The Cytotoxic Effect of Iraqi *Rumex Acetosella* against Breast and Esophagus Cancer Cells

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Abstract

Background: cell lines derived from cancer cells are frequently used in research, including use as a model to understand cancer and to identify potential new treatments. **The aim** of this article has been prepared to evaluate the anticancer effect of *Rumex Acetosella* that has been studied for their anticancer activity on esophagus and breast cancer cell line. **Method**: AMJ13 (new breast cancer *cell line* (*AMJ13*) has been established from an Iraqi breast cancer patient and SK-GT-4 cells (Human esophageal adenocarcinoma *cell line*) were treated with whole methanolic extract of the leaves of *Rumex acetosella.* the MTT assay to determine the anticancer activity was done using 96-well plates where cell lines were seeded at 1 × 104cells/well after 24 hrs.m the cells were treated with tested compounds at different concentrations. Cell viability was measured after 72 hrs of treatment by removing the medium, adding 28 µL of 2 mg/mL solution of MTT, and incubating the cells for 2.5 h at 37 °C. After removing the MTT solution, the crystals remaining in the wells were solubilized by the addition of 130 µL of DMSO (Dimethyl Sulphoxide) followed by 37 °C incubation for 15 min with shaking. The absorbency was determined on a microplate reader at 492 nm. **Conclusion**: Plant extract from *Rumex Acetosella* showed particularly strong anticancer capabilities since it inhibited actual tumor progression in a breast adenocarcinoma mouse model. Our results suggest that whole plant extracts are promising anticancer reagents.

Keywords*: Medicinal Plants, Phytochemicals, Natural Compounds, anticancer Activity.*

Introduction

The International Agency for Research on Cancer stated that the incidence of prevalence and mortality from different types of cancer for 184 countries were 14.1 million new cancer cases, 8.2 million cancer deaths, and 32.6 million people living with cancer (within 5 years of diagnosis) in 2012 worldwide [1]. By 2030, it is expected that there will be 26 million newly diagnosed cancer cases and 17 million cancer deaths [2]. Meanwhile, although considerable focus and effort, cancer still an aggressive killer over the world. In addition, during the last years, new and novel anticancer agents in use clinically have not succeeded in curing the malignant conditions despite the cost and time for their development. Therefore, there is a continuous demanding to develop new, effective, and affordable chemotherapeutic drugs [3]. Herbal products have received increasing attention

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over the last 30 years for their substantial anticancer potential [4,5]. In parallel, there is increasing evidence for the potential of plant-derived compounds as suppressor of several stages of tumorigenesis and associated inflammatory processes. Approximately 60% of drugs currently are used for cancer therapy isolated from natural products [6].

Currently, more than 3000 plants worldwide have been investigated to reveal anti-cancer features. Globally, the plant-derived products for cancer treatment are from 10% to 45% and can reach up to 50% in Asiatic patients [7–9].

Breast cancer is one of the major common cancer types in women worldwide, accounting for approximately 570,000 deaths in 2015. Over 1.5 million females (25% of all women with cancer) are diagnosed with breast cancer yearly throughout the world. Moreover, Breast cancer is a heterogeneous disease on the molecular level and most malignant cancer in woman. In the past 10–15 years, treatment concepts have been evolved to consider this heterogeneity into account, with the more focus on biologically- directed products and less aggressive treatment to reduce the adverse effects [10.11].

In The united states of America, it is estimated that 30% of most new cancer patients among women are breast cancer in 2017 [12]. Importantly, breast cancer is metastatic cancer and can commonly transfer to other organs such as the bone, liver, brain and lungs which known on their incurability. Interestingly, early diagnosis of the disease can lead to promising prognosis and a significant survival rate [13]. Moreover, in north American, the 5-year relative survival rate of breast cancer is up to 80% due to the early detection of this disease [13].

On the other hand, there're several risk factors such as sex, family history, estrogen, ageing, gene mutations, and unhealthy lifestyle, which can increase the possibility of inducing breast cancer [14]. Most breast cancers happen in women and the number of cases is 100 folds greater than that in men [12]. Although the incidence rate of breast cancer in America increases year after year, the mortality rate decreases due to the wide early screenings and developed medical therapies [14].

