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Research Article

CYTOTOXIC ACTIVITY OF ALKALOIDS EXTRACTED FROM THREE IRAQI PLANTS AGAINST BREAST CANCER CELL LINE

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ABSTRACT

Objectives: Screening for cytotoxic activity of total alkaloid extracts of *Eucalyptus camaldulensis*, *Aloe vera,* and *Capparis spinosa* against breast cancer cell line Michigan Cancer Foundation-7 (MCF-7) and nontumorigenic fetal hepatic cell line (WRL-68).

Methods: The plant powders were extracted separately with 80% methanol and chloroform at pH 2 and 10. Total alkaloids were detected qualitatively by Mayer's, Dragendorff's, and Hager's reagents and estimated quantitatively by bromocresol green spectrophotometry depending on the atropine calibration curve. The cytotoxic activity was evaluated by 3-[4, 5-dimethylthiazoyl]-2, 5-diphenyltetrazolium bromide assay.

Results: The extract of *E. camaldulensis* had highest total alkaloid content (24.50±1.70 mg/100 g plant dry weight) than the others. The total alkaloids (400 µg/ml) of *E. camaldulensis* reduced the cell viability of both cell lines MCF-7 and WRL-68 to 45.25±2.20% and 92.00±1.55%, respectively, and the inhibitory concentration 50% of cells were 375.50 µg/ml for MCF-7. The alkaloids of *C. spinosa* had effect 79.80±7.08% and 89.50±0.09% against MCF-7and WRL-68, respectively. While the total alkaloids of *A. vera* had slightly effect on both cell lines.

Conclusion: Plant alkaloids appeared variable cytotoxic activity against cancer and normal cell lines depending on the alkaloid contents, concentrations, purity, and cell line types.

Keywords: Alkaloids, *Eucalyptus camaldulensis* Dehnh., *Aloe vera* L., *Capparis spinosa* L., *In vitro*, Cytotoxicity, Breast cancer.

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INTRODUCTION

Cancer is a major global health problem and is currently classified as the third leading to death after infectious diseases and cardiovascular [1]. Breast cancer is the second most continual cancer effect on people worldwide and the most common cancer among females [2]. In Iraq, breast cancer is the first kind in female, accounting for approximately one-third of the registered female cancers, according to the latest Iraqi Cancer Registry [3]. The history of the plant as a source of anticancer agents started in serious in the 1950s with the detection and development of the vinca alkaloids [4] and the isolation of the cytotoxic podophyllotoxins. The investigations show that *Aloe vera* contains over 75 nutrient and 200 active phytochemical compounds, including phenol, alkaloid, flavonoid, vitamins, enzymes, minerals, sugar, lignin, anthraquinones, saponins, salicylic acid and amino acids, which are responsible for its medicinal properties [5]. This compound has multiple medicinal properties such as anti-inflammatory, antibacterial, antioxidant, immune boosting, anticancer, antiageing, sunburn relief, and antidiabetic potentials [6]. *Capparis spinosa* is recognized as a rich source of a wide array of phytochemical compounds in different parts has pharmacological actions, and therapeutic properties have also which include the treatment of diabetes, high blood pressure and liver, spleen and kidney disorders [7,8]. The plant seeds also contain a protein that inhibits the multiplication of hepatoma Hep G2 cells, colon cancer HT29 cells and breast cancer Michigan Cancer Foundation-7 (MCF-7) cells. The reported medicinal health functions and nutritional attributes of *C. spinosa* can be mainly attributed to the occurrence of alkaloids, glucosides, reducing sugars, essential fatty acids, organic acids, vitamin C, terpenoids, flavonoids, and resins in the fruit and leaves of this species [9]. *C. spinosa* is importance due to the presence of several classes of medicinally important alkaloids along with potential antioxidant compounds [10], it is a rich source of different classes of alkaloids which include spermidine, indole and pyrrole alkaloids along with indol-aldehyde and indol-nitrile type derivatives. Several new alkaloids and their glycosides have also been identified in *C. spinosa*. *Eucalyptus camaldulensis* has phytochemicals compound in various parts have medical properties were investigated using standard methods of phytochemicals screening this compound including tannins, saponins, glycosides, steroids and anthraquinones, alkaloids, flavonoids, and terpenoids [11]. The *Eucalyptus* extract may be considered as a potent anticancer. It reduced the tumor growth. Some this phytochemical compound in plants is toxic [12]. The objective of our study was investigated the cytotoxic activity of total alkaloid extracts of three plants, including *E. camaldulensis, A. vera,* and *C. spinosa* against breast cancer cell line MCF-7 and non-tumorigenic fetal hepatic cell line (WRL-68).

