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Cytotoxic Activity of *Ephedra alata* Extracts on Human Lymphocytes and Breast Cancer Cell Line *in Vitro*

L. Z. Mohammed*, R. K. Mohammed
Department of Biology, Collage of science, University of Baghdad, Baghdad, Iraq

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ABSTRACT

This study was conducted to use the local *Ephedra alata* plant as a model for extracting and detecting alkaloids in the stem of plant (alkaloids-rich extract and crude extract). Different extraction procedures were adopted for qualitative as well as the quantitative examination of the alkaloid extracts, as well as plant crude extract, the *best methods* for the extraction of the plant materials were applied. Simple, fast and accurate methods like TLC (thin layer chromatography) and HPLC (High-performance liquid chromatography), were used for the identification of the alkaloids (ephedrine) in different extracts of stems *E. alata* stems. Ephedrine alkaloid was detected in each alkaloids-rich and crude extracts of *E. alata* stems with 8 and 5 ppm concentrations respectively.

The assessment of plant stems extract was carried out as these extracts have effects on human breast cancer cell lines (MDA-MB-231) and normal human lymphocytes (HLs). The study showed that there was no risk in using Ephedra in medicines as it has low cytotoxicity on HLs, while on MDA-MB-231 cell lines the percentage of cell death was 50.11% at a concentration 75mg/ml for crude extract after the 72-hours incubation period and the concentration of 15 mg/ml during the 24-hours incubation period had 50.63% inhibition rate.

The study showed that *E. alata*, an important medicinal plant, has alkaloids-rich extracts that have low cytotoxic effects on cultured HLs, particularly when used at a concentration of 5 mg/ml considering its use at low concentrations, the local *Ephedra alata* plant has promising effects on cancerous lines.

Keywords: *Ephedra alata*; Alkaloids, Ephedrine, HPLC; TLC, MDA, HLs**.**

النشاط السام للخلايا لمستخلصات نبات العلندة على الخلايا اللمفاوية البشرية وخط خلايا سرطان الثدي خارج الجسم الحي

لبنى زهير محمد *, رشا كريم محمد
قسم علوم الحياة, كلية العلوم, جامعة بغداد، بغداد، العراق

الخلاصة :

تم اجراء الدراسة على النبات المحلي (العلندة) *Ephedra alata* كنموذج لاستخلاص المادة الخام والمستخلص القلويدي وتم اختيار افضل الطرق لاستخراج المواد النباتية بالاضافة الى الكشف عن مادة الافدرين

*Email: diyana.mushtaq1995@gmial.com

في المستخلص الغني بالقلويدات والمستخلص الخام خلال اجراء الفحص النوعي والكمي لمستخلصات القلويد وكذلك المستخلص الخام النباتي وتم استخدام طرق بسيطة وسريعة ودقيقة بواسطة TLC (كروماتوغرافيا الطبقة الرقيقة) و HPLC (كروماتوغرافيا سائلة عالية الأداء). لوحظ قلويد الايفيدرين في كل مستخلص غني بالقلويدات والمستخلص خام من *E. alata* بتركيزات 8 و 5 جزء في المليون على التوالي. وتم دراسة تاثير خلاصات نبات العلندة على خطوط خلايا سرطان الثدي البشرية (MDA–MB–231) والخلايا الليمفاوية البشرية الطبيعية (HLs). أظهرت الدراسة أنه لا يوجد خطر من استخدام الإيفيدرا في الطب ولديها نسبة منخفضة من السمية الخلوية على HLs ، بينما في خطوط الخلايا MDA–MB–231 كانت النسبة المئوية لموت الخلايا 50.11٪ عند التركيز 75 مجم \ مل للمستخلص الخام في فترة الحضانة لمدة 72 ساعة و كان للتركيز 15 مجم\ مل خلال فترة الحضانة التي تبلغ 24 ساعة معدل تثبيط 50.63%.

لقد أظهرت الدراسة أن *E. alata* ، نبات طبي مهم ، يحتوي على خلاصة غنية بالقلويدات والتي لها تأثيرات سامة منخفضة للخلايا على HLs المستنبتة ، خاصة عند استخدامها بتركيزات 5 مجم\ مل, كما ان نبات العلندة له تأثير واعد على السلالات السرطانية ، مع مراعاة استخدام التركيزات المنخفضة.