Esophageal cancer is considered a serious malignancy regarding its prognosis and death rate. Approximately more than 400000 death cases worldwide in 2005 [15]. Esophageal carcinoma is the 8th common type of cancer, and the 6th most common cause of cancer-related deaths worldwide in developing countries, which account for more than 80% of total cases and deaths [16]. Over 490000 newly diagnosed cases of esophageal cancer were discovered in 2005 only [16]. In contrast, several other kinds of cancer are expected to reduce in incidence in the next 10 years by 2025, however, the prevalence of esophageal cancer is expected to elevated by 140% [5]. According to the National Cancer Institute in USA, esophageal cancer cases are around 17990 new cases and 15210 deaths in 2013 [17]. Regarding the Prognosis in esophageal cancer is majorly based on local invasion as well as spread to regional and distant tissues within the body. Esophageal cancer is known with its aggressiveness, spreading by a various of pathways including lymphatic spread, direct extension, and hematogenous metastasis. Because of lacking of serosa in the esophageal wall plays an important role in the local spreading of esophageal cancer. As no anatomical barrier, the initial tumor can extend quickly into the adjacent organ of the thorax and neck including the thyroid gland, lung, trachea, larynx, pericardium, aorta, and diaphragm [18].

The Rumex species, belonging to the Polygonaceae family, including about 200 species widely-distributed worldwide. Moreover, the name Rumex comes from the word for dart, alluding to the shape of the leaves in Latin [19]. Many researches have been reported in different ethnobotanicals and ethnopharmacological literature the traditional uses of Rumex species [20]. In some areas, the leaves of Rumex species (e.g., *R. acetosa, R. tuberous, R. acetosella, R. abyssinicus, R. Crispus, R. sanguineus, and R. thyrsiflorus, R.*

vesicarius) are used as foods, mainly in the forms of sauces sour soups (usually with milk), and salads [21].

Rumex acetosella is known as red sorrel, field sorrel, sheep's sorrel and sour weed, is a flowering plant species in the buckwheat family Polygonaceae. R. acetosella is belong to native of Europe, the Middle East, Russia, and northern Africa. Also, R. acetosella is naturalized in New Zealand, and the southern tip of Africa, western South America, Iceland, and the USA. R. acetosella is characterized by green arrowhead-shaped leaves and red-tinted deeply ridged stems, and it sprouts from an aggressive and spreading rhizome and its flowers emerge from a tall, upright stem while flowers are maroon in color in female plant [22].

Sorrel is an important part as it is considered as a nutritional powerhouse, providing significant amounts of substantial micronutrients, including vitamin A, a fat-soluble vitamin that helping human to maintain healthy vision, reproductive health, skin, immune function, and growth. Also, vitamin C, an important antioxidant that helps your body to resist infection [23].

Furthermore, one American formula is known essiac which is a common anti-cancer treatment. The whole plant is used in the fresh status as refrigerant, diaphoretic, and diuretic. Moreover, tea makes from the leaves is utilized in the treatment of scurvy, fevers, and inflammation. While, the juice of the leaves is useful in the treatment of urinary and kidney diseases [24-26]. leaf poultice is also applied to different cancers, cysts, etc, while the folk usage for cancer. Additionally, tea produced from the roots is astringent which can be used to treat excessive menstrual bleeding and diarrhea [27]. A one-cup of sorrel provides about 4 grams of fiber to maintain cholesterol levels, regular bowel movements, and blood sugar. Fiber can also be used in certain health conditions including cancer, type 2 diabetes and obesity [27].

Sorrel is also sometimes useful for medicinal purposes such as sheep's sorrel which is a primary ingredient in Essiac tea, which is a herbal tea that was rumored to treat breast cancer and prevent other diseases including HIV/AIDS and diabetes [27]. Moreover, Essiac tea, containing R. acetosella, is investigated for its action to scavenge reactive oxygen species and for its mechanism on DNA damage. This formula is used in homeopathic cancer treatment and also to treat a variety of diverse allergies, osteoporosis, and hypertension. In vitro, Essiac tea has been shown to suppress cells proliferation and to improve differentiation in human prostate cancer cell lines [28,29].

Finally, many phytochemical compounds have also been isolated from R. acetosella which include Anthraquinones (emodin, Chrysophanol, physcion, emodin-8-O-β-D-glucopyranoside barbaloin, sennosides, and rhein) [30] , phenolic compound(gallic acid and p- coumaric), flavonoids (luteolin 7-O-glucoside, apigenin-C-glycosides quercetin derivatives, significant amounts of kaempferol derivatives, as well as myricetin and methylated derivatives of flavonols are present), stilbenoids and terpenoids [31-33].

So, in this study, it will be investigated anticancer activity of the leaves of *Rumex acetosella* naturally abundant tree found in every city in Iraq.

