free radicals is due to its TPC and TFC which were 30.88 ± 0.21 GAE/g E and 17.95 ± 0.17 RE/g E, respectively (Table 2). It is noted that the aqueous extract was the richest of these compounds, but it was not the best as an anti-oxidant (Table 4), which indicates that there is not always a direct relationship between TPC and TFC on the one hand and the antioxidant activity on the other hand. Ethyl acetate and *n*-hexane extracts showed low free radical scavenging ability (Table 4). Referring to Table 1, it is noted that they were poor in bioactive compounds, as Table 2 showed that they contained negligible amounts of total phenols and flavonoids, and therefore flavonoids and tannins did not appear in results of phytochemical qualitative screening (Table 1). However, these results indicate that latex has the highest antioxidant activity, and statistical study confirmed this result.

Anticancer Activity of *E. hyssopifolia* Latex against MDA-MB-231 Breast Cancer Cell Line

Cell cycle analysis

MDA-MB-231 cells were treated with various concentrations of *E. hyssopifolia* latex (1, 3, 5, and 10 µg/mL) for 24 hours. It should be noted that the mentioned concentrations depend on wet weight of latex. Figure 6 showed that the treatment of MDA-MB-231 cells with latex caused a concentration-dependent cell cycle arrest at G1 phase. The percentages of cells in G1 phase increased as a result of the treatment with concentrations (1, 3, 5, and 10) µg/mL by 0.87, 1.42, 1.63, and 2.35 times respectively compared to the percentage of G1 cells in untreated cells (control). In contrast, the percentages of cells in S phase decreased after treatment with the mentioned concentrations by 0.74, 1.38, 1.52, and 2.11 times, respectively, compared to the percentage of cells in S phase in the control. The percentages of cells in G2/M phase also decreased after treatment with the same concentrations by 1.81, 1.06, 0.97, and 1.63 times, respectively, compared to the percentage of cells in G2/M phase in the control. Thus, we conclude that the best results were obtained when cells were treated with a concentration of 10 µg/mL; so that the percentage of cells reached 61% in G1 phase, 21.69% in S phase and 17.31% in G2/M phase. The statistical study confirmed this result; there were significant differences in G1 phase and S phase between the concentration of 10% and other concentrations (Figure 6). These data suggest that treatment with latex induced cell cycle arrest at G1 phase.

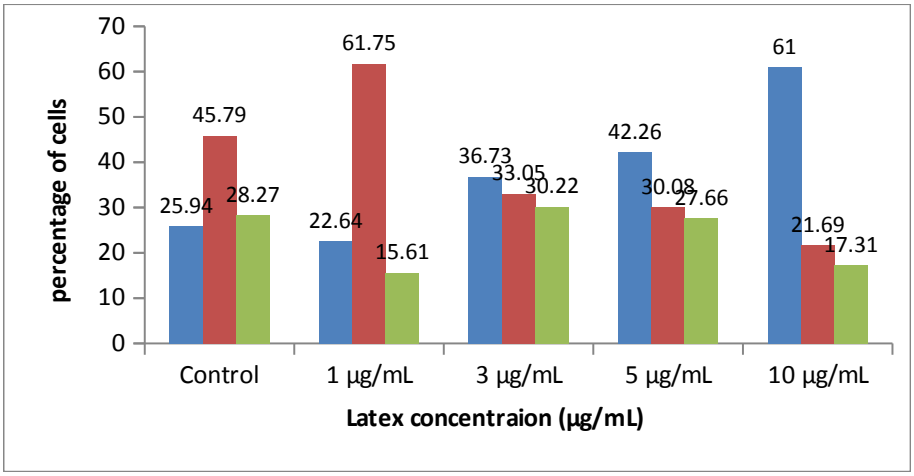
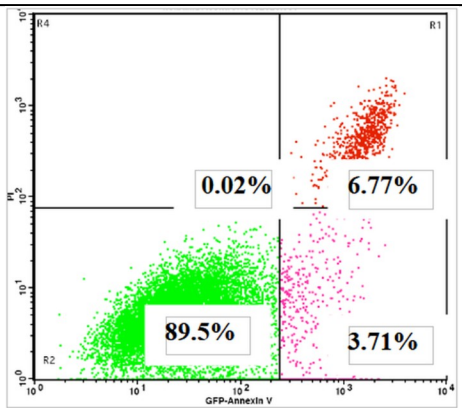
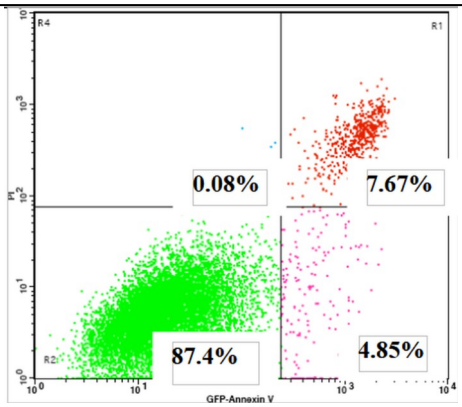


