INTRODUCTION

Cancer is one of the most life-threatening diseases and possesses many health hazards in both developed and developing characterized by irregular proliferation of cells. The toxicity of chemotherapeutic drugs sometimes creates a significant problem in the treatment of cancer using allopathy or established medicine. Plants still have enormous potential to provide newer drugs and as such are a reservoir of natural chemicals that may provide chemoprotective potential against cancer [1]. Recently, various therapies have been propounded for the treatment of cancer, many of which use plant-derived products. The medicines always played an important role in the global health. The health medicinal plants providing a new area of drug research [2]. The demand for plant-based medicines, food supplement, health products, pharmaceuticals, and cosmetics are increasing in both developing and developed countries due to the growing recognition that then a natural products are non-toxic, have less side effects and easily available [3]. Secondary metabolites have been developed in nature form of different plant species, insects, fungi, algae, and prokaryotes during evolution in enormous diversity. Plant secondary metabolites can be defined as the compounds that play an important role in the interaction of the plant with its environment, but have no such role in maintaining the fundamental life processes in plants [4]. The alkaloids represent a group of natural products that has had a major impact throughout history on the economic, medical, political, and social affairs of humans. Many of these agents have potent physiological effects on mammalian systems as well as other organisms, and as a consequence, some constitute important therapeutic agents [5]. In fact, alkaloids are among the most important active components in plants, and some of these compounds have already been successfully developed into chemotherapeutic drugs, such as camptothecin, a famous topoisomerase I inhibitor [6], and vinblastine, which interacts with tubulin [7]. Several alkaloids exhibit significant biological activities, such as the relieving action of ephedrine for asthma, the analgesic action of morphine, and the anticancer effects of vinblastine [7,8]. The objective of our study was to investigate the cytotoxic activity of total alkaloid extracts of three plants, including Pinus sabiniana L., Phoenix dactylifera L., and Ferocactus sp. L. against breast cancer cell line Michigan Cancer Foundation-7 (MCF-7) and non-tumorigenic fetal hepatic cell line (WRL-68).

MATERIALS AND METHODS

Plants collection

The plants (P. sabiniana L. leaves, P. dactylifera L. pollen grains, Ferocactus sp. L. leaves) were collected from the gardens of University of Babylon, Hilla, Iraq, during March 2015. 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 room temperature. Each dried part was ground and stored in airtight 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 [9]. 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 concentration was adjusted by a rotary evaporator. Next, the extract was partitioned with 10 ml chloroform three times. Then, the pH value of the extract was adjusted to 10 using NH4OH. 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 NH4OH, and partitioned with 10 ml chloroform three times.

RESULTS

The extract of P. sabiniana L. had highest total alkaloid content (164.62±2.8 mg/100 g dry weight of plant) than the other plants. The total alkaloids of Ferocactus sp. L. and P. dactylifera L., reduced the cell viability of both cell lines, the highest reduction occurred in the concentration 400 μg/ml was 46±2.20% (MCF-7) and 56.2±2.2% (WRL-68) for Ferocactus sp. L., followed by 56.2±2.2% (MCF-7) and 57.5±3.2% (WRL-68) for P. dactylifera L. The alkaloids of P. sabiniana was very lower effects on both cell lines MCF-7, and WRL-68 was 89.3±3.4±% and 90.16±7.7%, respectively, at the same concentration.

CONCLUSION

Plant alkaloids had variable effects against cancer and normal cell lines depending on the type of alkaloid compounds and their concentration in the extract.

Keywords: Alkaloids, Pinus sabiniana, Phoenix dactylifera, Ferocactus sp., In vitro, Cytotoxicity, Breast cancer.

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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 was 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 cream precipitate indicated the test was positive [10]. 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; then, the two solutions were mixed. 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 [11]. Hager’s test was done by adding a few drops of the reagent to plant extracts and appearing a yellow-colored precipitate that indicates the presence of alkaloids. Hager’s reagent is a saturated solution of picric acid [12].

Estimation of total alkaloid content
The total alkaloid content was estimated by bromocresol green (BCG) spectrophotometry method [13,14]. 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 NaHPO₄ in 11 distilled water) to 4.7 with 0.2 M citric acid (42.02 g citric acid in 11 distilled water).

BCG assay
A 10 ml of the plant extract was dissolved in 2 N HCl and then filtered. This solution (1ml) was transferred to separatory funnel and washed with 10 ml chloroform (3 times). The pH of the 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 was 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 (plant extract) [14]. The total alkaloids were calculated depending on the calibration curve of atopine.