INTRODUCTION

Many secondary metabolites such as alkaloids, terpenoids and phenylpropanoids are being considered for drug development. From time immemorial, the use of medicinal plants has always been part of human cultures. However, due to lack of access to better healthcare, affordable orthodox drugs and other factors, indiscriminate use of herbal preparations in developing countries has become common [1]. Many types of active compounds in plants have anticancer or cytotoxic activities like flavonoids, phenols, triterpenoids and vitamins (C, A and E) [2].

Ephedrine, pseudoephedrine, and nor pseudoephedrine are the main chemical compounds that have been identified and isolated from *Ephedra* extract as an alkaloids group [3]
The biological activity of the plant has indicated that the extract from aerial parts of the *Ephedra* species has antimicrobial, antioxidant activities and antiulcer properties [4].
In recent years, researchers have brought into light the importance of using some medicinal or toxic herbs in an attempt to treat cancer, hence drawing attention to the existing 250,000 plants species in nature of which more than 1,000 vincristine containing plants have effective anticancer compounds, including a substance that has been used effectively in treating breast and skin cancers.

Currently more than 50% of drugs are known as natural products that have the ability to control cancer cells through their effects on DNA division mechanisms (Cellular or pre-split such as DNA multiplication) [5].

E. alata is widely used as natural remedy in Palestine as well as in many Mediterranean countries [6]. Ephedra species are traditionally used to diminish obesity, decrease the blood glucose and to control blood pressure as diuretic [7] just as other medicinal plants that are traditionally used to treat cardiovascular diseases, and digestive system imbalances.
Various crataegus species are used as a remedy for cancer, diabetes and heart problems [8]. Other therapeutic mechanisms include antioxidant, antimicrobial, anti-inflammatory and antidiuretic activities [9, 10].
E. alata plant extract has revealed strong cytotoxic activity against the THP-1 and HepG2 cells [11].

The objective of the present study was to investigate the chemical composition of various extracts of *E. alata* and to correlate the chemical profile with the cytotoxic activity on normal

human lymphocytes and anticancer properties on MDA-MB 231 breast cancer cell line.

Material and Methods:

1. Plant material preparation:

The plant was collected from Al-Muthanna Governorate during October 2022 and was authenticated at Directorate of Seed Testing and Certification at the Ministry of Agriculture. The plant stems were washed and dried for 15 days at room temperature and grounded using an electric mill and weighed.

2. Extraction method:

1. Crude extract preparation.

Three hundred grams of the plant stems were ground and dissolved in 500 ml of ethanol: water (4:1 mixture) and then dried at room temperature to obtain a residual concentrated extract of 14 g, bringing the yield to 4.6% [12].

2. Alkaloids Extraction from *E. alata* Stems:

Minced fresh plant stems (250 gm) underwent intensive extraction with n-hexane for defatting. After that, the plant was extracted with 1000 ml of 5% hydrochloric acid for 24 hours and filtered twice. The acidic medium was mixed with chloroform ($CHCl_3$; 4 × 4 L) in a separation funnel to extract the weak base compounds. The aqueous phase of that mixture was evaporated to give a light brown residue (300 mg), subjected to the Dragendorff test, and unmerited as fraction number 2 [13].

The acidic medium was alkalized with NH_3OH to pH 10 and the white residue was precipitated and isolated separately (5g). Dragendorff test was performed on it and anointed as a fraction (e). This fraction had the most alkaloidal yield of the extraction. Basified medium was thoroughly extracted with chloroform ($CHCl_3$; 4 × 4 L) and the aqueous phase was evaporated to obtain 56 mg of dark brown residue. The obtained extract was Dragendorff test positive and was labeled as fraction 1. All fractions with crude extract were subjected to thin layer chromatography on neutral Al_2O_3 [14].

Detection of Alkaloids (Dragendroff test):

Sixty milligrams of Bismuth subnitrate was dissolved in 0.2ml HCL as solution (A). Another solution (B) contained 600 mg potassium iodide dissolved in 1 ml distilled water. Both solutions [A and B] were mixed before adding each extract sample [15].