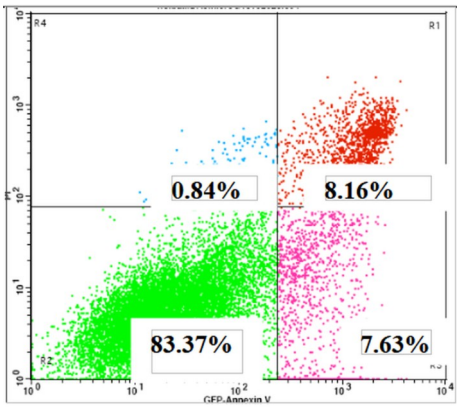
Figure 6: Bar graphs representing cell cycle analysis in cells treated with latex. Data are represented as mean ± SD.



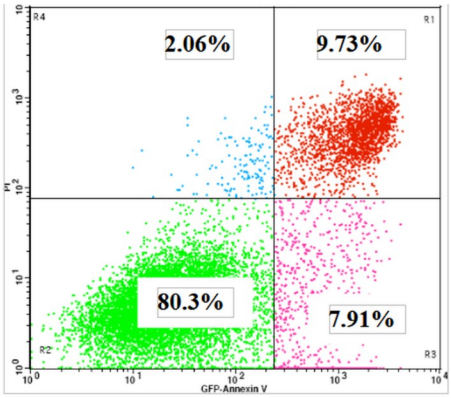
(a)



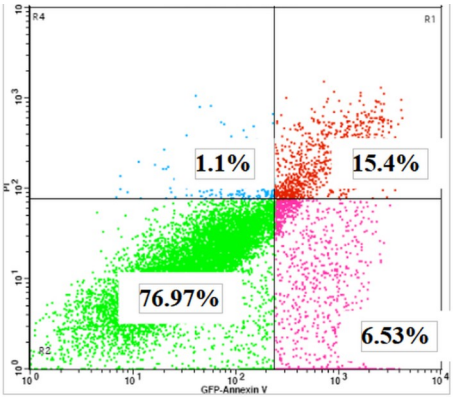
(b)



(c)



(d)



(e)

Figure 7: Fraction of viable, apoptotic, and necrotic MDA-MB-231 cells treated with different concentrations of latex for 24 h. a: control cells, b: cells treated with 1 µg/mL, c: cells treated with 3 µg/mL, d: cells treated with 5 µg/mL, e: cells treated with 10 µg/mL.

