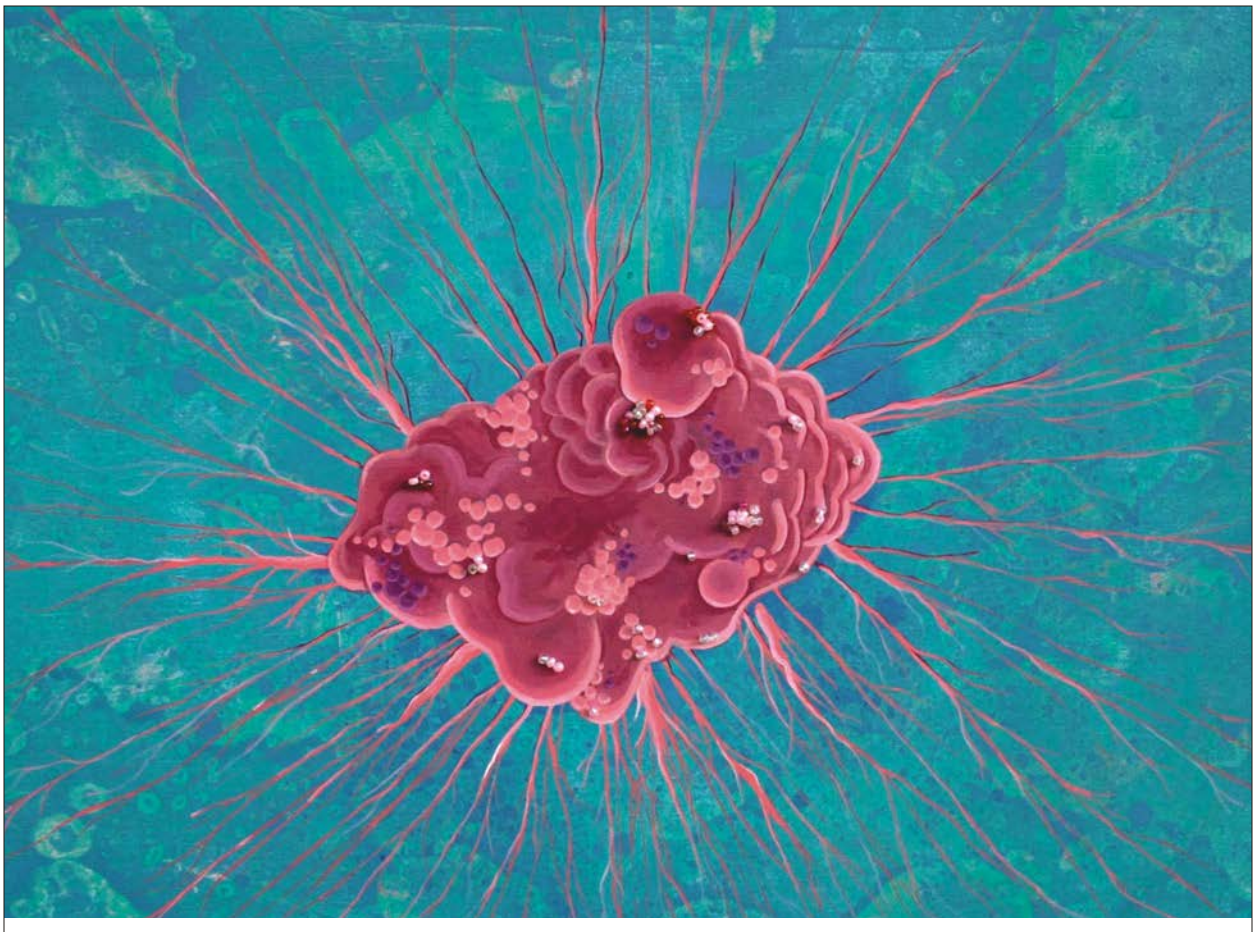

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Research Article
Cytotoxic Effect of Different Extracts of Euphorbia Lathyris Seeds on Peripheral Blood Mononuclear Cells in Leukemia