Material and Methods

Plant material collection.

The leaves of *Rumex acetosella* were collected from the peripheral of Sulaymaniyah of Kalo bazyan in March 2019. The plant was identified and authenticated by Prof. Dr. Sukaena Abass /Department of Biology /College of Sciences/ University of Baghdad. Leaves were washed thoroughly, dried under shade, and ground in a mechanical grinder to a fine powder.

Method of work

The air-dried powder of the leaves is weighted then defatted with N-hexane to get rid of chlorophyll and waxy material and then extracted by maceration with methanol for 72hr then the extract is combined and dried by a rotary evaporator the dry extract is

weighted and then used for anticancer activity.

Chemicals and Reagents

Table 1: chemical and reagent used

No.	Items	Company	Country
1	Trypsin/EDTA	Capricorn	Germany
2	DMSO	Santacruz Biotechnology	USA
3	RPMI 1640	Capricorn	Germany
4	MTT stain	Bio-World	USA
5	Fetal bovine serum	Capricorn	Germany

1-EDTA (Ethylenediaminetetraacetic acid) acts as a metal chelator, which is added to **trypsin** solutions to enhance activity. **EDTA** is added to remove the calcium and magnesium from the cell surface which allows **trypsin** to hydrolyze specific peptide bonds. The principal reason for using the **EDTA** along with **trypsin** is to remove cell to cell adhesion

2-The suggested mechanism for **DMSO** (Dimethyl Sulfoxide)

cytotoxicity is the effect on the physical properties of the phospholipids in membranes. As an amphipathic solvent, **DMSO** can interact with the plasma membrane allowing pores formation, which contributes to decrease membrane selectivity and increases **cell** permeability

3-RPMI 1640, also known as **RPMI medium**, is a growth medium used in cell culture.

4-**Molecular Targeted Therapies** (**MTT assay**) is used to measure cellular metabolic activity as an indicator of cell viability, proliferation, and cytotoxicity. ... The darker the solution, the greater the number of viable, metabolically active cells. This non-radioactive, colorimetric **assay** system using **MTT** was first described by Mosmann,

5-**Fetal bovine serum (FBS**) is a byproduct of harvesting cattle for the meatpacking industry—it's used extensively by both academic and industrial researchers as a supplement to the basal growth medium in cell culture applications. FBS is the liquid portion that remains after blood is drawn from the bovine fetus coagulates

Instruments

Table 2: instruments used.

No.	Item	Company	Country
1	CO2 incubator	Cypress Diagnostics	Belgium
2	Microtiter reader	Gennex Lab	USA
3	Laminar flow hood	K & K Scientific Supplier	Korea
4	Micropipette	Cypress Diagnostics	Belgium
5	Cell culture plates	Santa Cruz Biotechnology	USA

Maintenance of cell cultures

AMJ13 (new breast cancer ***cell line*** (***AMJ13***) has been established from an Iraqi breast cancer patient. It is considered unique because it is the first for an Iraqi population) and SK-GT-4 cells (Human esophageal adenocarcinoma *cell line*) were maintained in RPMI-1640 supplemented with 10% Fetal bovine serum, 100 units/mL penicillin, and 100 µg/mL streptomycin. Cells were passaged using Trypsin-EDTA reseeded at 80% confluence twice a week and incubated at 37 °C [34,35].

Cytotoxicity Assays

To determine the cytotoxic effect of *Rumex Acetosella*, the MTT assay was done using 96-well plates [36,37]. Cell lines were seeded at 1 × 104cells/ well. After 24 hrs. or a confluent monolayer was achieved, cells were treated with tested compounds at different concentrations. Cell viability was measured after 72 hrs of treatment by removing the medium, adding 28 µL of 2 mg/mL solution of MTT, and incubating the cells for 2.5 h at 37 °C. After removing the MTT solution, the crystals remaining in the wells were solubilized by the addition of 130 µL of DMSO (Dimethyl Sulphoxide) followed by 37 °C incubation for 15 min with shaking [38]. The absorbency was determined on a microplate reader at 492 nm; the assay was performed in triplicate. The inhibition rate of cell growth (the percentage of cytotoxicity) was calculated as the following equation [39,40].

Inhibition rate = A- B/A*100

where A is the optical density of control, and B is the optical density of the samples [41].