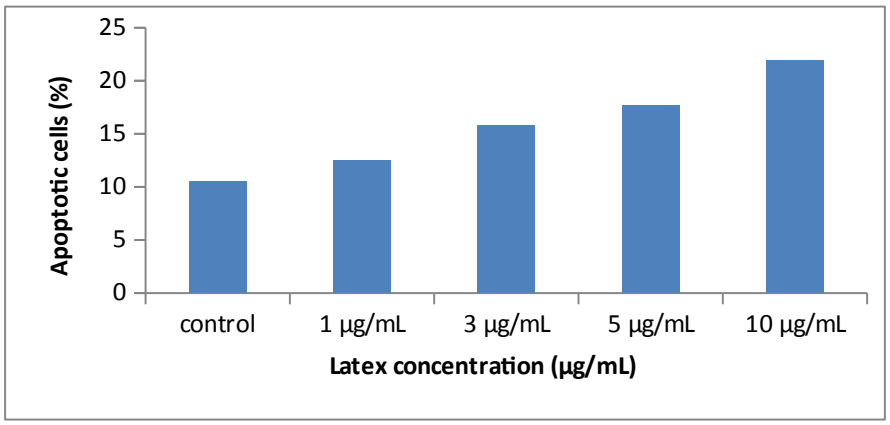


Figure 8: Apoptotic cells treated with *E. hyssopifolia* latex. Data are represented as mean ± SD.

Annexin V-FITC/PI assay confirmed that latex was able to induce apoptosis. As shown in Figures 6 and 7, cells treated with different concentrations of latex (1, 3, 5, and 10) µg/mL showed increasing of percentage of apoptotic cells by 1.19, 1.51, 1.68, and 2.09 times respectively compared to the percentage of apoptotic cells in control.

Annexin V/PI apoptosis assay
Particularly, cells were treated with different concentrations of *E. hyssopifolia* latex (1, 3, 5, 10 µg/mL) for 24 hours, and the double staining Annexin V /PI allowed to measure the percentage of live, apoptotic, and necrotic cells.

Figure 7 shows the percentages of apoptotic cells induced by treatment of MDA-MB-231 cells at different concentrations (0, 1, 3, 5, and 10) µg/mL of E. hyssopifolia latex for 24 hours. It was also observed that the percentage of necrotic cells increased, especially when treated with concentrations 5 and 10 µg/mL, reaching 2.06% and 1.1%, respectively. However, these percentages are relatively low compared to the percentages of apoptosis. Thus, treatment with a concentration of 10 µg/mL is better than treatment with a concentration of 5 µg/mL, because it resulted more apoptotic cells (21.93%) and less necrotic cells (1.1%), which in turn is better than treatment with the rest of the concentrations. The statistical study confirmed our results (Figure 8). The presence of necrotic cells after treatment with latex is due to the fact that Euphorbia species generally contain compounds that induce necrosis (52). The results of treatment with latex appear to be good. This is due to its content of compounds that have anti-cancer activity shown by GC-MS analysis, foremost of which are lupeol (found at 23.7089%) and pyrocatechol (found at 8.3532%) (Table 3). A study showed that lupeol acts as an anticancer agent against MCF-7 breast cancer cells by inducing apoptosis (53). Another study showed that pyrocatechol was able to induce DNA damage, apoptosis and G1 cell cycle arrest in MCF-7 and MDA-MB-231 breast cancer cells (41). Based on the results of the present and previous studies that evaluated anti-cancer activity of Euphorbia species against MDA-MB-231 breast cancer cells, it was found that E. hyssopifolia latex was able to induce apoptosis in a good percentage (21.93%) after treatment with a relatively low concentration (10 µg/mL), noting that the concentration would be less if latex was dried, and within a short period (24 hours). Also, the percentage of necrotic cells was relatively low in our study (1.1%). For reference, the effect of a hydroalcoholic extract of the aerial parts of E. szovitsii on MDA-MB-231 breast cancer cells was studied. Results revealed that treatment with this extract increased the percentage of cells in G1 phase and decreased in S phase with increasing concentration. The study also found that treating cells with concentration of 50 µg/mL for 24 hours induced apoptosis of 20.65% and necrosis of 0.58% compared to the control (54). By comparing the results of this study with ours, it is clear that the percentages of apoptotic and necrotic cells are similar, but the concentration used in our study is lower.