MATERIALS AND METHODS

Plants collection

The plants (*E. camaldulensis* Dehnh., *A. vera* L., *C. spinosa* L.) were collected from the gardens of University of Babylon, Hilla, Iraq, during March and May 2015.

The plants were classified by specialists in the Botanical Garden at University of Babylon (Table 1).

The plant parts were washed with tap water to remove dust and then with distilled water (DW), and dried under shade for 10 days at

Table 1: The studied plants

Plant	Family	Common name	Part used
Aloe vera L.	*Asphodelaceae*	Aloe	Leaves
Capparis spinosa L.	*Capparaceae*	Caper	Fruits
Eucalyptus camaldulensis Dehnh.	*Myrtaceae*	Murray red gum	Bark

room temperature. Each dried part was ground and stored in an air-light container to prevent the humidity effect and then stored at room temperature until further use.

Total alkaloid extraction

Total alkaloids were extracted according to Harborne [13]. Briefly, 20 g of plant dry powder was extracted with 80% methanol for 24 h in a continuous extraction by soxhlet apparatus 250 ml volume. The extract was filtered by Whatman No. 1 filter paper, and then, the filtrate was concentrated by a rotary evaporator under vacuum at 45°C until the solution reached to 10 ml. Subsequently, the concentrated extract was transferred to a separating funnel and 2 N HCl was added gradually to adjust the pH value up to 2, after that the extract was washed with 10 ml chloroform three times. Then, the pH value of the extract was adjusted to 10 using NH_4OH, and partitioned with 10 ml chloroform 3 times. The chloroform portion was dried to obtain the total alkaloid extract. The dried extract was weighed and preserved in a clean container at 4°C for further investigation.

Qualitative detection of alkaloids

To detect the presence of alkaloids in plant extracts some qualitative tests were performed using Mayer's, Dragendorff's and Hager's reagents. Mayer's reagent used to screen all types of alkaloids, prepared by dissolving 13.5 g of mercuric chloride and 5 g of KI in 1000 ml distilled water. The test was done by adding 1-2 ml of the reagent to 5 ml of plant extract. The formation of white or creamy precipitate indicated the test was positive [14]. Furthermore, Dragendorff's reagent was used to investigate alkaloids in plant extract. The reagent was prepared by dissolving 20 g of bismuth nitrate in 40 ml distilled water and 16 g of sodium iodide in 40 ml distilled water, and then the two solutions were mixed together. The test was performed by adding 1-2 ml of Dragendorff's reagent in 5 ml of the plant extract; the formation of a prominent orange color indicated the test was positive [15]. Hager'stest, Hager's reagent is saturated solution of picric acid, was done by adding a few drops of the reagent to plant extracts and appeared a yellow color precipitate that indicate to the presence of alkaloids [16].

Estimation of total alkaloid content

The total alkaloid content was estimated by bromocresol green (BCG) spectrophotometry method [17,18]. The BCG reagent was prepared by heating 69.8 mg of BCG with 3 ml of 2 N NaOH and 5 ml distilled water until completely dissolved, and then, the solution was diluted to 1000 ml with distilled water. Phosphate buffer solution (pH 4.7) was prepared by adjusting the pH of 2 M sodium phosphate (71.6 g Na_2HPO_4 in 1 distilled water) to 4.7 with 0.2 M citric acid (42.02 g citric acid in 1 distilled water).