To visualize the shape of the cells under an inverted microscope, the cell was seeded into 24-well micro-titration plates at a density of 1×105 cells mL−1 and incubated for 24 h at 37 °C. Then, cells were exposed to *Rumex Acetosella* at IC50 concentration for 24hr. After the exposure time, the plates were stained with crystal violet stain and incubated at 37 °C for 10–15 min [39]. The stain was washed off gently with tap water until the dye was completely removed. The cells were observed under an inverted microscope at 100× magnification and the images were captured with a digital camera attached to the microscope [42,43].

Statistical Analysis

The obtained data were statically analyzed using an unpaired t-test with GraphPad Prism 6 [44]. The values were presented as the mean ± SD of triplicate measurements and P value < 0.05 considered significant [45].

Results and Discussion

In this study, the cytotoxic effect of *Rumex Acetosella* against cancer cells was evaluated. The antitumor activity of the *Rumex Acetosella* was assessed by studying their ability to inhibit the proliferation of cancer cells through cancer cells line of esophagus and breast. The results of this research are showed that highly significant cytotoxic activity against the human cancer cell lines as showed in the Figures below (1,2). These data suggest that there is an ability of *Rumex Acetosella* to suppress the growth of cancer cell lines and importantly, this effect is concentration-dependent manner (Fig. 1,2). The previous result is coordinated with traditionally used plant in folk medicine for long time as natural remedies with significant therapeutic effects in many areas including prevention of antimicrobial, cardiovascular diseases, anti-inflammatory, and anticancer activity. Also, the emergence of resistance to cancer chemotherapy and its substantial adverse effect has forced researchers toward natural products of plant and marine origin.

Although many compounds isolated from plants are being rigorously tested for their anticancer properties, it is becoming clear that the beneficial effects of whole plants are due to its complex interplay of the mixture of compounds which present in the whole plant (additive/synergistic and/or antagonistic) rather than single constituent alone [46, 47].