CONCLUSION

The current study showed that E. hyssopifolia contains flavonoids, tannins, alkaloids, diterpenes, steroids, and cardiac glycosides, whereas saponins were absent. Methanolic 70% extract was the richest in secondary metabolites, especially flavonoids and tannins. The maximum yield extraction was obtained for aqueous extract, and it had the highest TPC and TFC. Latex significantly outperformed methanolic 70% extract in terms of TPC and TFC, even though it had the lowest yield. GC-MS analysis led to the identification of 26 bioactive compounds in E. hyssopifolia latex, of which triterpenoids constitute 67.0172%, led by lupeol and betulin. Latex had strong antioxidant activity which outperformed significantly ascorbic acid and all extracts, which indicates that latex is an excellent source of antioxidants. E. hyssopifolia latex induced cell cycle arrest at G1 phase and apoptosis of MDA-MB-231 cells after treatment for 24 hours at relatively low concentrations. However, more studies should be performed to isolate and purify bioactive compounds in E. hyssopifolia, as well as to determine the underlying mechanism its latex anti-breast cancer effects.

CONFLICT OF INTEREST

Authors have no conflicts of interest to disclose.

ACKNOWLEDGEMENTS

All the authors are thankful to Dr. Adnan Ikhtiar (main researcher in Biotechnology Department in Atomic Energy Commission of Syria) for his support and encouragement.

REFERENCES

1. Mabberley DJ. The plant book. Cambridge: Cambridge University Press; 1997.

2. Prenner G, Rudall PJ. Comparative ontogeny of the cyathium in Euphorbia (Euphorbiaceae) and its allies: exploring the organ flower inflorescence boundary. American Journal of Botany. 2007;94(10):1612–29. <DOI>.

3. Muthu C, Ayyanar M, Raja N, Ignacimuthu S. Medicinal plants used by traditional healers in Kancheepuram District of Tamil Nadu, India. Journal of Ethnobiology and Ethnomedicine. 2006;2(1):43. <DOI>.

4. Kitajima S, Kamei K, Taketani S, Yamaguchi M, Kawai F, Komatsu A, et al. Two chitinase-like proteins abundantly accumulated in latex of mulberry show insecticidal activity. BMC Biochemistry. 2010;11(1):6. <DOI>.

5. Mouterde P. Tome II. In: Nouvelle flore du Liban et de la Syrie. Beyrouth: Éd. de l'Impr. Catholique; 1966. p. 475–98.

6. Bolaji AO, Olojede CB, Famurewa AA, Faluyi JO. Morphological and cytological studies of Euphorbia hyssopifolia L. and Euphorbia heterophylla L. from Ile-Ife, Nigeria. Nigerian Journal of Genetics. 2014;28(2):15–8. <DOI>.

7. Ma J-S, Gilbert M. 4. Euphorbia hyssopifolia Linnaeus, Syst. Nat., ed. 10. 2: 1048. 1759. Flora of China; FOC. 2016;11:289–93. <URL>

8. Babojian G. Recordation of Euphorbia hyssopifolia L. (Euphorbiaceae) a new alien Species for Flora of Syria. Damascus University Journal for Basic Sciences. 2018;34(1):121–47.

9. Abo KA. Characterisation of ingenol: an inflammatory diterpene from some Nigerian Euphorbia and Elaeophorbia species. African Journal of Medicine and Medical Sciences. 1994;23(2):161–3.

10. Adedapo AA, Abatan MO, i Olorunsogo OO. Toxic effects of some plants in the genus Euphorbia on haematological and biochemical parameters of rats. Veterinarski Arhiv. 2004;74(1):53–62.

11. Igwenyi IO, Agwor AS, Nwigboji IU, Agbafor KN, Offor CE. Proximate Analysis, Mineral and Phytochemical Composition of Euphorbia Hyssopifolia. IOSR Journal of Dental and Medical Sciences. 2014;13(6):41–3. <DOI>.

