Egypt. J. Chem. **Vol. 64**, No. 10, pp. 5493- 5502 (2021)

Egyptian Journal of Chemistry

http://ejchem.journals.ekb.eg/

Assessment of the Chemical Composition, Antimicrobial Potential and Cytotoxic Activity of *Eriobotrya japonica* fruits

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Abstract

The present study was carried out to evaluate the cytotoxic and antimicrobial activities of both 70% methanol extract and the isolated polysaccharides from *Eriobotrya japonica* fruits as well as to investigate their chemical composition. The cytotoxic activity was evaluated against ovarian (SKOV-3), liver (HepG2) and prostate cancer cell lines (PC-3) by SRB (Sulforhodamine B) assay. The antimicrobial activity was evaluated by the well diffusion method against *Escherichia coli*, *Staphylococcus aureus*, *Bacillus cereus*, *Salmonella typhimurium*, *Candida albicans* and *Aspergillus brasiliensis.* The results revealed that the extract was more potent than the isolated polysaccharide against the tested human cell lines. Moreover, the methanol extract was highly potent against (SKOV-3) with IC_{50} of (**0.012** µg/mL) more than that of Doxorubicin (IC_{50} =**0.86** µg/mL). In addition, the results revealed that the 70% methanol extract showed a reasonable antibacterial activity against all tested bacteria. While the isolated polysaccharides exhibited antibacterial activity against *Staphylococcus aureus* and *Bacillus cereus* only. In addition, *Eriobotrya japonica* fruits contain 41% (wt/wt) carbohydrate as glucose and 37% (wt/wt) as mucilage. These results may help to discover new classes of a natural cytotoxic and antimicrobial drug after applying several clinical trials to confirm that it can be safely applied in living systems.

Keywords: Antimicrobial; Cytotoxic activity; *Eriobotrya japonica;* Polysaccharides.

1. Introduction

Cancer causes the highest death rate globally, especially for the undeveloped countries. Though synthetic drugs have been known to be standard treatments for cancer, their toxicity to normal cells are the most common concern. On the contrary, natural products exhibit few adverse effects on normal cells in the treatment of cancer **[1].** Medicinal plants used in traditional folk medicine provide diversity of medically beneficial drugs to treat different diseases **[2].** Epidemiological researches revealed that diets rich with fruits and vegetables offer a mean of cancer chemoprevention as well as antimicrobial potential due to their phytoconstituents **[3].** Medicinal plants contained diversity of phytoconstituents such as carbohydrates, terpenoids, tannins, alkaloids and flavonoids which possess antimicrobial and anticancer properties **[4].** Much focus has been devoted in recent years to polysaccharides extracted from natural sources such as plants, algae, fungi and bacteria **[5,6].** These polysaccharides exhibited diversity of biological activities and have received much interest in the medical field **[7].** *Eriobotrya japonica* (Loquat), family Rosaceae, is a subtropical tree and is commercially cultivated in different countries worldwide. It is a well-known medicinal plant in China and Japan **[8].** *Eriobotrya japonica* showed diversity of medicinal benefits, the leaves have beneficial effects in numerous diseases including anti- inflammatory hypoglycemic antitumor, antiviral activities, as well as in chronic bronchitis, gastroenteric discorders and asthma **[9-11].** *Eriobotrya japonica* extracts can inhibit cell carcinogenesis at different progression stages **[12].** The polyphenolic compounds of Loquat leaves play a crucial role as cytotoxic agents against human oral tumor cell line **[13].** A study revealed that the methanol extract of loquat exhibited a potent inhibitory effect against invasiveness of estrogen receptor-negative breast cancer (MDA-MB-231) by suppressing the enzymatic activities of matrix

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Receive Date: 15 May 2021, Revise Date: 30 May 2021, Accept Date: 31 May 2021

DOI: 10.21608/EJCHEM.2021.76278.3729

metalloproteinases (MMPs) **[11].** Another research showed the anticancer activity of ethanol extract of *Eriobotrya japonica* fruits against bladder cancer EJ cells (Human endometrial adenocarcinoma) [14]. Previous researches on *Eriobotrya japonica* revealed the presence of diversity of phytoconstituents in different organs; the kernel is rich in minerals, tannins, starch and proteins; fruit is a good source of vitamins, phenolic acids, flavonoids, organic acids, carotenoids and sugars; flowers and leaves are rich in triterpenes and phenolics **[15-17].** These compounds have a crucial role in the bioactivity of the plant [13, 10, 11].

