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STUDY OF THE INHIBITORY EFFECTS OF *RHEUM RIBES* EXTRACTS ON A PATHOGENIC FUNGI AND CANCER CELL LINE

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Abstract

The present study examined the anti-fungal and anti-growth role of cancer cell lines for the roots and stems of the *Rheum ribes*, in addition to evaluating its biological and antioxidant efficacy. This plant is very important in research because it is considered the source of one of the most important raw medicines in the Middle East, which belongs to the plant family Polygonaceae and is a plant known in the medical and therapeutic uses against many diseases such as Diabetes mellitus, intestinal parasites, Ulcer ulcers, Cancer Inflammation, as plant roots are used to treat intestinal worms, diabetes, high blood pressure, obesity, ulcers and diarrhea, and it has been found to contain chemicals that have an anti-oxidant, anti-inflammatory and bacterial growth. The diameter of the colonies was measured 48 hours after implantation at a temperature of 37°. The aqueous extract of the root shows that the higher the concentration of the extract, the greater the inhibition diameter. Also, the aqueous extract of the stem appears as the concentration of the extract increases, the diameter of the inhibition increases. But the aqueous extract of the stem showed greater efficacy than the aqueous extract of the root in inhibition and for all concentrations. The alcoholic root extract was more effective than the aqueous extract of the root and for all concentrations as well as for the alcoholic extract of the stem it showed higher efficacy than the aqueous extract of the stem. The effectiveness of the alcohol extract of the root and stem was close at concentrations 10 and 20, while the concentrations of 30 and 40 of the alcoholic extract of the stem gave the same effect despite the increased concentration.

Key words : *Rheum ribes*, Fungal infection, Yeast, Medical and therapeutic uses against diseases.

Introduction

There are large numbers of fungi in different environments, some of which are pathogenic, and the other part is coexisting with a natural state Norma flora, as there are approximately 180 types of 250,000 types of fungi known to be a major cause in human and animal disease, and that most of these fungi are filamentous and part of them is Satisfactory yeasts, including Dimorphic (Villaro *et al.,* 2007, Rassin *et al.,*2015). It has been possible to observe 150 types of yeasts, the majority of which can coexist with a temperature of 37°C in order to settle in the human host. Recent decades have witnessed an increase in the importance of research on fungal disease and its recurrence in all its forms (Ramani *et al.,*1997, Panackal *et al.,*2009). The cause of the high rate of infections may be attributed to changes in medical practice with increased use of broad-spectrum antibiotics and exposure to HIV / AIDS. From the above, we find that Candidiasis is a common opportunistic disease that is caused by several types that belong to *Candidia Spp.* (Lionakis *et al.,*2008, Nouri *et al.,* 2015). Oral thrush, skin infection, and systemic disorder are among them. The white ovaries *C. albicans* is one of the most important types of ovaries in the pathogenic events of being opportunistic, as they are present in the form of Norma flora at the site of their presence before the injury, followed by the type *C. glabrata* responsible for 16% of the circulatory system injuries, in addition to the type *C. famata C. krusei, C. tropica and other yeasts* (Kim, 2011, Lee *et al.,* 2014). The plant kingdom is the actual source of most drugs and other pharmacologically active molecules waiting to be discovered, and during the past

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decade the therapeutic use of herbal medicines has gained significant momentum in the world due to the side effects and high toxicity of many of the laboratory drugs manufactured, which led to a sudden increase in the number of herbal drug manufacturers, Herbal medicine has been used as a main treatment in the traditional medicine system since ancient times and to this day because of its biomedical benefits as well as its place in cultural beliefs in many parts of the world which made its contribution clear and significant towards preserving human health (Jain *et al.,* 2010, Ibraheem *et al.,* 2017). In some Asian and African countries, the World Health Organization estimates that up to 80% of the population depends, Traditional medicine must meet their primary health care needs. Treatment involves the use of plant extracts and their active ingredients, which are non-food chemicals that have been created from plants and have satisfactory protective properties (Hussain *et al.,* 2017, Hussain *et al.*,2019,). The aims of study : Qualitative assessment of secondary metabolites (sterols, flavonoids, clikoside, phenolic compounds, alkaloids, tannins, saponins, sugars) in *Rheum ribes*, isolation and diagnosis of flavonoids, fatty acids and phenols from the plant using HPLC technique and estimation of some mineral elements in a *Rheum ribes* and evaluate the effect of secondary metabolic compounds isolated as antioxidants outside the body (Kim, 2011, Lee *et al.,* 2014). Finally, an evaluation of the effect of flavonoids, phenols and fatty acids isolated on the growth of fungi (Villaro *et al.,*2007, Rassin *et al.*,2015).