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Abstract

Background: Leukemia is a cancer of blood cells which treated by different anticancer agents, Plants nowadays considered a new source for the development of new anticancer agent. Euphorbia Lathyris use in folklore to treat skin cancer. Objective Euphorbia lathyris seeds phytochemically investigated then examined its cytotoxic activity against leukemia cells. **Materials and Methods:** Euphorbia lathyris seed was extracted by maceration with different organic solvents (petroleum ether, chloroform and methanol), each organic extract submitted to phytochemical investigation and evaluated its cytotoxic activity on Leukemic blood with three different doses (50,100 and 150 μg mL^{-1}) and three different intervals. The data were evaluated by two-way analysis of variance (ANOVA). **Results:** In the present study, the major active constituents for petroleum ether and chloroform fractions were steroids and cholesterol while methanol fraction contains flavonoids as a major active constituent. Percent of inhibition for Petroleum ether and chloroform fractions showed a decrease in the percent of inhibition of leukemic cell proliferation with an increase of time but the same fractions showed an increase in the percent of inhibition with an increase of dose. Methanol fraction showed an increase in the percent of inhibition with increasing the dose and time. **Conclusion:** It had been concluded that Stepwise fractionation of Euphorbia lathyris offering a separation of active constituents according to polarities, this give a different cytotoxic effect to leukemia cells according to the active constituent that contains.

Key words: Leukemia, euphorbia lathyris, phytochemical investigation, percent of inhibition, cytotoxic effect

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Cancer is a very serious disease, one of the most famous example is Leukemia. Leukemia could be classified into acute or chronic malignant diseases that involve the blood-forming organs (bone marrow)[1]. Leukemia is characterized by different pathological features which occur due to the abnormal proliferation, differentiation and excessive production of white blood cells and their precursors in the bone marrow, with or without a corresponding increase of those in the circulating blood[2]. The consequences of leukemia are the decrease in the production and function of normal blood cells. Leukemia like another type of cancer could be metastasis to other organs like lymph nodes, spleen, liver, central nervous system and other organs[3]. To date, the development of highly sophisticated technologies and new diagnostic tool make the diagnosis and treatment of leukemia made the surviving rate higher than in the past[4]. Chemotherapy is one of the methods used most frequently in the treatment of leukemia. It works by limiting leukemic cells from growing or multiplying and then causing their disruption[5].

There are several limitations in the use of chemotherapy, the most important limitation is manifested by low specificity and resistance of drugs chemotherapy which considered the major problem of oncology, because it determines the development of side effects in patients. There is search fuscous on the increased efficiency and specificity with low resistance tendency anticancer drugs is an urgent problem[6].

For decades, plants extracts considered a cornerstone in the treatment of several diseases[7]. Cancer chemotherapy is one approach in developing a new medicine with plants source. Nowadays, taxol, methotrexate and vinblastine are approved for cancer treatment as a chemotherapy medicine with plant sources[7].

Plants use in everyday food like herb, spices and vegetables are rich in active constituents[8]. These chemical components, in the past, had great values in the folklore treatment, nowadays, these dietary foods used in the prevention and treatment of several diseases like cancer[9].

Euphorbia lathyris (Euphorbiaceae) is one species of spurge native different region in the world. *Euphorbia lathyris* has different names like Gopher Spurge, Gopher Plant or Mole Plant[10].

Euphorbia has wide different genus belongs to the very diverse with at least 7,500 species. The variation within this genus is incredible; from low-growing garden weeds called spurges to giant, cactus-like succulents that rival in size[11].

Traditional usages of the milky sap of specific type of euphorbia used in Australia have used to cure cancerous spots on people skin. In the past, they found that the application of a fresh milky sap on skin cancer had the ability to kill cancer and cure the patient[12]. Nowadays, specific research study these curative abilities of euphorbia, they found that milky sap penetrate and killed skin cancer as similar as liquid nitrogen that uses by the dermatologist[13].

Recently, several studies focus on several types of euphorbia. They found that it had broad pharmacological effects, this effect includes; Antibacterial activity[14], Antimalarial activity[15], Anti-inflammatory activity[16], Antidiarrheal activity[17], Antioxidant activity[18], Anti-amoebic activity[19], Antifungal activity[20], Euphorbia lathyris produce many types of active constituents. It contains quercetin, kaempferol, diterpenoids, daphnetin, ingenol, b-sitosterol, p-coumaric acid, ferulic acid, lathyrol, esculetin, glycerides, isoprenoids, protein, rubber, oleic acid and wax[21].