The current research aimed to evaluate the cytotoxic and antimicrobial activities of both 70% methanol extract and the isolated polysaccharides from *Eriobotrya japonica* fruits focusing on the content of 70% methanol extract and the isolated polysaccharides which may be responsible for their bioactivities.

2. Materials and Methods

2.1. Phytochemical Study

2.1.1. Plant Materials

Fresh fruits of *Eriobotrya japonica* were collected from the Agricultural Research Centre, Giza, Egypt, and identified by Dr Mohammed El-Gebaly, Department of Botany, National Research Centre (NRC), Egypt. The fruits were freed from seeds, dried, powdered, and kept in dark well-closed containers.

2.1.2. Preparation of the plant extract

Dried powder of *Eriobotrya japonica* fruits (1Kg) was defatted with n-hexane then it was percolated with 70% methanol till exhaustion. The extract was filtered and concentrated to dryness under reduced pressure at 40°C by using rotatory evaporator, and then it was kept in refrigerator for the further studies.

2.1.3. Phytochemical screening

Chemical tests were carried out on the plant extract using standard procedure to identify the constituents as described by **[18, 19].**

2.1.4. Total Phenolic Assay

The total phenolic content (TP) was determined applying the Folin–Ciocalteu colorimetric method using gallic acid as a standard **[20, 21].** TP was expressed as milligrams of gallic acid equivalents(GAE)/g of the dry plant materials.

2.1.5. Total Flavonoid Assay

Total flavonoid content (TFC) was measured using an aluminum chloride colorimetric assay **[20, 21].** A calibration curve was established using rutin as a standard. TFC was expressed as mg rutin equivalent(RE)/g of the dry plant material.

2.1.6. Proximate analysis

The official methods were used to calculate the percentages of crude fibre, moisture content, total ash, acid-insoluble ash and water soluble ash **[22].**

2.1.7. HPLC analysis for both flavonoids and phenolics of 70% methanol extract of *Eriobotrya japonica* fruits

HPLC analysis was carried out according to Matilla *et al.,* **[23]** using an Agilent 1260 series. Using Kromasil C18 column. The column temperature was maintained at 35 °C. The multi-wavelength detector was monitored at 280 nm. The mobile phase was programmed consecutively in a linear gradient using water (A) and 0.05% trifluoroacetic acid in acetonitrile (B) as mobile phase at a flow rate 1 ml/min. The injection volume was 10 µl for each of the sample solutions. Peaks were identified by congruent retention times and UV spectra in comparison with those of the standards.

2.1.8. Investigation of carbohydrate profile

Extraction and purification of the polysaccharides were carried out according to the method of Samee *et al.*,**[24]** The dried polysaccharide precipitate isolated from the plant was tested using KOH, Ruthenium red, and subjected to gel formation test to confirm their nature, and then kept in the refrigerator for chemical and biological evaluations. The total polysaccharide and sugar contents were measured using phenol sulfuric acid method **[25].** Authentic sugars were obtained from Fluka, Switzerland. Paper chromatography Whatman No.1, (Whatman Led. Maid Stone, Kent, England) for qualitative detection of sugars. The developing system was BAW (n-butanol/acetic acid/ water) 4:1:5, (v/v/v) upper phase. After development, the chromatogram was dried, sprayed with aniline hydrogen phthalate reagent (0.93 g aniline and 1.66 g O-phthalic acid dissolved in 100 ml n-butanol saturated with water), and heated at 105 °C for 5 min **[26].** GLC analyses (HP 6890, USA), after derivatization using the trimethylsilylation reagent (Merk), under the following condition: ZB-1701 capillary column, 30 m in length, 0.25mm i.d; 0.25 µm film thickness, carrier gas, helium at a flow rate at 1.2 ml/min, temperature programmed 150-200 °C at a rate of 7 °C/min, flame ionization detector. The retention times of the peaks were compared to those of the authentic sugars for qualitative identification, while peak area measurements were used for quantitative determination.

2.2. Material for cytotoxic study

2.2.1. Human tumor cell lines

Liver cancer cell line (HepG2), Ovarian cancer cell line (SKOV-3) and Prostate cancer cell line (PC-3) were obtained from Nawah Scientific Inc., (Mokatam, Cairo, Egypt).

