Chemical Evaluation of Some Bio Extracts with Pathological Study on Tumor Cell in Mice Model

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Abstract— Our study objective was to chemically evaluate some bio extracts regarding Chemical composition, Minerals contents, and their effects on tumor cells. Seventy seven adult Swiss female albino mice (20-25g) were procured from the animal house of Faculty of Pharmacy, Mansoura University, Egypt, a model of Solid Ehrlich Carcinoma (SEC) were implanted subcutaneously into the right thigh of the lower limb of 70 mice and a palpable solid tumor mass (about 100 mm3) was developed within 12 days. Female Swiss albino mice were divided into 11 groups with 7 animals of each. Moisture content for air dried investigated flowers was 11.16, 9.45, 12.62 and 9.76% of Cynara cardunculus, Achillea millefolium, Calendula officinalis, and Matricaria chamomilla, respectively. Ash, crude fiber, crude lipids, crude protein, total sugars, reducing sugar and non-reducing sugars were also determined and calculated as (g/100g dry weight). Cynara cardunculus and Achillea millefolium flowers have the highest values of crude fiber contents which were 33.72, 36.31 and 37.44 %, respectively. Calendula officinalis flowers have the lower value of crude fiber which was 28.15%.While, the same plant have the highest values of lipids and crude protein which were 2.19 and 19.58%, respectively. On the other hand, M. chamomilla flowers have the higher values of ash, total sugars, reducing sugar and non-reducing sugar which were 6.77, 49.50, 22.52 and 26.89%, respectively. These values were higher than those of A. millefolium flowers which were 36.31, 26.60, 20 and 46.62% for crude fiber, total sugars, reducing sugar and non-reducing sugar contents, respectively. Calcium was the main element in all samples which ranged from 625 to 972 mg/100g on dry weight basis in A. millefolium and C. officinalis, respectively. Also, Magnesium content, of C. cardunculus, A. millefolium and C. officinalis was 525, 298 and 245.1 mg/100gm, respectively. Moreover, the methanolic and aqueous extract of all plants showed the effects as antitumor in mice when compared with doxorubicin.

Key words: Achillea (*Achillea millefolium*), Chamomile (*Matricaria chamomilla*), Chemical composition, Globe Artichoke (*Cynara Cardunculus*), Histopathology, Mice, Minerals contents, Pot marigold (*Calendula officinalis*), Tumor Cell

1 INTRODUCTION:

Since antiquity, plants play an important role in both food and medicine. Higher plants represent the only source for feeding humans or animals such as fresh or cooked legumes, oil crops, fiber crops, fruits and vegetative crops. It is worthy to state that some of plant organs are responsible for synthesizing of some compounds by means of green leaves synthesize carbohydrates which are converted to another components via some specific cycles.

Medicinal plants are considered as very important source for obtaining natural crude materials which are used in pharmaceutical industries. Nowadays, many investigators focused their studies for Medicinal plants in order to extract components which have effects as antibacterial and antioxidant activities, also, some diseases such as diabetes, cardiovascular, diarrhea, antitumor and anticancer, etc.

Globe Artichoke (*Cynara Cardunculus*) belongs to Family *Compositeae* are grown in the Mediterranean area, North Africa, North America and Italia. It is widely used in Greeks and Romans as food and medicine**.** Folk medicine, Artichoke flowers and leaves extract has shown antioxidant, antibacterial, anti-HIV, bile-expelling, hepatoprotective, urinative and choleretic activities, as well as the ability to inhibit cholesterol biosynthesis and LDL oxidation [1].

Achillea (*Achillea millefolium*) belongs to Family Compositeae contain one of the most important plants namely which grown widely all around Europe, Asia, North Africa and North America in it is widely used in Italian folk medicine. Plant properties have been known since antiquity in different uses in many cultures from Europe to Asia. For instance, in Greece, A. millefolium is recommended for the treatment of many different ailments. Also, in west Azerbaijan and Iran, the infusion of dried flowers are considered suitable for treatment of antioxidant, antifungal, anti-inflammatory, anti-edematous, antiviral, antimicrobial, hemorrhoids, dyspepsia, dysmenorrhea, gastritis and immune modulatory properties [2].