This study was designed to evaluate the cytotoxic activity of a different organic extract of *Euphorbia lathyris* seeds with three different doses (50, 100, 150 μg kg^{-1}) on Peripheral Blood Mononuclear Cells in a Leukemia cell.

MATERIALS AND METHODS

Plants collection: The plant was brought from the Iraqi market and authenticated by the Department of Pharmacognosy and Medicinal plants, College of Pharmacy/ University of Baghdad. A voucher sample was kept at the Department of Pharmacognosy and Medicinal plants, College of Pharmacy/University of Baghdad. The duration of this study extended for three months from July, 2017 to October, 2017 and all chemicals used through the experiment were analytical.

Extraction and fractionation of extract of *Euphorbia lathyris*: Seeds were smashed by mortar and 500 g of the powder was macerated with 1500 mL of petroleum ether (B.p. 40-60°) for 24 h, then filtration by Whatman paper and the filtrate was evaporated to dryness. The mark was re-extract by maceration with 1500 mL of 80% ethanol for 24 h, then filtered and the mark was re-macerated with another 1500 mL of the same solvent for another 24 h, filtered and the filtrates were combined together and concentrated to about 200 mL by evaporation under vacuum using rotary evaporator. The concentrated extract was introduced in a separatory funnel and partitioned with chloroform (300 mL×3), the organic layers were combined together, dried over anhydrous sodium sulfate, filtered and evaporated to dryness under vacuum. The aqueous layer was evaporated to dryness and the residue was

dissolved in 500 mL methanol, warmed in a water bath with swirling, filtered and evaporated to dryness under vacuum[22].

Phytochemical investigation: Each fraction was screened qualitatively for phytochemical constituent utilizing standard methods for analysis[23].

Peripheral blood mononuclear cells (PBMCs) isolation: About 150 blood samples have been collected from the national center of hematology, each peripheral blood samples were collected in EDTA vacationer tubes from patients with acute lymphoblastic leukemia and PBMCs were isolated using the Ficoll Hypaque™ density-gradient centrifugation technique (Sigma). For cytotoxic tests, different organic extracts of *Euphorbia lathyris* are tested in concentrations of 50, 100 and 150 μg mL^{-1} were added (except for the control well). The cells were incubated in 96-well culture plates and the optical density was measured for each well after 24, 48 and 72 h[24].

Statistical analysis: All data expressed as the Mean±standard deviation (STD), significant differences between the treatment and control groups were evaluated by two-way analysis of variance (ANOVA) (Graph pad prism 5.02 software) Differences were considered significant at $p<0.05$.

Percent of inhibition is calculated according to the following Eq.:

$$\text{Cell inhibition (\%)} = 100-\left\{\frac{\text{At-Ab}}{\text{Ac-Ab}}\right\}\times 100$$

Where:

At = Absorbance value of test compound

Ab = Absorbance value of blank

Ac = Absorbance value of the control

RESULTS

Phytochemical investigation

Yield value calculation: The yield values for each fraction have been calculated for different fraction after evaporation of the solvent. It have been found that the yield value for petroleum ether was 153.1 g, for chloroform fraction 76.2 g and for methanol fraction 34.3 g.

Chemical tests: In the Table 1, chemical tests have been achieved for each fraction to detect the presence of specific active ingredient. It have been found that petroleum ether and chloroform fraction contain steroidal compounds (Liebermann test positive) meanwhile methanol fraction contain polyphenol compound (flavonoids) (5% alcoholic KOH test and ferric chloride test was positive).

Cytotoxic activity of *Euphorbia lathyris*: In the Table 2, percentage of inhibition for leukemic cell proliferation after exposure to petroleum ether extract of *Euphorbia lathyris* showed a significant difference between the doses (50, 100, 150 μg mL^{-1}) within the same time (24, 48, 72 h) (31.38±7.74, 46.46±5.3, 60.9±8.7) (27.5±5.2, 43.9±5.4, 58.8±7.3) (26.5±7.1, 42.1±7.9, 57.63±5.5) respectively ($p\leq0.05$). At the same time, percentage of inhibition for leukemic cell proliferation after exposure to petroleum ether extract of *Euphorbia lathyris* showed a significant difference at a dose (50 μg mL^{-1}) at different time intervals (24, 48, 72 h) (31.38±7.74, 27.5±5.2, 26.5±7.1) ($p\leq0.05$), but at doses (100, 150 μg mL^{-1}) percentage of inhibition for leukemic cell proliferation after exposure to petroleum ether extract of *Euphorbia lathyris* showed not significant differences at different time intervals ($p\geq0.05$).