2.2.2. Chemicals

Doxorubicin, (Pharmacia, Sweden), was used as a reference anticancer agent, Sulphorhodamine B stain, from Sigma Co, Egypt and Tris EDTA buffer, from Sigma Co, Egypt.

2.2.3. Cell culture

Cells were maintained in Roswell Park Memorial Institute (RPMI) media, supplemented with 100 units/ml of penicillin, 100 mg/ml of streptomycin and 10% of heat-inactivated fetal bovine serum in humidified, 5% (v/v) CO_2 atmosphere at 37 °C.

2.2.4. Assay method for cytotoxic activity [27, 28].

Cell viability was assessed by SRB assay. Aliquots of 100µL cell suspension (5x10^3 cells) were in 96-well plates and incubated in complete media for 24 h. Cells were treated with another aliquot of 100µL media containing drugs at various concentrations ranging from (0.01, 0.1, 1, 10,100ug/ml). After 72 h of drug exposure, cells were fixed by replacing media with 150µL of 10% TCA and were incubated at 4 °C for 1 h. The TCA solution was removed, and the cells were washed 5 times with distilled water. Aliquots of 70µL SRB solution (0.4% w/v) were added and were incubated in a dark place at room temperature for 10 min. Plates were washed 3 times with 1% acetic acid and allowed to air-dry overnight. Then, 150µL of TRIS (10mM) were added to dissolve protein-bound SRB stain; the absorbance was measured at 540 nm using a BMG LABTECH®- FluoStar Omega microplate reader (Ortenberg, Germany).

2.3. *Methods for Antimicrobial Activity*

The antimicrobial activity and minimum inhibitory concentration (MIC) of the extract were evaluated by the well diffusion method **[29].**

2.3.1. Preparation of the stock solution for antimicrobial assay

A stock solution was prepared by dissolving 500mg of the plant extract in 1ml of distilled water.

2.3.2. Antibacterial Assay

The antimicrobial activity of the plant extract was evaluated by the well diffusion assay. 0.2mL of a bacterial cell suspension matching a 0.5 McFarland standard solution was inoculated into petri dishes containing 20ml nutrient agar culture medium. A sterile swab was used to spread the suspension over the medium's surface. With a sterile glass Pasteur pipette, wells of 8 mm diameter were rendered in the agar plates and 0.1ml of each compound from the stock solution was applied to the wells, using Amoxicillin as a positive control while DMSO was used as a negative control. The plates were then incubated at 37ºC for 24 h. The diameter of the inhibition zone that developed around the wells in mm was used to determine the antimicrobial activity. Each assay was carried out at least twice.

2.3.3. Antifungal assay

The *Candida albicans* was cultured for 24 hours on sabouraud dextrose agar and incubated at 30°C, while the other fungus was cultured for 5 days on potato dextrose agar slant. Pure colonies of the *Candida* species was transferred by a sterile loop into a tube contained sabouraud dextrose broth cultured at 30°C for 24h . For the other fungus spore suspension was prepared in 10ml distilled water. The suspension was adjusted to $2–5\times10^6$conidia/ml using a hemocytometer. 0.2ml of the strains was inoculated into petri dishes containing 20ml sabouraud dextrose agar for *Candida albicans* or potato dextrose agar for other fungus. A sterile swab was used to spread the suspension over the medium's surface. With a sterile glass Pasteur pipette, wells of 8 mm diameter were rendered in the agar plates and 0.1ml of each extract of the stock solution was applied to the wells, using DMSO as a negative control and diflucan (the antifungal drug) (10 mg/ml) as a positive control and . The plates were incubated for 48 h at 30ºC . The inhibition zone was estimated in millimeter.

2.3.4. Estimation of the minimal inhibitory concentration

The MIC is the lowest concentration of the compound that can inhibit the microbial growth in the culture medium. As previously described, the strains were inoculated into the Petri dishes . The tested extracts were diluted at various concentrations and each concentration was placed in each well.

3. Result

3.1. Phytochemical screening

Table 1 showed the results of the phytochemical screening of 70% methanol extract of *Eriobotrya japonica* fruits. It revealed the presence of the alkaloids, triterpenes and/or sterols, flavonoids, carbohydrate and/or glycosides and coumarins in the plant extract. While, it revealed the absence of saponins and tannins in the fruits of *Eriobotrya japonica.*

Table 1. Phytochemical screening of 70% methanol extract of *Eriobotrya japonica* fruits.