Pot marigold (*Calendula officinalis*) belongs to Family Compositeae has been a subject of several chemical and pharmacological studies of sesquiterpenes, triterpenes, saponins, carotenoids, flavonoids, hydroxyl coumarins, tannins, and volatile oils for traditional medicine uses in Europe, China and India amongst several places in the world for cutaneous wound healing, collagen efficiency, jaundice, blood purification, cytotoxic, antitumor and antispasmodic effects [3].

Chamomile (*Matricaria chamomilla*) belongs to Family *Compositeae* is as an annual herbaceous flowering plant native to Europe. It has been used traditionally as a medicinal and pharmaceutical preparation due to its anti-inflammatory and antispasmodic properties. Apigenin 7-glucoside is one of the

main components of flowers and as such has become the standard flavonoid to establish extract potency. Phenolic acids including caffeic, chlorogenic and ferulic acids derivatives have been also found in flowers of chamomile [4].

Medicinal plants containing flavonoids and terpenoids products that have diverse pharmacological properties including antioxidant and cancer chemo preventive effects, e.g. solid Ehrlich tumor and ascetic Ehrlich tumor [5].

Cynara Cardunculus

Achillea Millefolium

Calendula Officinalis

Matricaria Chamomilla

2 MATERIALS AND METHODS:

2.1 Plant Materials

Flowers samples of C. cardunculus, A. millefolium, C. officinalis, and M. chamomilla, were kindly obtained from Agricultural Research Center, Giza, Egypt. Samples from two species were air dried in the shade and ground into a fine powder.

The powdered air dried flowers were divided into dry part and two extracts. First extract; Powdered air dried flowers (1Kg) from each samples were extracted by soaking at room temperature for six hours with methanol (10L), then the methanolic extracts were concentrated to nearly dryness under reduced pressure using the rotary evaporator at 45°C to achieve the crude methanol extract which kept for further investigation [6]. Second extract; Powdered air dried flowers (1Kg) of dried samples were extracted with distilled water by boiling at temperature from 80 to 100°C in reflux for 3h to achieve an initial extract, the extract was filtered after cooling to room temperature, Finally, the extract was lyophilized and preserved at −20°C until further use according to [7]. All tests were conducted in and were obtained from Science Academy of Experimental Researches, Mansoura, Egypt.

2.2 Chemical composition of investigated flowers:

A known weight of air dried leaves (2g) was dried at 105°C in an air drying oven to a constant weight, and the percentage of moisture content was calculated [8].

Determination of ash content: Ash content was determined according to **[8]**, as follow: Exactly 2g of air dried leaves were placed in a silica crucible and ignited at 600°C in a muffle furnace till a constant weight then percentage of ash content was calculated.

Determination of crude fiber content: Crude fiber is a mixed material and defined as the sum of lignin and polysaccharide contents which not digested by dilute acid and alkali. Crude fiber was estimated according to the method described by **[8]**, A known weight of the air dried leaves (2g) was mixed with 0.5g asbestos, then 200ml of sulphuric acid (1.25%v/v H_2SO_4), were added, the mixture was boiled under reflex for 30 minutes, followed by filtration through Gooch crucible, the residue was boiled again with aqueous sodium hydroxide solution (200ml, 1.25%w/v NaOH) for 30 minutes then filtration was repeated in the same manner, Finally the residue was washed with hot water followed by diethyl ether and dried at 110°C to a constant weight. The content of Gooch crucible was then ignited in the muffle furnace at 600°C to a constant weight. Fiber content was calculated by subtraction of ash content from the weight of digested sample. Percentage of crude fiber content was then calculated.

Determination of soluble carbohydrate content: The soluble carbohydrate contents were determined with a slightly modified phenol–sulphuric acid method according to **[9]**. The colour reaction was initiated by mixing 50µml of crude polysaccharide solution with 150 µml of concentrated sulphuric acid, followed immediately with 30µml of 5% phenol, and the reaction mixture was kept at 90◦C for 5 min. After cooling to room temperature, the absorbance of the mixture was measured at 490 nm, using a Spekol 11 (Carl Zeiss-Jena) spectrophotometer. The total carbohydrate content was calculated with D-glucose as a standard material.