Materials and Methods

Materials and devices used -Chemicals (High purity materials were used for example (Acetic anhydride, Potassium ferricyanide, Hydrogen peroxide, Ascorbic acid and Glacial acetic acid. Instruments **(**ELISA, Ultrasonic, Centrifuge, Rotary Evaporator, spectrophotometer, Atomic absorption, Flameless Atomic absorption and HPLC (Ramani *et al.*,1997, Panackal *et al.*,2009).

- Agricultural circle (Potato Dextrose Agar (PDA), Sabouraud's, Sabouraud Dextrose Broth *(SAB)*, Roswell Park Memorial Institute Medium (RPMI-1640) and Complete Culture Medium (CCM).

Methods

Collection of *Rheum ribes* rhizome and stalk

The roots and stems of *Rheum ribes* were obtained from the local markets of Kirkuk and the roots were cut into small pieces, left to dry and then ground using an electric mill to fine powder and then stored in a sealed carton until used in the experiment.

Prepare the alcoholic extract of the roots and stems of *Rheum ribes*

(100) g of *Rheum ribes* roots and stems were weighed and soaked in (1000) cm^3 of ethanol alcohol and the root powder mixture was left for (24) hours and leg powder for (72 hours) with continuous shaking every hour. After the necessary period had passed, the solutions were filtered by leaves Filtration, then the solutions were poured into the rotary evaporator under vacuum and at a low temperature, and dry powder was obtained and placed in sterile containers with sealed lids (Jain *et al.,*2010, Ibraheem *et al.*,2017).

Prepare hot aqueous extract for the roots and stems of *Rheum ribes*

(50) grams of root powder and stems were weighed and soaked in (1000) cm^3 of hot distilled water and left the root mixture for (24) hours and leg mixture for ((72 hours) with continuous shaking every hour, and after the end of time the solutions were filtered by filter papers, Then the solutions were poured into glass dishes and placed in an electric oven at a temperature (40°C). Dry powder was obtained and the powder was placed in sterile containers with sealed lids (Ramani *et al.*,1997, Panackal *et al.*,2009).

Phytochemical Screening

Initial chemical disclosures were performed on *Rheum ribes* root extracts (aqueous and alcoholic) to identify secondary metabolic compounds as follows: - Alkaloids statements, Tests for Phytosterols, Tests for Carbohydrates, Tests for Glycosides, Tests for Flavonoids, Test for Tannins and phenols, Tests for amino acids and proteins (Jain *et al.*,2010,Ibraheem *et al.*,2017).

Determination of some mineral In *Rheum ribes*

The dry ashing method was used to quantify the elements in *Rheum ribes* using an Atomic absorption spectrophotometer.

Evaluation of antioxidant activity of Ethanolic extract and Etheric extract *in Vitro*

The effectiveness of the alcoholic and hot water extract against oxidation of the roots and stems of rhubarb (outside the body of the organism) was estimated using the following methods:-Reducing power assay,-Stock Standard Solution, It was prepared with a concentration of (250 g /cm^3), by dissolving (25 mg) of ascorbic acid in (75 cm^3) water without ions, then mixing the solution well and in case of not completely dissolving it is placed in the wave apparatus above Acoustic for (10 minutes) at a temperature (35°C) after thawing, complete the volume to (100 cm^3) using ion-free water. Vital efficacy, In tissue

culture. RPMI-1640 (Rosswell Park Memorial Institute)The liquid media used in this study was prepared and stored at 4ºC for the short-term use, while the long-term storage for use is at -20ºC.Agricultural circles used, Sabouraud Dextrose Agar Chloramphenicol (SDAC), Potato Dextrose Agar Chloramphenicol (SDAC), Sabouraud Sucrose Broth (SSB), Roswell Park Memorial Institute Medium (Kim, 2011, Lee *et al.*,2014).