BCG assay

A 10 mg of the plant extract was dissolved in 2 N HCl and then filtered. 1 ml of this solution was transferred to separator funnel and washed with 10 ml chloroform (3 times). The pH of this extract was adjusted to neutral with 0.1 N NaOH. Then, 5 ml of BCG solution and 5 ml of phosphate buffer were added to the extract. The mixture was shaken and the complex extracted with 1, 2, 3, and 4 ml chloroform by vigorous shaking; the extract was then collected in a 10 ml volumetric flask and diluted with chloroform. The absorbance of the complex in chloroform was measured at 470 nm against blank prepared as above but without alkaloid or plant extract. The total alkaloids were calculated depending the calibration curve of atropine [17].

Cytotoxic activity

This assay was held at the Centre for Natural Product Research and Drug Discovery, Department of Pharmacology, Faculty of Medicine, University of Malaya/Kuala Lumpur, Malaysia.

The cytotoxic activity was assayed against two kinds of cell lines including breast cancer cell line MCF-7 and nonmutagenic fetal hepatocyte WRL-68 using 3-[4, 5-dimethylthiazoyl]-2, 5-diphenyltetrazolium bromide (MTT dye). Briefly, 100 µl cell suspension was added onto the flat-bottomed micro-culture plate wells, separated plate for each cell line in triplicate, and treated them with 100 µl partially purified plant extract, incubated for 24 h, centrifuged to remove the dead cells. Aliquot of 100 µl of 2 mg/ml MTT dye was added to each well, and the incubation was continued for a further 4 h, then 50 µl of solubilization solution of dimethyl sulfoxide was added into each well. After complete solubilization of the dye, the absorbance of was read at 620 nm with an enzyme-linked immunosorbent assay reader. The mean absorbance for each group of replicates was calculated. The percentage viability of cells exposed to various treatments was calculated as follows [19]:

$$\%\,\text{Cell viability} = \frac{\text{Mean absorbance of treated sample}}{\text{Mean absorbance of non-treated sample}} \times 100$$

The control was the non-treated cultures in all experiments that contained cells in the medium only.

Statistical analysis

Statistical analysis of the data was performed using SPSS using one-way analysis of variance according to the method described by Levesque [20] numerical data were expressed as mean ± standard deviation. $p<0.05$ was considered to be statistically significant.

RESULTS

Table 2 showed the qualitative detection of alkaloids present in three plant extracts using different reagent. The qualitative analysis of all extracts appears the presence of alkaloids by changing color in each reagent. The results of extraction yields of chloroform extracts were presented in Table 3. The soxhlet extraction procedure using the chloroform and methanol solvent showed that the total alkaloid of the *E. camaldulensis* bark was 24.50±1.70 mg/100 g DW of plant, which was higher than *A. vera* leaves and *C. spinosa* fruits were 16.50±1.00 and 13.35±1.00, respectively. Fig. 1 showed the effects of the total alkaloid extract of *E. camaldulensis* bark treatment against MCF-7 and WRL-68. The growth of cancer cell was inhibited at low concentration with an eventual decline at the highest concentrations tested. It was given cell viability 45.25±2.20%, 78.70±2.67%, 92.00±3.19%, 94.90±6.20% at alkaloid concentrations 400, 200, 100, 50 µg\ml respectively, in comparison with the cell viability of normal cell (92.00±2.10%, 97.29±0.67%, 94.20±2.90%, and 97.87±1.50%, respectively) in the same concetration. The inhibitory concentration 50% (IC_{50}) of MCF-7 cell line equal 375.5 µg/ml of alkaloid extract. The second alkaloid extract (*C. spinosa* fruits) in this study reduced the cell viability of the MCF-7 cell line was 79.80±7.08% with 400 µg\ml, and the IC_{50} was 991.50 µg\ml (Fig. 2). Whereas, Fig. 3 showed the cell viability of the cancer cells did not inhibit in comparison with the normal cell when

Table 2: Qualitative detection of alkaloids in plant extract using different reagents

Reagent	Result	Resulted color
Mayer's reagent	+	Creamy precipitate
Dragendorff's reagent	+	Orange color
Hager's reagent	+	Yellow color