Determination of reducing sugar: The reducing sugar was determined by the modified method of **[10]**. Briefly, 0.5 ml of 1% 3, 5-dinitrosalicylic acid (DNS) was added to an aliquot of sample (20–500 µml), and the volume adjusted to 5 ml with distilled water. After shaking, the mixture was heated in boiling water for 5 min and cooled to room temperature; 2.5 ml of distilled water were added to the mixture. The absorbance was measured at 540 nm, using a Spekol 11 (Carl Zeiss-Jena) spectrophotometer. The total reducing sugar was calculated with D-glucose as a standard reducing sugar.

Calculation of non-reducing sugars: Insoluble sugars were calculated according to the following equation:

Non-reducing % = Total sugars % - Reducing sugars %

2.3 Minerals content of investigated Flowers:

Ashed air dried flowers sample was dissolved individually in 1ml of concentrated hydrochloric acid solution and the volume was completed to 100 ml with distilled water. Sodium and potassium were determined using flame photometer according to **[11]**. Magnesium, calcium, copper, zinc, manganese, and iron were determined using atomic absorption (Perkin-Elmer 2380) according to **[12]**. Phosphorous was determined colorimetrically as described by Page (1982). Previous determinations were achieved at Agricultural Research Center, Mansoura, Egypt.

2.4 Effect of crude methanolic and aqueous extracts on cancer cell in mice:

2.4.1. Experimental animals:

Seventy seven adult Swiss female albino mice (20-25g) were

procured from the animal house of Faculty of Pharmacy, Mansoura University, Egypt. All mice were housed in microlon boxes in a controlled environment (temperature 25±20°C and 12 h dark/ light cycle) with standard laboratory diet and water ad libitum [13].

2.4.2. Solid Ehrlich Carcinoma (SEC) Tumor Model:

A model of SEC where 0.2 mg (1×10^6) of the Ehrlich carcinoma cells obtained from Experimental Oncology Unit of National Cancer Institute, Cairo University, Egypt, were implanted subcutaneously into the right thigh of the lower limb of 70 mice. A palpable solid tumor mass (about 100 mm^3) was developed within 12 days **[14].**

2.4.3. Experimental protocol:

Female Swiss albino mice were divided into 11 groups with 7 animals of each, as follows;
Group 1 (normal): represents normal mice (non tumors).
Group 2 (control): SEC-bearing untreated mice.
Group 3: SEC-bearing mice treated with doxorubicin (DOX), (molecular weight: 543.5262 and chemical formula: C27-H29-NO11) in concentration of 15 mg/kg.
Group 4, 5, 6 and 7: SEC-bearing mice treated with methanolic extract of A. millefolium, C. officinalis, A. millefolium, and C. officinalis flowers 200 mg/kg.
Group 8, 9, 10 and 11: SEC-bearing mice treated with aqueous extract of A. millefolium, C. officinalis, A. millefolium and C. officinalis flowers 200 mg/kg.

2.4.4. Histopathological Examination:

At the end of the experimental period after 21 days, selected organs (right thigh) were excised, rinsed in isotonic saline solution and stored in 10% formalin, then treated with conventional grades of alcohol and xylol, embedded in paraffin and sectioned at 4 to 6µ thickness. Sections were stained with Haematoxylin and Eosin (H and E) stain for studying the histopathological changes in organs tissues [15].

2.5. Statistical analysis: Statistical analyses of all experimental data were done using the statistical software package **[16]**. All comparisons were first subjected to one way analysis of variance (ANOVA) and significant differences between treatment means were determined using Duncan's multiple range test at $P< 0.05$ as the level of the significance **[17]**.

3. RESULTS AND DISCUSSION:

3.1. Chemical composition of investigated flowers:

As showed in **table (1)** and **figure (1),** the percentages of moisture content for air dried investigated flowers were 11.15, 9.44, 12.61, and 9.75% for *Cynara cardunculus, Achillea millefolium, Calendula officinalis* and *Matricaria chamomilla,* respectively. These results were in accordance with those obtained by **[18]**, who found that the percentage weight loss on drying of *Achillea millefolium* flowers was 9.50%. Also, our findings coincided with those obtained by **[19]**, who found that the percentage of moisture was 9.81% for *Matricaria chamomilla* flowers. While the results were lower than those reported by **[20]**, who stated that percentage of moisture was 18.79% for *Calendula officinalis* flowers. On contrary, the data were higher than those reported by **[21]**, who found that moisture content was 75.80% for *Cynara cardunculu* fresh flowers.