3. Thin Layer Chromatography:

Ready-made 20x20 cm aluminum oxide TLC plates with 0.25mm thickness were used for routine work. Four solvent systems were used as shown in Table 1

Table 1: Different types of solvent used in the experiment

S1	S2	S3	S4
Methanol 100 ml	**Methanol 50 ml**	**N_butanol 40 ml**	**Ethanol 50 ml**
HCL 2 ml	**Ethanol 50 ml**	**A.A 10 ml**	**Methanol 50 ml**
—	**Hcl 2 ml**	**H20 5 ml**	**A.A 2 ml**

2. Separation and Purification of Alkaloids with TLC (Thin Layer Chromatography):

TLC is an easy, quick and an efficient process that provides the chemist with an immediate answer regarding the number of components in a combination. When the Rf of a chemical is

compared with the Rf of a known compound, TLC is used to establish the identity of a component in a mixture. In order to achieve a high level of saturation solvent separation system was placed in a covered jar and preferably lined with the filter paper in contact with the solution on the inner wall of the jar (Table 1). It appeared that the medium s4 was the best at obtaining more than one band when it came to achieving it under UV light (254 and 366) on preparative aluminum oxide TLC plates as in the standard reference. Little amounts of each fraction (E), (1), (2) and crude obtained from the extraction were dissolved in ethanol and then passed with the ephedrine standard purchased from BDH-England in more than one mobile phase [16]. The study of the extracts used four developing systems (S1–S4). Among all fractions, only S4 demonstrated the presence of ephedrine alkaloids.

The standard ephedrine (~1 µL) was put onto the aluminum oxide TLC plates (20 cm × 20 cm, height x weight), spaced 10 mm apart and 1/2 cm from the bottom. The plate was developed to a distance of 15 cm inside a TLC developing chamber that had been saturated with ethanol, methanol and acetic acid (5:5:0.2v/v/v) as S4 for 25 minutes.
The rf value was calculated by using the following equation:
RF value = Distance from baseline to spot / Distance from baseline to solvent front.

3. High Performance Liquid Chromatography (HPLC):

Ephedrine standard and each extract test were diluted to 0.01 mg in a 25 ml volumetric flask of distilled water. A Shimadzu LC- 6A high-performance liquid chromatography system with 6A-UV spectrophotometers was used to measure the samples. Concentration (ppm) = (sample area/standard model area) x standard concentration was used to analyze the samples at 254 nm in comparison to extraction samples in terms of peak area and retention duration [17].

Preparation of Serial Dilution:

Serial dilutions were made from the greatest extract yield in a series (normal whole plant crude and alkaloids rich extract).
The formula used for calculating a dilution was (C1) (V1) = (C2) (V2) where:
•C1 is the concentration of the starting solution.
•V1 is the volume of the starting solution.
•C2 is the concentration of the final solution.
•V2 is the volume of the final solution

Lymphocyte Isolation and Culture:

Lymphocytes were isolated from the healthy individuals according to method used in Biotechnology Research Center, Nahrain University. To achieve pure cells, the lymphocytes from healthy persons were collected and centrifuged repeatedly for 30-45 minutes. The EDTA tube containing 5 ml of blood and phosphate saline solution (PBS), a balanced solution used to keep cells out of natural and fast-growing habitats was then added. Around 5 ml of the mixture (PBS + blood) was added to another 5 mL container of Ficoll solution (white blood cells and red blood cells) and then centrifuged. Layers of the sample were noted to be arranged top to bottom as following: plasma and other components; the transparent casing layer of monochrome nuclei; red blood cells; and granular cells. After washing, the lymphocytes were repeatedly harvested to obtain only pure cells. Before removing the cells into a new flask and in order to support the growth of living cells, RPMI-1640 containing L-Glutamine 9% and 1% Fetal bovine were added to the live cells serum. It was then incubated at 37°C for 48 hours with 5% CO_2 and 95% relative humidity [18].

Breast Cancer MDA-MB-231 Cell Lines Culture:

The cell line was taken from the Biotechnology Research Center, Nahrain University. To work subculture for MDA-MB-231 cell line, the cells were taken from the incubator and exhaled off the media before checking confluence of cells and removing spent medium.

The flask was washed with PBS solution and discharged. The amount of trypsin-EDTA was added until the surface area was sufficiently covered and resuspended in serum-containing media, and then transferred to the culture flask and passage cells and resuspend into another serum containing media (17 times) before centrifuging and aspirating supernatant. resuspend cells in (10% Dimethyl sulfoxide + 90% fetal bovine serum). The cells were distributed into a new flask, according to the recommended ratio of the selected cell line before observing the cells under the microscope and then returned to the incubator at 37°C with 5% CO2 [19].