12. Araújo S de S, Fernandes TCC, Cardona YT, Almeida PM de, Marin-Morales MA, dos Santos AV, et al. Cytotoxic and genotoxic effects of ethanolic extract of Euphorbia hyssopifolia L. on HepG2 cells. Journal of Ethnopharmacology. 2015;170:16–9. <DOI>.

13. Igwenyi I, Nwachukwu N, Mba O, Offor CE, Aja P, Ugwu O. Hepatoxicity effects of aqueous extract of Euphorbia hyssopifolia on selected tissues of albino rats. Fermentation Technology and Bioengineering. 2011;2:7–10.

14. McGuire A, Brown J, Malone C, McLaughlin R, Kerin M. Effects of Age on the Detection and Management of Breast Cancer. Cancers. 2015;7(2):908–29. <DOI>.

15. Bigoniya P, Shukla A, Singh CS. Dermal irritation and sensitization study of Euphorbia neriifolia latex and its anti-inflammatory efficacy. International Journal of Phytomedicine. 2010;2:3. <DOI>.

16. Yener İ, Ertaş A, Yilmaz MA, Tokul Ölmez Ö, Köseoğlu Yılmaz P, Yeşil Y, et al. Characterization of the Chemical Profile of Euphorbia Species from Turkey by Gas Chromatography–Mass Spectrometry (GC-MS), Liquid Chromatography–Tandem Mass Spectrometry (LC-MS/MS), and Liquid Chromatography–Ion Trap–Time-of-Flight–Mass Spectrometry (LC-IT-TOF-MS) and Chemometric Analysis. Analytical Letters. 2019;52(7):1031–49. <DOI>.

17. Shashikala M, Shah M, Pande M. Determination of Total Phenols and Flavonoid Content of Bryonia laciniosa by Spectrophotometric Method. Journal of Chemical and Pharmaceutical Research. 2018;10(12):30–4.

18. Borah R, Biswas SP. Tulsi (Ocimum sanctum), excellent source of phytochemicals. International Journal of Environment, Agriculture and Biotechnology. 2018;3(5):1732–8. <DOI>.

19. Gonfa T, Teketle S, Kiros T. Effect of extraction solvent on qualitative and quantitative analysis of major phyto-constituents and in-vitro antioxidant activity evaluation of Cadaba rotundifolia Forssk leaf extracts. Yildiz F, editor. Cogent Food & Agriculture. 2020;6(1):1853867. <DOI>.

20. Archana P, Samatha T, Mahitha B, Chamundeswari NR. Preliminary phytochemical screening from leaf and seed extracts of Senna alata L. Roxb-an ethno medicinal plant. Int J Pharm Biol Res. 2012;3(3):82–9.

21. Tadesse G, Reneela P, Dekebo A. Isolation and characterization of natural products from Helinus mystachnus (Rhamnaceae). J Chem Pharm Res. 2012;4(3):1756–62.

22. Das SS. Studies on Qualitative Determination of Phytochemical Constituents and Antimicrobial Activities of Five Mangrove Plants. In: Mangroves of Indian Sundarban: Ecological, Biochemical and Molecular Aspects. Lulu Publication; 2021. p. 31.

23. Kumar Bargah R. Preliminary test of phytochemical screening of crude ethanolic and aqueous extract of Moringa pterygosperma Gaertn. Journal of Pharmacognosy and Phytochemistry. 2015;4(1):07–9.

24. Sawant RS, Godghate AG. Qualitative phytochemical screening of rhizomes of Curcuma longa Linn. International Journal of Science, Environment and Technology. 2013;2(4):634–41.

25. AlHafez M, Kheder F, AlJoubbeh M. Polyphenols, flavonoids and (-)-epigallocatechin gallate in tea leaves and in their infusions under various conditions. Nutrition & Food Science. 2014;44(5):455–63. <DOI>.

26. Karimi A, Mohammadi-Kamalabadi M, Rafieian-Kopaei M, Amjad L, Salimzadeh L. Determination of antioxidant activity, phenolic contents and antiviral potential of methanol extract of Euphorbia spinidens Bornm (Euphorbiaceae). Tropical Journal of Pharmaceutical Research. 2016;15(4):759. <DOI>.