Table 1: Chemical composition of investigated Flowers

Plant flowers	Moisture %	Crude Ash %	Crude Fiber %	Crude Lipids %	Crude Protein %	Soluble carbo-hydrate %	R-Sugar %	NR-Sugar %
C. Cardunculus	11.15	4.34	33.72	1.71	16.93	43.28	18.07	25.16
A. millefolium	9.44	3.71	36.31	1.83	11.49	46.62	20	26.60
C. officinalis	12.61	5.02	28.15	2.19	19.58	45.04	19.85	25.15
M. chamomilla	9.75	6.77	37.44	1.46	4.82	49.50	22.52	26.89

Furthermore, ash, crude fiber, crude lipids, crude protein, total sugars, reducing sugar and non-reducing sugars were determined in *Cynara cardunculus, Achillea millefolium, Calendula officinalis,* and *Matricaria chamomilla* flowers. All results were calculated as (g/100g dry weight) and recorded in table (1).

From table (1) and figure (1), it was clear that *Cynara cardunculus* and *Achillea millefolium* flowers have the lowest percentage values for ash which were 4.34 and 3.71%, respectively. While, the lowest percentage value of protein was 4.82% for *Matricaria chamomilla* flowers. On the other hand, the same

flowers have the highest values of crude fiber contents which were 33.72, 36.31 % for *C. cardunculus, A. millefolium* flowers respectively.

Data in table (1) and figure (1) showed that *Calendula officinalis* flowers have the lower value of crude fiber which was 28.15%. While the same plant values of lipids and crude protein were 2.19 and 19.58%, respectively. On the other hand, *Matricaria chamomilla* flowers have the lowest values of crude lipids and crude protein which were 1.46 and 4.82%, respectively. Though, the same plant have the highest values of ash, crude fiber, total sugars, reducing sugar, and non-reducing sugar which were 6.77, 37.44, 49.50, 22.52, and 26.89%, respectively.

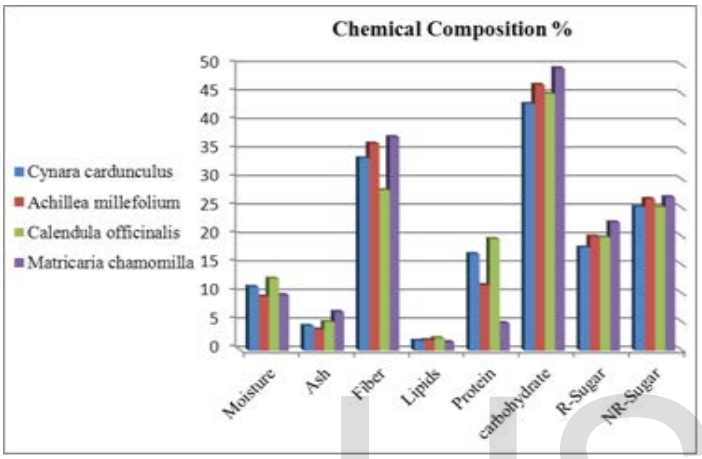

Figure 1: Chemical composition of investigated Flowers
R-Sugar = Reducing SugarNR-Sugar = Non-Reducing Sugar

The percentages of ash, crude fiber, crude lipids and crude protein were 3.8, 17.1, 24.05, and 21.6% for Cynara Cardunculus seeds, respectively [1].

The data were not in the same line with those reported by [21], who mentioned that the percentages of ash, crude fiber, crude lipids and crude protein were 1.56, 63.76, 76.34, and 13.84 %, for fresh Artichoke, respectively.

Previous data were in the same line with those reported by [22], for Achillea millefolium flowers protein which ranged from 12.53 to 19.53 g/100g based on dry weight. Also, they stated that data of crude lipids and total sugars were not agreed with the same authors who reported that fat content was ranged from 5.20 to 8.03 g/100g and carbohydrates was in average of 63.90 to 75.84 g/100g on dry weight basis.