Table 3 showed that for specific time, the increasing in the doses (50, 100, 150 μg mL^{-1}) of chloroform extract caused a significant differences in cytotoxicity toward leukemia cells between these doses (35.2±2.9, 56.13±3.66, 78.61±9.1) (30.2±7.2, 39.5±5.7, 45.7±8.1) (21.8±7.1, 23.5±8.1, 37.6±6.5) ($p\leq0.05$). Meanwhile, for each doses, the percentage of inhibition for leukemic cell proliferation after exposure to this extract did not show any significance differences at dose (50 μg mL^{-1}), except when time was increased to 72 h ($p\geq0.05$), but at doses (100, 150 μg mL^{-1}) percentage of inhibition showed significant differences at different time intervals (56.13±3.66, 39.5±5.7, 23.5±8.1) (78.61±9.1, 45.7±8.1, 37.6±6.5) ($p\leq0.05$).

Table 1: Chemical test that achieved on different fractions of *Euphorbia lathyris*

Chemical test	Petroleum ether fraction	Chloroform fraction	Methanol fraction
5% alcoholic KOH test	Negative	Negative	Positive
Vanillin/H_2SO_4 test	Positive	Positive	Negative
Ferric chloride test	Negative	Negative	Positive
Liebermann test	Positive	Positive	Negative

Table 2: Percentage of inhibition of leukemic cell proliferation after exposure to a different concentration of petroleum ether extract of *Euphorbia lathyris* during different intervals

Doses (μg mL^{-1})	After 24 h	After 48 h	After 72 h
Percentage of inhibition petroleum ether fraction of *Euphorbia lathyris*			
50	31.38±7.74^{Aa}	27.5±5.2^{Ab}	26.50±7.1^{Ac}
100	46.46±5.3^{Ba}	43.9±5.4^{Ba}	42.10±7.9^{Ba}
150	60.90±8.7^{Ca}	58.8±7.3^{Ca}	57.63±5.5^{Ca}

All values are represented as the Mean±SEM, A, B, C indicate significant differences when compared between treated group within same time ($p \leq 0.05$), a, b, c indicate significant differences when compared between treated group within same concentration ($p \leq 0.05$)

Table 3: Percentage of inhibition of leukemic cell proliferation after exposure to a different concentration of chloroform extract of *Euphorbia lathyris* during different intervals

Doses (μg mL^{-1})	After 24 h	After 48 h	After 72 h
Percentage of inhibition chloroform fraction of *Euphorbia lathyris*			
50	35.20±2.9^{Aa}	30.2±7.2^{Aa}	21.8±7.1^{Aa}
100	56.13±3.66^{Ba}	39.5±5.7^{Bb}	23.5±8.1^{Bc}
150	78.61±9.1^{Ca}	45.7±8.1^{Cb}	37.6±6.5^{Cc}

All values are represented as the Mean±SEM, A, B, C indicate significant differences when compared between treated group within same time ($p \leq 0.05$), a, b, c indicate significant differences when compared between treated group within same concentration ($p \leq 0.05$)

Table 4: Percentage of inhibition of leukemic cell proliferation after exposure to a different concentration of methanol extract of *Euphorbia lathyris* during different intervals

Doses (μg mL^{-1})	After 24 h	After 48 h	After 72 h
Percentage of inhibition methanol fraction of *Euphorbia lathyris*			
50	24.1±6.4^{Aa}	27.2±6.9^{Aa}	29.7±6.0^{Aa}
100	42.2±4.0^{Ba}	43.7±8.2^{Ba}	48.4±7.9^{Ba}
150	49.1±6.3^{Ca}	59.2±7.2^{Cb}	64.6±4.1^{Cc}