Solution and Reagents

A number of solutions and reagents were prepared, and those needing sterilization were sterilized and not thermally damaged with the conductor at a temperature of (121°C) and pressure (15 pounds / 2 inches) for 15 minutes (Ramani *et al.*,1997, Panackal *et al.*,2009).

Solution: Physiological Saline Solution, McFarland Standard**,** The effect of plant extracts on the growth of pathological yeasts. The agar –well diffusion method was used for testing as follows: Sabouraud's Dextrose agar (SDA), a sterile swab from a yeast transplant containing (1.5X108) cells / ml, compared with a fixed turbidity solution, then left the plates to dry at room temperature(Kim, 2011, Lee *et al.*,2014). I made a drill (5 mm) in diameter, implanted by Cork Borer. Prepared gradient concentrations of dry vegetable extract for both aqueous and alcoholic extracts using the organic dimethyl sulfoxide (1) g of the dry extract was dissolved in (5) ml of the solvent solution to obtain a concentration of (200) mg / ml. Sterilized with a Millipore Filter Paper (0.22m) and then attended gradient concentrations of 200,180,160,140 mg / ml. (0.1) ml of the aforementioned extract concentrations were added to each hole by means of a micropipete pipette in a sequence. The control hole represented by adding the organic solvent DMSO was made. To allow the concentrations of the extracts to spread through the medium, the plates were placed in the refrigerator at a temperature of (4)°C and for half an hour as described. The dishes were incubated at a temperature of (37)°C. for 24 hours. The efficacy of each concentration was determined by measuring the diameter of the Inhibition Zone around each hole (Villaro *et al.*,2007, Rassin *et al.*,2015).

Effect of Rheum ribes extract on hepG2 cell lines**:** Study of the effect of *Rheum ribes* extract on hepG2 cell lines in Tissue Culture Laboratory, Biotechnology Research Center, University of Al-Nahrain. The research project methodology includes extraction, purification, characterization and anti-cancer activity.

Detection of cell growth inhibition: To detect HepG2 cell line growth inhibition, the cultured cell line was incubated with different concentrations (12.5, 25, 50, 100, 200, 400, 800) and six iterations of rhubarb extract (1.17µg / ml) from the purification of each treatment (Hussain *et al.*,2017, Hussain *et al.*,2019,).

Results and Discussion

Identification and quantitative determination of flavonoids and phenoles in *rehum* by HPLC

The results of the analysis with a high-performance chromatographic technique indicated the emergence of five bundles indicating the presence of five flavonoids and phenolic compounds of the extract (hot water) of the root of the *Rheum ribes* and four of them were identical to the standard compounds approved by the chromatographic analysis and as in Fig. (1) that shows the peaks and retention time Rt Rwandan flavonoids separated by the technique of photography were as follows (2.153,2.147,3.825,5.47,6.462) per minute.

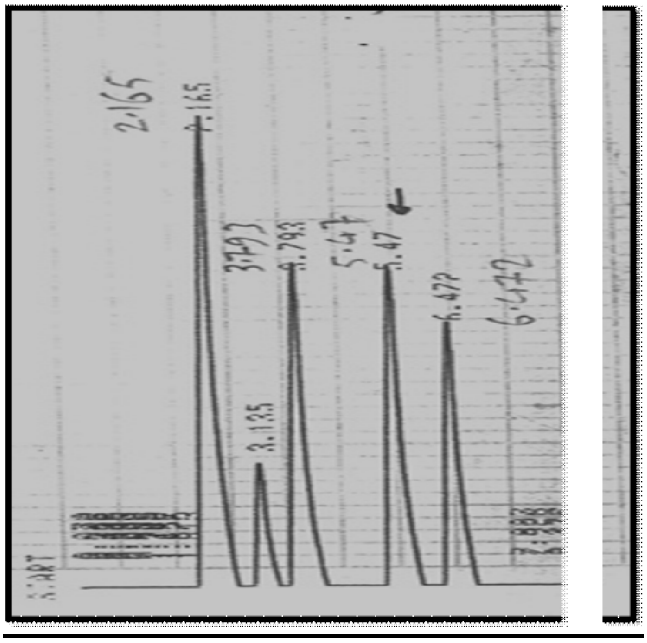

Fig. 1: HPLC analysis of isolated *Rheum ribes* phenols and flavonoids.