The results of Calendula officinalis flowers for ash and soluble carbohydrate were 5.02 and 45.04% based on dry weight, respectively. These findings were not in the same line with those reported by [20], who found that ash content was 3.67%. Also, the total carbohydrates content was 28% [23].

Previous data of Matricaria chamomilla flowers for crude protein was 4.82% which accordance with those obtained by [24], who stated that crude protein was 4.02%. On contrary, our results for ash, crude fiber and crude lipids were 6.77, 37.44, and 1.46%, respectively, which were higher than those achieved with [24], who found that ash, crude fiber, and neutral detergent essential oil were in average values of 2.02, 2.11, and 0.759 %, respectively.

3.2 Minerals content of investigated flowers:

Minerals or elements play an important role in either plant or human nutrition. For example, calcium is an essential element for animal bones skeleton and iron represent an important physiological function in the hemoglobin and other elements are important for activity of some enzymes and vitamins.

From table (2) and figure (2), it could be seen that calcium was the main element in all samples which ranged from 76.4 to 972 mg/100g based on dry weight in *Matricaria chamomilla* and *Calendula officinalis* flowers respectively.While Magnesium content was ranged from 40.8 to 525 mg/100g based on dry weight in *Matricaria chamomilla* and *Calendula officinalis* flowers respectively.

On the other hand, the copper content in *Cynara cardunculus* was 0.93 mg/100g on dry weight. While copper content in *Matricaria chamomilla* was (n.d). Also, the zinc and Manganese content in all samples which ranged from 0.036 to 3.37 mg/100g and 0.098 to 13.64 mg/100g on dry weight basis in *Matricaria chamomilla* and *Calendula officinalis* flowers respectively, table (2) and figure (3). The iron content in *Cynara cardunculus, Achillea millefolium, Calendula officinalis,* and *Matricaria chamomilla* flowers were 5.39, 7.485, 6.02 and 0.437 mg/100g on dry weight respectively.

Data in table (2) showed clearly that *Cynara cardunculus, Achillea millefolium, Calendula officinalis,* and *Matricaria chamomilla* flowers contain some elements in considerable amounts. These elements are calcium, magnesium, zinc, manganese, and iron, except copper of *M. chamomilla* as shown in table (2).

Table (2): Minerals content of investigated Flowers (mg/100g) on air dry weight basis

Minerals (mg/100g) based on dry weight					
	iron	5.39	7.485	6.02	0.437
	Manganese	1.36	3.11	13.64	0.098
	zinc	2.24	1.52	3.37	0.036
	Copper	0.93	0.49	0.67	n.d
	Magnesium	245.1	298	525	40.8
	Calcium	178.6	625	942	76.4