Cytotoxicity Assay:

For both MDA-MB-231 and lymphocyte cell lines, the cells were seeded into 96-well plates at a starting density of 1×10^4 cells/well and cultured overnight in humidified and 5% CO2 atmosphere at 37°C. The cells were treated after modifying their viability after incubation time (24 and 72 hr). Next the medium was removed from the wells and incubated for 3 hours with 0.5% water soluble mitochondrial dye (3-(4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide (MTT+) dye. 20 µL of serum-free media and 20 µL of MTT solution into each well then added. Afterwards, 60 µL of MTT solvent (DMSO) then was finally added into each well

The optical density was determined by an ELISA reader at 590 nm wavelength. The percentage of cell inhibition was then measured by applying the following equation:
Cytotoxicity (Growth Rate inhibition %) = control-treated cell/control *100 [20].

Morphology Examination:

Inverted light microscopic observation of the ephedra alkaloids plant extract at (15 mg/ml) alkaloids extract 24 hr treated MDA-MB-231 cell line showed typical cells which were treated and stained with crystal violet only (original magnification x200) and captured by iPhone eight plus [21].

Statistical Analysis:

The Statistical Package for Social Sciences (SPSS) software, version 25 was used for the purpose of analyzing the comparisons between totals, concentrations and control, as well as the analysis of descriptive statistics for the study samples using Kruskal-Wallis test [22].

Results and Discussion:

Table 2 shows the Dragendroff reagent results. All fractions passed the test. These results are consistent with another study [23].

Table 2: Dragendroff test for different extraction fractions

Extraction Fraction	Dragendroff Test
Crude fraction	+
Fraction 2	+
Fraction 1	+
Alkaloids precipitate fraction(E)	+

TLC results:

Different extraction techniques were used to examine the alkaloids qualitatively and quantitatively, and to find the ephedrine alkaloid in each extract. The overall approach was

determined to be the most effective way to extract plant resources. Under the specified chromatographic conditions, the developed TLC plate was air-dried at room temperature utilizing an image analysis approach, as shown in Figure 1. Observation and recording of the plates were carried out at 254 and 366 nm accordingly [24]. As shown in Figure 1, the TLC spots of each component could be used as markers to make them easier to distinguish in the future.

All results from crude were above the standard ephedrine (s) Rf value, with the exception of result number 7 which was similar to the standard ephedrine. The recorded maximum Rf value of the crude extract was 0.8 and the lowest recorded value was 0.4.
The highest Rf value of crude extract was 0.8 and the lowest was 0.4; all results from crude were below the standard ephedrine (s) Rf value, with the exception of result number 7, which was similar.

While number 3 of the fraction (E and 1) was similar to the Rf value (S) of ephedrine and number 1 and 2 were detected to be different compounds, ephedrine and other alkaloids were isolated in other research [25]. Number 1 of the fraction 2 was in (0.8) Rf value, while number 2 and 3 were in a different range, indicating other unknown compounds, as can be seen in Table 3.

Table 3: Represents Rf values of samples as compared with the standard reference in s4 solvent system

Ephedrine	Crude extract		(E) Fraction	(1) Fraction	(2) Fraction
0.806	1	0.467	0.21	0.12	0.806
	2	0.516	0.72	0.72	0.725
	3	0.56	0.806	0.806	0.677
	4	0.612	---	---	---
	5	0.677	---	---	---
	6	0.777	---	---	---
	7	0.8	---	---	---

HPLC (High Performance Liquid Chromatography):

In the modern pharmaceutical industry, high-performance liquid chromatography (HPLC) is the major and integral analytical tool applied in all stages of drug discovery, development and production.

First ephedrine standard components are injected in an HPLC device to identify a peak area. The retention and height of the peaks of standard are calculated by using a column type (C18) (2.1mm*50mm) dimension and (Acetonitrile / Sodium citrate) mobile phase solution. The resulting sample of crude and alkaloids from ephedra plant which had the most extraction yield, were combined with the resulting standard solution (Figure 2) packages under the same conditions before measuring the complex concentrations.

The mobile phase was used for 40 minutes under isocratic conditions, and ephedrine standard was detected at 4.789 min with peak area = 405897, height = 13546 (Figure 2), which have the maximum peak area regarding to peak at 2.373 min which were detected as impurities. The alkaloids extract sample detected the presence of ephedrine on 5.212 min (peak area =7905, height = 385). Crude extract sample detected the presence of ephedrine alkaloid at 5.363 min ((peak area =4946, height = 246) by comparing the retention time and absorbance spectrum of

standard. Figure 2 shows the difference in retention time to be15.64 min just as other previous research [26].