+: Indicate the positive results

Table 3: The total alkaloid contentsof tested plant

Plant	Parts	Total alkaloid content mg/100 g of plant DW±SD
Eucalyptus camaldulensis Dehnh.	Bark	24.50±1.70
Aloe vera L.	Leaves	16.50±1.00
Capparis spinosa L.	Leaves	13.35±1.00

The results represent as a mg/100 g of plant DW±standard deviation , the mean difference is significant at the 0.05 level. DW: Distilled water

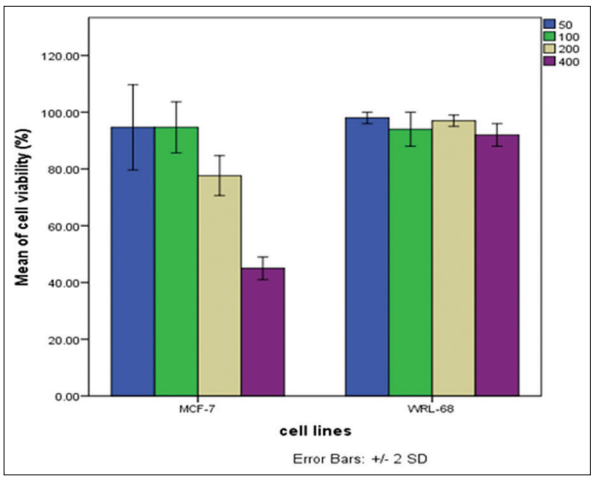

Fig. 1: Cytotoxic activity of the total alkaloids of *Eucalyptus camaldulensis* bark against the WRL-68 and Michigan Cancer Foundation-7 (MCF-7) cell lines. Extract concentrations ranged from 50 to 400 µg/ml and inhibitory concentration 50% of MCF-7 was 375.50 µg/ml

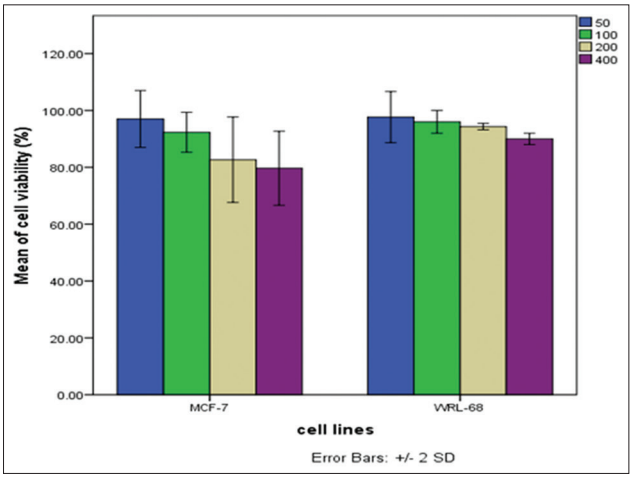

Fig. 2: Cytotoxic activity of the total alkaloids of *Capparis spinosa* leaves against the WRL-68 and Michigan Cancer Foundation-7 (MCF-7) cell lines. Extract concentrations ranged from 50 to 400 µg/ml and inhibitory concentration 50% of MCF-7 was 879.20 µg/ml

treated with the alkaloid extract of *A. vera* leaves; it was 97.70±2.67%, 98.37±3.00%, 104.00±2.70%, and 102.00±0.75% with treating 400, 200, and 100, 50 g\ml.

DISCUSSION

Medicinal plants contain some organic compounds which provide definite physiological action on the human body, and these bioactive substances include tannins, alkaloids, carbohydrates, terpenoids, steroids, and flavonoids [21]. Alkaloid in three plants was extracted using methanol and chloroform solvents, and the total alkaloids were detected by changing the color of specilized reagents. Alkaloid quantities were different among three plants; the higher content was *E. camaldulensis* bark extract and the *C. spinosa* leaves extract had lower content. The results were not consistent with the previous study which revealed that the total alkaloid in the ethanolic extract of *E. camaldulensis* bark was 400±0.03 mg/100 g and it higher than other parts [22]. Not all alkaloids can react with the BCG dye, due to the lack of a general method to estimate all types of alkaloids [23], the method described in this study can be used for the determination of a