Plant flowers	C. Cardunculus	A. millefolium	C. officinalis	M. chamomilla

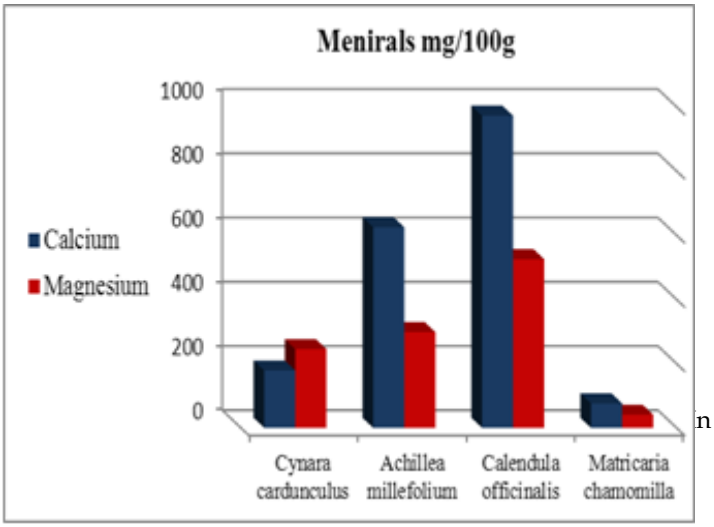

Figure (2): Minerals content of investigated Flowers (mg/100g) of Ca and Mg

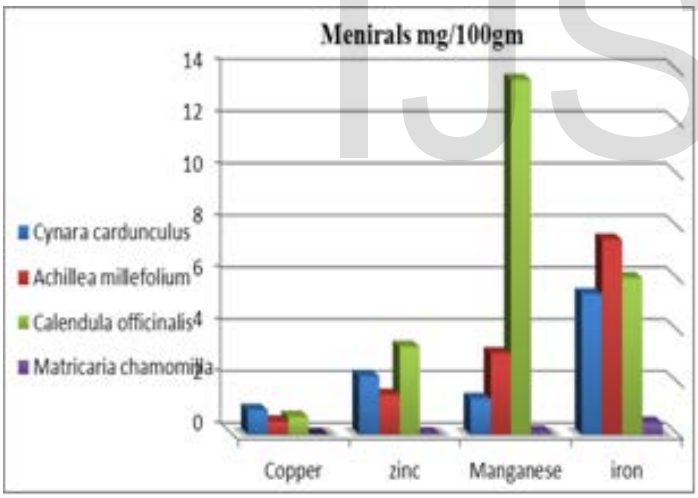

Figure (3): Minerals content of investigated Flowers (mg/100g) of Cu, Zn, Mn and Fe

[25] Studied Cynara cardunculus minerals and they found that the content of elements e.g. calcium, sodium, magnesium, potassium, manganese, copper, zinc, and iron were 2.7, 1.7, 0.3, 0.5, 0.1, 0.01, 0.1, and 1.5mg/100gm respectively. While [21] stated that elements content e.g. potassium, sodium, magnesium, and calcium were 364.30, 92.53, 59.07, and 43.31mg/100g in fresh artichoke respectively, reduced to 283.20, 74.56, 28.75, and 24.25mg/100gm, for cooked artichoke respectively.

[26] Estimated the minerals content of some medicinal herbs. They found that total micro elements content in Achillea millefolium were iron, copper, manganese, zinc, chrome, and nickel were179, 9.88, 84.3, 40.2, 0.26, and 5.54 mg/kg respectively. While minerals content of Calendula officinalis e.g. iron, copper, manganese, zinc, chrome, and nickel were 533, 15.51, 85.9, 49.9, 4.75, and 5.68 mg/kg. Also, minerals content of Matricaria chamomilla were 1440, 10.23, 135, 45.6, 1.25, and 4.39 mg/kg for the same elements, respectively.

3. 3. Effect of crude methanolic and aqueous extracts on histopathological changes of Solid Erlich Tumor (SET):

Histopathological examination of the Tumor tissue confirmed that the normal organs architecture **(figure 4)** was damaged after bearing tumor (0.2 ml of 1×10^6 cells/mice, subcutaneously except the normal group) and revealed larger sized tumour mass with infiltration of malignant cells with criteria of malignancy including; pleomorphism and frequent atypical mitotic figures **(figure 5)**. However, injection of doxorubicin in concentration of 15mg/kg body weight, methanolic, and aqueous extracts at a dose of 200mg/kg body weight significantly minimized the tumour mass compared with the tumor induced mice group **(figure 6, 7, 8).**

In the other hand, methanolic and aqueous extracts of *C. cardunculus* and *A. millefolium* flowers 200mg/kg body weight were less effective to heal the degenerative changes in the organs tissues. The results of the biochemical tests and histopathological observations suggested that treated mice with doxorubicin, methanolic, and aqueous extracts for *C. officinalis* followed by *M. chamomilla* were the most effective treatment.

These findings recommended that all crude methanolic and aqueous extracts of investigated flowers have comparable abilities and more effective in bringing the functional improvement of tissue.

Hence, our study suggested that crude methanolic extracts of investigated flowers play a beneficial role in the treatment of SEC-tumor mice induced tissues damage which could be one of their therapeutic values.