27. Saka F Al, Karabet F, Daghestani M, Soukkarieh C. Composition, in vitro antioxidant and antileishmanial activities of Vitex agnus-castus L. and Thymus syriacus boiss. Essential Oils. International Journal of ChemTech Research. 2015;8(8):53–60.

28. Re R, Pellegrini N, Proteggente A, Pannala A, Yang M, Rice-Evans C. Antioxidant activity applying an improved ABTS radical cation decolorization assay. Free Radical Biology and Medicine. 1999;26(9–10):1231–7. <DOI>.

29. Evans FJ, Taylor SE. Pro-Inflammatory, Tumour-Promoting and Anti-Tumour Diterpenes of the Plant Families Euphorbiaceae and Thymelaeaceae. In: Fortschritte der Chemie organischer Naturstoffe/Progress in the Chemistry of Organic Natural Products. 1983. p. 1–99. <DOI>.

30. Mahomoodally MF, Dall'Acqua S, Sinan KI, Sut S, Ferrarese I, Etienne OK, et al. Phenolic compounds analysis of three Euphorbia species by LC-DAD-MSn and their biological properties. Journal of Pharmaceutical and Biomedical Analysis. 2020;189:113477. <DOI>.

31. Majid M, Khan MR, Shah NA, Haq IU, Farooq MA, Ullah S, et al. Studies on phytochemical, antioxidant, anti-inflammatory and analgesic activities of Euphorbia dracunculoides. BMC Complementary and Alternative Medicine. 2015;15(1):349. <DOI>.

32. Dai J, Mumper RJ. Plant Phenolics: Extraction, Analysis and Their Antioxidant and Anticancer Properties. Molecules. 2010;15(10):7313–52. <DOI>.

33. Galanakis CM, Goulas V, Tsakona S, Manganaris GA, Gekas V. A Knowledge Base for The Recovery of Natural Phenols with Different Solvents. International Journal of Food Properties. 2013;16(2):382–96. <DOI>.

34. Abdel-Aty AM, Hamed MB, Salama WH, Ali MM, Fahmy AS, Mohamed SA. Ficus carica, Ficus sycomorus and Euphorbia tirucalli latex extracts: Phytochemical screening, antioxidant and cytotoxic properties. Biocatalysis and Agricultural Biotechnology. 2019;20:101199. <DOI>.

35. Guo M-B, Wang D-C, Liu H-F, Chen L-W, Wei J-W, Lin Y, et al. Lupeol against high-glucose-induced apoptosis via enhancing the anti-oxidative stress in rabbit nucleus pulposus cells. European Spine Journal. 2018;27(10):2609–20. <DOI>.

36. Gallo MBC, Sarachine MJ. Biological activities of lupeol. Int J Biomed Pharm Sci. 2009;3(1):46–66.

37. Zhao J, Li R, Pawlak A, Henklewska M, Sysak A, Wen L, et al. Antitumor Activity of Betulinic Acid and Betulin in Canine Cancer Cell Lines. In Vivo. 2018;32(5):1081–8. <DOI>.

38. Shamsabadipour S, Ghanadian M, Saeedi H, Rahimnejad MR, Mohammadi-Kamalabadi M, Ayatollahi SM, et al. Triterpenes and Steroids from Euphorbia denticulata Lam. With Anti-Herpes Symplex Virus Activity. Iranian Journal of Pharmaceutical Research : IJPR. 2013;12(4):759–67.

39. Delinassios G. UVA-induced oxidative stress and DNA damage in human skin cells and photoprotection by antioxidant compounds. King's College London (University of London); 2012.

40. Kosobutskii VS. Pyrocatechol and its derivatives as antioxidants and prooxidants. Russian Journal of General Chemistry. 2014;84(5):839–42. <DOI>.