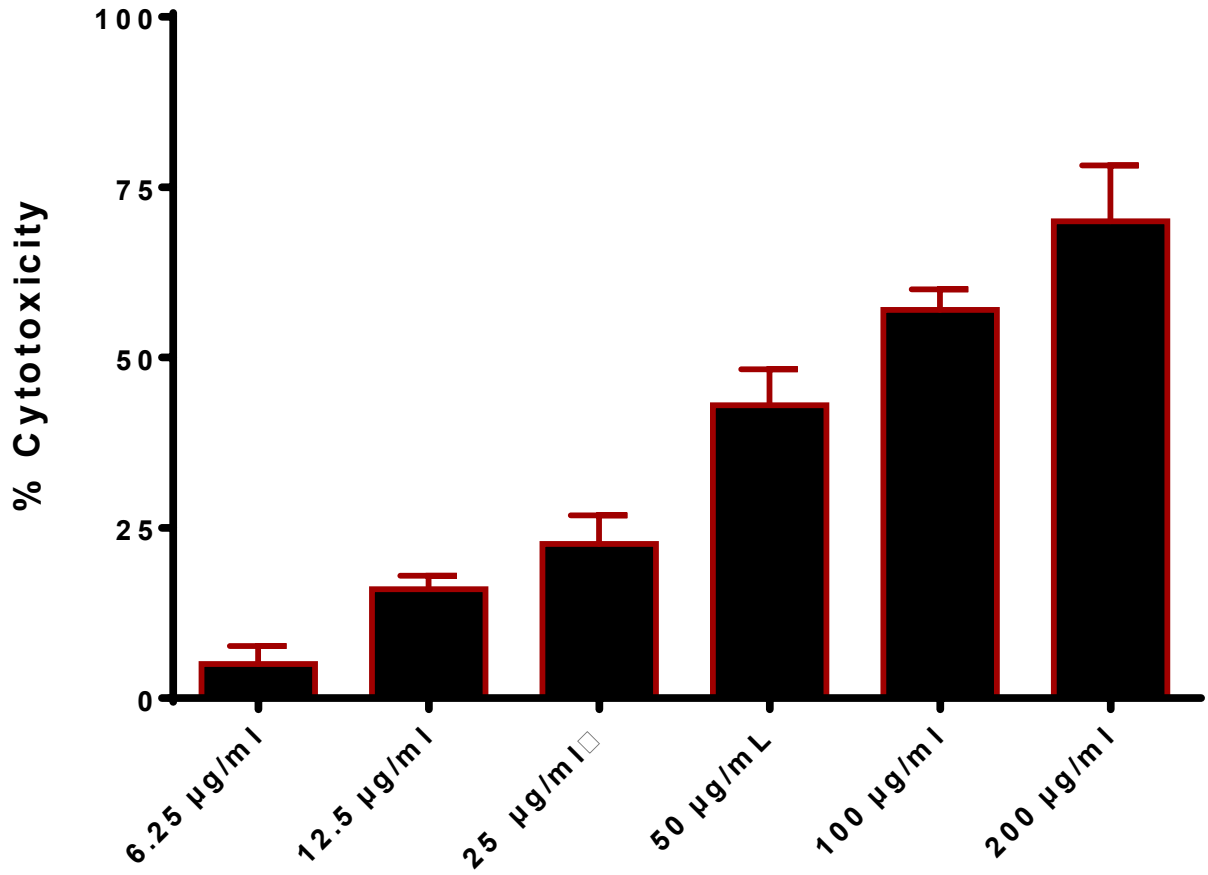

Figure 1: Cytotoxic effect of *Rumex Acetosella* in SK-GT-4 cells. IC50= 42.62 µg/ml

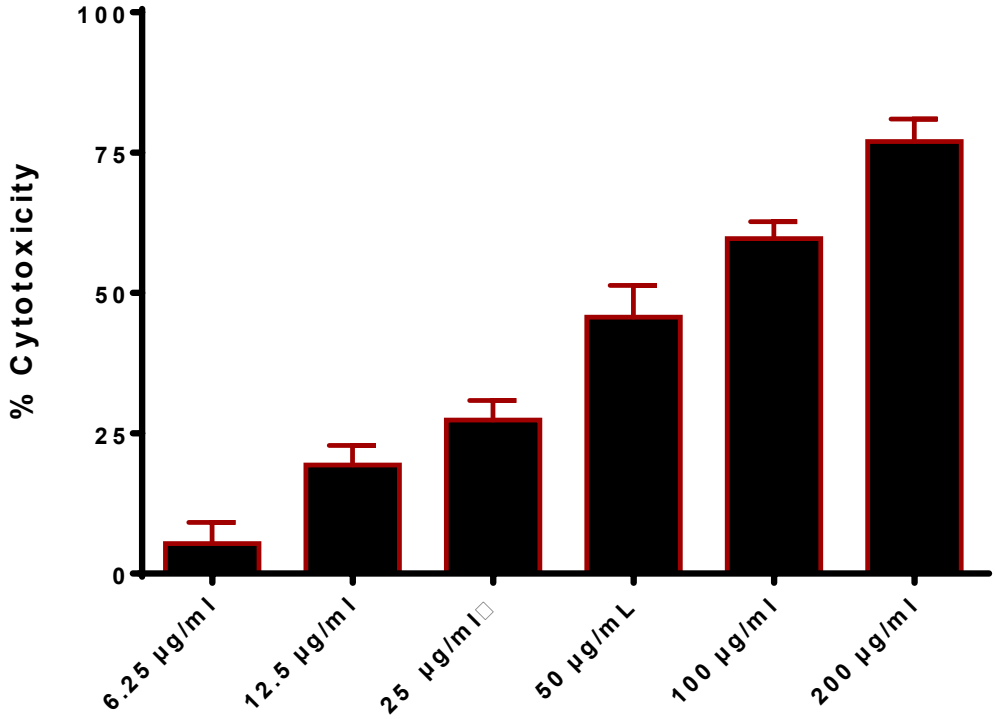

Figure 2: Cytotoxic effect of *Rumex Acetosella* in AMJ13 cells. IC50= 29.33 µg/ml