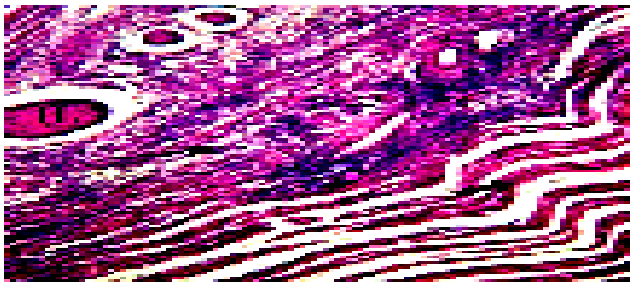

Figure (4): Normal thigh tissue composed of normal tissue. (H&E X 400)

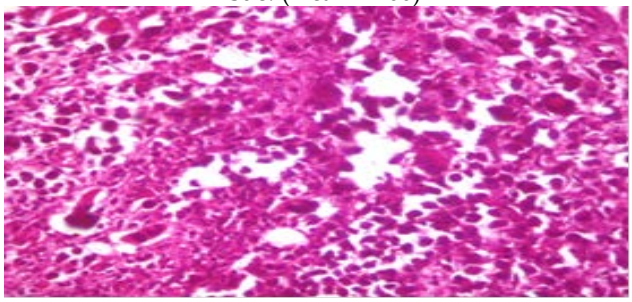

Figure (5): Higher magnification of Solid Ehrlich Tumor

larger sized tumour mass with infiltration of malignant cells with criteria of malignancy including; pleomorphism and frequent atypical mitotic figures. (H&E X 400)

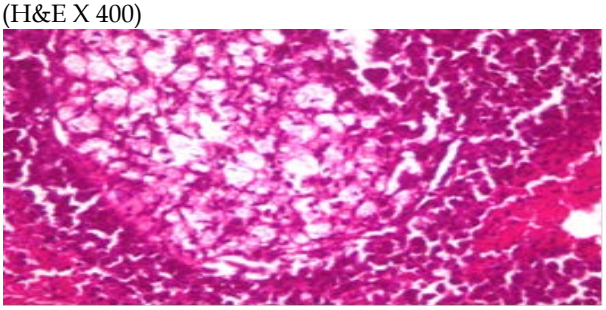

Figure (6): Tumor tissue of Solid Ehrlich Tumor bearing mice treated with doxorubicin showing large sized tumour mass with infiltration of malignant cells. (H&E X 400)

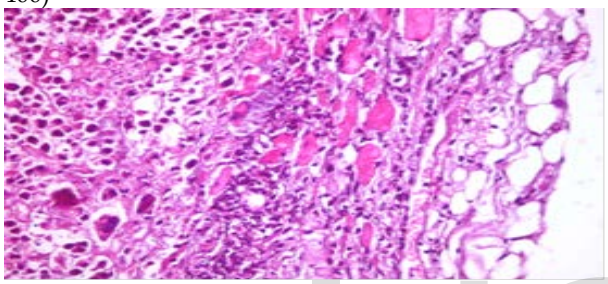

Figure (7): Tumor tissue of Solid Ehrlich Tumor bearing mice treated with methanolic extracts showing small sized tumour mass infiltrated with mononuclear cells. (H&E X 200)

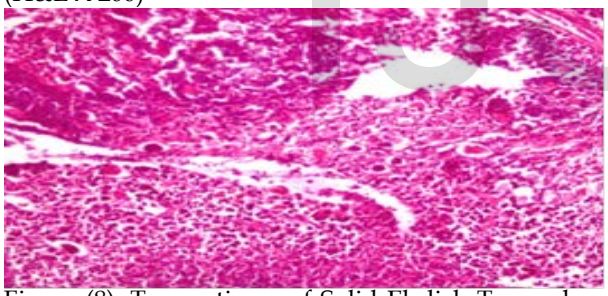

Figure (8): Tumor tissue of Solid Ehrlich Tumor bearing mice treated with aqueous extracts showing medium sized tumour mass infiltrated with mononuclear cells. (H&E X 200)

4. CONCLUSION

Chemical composition of the four investigated plants showed the highest content of moisture, ash, fiber, protein, lipid, total sugars, and minerals. Medicinal plant extract containing high percentage of active compounds such as polyphenols and flavonoids of *Cynara cardunculus, Achillea millefolium, Calendula officinalis*, and *Matricaria chamomilla* revealed antitumor activity [6], which confirmed by histopathology. Moreover, methanolic extracts were the most effective as antitumor followed by aqueous extract on mice if compared with chemotherapy (doxorubicin).

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