According to a statistical calculation, the curve area of alkaloid rich crude extract had higher ephedrine than regular unpurified plant crude extract, giving values of 1.94% for alkaloid rich extract and 1.2% for normal plant crude extract, each equaling 8 and 5ppm respectively.

Table- 4: Quantitative study of ephedrine alkaloids in stem crude extract and alkaloid extracts.

Extract	Extract Weight	Area of Sample	Area of Standard Ephedrine	Concentration of Ephedrine for Each Sample
Crude extract	14gm	4946	4058097	5 ppm
Alkaloids _ extract	5gm	7904		8 ppm

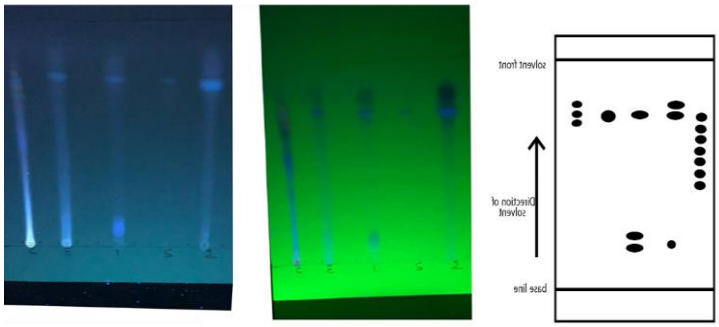

Figure 1: TLC separation of ephedrine in 4 different extraction fractions.

(a) (b)

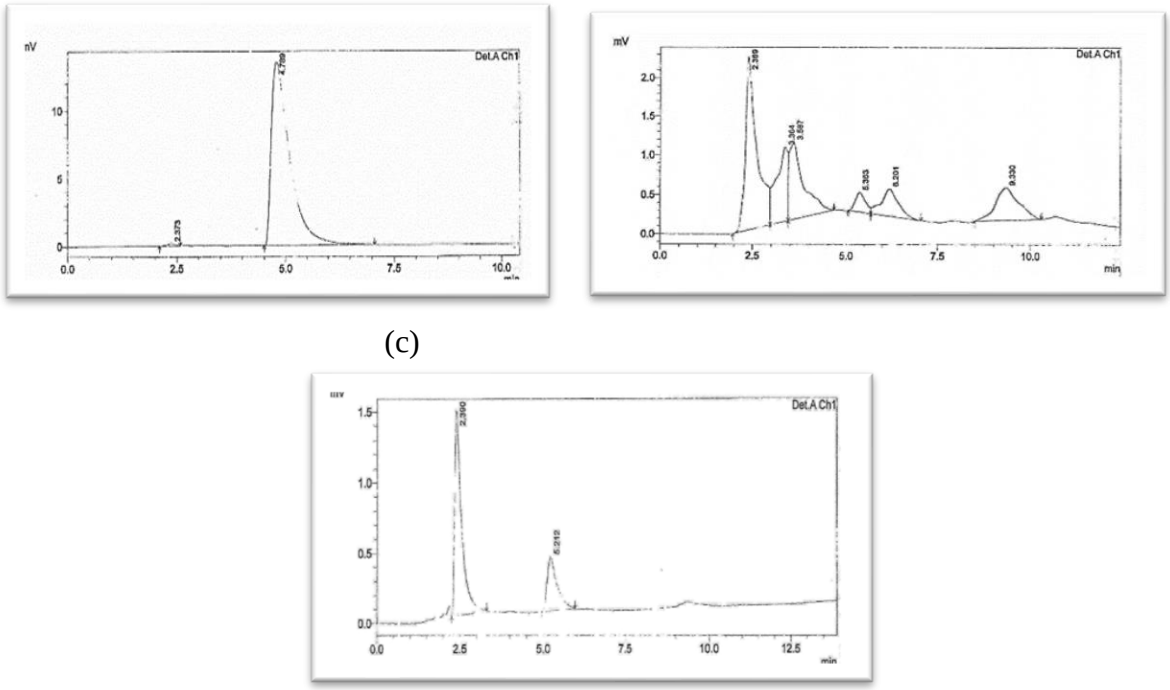

Figure 2: HPLC chromatogram for (a) ephedrine, (b) alkaloids extract(E), (c) crude extract

Cytotoxic activity on HLs:

Cytotoxic effects of different concentrations of the extract on HLs were assessed by MTT assay tests. During the study it was noted that inhibition rate increased with an increase in the concentration of each extract and standard ephedrine (Table 5). It was also noted that the highest value was found in the crude extract of ephedra plant at 75 mg/ml, where the inhibition rate was 8.25% and 7.09% for each of the 24 and 72hr incubation periods respectively. The inhibition rate increased significantly with each concentration of crude extract at 24 and 72hr (Table 5). Least inhibition rate (IR) value was found at 5 mg/ml concentration for standard ephedrine (2.10%) (Table 5). Other studies have recorded that the viability in human lymphocytes decreases depending on the concentration [27].