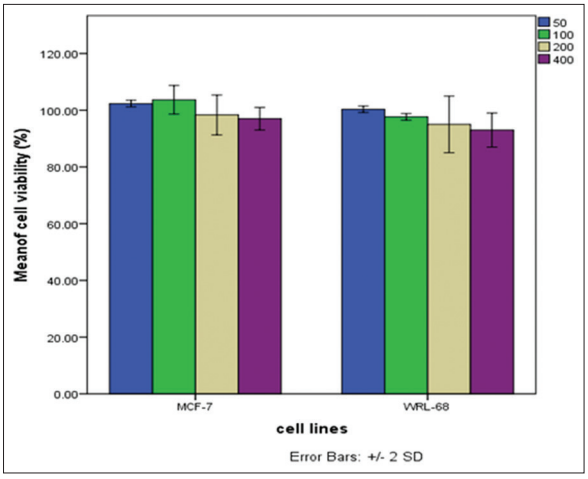

Fig. 3: Cytotoxic activity of the total alkaloids of *Aloe vera* leaves against the WRL-68 and Michigan Cancer Foundation-7 (MCF-7) cell lines. Extract concentrations ranged from 50 to 400 µg/ml and inhibitory concentration 50% of MCF-7 was 991.50 µg/ml

special group of alkaloids [18]. The BCG can react with a certain class of alkaloids and some alkaloids do not react with this reagent [17].

Cytotoxicity of total alkaloid against MCF-7 and WRL-68 cell lines was performed using a MTT assay, which it is reduced by metabolically active cells, in part by the action of dehydrogenase enzymes, to generate reducing equivalents such as nicotinamide adenine dinucleotide (NAD) and NAD phosphate. The resulting intracellular purple formazan can be solubilized and quantified by spectrophotometer [24].

E. camaldulensis contains many phytochemical compounds have biological activities [11,25]. This study implicit the observation that petroleum ether and chloroform extracts of *Pinus* and *E. camaldulensis* showed a promising anticancer activity this compounds that inhibit cancer initiation are traditionally termed (blocking agents). Bioactive components present in plants can prevent carcinogenesis by blocking metabolic activation, increasing detoxification, or providing alternative targets for electrophonic metabolite [26]. They may act by preventing the interaction between chemical carcinogens orendogenous free radicals and DNA; some alkaloid has an activity to inhibits the proliferation of multiple cancer cell lines by inducing cell cycle arrest at the G1 or G2/M phases and by apoptosis [27,28]. Furthermore, another study revealed that alkaloid have ability inhibits breast stem cell self-renewal without cause toxicity to differentiated cells [29], interacts with DNA or RNA to form an alkaloid-DNA or a alkaloid-RNA complex, respectively, to prevent damage such as berberine make as anticancer by formation complex with DNA and RNA to prevent damage [30,31]. Thereby reducing the level of damage and resulting mutations which contribute not only to cancer initiation but also progressive genomic instability and overall neoplastic transformation. Protection may be achieved as a consequence of decreased cellular uptake and metabolic activation of procarcinogens and/or enhanced detoxification of reactive electrophiles and free radical scavenging, as well as induction of repair pathways [32-35].

Similar studies have found that the methanolic extract of *E. camaldulensis* bark had cytotoxicity and antitumor activity against Ehrlich's ascites carcinoma (EAC) in Swiss albino mice [36]. Some alkaloid compound, in other hand, had toxic activity to increase proliferation cancer cell at low concentration of an alkaloid extract as shown in Fig. 3. This result was in agreement with the previous studies which revealed the alkaloids of natural herbs that had a side effect lead to the toxicity [37]. Such as piperine lead to neurotoxicity, immunotoxicity, and reproductive toxicity have been reported [38], and hepatotoxicity and embryonic toxicity can also be induced by sanguinarine [39]. The inhibition

difference in cell viability may be due to the nature of the compounds found in each crude extract and their interaction with metabolic nature of each type cells or may be due to the effectiveness of some enzymes that act as antioxidants especially in cancer cells [40].

CONCLUSION

Plant alkaloids appeared variable cytotoxic activity against cancer and normal cell lines depending on the alkaloid contents, concentrations, purity, and type of cell lines.

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