41. Vazhappilly CG, Hodeify R, Siddiqui SS, Laham AJ, Menon V, El-Awady R, et al. Natural compound catechol induces DNA damage, apoptosis, and G1 cell cycle arrest in breast cancer cells. Phytotherapy Research. 2021;35(4):2185–99. <DOI>.

42. Velika B, Kron I. Antioxidant properties of benzoic acid derivatives against Superoxide radical. Free Radicals and Antioxidants. 2012;2(4):62–7. <DOI>.

43. Sawale JA, Patel JR, Kori ML. Antioxidant Properties of Cycloartenol Isolated from Euphorbia neriifolia Leaves. Indian Journal of Natural Products. 2019;33(1):60–4.

44. Zhang Z-L, Luo Z-L, Shi H-W, Zhang L-X, Ma X-J. Research advance of functional plant pharmaceutical cycloartenol about pharmacological and physiological activity. Zhongguo Zhong Yao Za Zhi= Zhongguo Zhongyao Zazhi= China Journal of Chinese Materia Medica. 2017;42(3):433–7.

45. Hong G-L, Liu J-M, Zhao G-J, Wang L, Liang G, Wu B, et al. The reversal of paraquat-induced mitochondria-mediated apoptosis by cycloartenyl ferulate, the important role of Nrf2 pathway. Experimental Cell Research. 2013;319(18):2845–55. <DOI>.

46. Romero-Estrada A, Maldonado-Magaña A, González-Christen J, Bahena SM, Garduño-Ramírez ML, Rodríguez-López V, et al. Anti-inflammatory and antioxidative effects of six pentacyclic triterpenes isolated from the Mexican copal resin of Bursera copallifera. BMC Complementary and Alternative Medicine. 2016;16(1):422. <DOI>.

47. Simão da Silva KAB, Paszcuk AF, Passos GF, Silva ES, Bento AF, Meotti FC, et al. Activation of cannabinoid receptors by the pentacyclic triterpene α,β-amyrin inhibits inflammatory and neuropathic persistent pain in mice. Pain. 2011;152(8):1872–87. <DOI>.

48. Duke JA. Dr. Duke's Phytochemical and Ethnobotanical Databases. 1992.<URL>.

49. Júnior JSC, Ferraz ABF, Feitosa CM, Cito A, Freitas RM, Saffi J. Evaluation of antioxidant effects in vitro of garcinielliptone FC (GFC) isolated from Platonia insignis Mart. Journal of Medicinal Plants Research. 2011;5(2):293–9.

50. Costa Júnior JS, de Almeida AAC, Ferraz A de BF, Rossatto RR, Silva TG, Silva PBN, et al. Cytotoxic and leishmanicidal properties of garcinielliptone FC, a prenylated benzophenone from Platonia insignis. Natural Product Research. 2013;27(4–5):470–4. <DOI>.

51. Won S-J, Lin T-Y, Yen C-H, Tzeng Y-H, Liu H-S, Lin C-N, et al. A novel natural tautomeric pair of garcinielliptone FC suppressed nuclear factor κB and induced apoptosis in human colorectal cancer cells. Journal of Functional Foods. 2016;24:568–78. <DOI>.

52. Huerth KA, Hawkes JE, Meyer LJ, Powell DL. The Scourge of the Spurge Family—An Imitator of Rhus Dermatitis. Dermatitis. 2016;27(6):372–81. <DOI>.

53. Pitchai D, Roy A, Ignatius C. In vitro evaluation of anticancer potentials of lupeol isolated from Elephantopus scaber L. on MCF-7 cell line. Journal of Advanced Pharmaceutical Technology & Research. 2014;5(4):179. <DOI>.

54. Asadi-Samani M, Rafieian-Kopaei M, Lorigooini Z, Shirzad H. The effect of Euphorbia szovitsii Fisch. & C.A.Mey extract on the viability and the proliferation of MDA-MB-231 cell line. Bioscience Reports. 2019;39(1):1–8. <DOI>.