While the results of the analysis using high-performance chromatographic technique indicated the emergence of five packages indicating the presence of five flavonoids and phenolic compounds of the extract (hot water) of the stem of the *Rheum ribes* plant and four of them were identical to the standard compounds approved by the Chromatography and as in Fig. (2) that shows peaks and retention time Rt is for Rwandan flavonoids separated by photographic technology and was as follows (2.A38, 3.A78, 3.76, 5.427, 6.445) per minute.

As for the alcoholic extract, the results of the analysis with a high-performance chromatographic technique

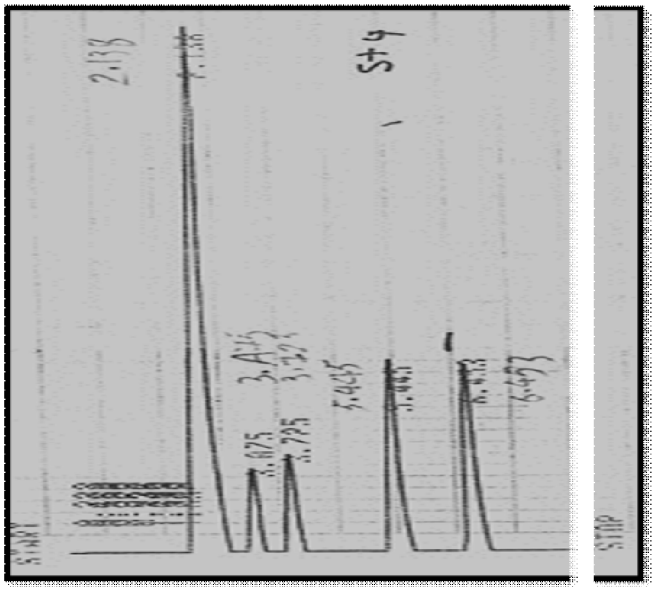

Fig. 2: HPLC analysis of isolated *Rheum ribes* phenols and flavonoids.

indicated the emergence of five bundles indicating the presence of five flavonoids and phenolic compounds for the extract (alcoholic) for the root of *Rheum ribes*, and four of them were identical to the standard compounds approved by the Chromatographic analysis, as in Fig. (3) that shows The peaks and retention time Rt of flavonoids and Rwanda phenols separated by the technique of photography were as follows (2.165,3.135, 3.793, 5.47, 6.47) per minute.

While the results of the analysis using high-performance chromatography technique for the rhubarb alcoholic extract for the *Rheum ribes* stem indicated the

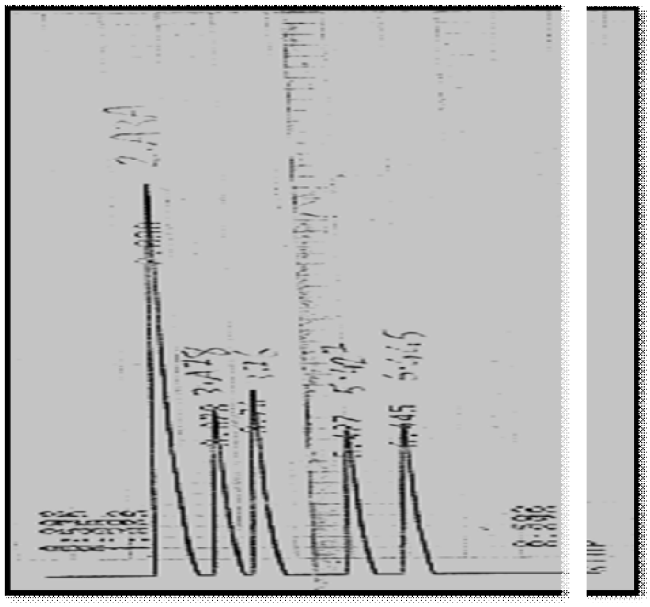

Fig. 3: HPLC analysis of isolated *Rheum ribes* phenols and flavonoids.