Alkaloids showed IR of 24 and 72hr treatment for all 5 concentrations and, have been indicated by reports from other authors that HLs do not significantly impair the viability of treated cells which detect low cells death potential at the tested concentrations. It is likely that at the highest concentration tested some of the pro-oxidative constituents that were present in amounts capable of inducing higher cell death. As mentioned in a previous research, it seems that plant alkaloidal containing extracts presented better protective toxicity effects [28]. Some alkaloid compounds are toxic to several organisms and others are used as pharmacological drug [29].

At 24hr incubation period, least IR value of 2.10% was found at 5 mg/ml concentration for standard ephedrine (Table 5). The current study showed that pure ephedrine had less cytotoxic effects for all 5 concentrations after cell treatment which had a significant difference between both plant extracts, except at 75 mg/ml after 72hr incubation period which had no significant difference (Table 5). Another research showed that that short-term exposure to ephedrine does not induce genotoxic effects in the comet assay. Bearing in mind previous study on ephedrine genotoxicity and this study on ephedrine cytotoxicity, probably there is no risk in using Ephedra in medicine [30].

Table 5: Cytotoxic effects of ephedra plant extract and ephedrine on human lymphocyte at 24

h and 72 h in incubation time.

Con. mg/ml	Growth Inhibition % / 24hr			Growth Inhibition % / 72hr		
	Crude	Alkaloid	Pure-ephedrine	Crude	Alkaloids	Pure-ephedrine
5	4.51	3.15	2.17	5.45	3.38	2.99
15	5.30	4.61	3.51	5.78	5.00	4.14
35	7.15	6.09	4.56	7.86	6.21	5.62
55	8.20	6.65	5.81	6.50	6.93	6.01
75	8.25	7.08	6.07	7.10	7.14	6.67
p-value	P≤0.01			P≤0.01		

Test Statistics[a,b]	IR	**Test Statistics[a,b]**	IR
Kruskal-Wallis H	107.169	Kruskal-Wallis H	94.326
df	14	df	14
Asymp. Sig.	0.000002	Asymp. Sig.	0.00003

Cytotoxic Activity on MDA-MB-231 Cell Lines and Breast Cancer Cell Line:

The results of the MTT assay showed that the stock extract of the *E. alata* plant was cytotoxic against the MDA-MB-231 cell line when compared to all of the concentrations that were examined (5, 15, 35, 55, and 75mg/ml) of the three solvents, though at varying rates of inhibition in 24 and 72 hours. The statistical analysis indicated that the concentrations increased cytotoxic effects on MDA-MB-231 after 24 and 72 hours incubation periods at levels (P≤0.01) [31, 32].

The cytotoxicity was higher and inhibition rate increased in standard ephedrine more than both alkaloids and crude extracts. The highest inhibition rates of 80.09% and 74.17% were noted for the extracted alkaloids at a concentration of 75 mg ml during the 72-hour incubation period. Both rates, however, gradually decreased during the 24-hour incubation period (P≤0.01) (Table 6).

It was noted that the percentage of cell death was 50.11% at a concentration of 75mg/ml for crude extract after the 72-hour incubation period and the concentration of 15 mg/ml during the 24-hour incubation period had 50.63% inhibition rate. The lowest killing rate was observed at the concentration of 5 mg of the crude extract which gave the lowest killing rate of the standard alkaloid and ephedrine extract.

The microscopic examination with crystal violet showed that the exposure of MDA-MB-321 *E. alata* alkaloids extract for 24 hours led to an increasing number of dead cells in a concentration-dependent manner (Figure 3). At a concentration of 15mg /ml this fraction inhibited MDA-MB-321 cells by 50.63%.

In the current study high concentration was used which could have missed the effects at lower doses (i.e., 1mg/ml and below). Therefore, the study on lower concentrations of the *ephedra* plant to know its anti-tumor effect on MDA-MB-231 cell lines is highly recommended.