All values are represented as the Mean±SEM, A, B, C indicate significant differences when compared between treated group within same time ($p \leq 0.05$), a, b, c indicate significant differences when compared between treated group within a concentration ($p \leq 0.05$)

Table 4 showed that, at specific time, cytotoxicity of methanol extract showed significant differences between the doses (50, 100, 150 μg mL^{-1}) (24.1±6.4, 42.2±4, 49.1±6.3) (27.2±6.9, 43.7±8.2, 59.2±7.2) (29.7±6, 48.4±7.9, 64.6±4.1) respectively ($p \leq 0.05$). Meanwhile at doses (50, 100 μg mL^{-1}) of methanol extract increasing of time didn't cause any significant differences between time intervals ($p \geq 0.05$), but at dose (150 μg mL^{-1}) percentage of inhibition show significant differences at different time intervals (49.1±6.3, 59.2±7.2, 64.6±4.1,) ($p \leq 0.05$).

DISCUSSION

Leukemia is a serious hematological disease all over the world. There are several chemotherapeutic agents used to treat this cancer[1]. These agents besides its therapeutic activity, it has significant side effect so that an alternative should be found[25]. One of most successful alternative is using plants extract to treat this cancer[26]. One of the most promising medicinal plants used to treat leukemia is Euphorbia lathyris seeds. In the present study, after extraction of seeds with different organic solvents, extracts phytochemical investigations have been shown the presence of terpenoids and phytosterols in both petroleum ether and chloroform extract meanwhile flavonoids and terpenoids are present in methanol fraction. The separation of these active constituents was return to the use of solvents with different polarities which separate the active constituents according to polarity, this explain the differences in the cytotoxic activity between these fractions[27].

Phytosterols compounds are present in petroleum ether and chloroform fractions with different quantity in *Euphorbia lathyris* seeds which cause the increasing in cytotoxicity when the doses was increased and decreasing when the time was decreased. These phytosterols compounds that presents in these fractions could be responsible for cytotoxicity. Several studies confirm that, phytosterols have been shown similar cytotoxic activity in another type of human cancer[28,29]. Meanwhile anti-proliferative activity of methanol fraction of *Euphorbia lathyris* seeds was increased when both doses and time increase. According to phytochemical investigations, flavonoids was present within this fraction that maybe responsible for its cytotoxic activity. Several studies focus on the cytotoxic effect of flavonoids on cancer cells, they found that these agents attenuated cell division and induced apoptosis *in vitro* study, besides it caused a repression of tumor angiogenesis *in vivo*[30], besides flavonoids greatly influence the cascade of immunological events associated with the development and progression of cancer[31]. One should understand the mechanism how these flavonoids got accumulated in cellular organelles and tissues once they enter inside. Flavonoids had the potential of modulating many biological events in cancer such as apoptosis, vascularization, cell differentiation, cell proliferation, etc.[32,33]. All of these previous study finding matching with the results of present study. The importance of the current study was manifested by the high percent of leukemic cell proliferation inhibition which could be a very promising results in the development of newly supplement and/or therapy for leukemia besides it have been discovered a new activity for *Euphorbia lathyris* seeds which is only use to treat a skin carcinoma (melanoma). There are two important limitations need further investigations one of them was the needs for more phytochemical investigations for Euphorbia lathyris seeds other limitation the need for the molecular mechanism behind the anti-cancer activity.

CONCLUSION AND FUTURE RECOMMENDATIONS

In the light of the results obtained, it could be concluded that, extraction of Euphorbia lathyris seeds with a different organic solvent which had different polarity offering a fractionation of active constituents according to polarity. According to phytochemical investigations, terpenoids are present in petroleum ether and chloroform make the cytotoxic activity against leukemic cells is dose-dependent meanwhile the flavonoids that present in methanol fraction make the cytotoxic activity against leukemic cells is dose and time-dependent. Further studies should focus on isolation and identification of these active constituents then examine on leukemic cell line and/or on bone marrow cells are taken from leukemia patients.

SIGNIFICANT STATEMENT

This study discovers that different extracts of Euphorbia lathyris seeds contain different active constituents, these active constituents play an important role in the treatment of a serious hematological disease which is leukemia. This discovery will open a new line of research for developing a new drug could be used in the treatment of leukemia with maximum efficacy and minimum or no adverse effects.

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