The standard curve was constructed using (0.4, 0.8, 1.2, 1.6, and 2 ml) of atopine standard solution (1 mg/10 ml) and each of them was transferred to different separating funnels as the previous method. The absorbance of the complex in chloroform was measured at 470 nm against blank prepared as above but without atopine [13].

Cytotoxic activity
To determine the cytotoxic activity against two kinds of cell lines including breast cancer cell line MCF-7 and non-mutagenic fetal hepatocyte WRL-68 using 3-[4,5-dimethylthiazol]-2, 5-diphenyltetrazolium bromide (MTT dye). Briefly, 100 µl cell suspension was added to 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 another 4 h, then 50 µl of solubilization solution of dimethyl sulfoxide was added to each well. After complete solubilization of the dye, the absorbance 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 [15]:

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\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. 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.

Statistical analysis
Statistical analysis of the data was performed using SPSS 14.0 version using one-way analysis of variance according to the method described by Levesque [16] numerical data were expressed as mean±standard deviation. p<0.05 was considered to be statistically significant.

RESULTS
The qualitative analysis of all extracts appears the presence of alkaloids by changing the color in each reagent. The quantitative contents of alkaloid compounds were estimated according to bromocresol green (BCG) spectrophotometry method [13,14], and the total alkaloids were calculated depending on the calibration curve of atopine (Fig. 1). Tables 1 and 2 show the total alkaloid contents, which revealed the highest concentration of alkaloids in P. sabiniana leaves were 164.62±2.80 mg/100 g DW and P. dactylifera pollen grains were 66.50±2.00 mg/100 g DW, while the Ferocactus sp. leaves were 50.37±2.50 mg/100 g DW. The results of cell viability assay based on the MTT assay using MCF-7 and WRL-68 cell lines which treated with total alkaloid extracts appeared the percentage of cytotoxicity increased with increasing concentration of alkaloids. The Ferocactus sp. and P. dactylifera L. total alkaloids extracts had cytotoxicity effect on both cancer and normal cell as shown in Tables 3 and 4. The highest reduction of viability was observed at the highest concentration (400 µg/ml) of Ferocactus sp. alkaloid extract was 46.87±1.80% and 56.20±2.20% for the breast cancer and normal cell lines, respectively (Table 3), whereas P. dactylifera alkaloid extract reduced the cell viability to 56.20±2.20% for breast cancer cell line and 57.50±3.20% for normal cell line (Table 4). While P. sabiniana alkaloid extract had less effects on both cell lines were 89.30±3.44% and 90.16±2.70% for cancer and normal cells, respectively, at 400 µg/ml (Table 5).

DISCUSSION
The quantitative contents of alkaloid compounds showed different concentrations among three plants, the highest concentration of alkaloids was P. sabiniana leaves, and the less concentration was Ferocactus sp. leaves., P. dactylifera pollen grains also had alkaloids. These results were in agreement with Al-Samarai et al. [17] how found the alkaloid concentration in P. dactylifera pollen grains were higher than another phytochemical compound. The range of alkaloid concentration necessary to elicit the anticancer effects is wide [6,7,18], and not all alkaloids can react with BCG dye [14]. Therefore, due to the lack of a general method to estimate all types of alkaloids [19], the method described in this study can be used for the determination

**Table 1: Qualitative detection of alkaloids in plant extract using different reagent**

| Reagent                  | Result          | Resulted color   |
|--------------------------|-----------------|------------------|
| Mayer’s reagent          | +               | Creamy precipitate |
| Dragendorff’s reagent    | +               | Orange color     |
| Hager’s test             | +               | Yellow color     |

**Table 2: The total alkaloid contents of tested plant**

| Plant          | Family      | Common name       | Part used | Amount in mg/100 g of plant DW±SD |
|----------------|-------------|-------------------|-----------|----------------------------------|
| Ferocactus     | Cactaceae   | Glauces cactus    | Leaves    | 50.37±2.50                       |
| Pinus          | Pinaceae    | Aleppo pine      | Leaves    | 164.62±2.80                      |
| P. sabiniana   | Cactaceae   | Date palm         | Pollen grains | 66.50±2.00                    |
| P. dactylifera | Cactaceae   | Pollen grains     | Pollen    | 66.50±2.00                       |