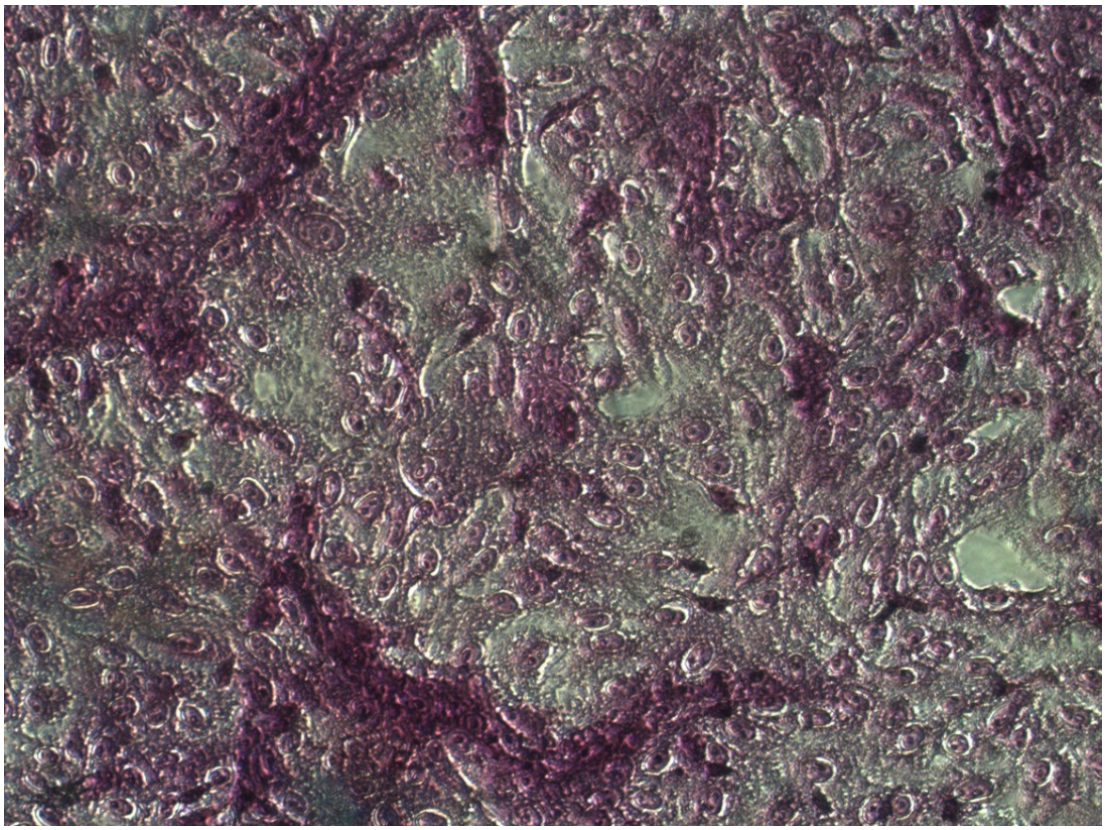

Figure 3: Control untreated SK-GT-4 cells

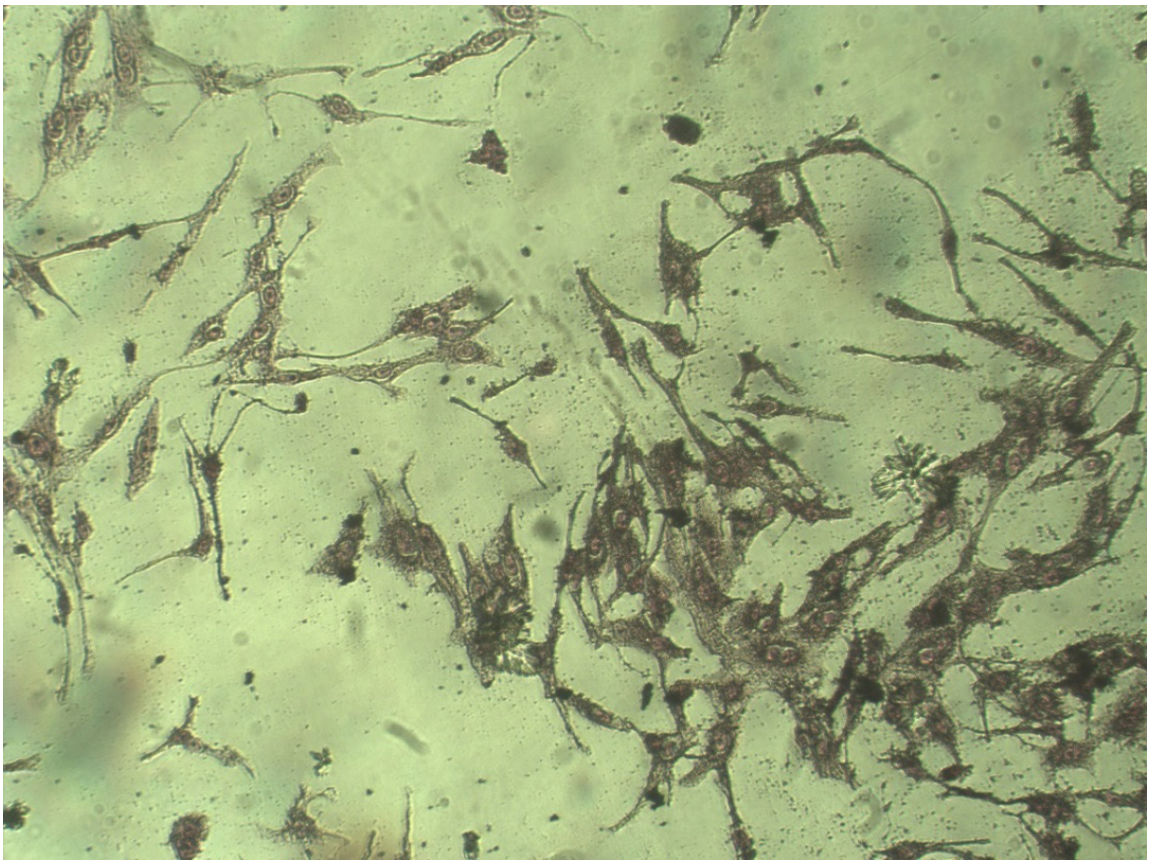

Figure 4: Morphological changes in SK-GT-4 cells after treated with *Rumex Acetosella* extract