emergence of five bundles indicating the presence of five flavonoids and phenolic compounds for the rhizome of the rhubarb stem and four of them were identical to the standard compounds approved by the Chromatographic analysis and as in Fig. (4) that shows the peaks The holding time Rt for Rwanda flavonoids separated by the technique of photography was as follows (2.138, 3.875,3.725.5.445,6.453) per minute.

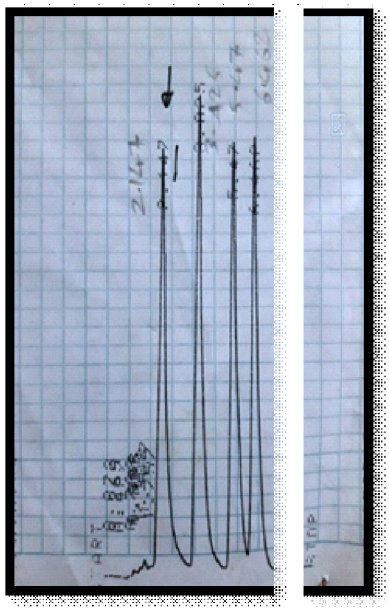

Fig. 4: HPLC analysis of isolated *Rheum ribes* phenols and flavonoids.

The standard flavonoids and phenols used in chromatographic analysis are (Aloe-emodin, Emodin, physcion, Chrysophanol) as in Fig. (5) which shows the peaks and retention times for standard flavonoids and the holding time for standard compounds was (2.14, 3.82, 5.49, 6.48) per minute.

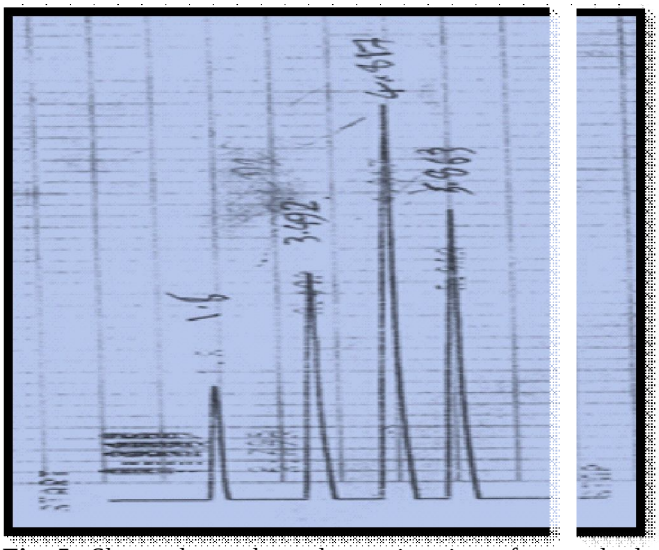

Fig. 5: Shows the peaks and retention times for standard flavonoids.

Cellular toxicity on the line of cancer cells

The effect of purified cellular toxicity extracted from p was studied. Sativum on the tumor cell line by assessing its effect on the Hep G2 (passage 18) cell line on a 48-hour exposure time at different concentrations of (150.0, 75.0, 37.5, 18.75, 9.37, 4.68, 2.34, and 1.17 µg / ml) using examination Neutral red. Optical density was measured at 450nm and 492nm wavelength.

Red examination is the cell viability and depends on the ability of viable cells to integrate and bind to the neutral red dye. The neutral red dye absorption test provides one of the most widely used cytotoxic tests with many biomedical and environmental tests, along with several tests that have been identified to determine applications, so *Rheum ribes* extract was selected to determine cytotoxicity. After exposure to the enzyme, the cells were incubated in the presence of a neutral red dye. The dye easily penetrates into cell membranes and accumulates inside cells in the particles. Since neutral red is a vital dye, it has been used to stain living cells. The changes in the cells resulting from the action of the purified rhubarb extract cause a decrease in the absorption of the neutral red pigment. After washing the cells using PBS and treating them with a dye removal solution to release any excess of the dye covered, the damage at the cell level was assessed by measuring the optical density of the treated cell solution and comparing it with the untreated negative control samples. The results shown in the Fig. 6 below showed a gradual decrease in the ability of cells compared to negative control.