DW: Distilled water; SD: Standard deviation

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Table 3: Cytotoxic activity of the total alkaloids of Ferocactus sp. L. against the breast cancer cell line MCF7 and normal cell line WRL-68

| Alkaloid extract concentration µg/ml | % Viability of WRL±SD  | % Viability of MCF7±SD | IC_{50} of MCF7 µg/ml |
|--------------------------------------|------------------------|------------------------|-----------------------|
| 400                                  | 54.68±0.78             | 46.87±1.80             | 5.09±006              |
| 200                                  | 84.36±1.30             | 77.79±1.20             |                       |
| 100                                  | 86.60±2.00             | 89.24±5.00             |                       |
| 50                                   | 91.49±0.70             | 94.32±3.50             |                       |

MCF-7: Michigan Cancer Foundation-7, SD: Standard deviation, IC_{50}: Inhibitory concentration

Table 4: Cytotoxic activity of the total alkaloids of P. dactylifera L. pollen grains against the breast cancer cell line MCF7 and normal cell line WRL-68

| Alkaloid extract concentration µg/ml | % Viability of WRL±SD | % Viability of MCF7±SD | IC_{50} of MCF7 µg/ml |
|--------------------------------------|-----------------------|------------------------|-----------------------|
| 400                                  | 57.50±3.20            | 56.20±2.20             | 2.09±006              |
| 200                                  | 82.68±3.70            | 82.60±2.67             |                       |
| 100                                  | 94.30±2.20            | 86.40±3.19             |                       |
| 50                                   | 93.70±2.12            | 90.70±6.20             |                       |

P. sabiniana: Pinus sabiniana, MCF-7: Michigan Cancer Foundation-7, SD: Standard deviation, IC_{50}: Inhibitory concentration

Table 5: Cytotoxic activity of the total alkaloids of P. sabiniana L. leaves against the breast cancer cell line MCF7 and normal cell line WRL-68

| Alkaloid extract concentration µg/ml | % Viability of WRL±SD  | % Viability of MCF7±SD | IC_{50} of MCF7 µg/ml |
|--------------------------------------|------------------------|------------------------|-----------------------|
| 400                                  | 90.16±2.70             | 89.30±3.44             | ~                     |
| 200                                  | 91.74±1.00             | 92.10±2.33             |                       |
| 100                                  | 92.78±0.65             | 93.80±2.92             |                       |
| 50                                   | 94.50±3.23             | 95.50±3.57             |                       |

P. sabiniana: Pinus sabiniana, MCF-7: Michigan Cancer Foundation-7, SD: Standard deviation, IC_{50}: Inhibitory concentration

Fig. 1: Calibration curve of the atropine using bromocresol green method at 470 nm

of a special group of alkaloids [13,20,21]. The BCG can react with a certain class of alkaloids, and some alkaloids do not react with this reagent [14,22].

The alkaloids of Ferocactus sp. leaves and P. dactylifera pollen grains had an anticancer activity to reduce the growth of cancer cell; also, it had inhibited effect on normal cell. The compounds that inhibit cancer initiation are traditionally termed (blocking agents), this bioactive component present in plants can prevent carcinogenesis by blocking metabolic activation, increasing detoxification, or providing alternative targets for electrophilic metabolites [23]. They may act by preventing the interaction between chemical carcinogens or endogenous free radicals and DNA, 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 [24-26]. This activity of inhibition may be due to the nature of the compounds found in each crude extract and their interaction with metabolic nature of each type of cancer cells or may be due to the effectiveness of some enzymes that act as antioxidants especially in cancer cells [27,28]. Alkaloid extract of Ferocactus sp. and P. dactylifera may be contained another phytochemical compounds having effect on normal cell lead to reduce the viability alkaloids isolated from natural herbs are not always safe have side effect lead to the toxicity [29], such as Neurotoxicity, immunotoxicity, and reproductive toxicity induced by piperine have been reported [18,30,31], and hepatotoxicity and embryonic toxicity also can be induced by sanguinarine [32,33]. The present study indicates that the chloroform alkaloid extract of P. sabiniana had slightly effect on the cell viability of both cell lines. In the other words, it had not anti-cancer activity against MCF-7.

CONCLUSION

Plant alkaloids had variable effects against cancer and normal cell lines depending on the type of alkaloid compounds and their concentration in the extract. Furthermore, these alkaloids need to further purification and tested against different cell lines to determine their effectiveness.

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