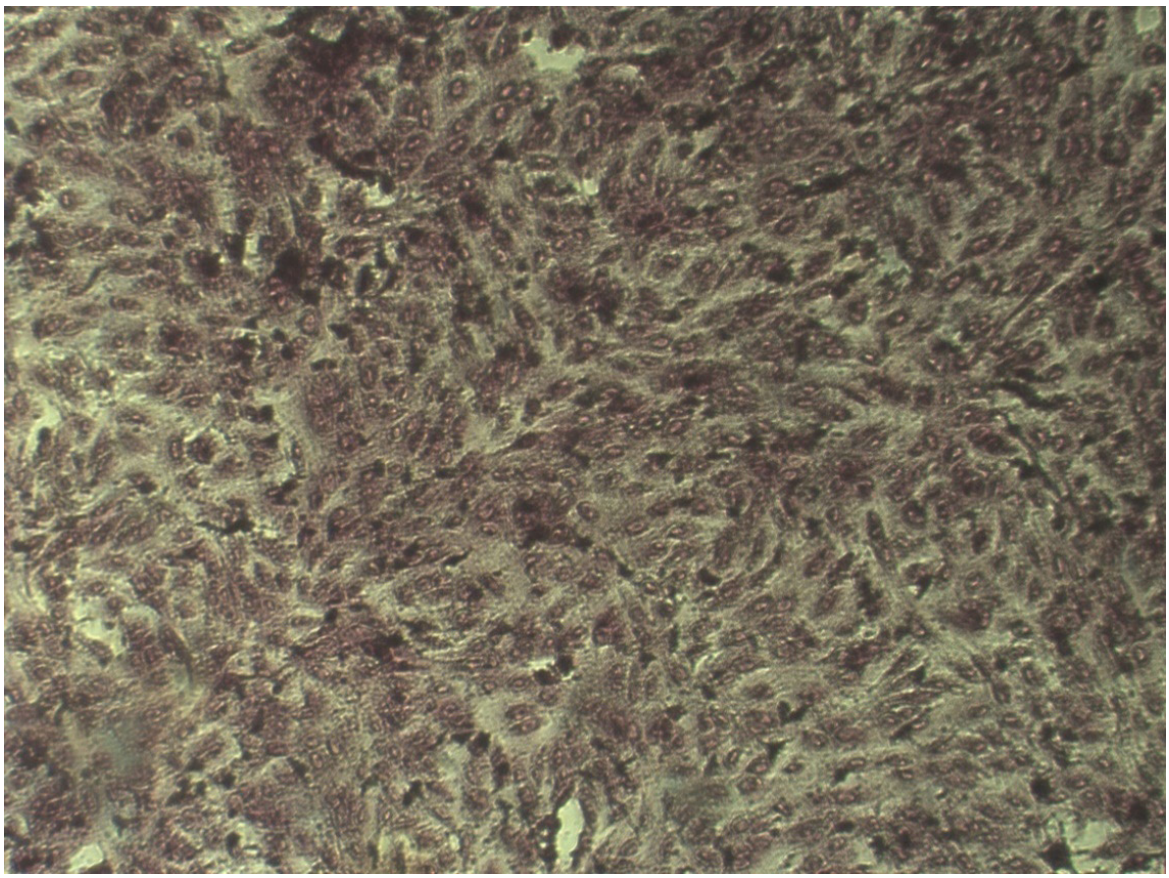

Figure 5: Control untreated AMJ13 cells

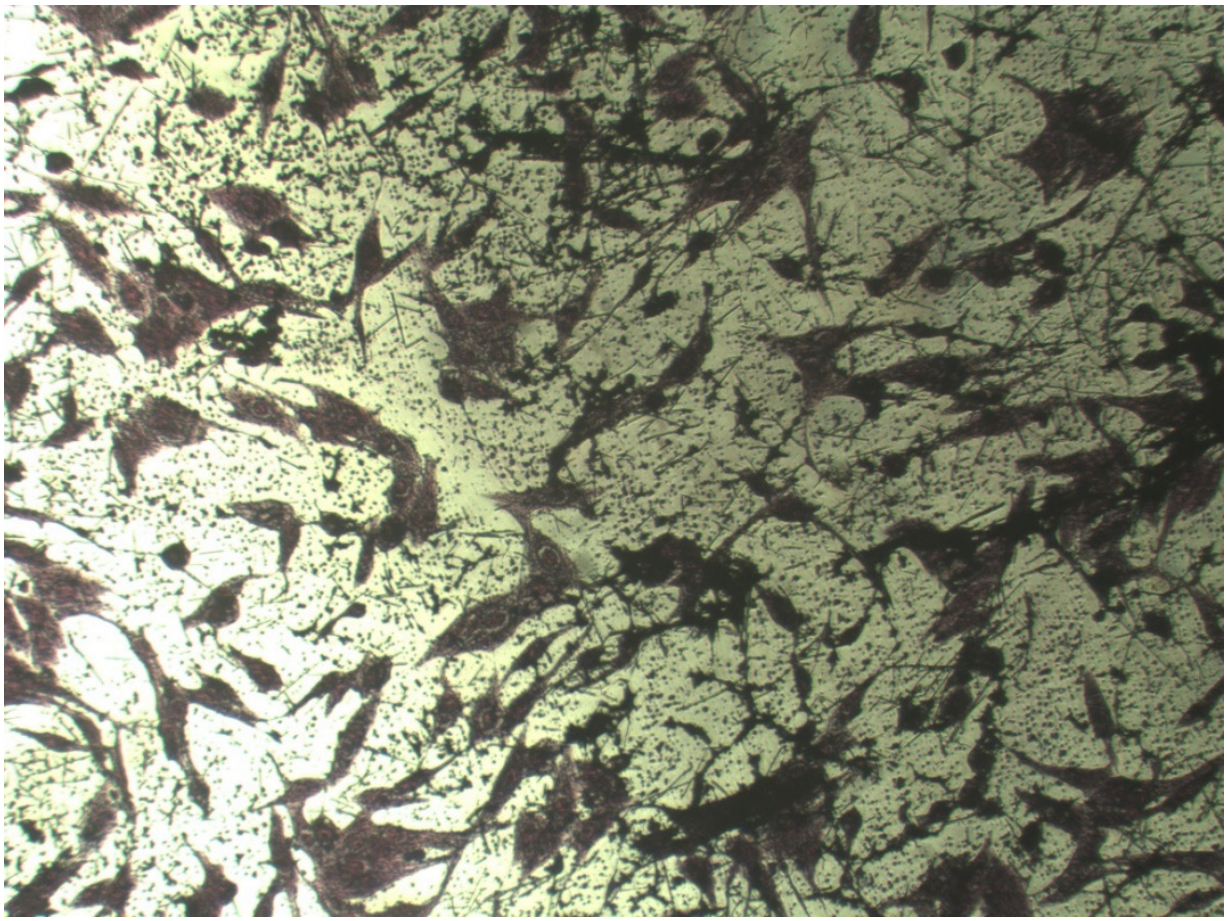

Figure 6: Morphological changes in AMJ13 cells after treated with *Rumex Acetosella* extract.

Conclusions

Whole-cell extracts (methanolic extract) from *Rumex Acetosella*, plants indigenous to the coastal plain and desert areas of Iraq, exhibited dose and time-dependent killing capabilities on various human-derived hematological and solid tumor cell lines and primary cultures established from patients 'biopsies. The killing activity was specific toward tumor cells, as the plant extracts did not affect primary cultures of healthy cells line. Cell death caused by the whole plant extracts was via apoptosis. Plant extract from *Rumex Acetosella* showed particularly strong anticancer capabilities since it inhibited actual tumor progression in a both breast and esophagus cancer cell line. Our results suggest that whole plant extracts are promising anticancer reagents.

Conflict of Interests: The authors declare that there is no conflict of interests regarding the publication of this paper

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Ethical Clearance: According to ethical committee of college of pharmacy/ University of Baghdad

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