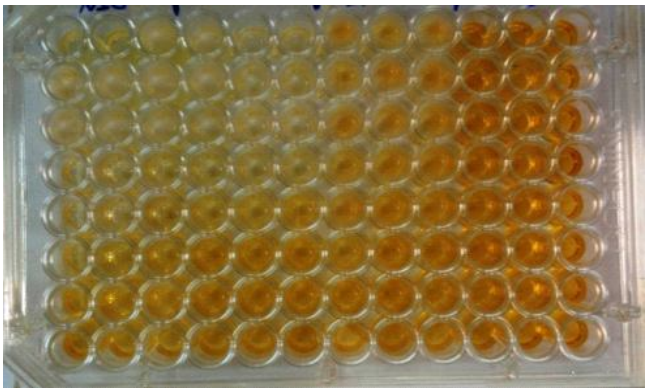

Fig. 6: The cytotoxicity of the purified *Rheum ribes* extract from the inst on the hepG2 cell line using a neutral red assay.

The 96-microtiter plate was used as a standard tool for cytotoxic analysis as shown in Appendix I which reviewed the example map (plate composition) of the Microtiter plate. The effect of cytotoxicity depends on the percentage of inhibition growth rate (I.R) that represents the cytotoxicity of *Rheum ribes* extract and the ratio of residual activity and cell survival rate. The (Table 1, 2) below shows the effect of cytotoxicity of different concentrations of purified *Rheum ribes* extract on HepG2 cell line after 48-hour incubation measured at 492nm. HepG2 after 48-hour incubation measured at 492nm.

Table 1: The effect of cytotoxicity of different concentrations by purified *Rheum ribes* extract from HepG2 cell line after incubation for 48 h measured at 492nm.

2(%)	1(%)	Rheum ribes extract Concentration (µg/ml)
74.8	76.9	150
73.2	60.7	75
72.7	65.9	37.5
73.0	70.2	18.75
74.5	82.2	9.37
70.8	78.9	4.68
75.9	77.6	2.34
75.2	83.3	1.17

Table 2: The effect of cytotoxicity of different concentrations of purified *Rheum ribes* extract from pds on the hepG2 cell line after incubation for 48 h measured at 492nm.

4(%)	3(%)	Rheum ribes extract Concentration (µg/ml)
74.5	75.7	150
76.1	65.6	75
75.5	71.6	37.5
75.3	73.0	18.75
77.5	85.4	9.37
72.0	78.6	4.68
81.2	81.4	2.34
77.1	80.6	1.17

The diameter of the colonies was measured 48 hours after implantation at a temperature of 37°. The aqueous extract of the root shows that the higher the concentration of the extract, the greater the inhibition diameter. Also, the aqueous extract of the stem appears as the concentration of the extract increases, the diameter of the inhibition increases. But the aqueous extract of the stem showed greater efficacy than the aqueous extract of the root in inhibition and for all concentrations. The alcoholic root extract was more effective than the aqueous extract of the root and for all concentrations as well as for the alcoholic extract of the stem it showed higher efficacy than the aqueous extract of the stem. The effectiveness of the alcohol extract of the root and stem was close at concentrations 10 and 20, while the concentrations of 30 and 40 of the alcoholic extract of

Inhibitory efficacy of *Rheum ribes* extract on fungi

Table 3: Represents the average damping diameters of *Rhum ribes* extract against *C. albicans* yeast.

Extract type	Concentrations used mg / ml				Positive control
	10	20	30	40	
Aqueous (hot) extract of the root	2.0 *K	3.50 J	4.50I	6.66G	0.00L
Leg (hot) aqueous extract	4.50 I	5.66H	6.16GH	8.50F	0.00L
Alcoholic root extract	15.5E	20.0D	24.5C	28.0B	0.00L
Leg alcohol extract	16.5E	20.0D	22.5C	22.0B	0.00L

Table 4: Shows the effect of a hot (root) aqueous extract on stem and stem against Mushrooms *A. fumigatus*.