The species and the solvents employed for extraction are two aspects that can have an impact on the cytotoxic effect. For instance, in south-eastern Europe, breast cancer patients receive

treatment using extracts of *E. foeminea* and *E. alata* [33]. The chloroform extract of *E. viridis* exhibits cytotoxic action against leukemia cells, while *E. alata* extract has no cytotoxic impact against human liver cancer or the leukemia cell line [34]. Alcoholic extracts have a mild cytotoxic effect against melanoma and non-cancer cell lines [35]. According to the American National Center Institute, the results for the MCF-7 and PC-3 cancer cell lines suggest encouraging anticancer properties for potential drug research and development [36]. Ephedra herb reduces HGF-induced cancer cell motility by preventing c-Met phosphorylation by inhibiting its tyrosine kinase activity [37].

The differences in MDA and HLs cell lines response toward different treatments for different durations (24, 72hr) which might indicate a presence or absence of specific cellular receptors in each type of cell lines; making the cells interact at same concentration in different manner.

Table 6: Cytotoxic effects of ephedra plant extract and ephedrine on MDA-MB-231 breast cancer cells at 24hr and 72hr incubation times.

Con. mg/ml	24hr			72hr		
	Crude	Alkaloid	Pure Ephedrine	Crude	Alkaloid	Pure Ephedrine
5	31.32	41.56	60.95	33.84	46.24	50.20
15	36.02	50.62	62.86	40.07	55.87	50.79
35	41.07	53.28	62.61	42.68	54.30	61.69
55	43.40	64.85	71.76	44.14	68.38	75.05
75	46.77	67.14	75.89	50.11	74.17	80.09
p-value	P≤0.01			P≤0.01		

Test Statistics[a,b]	IR	**Test Statistics[a,b]**	IR
Kruskal-Wallis H	114.414	Kruskal-Wallis H	110.797
df	14	df	14
Asymp. Sig.	0.000	Asymp. Sig.	0.000

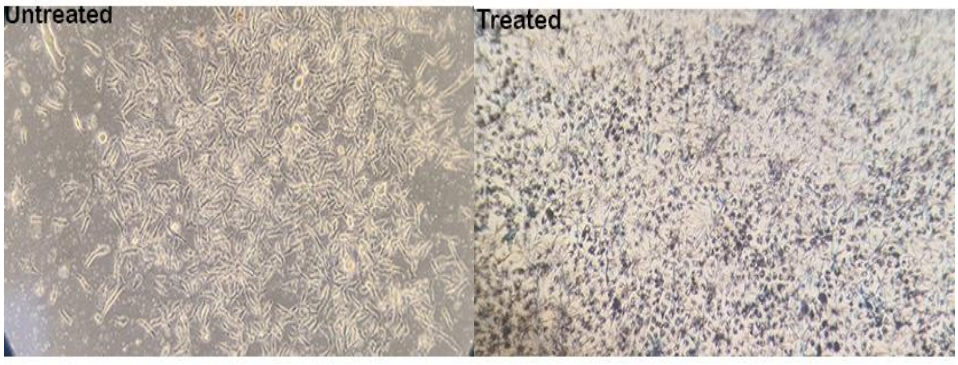

Figure 3: The MDA-MB-321 cells were viewed on an optical microscope before and after treatments, finding greater cellular damage with (15 mg/ml) *E. alata* alkaloids extract for 24 h duration, scale bar 10µm.

Conclusions:

TLC technique was used for pre-indication and semi-purification of compound in alkaloids as ephedrine of *E. alata* plant extract. Alkaloids-rich crude extract was obtained through several

extraction methods and detected through the HPLC device which indicated the presence of ephedrine alkaloids. Higher percentages of alkaloids compounds such as ephedrine can be more purified by providing newer conditions and extraction techniques. The current study showed that *E. alata*, an important medicinal plant, has alkaloids-rich extracts that have low cytotoxic effects on cultured HLs, particularly when used at a lower concentration of 5 mg/ml. Hence, considering the use of low concentrations, the local *Ephedra alata* plant has promising effects on cancerous lines.

Additionally, it is necessary to evaluate the antibacterial, antioxidant and cytotoxic capabilities of other sections of the investigated plant. Moreover, other parts of the examined plant are also needed to be assessed for their antimicrobial, antioxidant and cytotoxic activities.

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