Concentration	Aqueous (hot) extract of the root		Leg (hot) aqueous extract	
	Colony diameter rate (mm) (m ± SE)	Percentage For inhibition	Diameter rate Colony (mm)(m ± SE)	Percentage For inhibition
Control	A , a 40 ± 2	0%	A , b 8 ± 1	0%
100	B , a 32 ± 3	20%	B , b 7.2 ± 1	10%
125	C , a 27 ± 1	32.5%	B , b 6.8 ± 2	15%
150	D , a 21.6± 4	46%	C , b 6 ± 2	25%
175	D , a 17.8 ± 2	55.5%	D , b 5.4 ± 1	33%

Table 5: Explains the effect of root and stem alcohol extracts against fungus *A. fumigatus.*

The name of the mushroom / Concentration	Alcoholic root extract		Leg alcohol extract	
	Colony diameter rate (mm) (m ± SE)	Percentage For inhibition	Diameter rate Colony (mm)(m ± SE)	Percentage For inhibition
Control	A , a 40 ± 2	0%	A , b 8 ± 1	0%
100	A , a 38 ± 2	10%	B , b 7.5 ± 3	12%
125	B , a 35 ± 3	22%	B , b 7.1 ± 1.9	22%
150	C , a 29.2 ± 1	33%	C , a 6.6 ± 0.6	40%
175	D , a 23.2 ± 2	56%	D , a 5.8 ± 0.6	69%

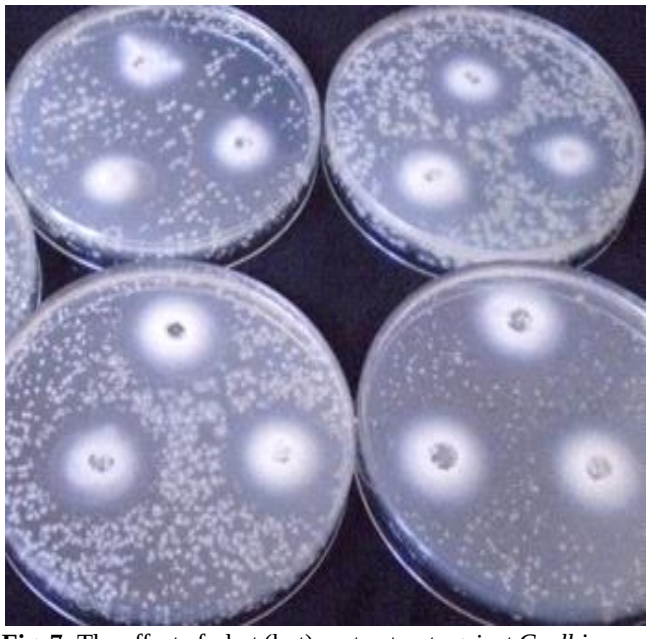

Fig. 7: The effect of a hot (hot) root extract against *C. albicans.*

the stem gave the same effect despite the increased concentration().

The diameter of the colonies was measured 5 days after incubation at a temperature of 37°. The table shows that by increasing the concentration of extracts, Aqueous (hot) extract of the root is more inhibitory than the aqueous (hot) extract of the stem (Hapcioglu *et al.*,2005, Hee *et al.*,2014).

The diameter of the colonies was measured 5 days after incubation at a temperature of 37°. The table shows that by increasing the concentration of extracts, the growth diameter of the fungus decreases and the percentage of inhibition increases (Grover *et al.*,2003, Gallardo *et al.*,2004, Dheeb.,2016), It is shown that the alcoholic extract of the stem has the highest inhibitory activity from the alcoholic root extract, but the inhibitory activity of both extracts was equal at 125 concentration which was 22% (Abdulbaqi *et al.*,2018, El hilali.,2016, Dheeb.,2015).

Conclusion

We concluded from the study the *Rheum ribes* extracts have high activity against cancer cell line and strong effectiveness on studied Fungi.

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