Phytoconstituents	*Eriobotrya japonica* fruits
Tannins	-
Alkaloids	+
Saponins	-
Triterpenes and/or sterol	+
Flavonoids	+
Carbohydrate and/or glycosides	+
Coumarins	+

(+)Present (-) Absence

3.2. Total Phenolic and flavonoid contents

The total phenolic content was expressed as gallic acid equivalent was found to be **18.15 mg** gallic acid equivalents (GAE)/g, while the total flavonoid content as rutin equivalent was **4.26 mg** rutin equivalent (RE)/g

3.3. *Proximate analysis*

The result of proximate analysis of *Eriobotrya japonica* fruits was compiled in Table 2. From these results, it was found that these constants should be used as pureness criteria for the fruits of *Eriobotrya japonica.*

Table 2. Proximate analysis of the fruits of *Eriobotrya japonica*

Pharmacopoeial constants	*Eriobotrya japonica* fruits
Crude fibre %	10.76
Total Ash (%)	4.77
Acid-insoluble ash (%)	0.88
Water-soluble ash (%)	3.52
Moisture (%)	9.84

3.4. HPLC analysis of polyphenolic compounds

The result of HPLC analysis of polyphenolic compounds was compiled in table 3. It showed that ten polyphenolic compounds were identified representing 84.6% of the total area. Chlorogenic acid was the major compound representing (28.3%) followed by catechin (19.4%), syringic acid (6.8%) and gallic acid (6.1%).

3.5. Investigation of carbohydrate content

The results confirmed the mucilaginous nature of the isolated polysaccharides as it gave a red stain with ruthenium red and with potassium hydroxide no gelatinous precipitate was formed. Moreover, the results revealed that the total carbohydrate content in the aqueous extract of *Eriobotrya japonica* fruits calculated as glucose was 41% (wt/wt). While, the the isolated mucilage represents 37% (wt/wt). Table 4 showed the result of PC investigation of the polysaccharide hydrolysate; it revealed the presence of mannose, rhamnose, xylose, arabinose, glucose and galactose.

Table 3. HPLC analysis of polyphenolic compounds in 70% methanol extract of *Eriobotrya japonica* fruits

No.	Polyphenols	Rt (min.)	Area %
1	Gallic acid	3.23	6.1
2	Chlorogenic acid	2.42	28.3
3	Catechin	4.02	19.4
4	Methyl gallate	4.99	3.1
5	Caffeic acid	6.02	2.6
6	Syringic acid	6.50	6.8
7	Ellagic acid	7.98	5.6
8	Vanillin	9.88	4.1
9	Ferulic acid	10.23	4.8
10	Naringenin	10.44	3.8
% of total identified compounds		**84.6%**	

Rt: Retention time in minutes.

Table 5 showed the result of GLC analysis which confirmed the presence of six free sugars in the mucilage hydrolysate which represent 95.05% of the total hydrolysate. The major sugar in the mucilage hydrolysate was rhamnose which represents 35.76% of the total hydrolysate followed by xylose (21.43%), mannose (19.46%), arabinose (14.65%), glucose (2.76%) and galactose (0.99%). Various researchers suggest that polysaccharides are effective in managing cancer and a variety of microbial infections **[30].**

Table 4. PC investigation of the polysaccharide hydrolysates in the aqueous extract of *Eriobotrya japonica* fruits

Authentic Sugars	R_f	Color with aniline phthalate	Result
Mannose	0.29	Brownish yellow	+
Glucose	0.19	Brown	+
Galactose	0.17	Yellowish brown	+
Glucuronic acid	0.15	Yellowish brown	-
Fructose	0.26	Yellowish brown	-

Rhamnose	0.38	Brown	+
Galacturonic acid	0.12	Yellowish brown	-
Ribose	0.37	Brown	-
Xylose	0.33	Reddish brown	+
Arabinose	0.28	Brown	+

+: present; - : absent ; PC: Paper chromatography

Table 5. Sugar components (%) in the crude polysaccharide extract of the aqueous extract of *Eriobotrya japonica* fruits analyzed by GLC

Sugars	Rt (min.)	Relative percentage (%)
Arabinose	8.41	14.65
Xylose	8.72	21.43
Rhamnose	9.57	35.76
Galactose	13.95	0.99
Mannose	14.24	19.46
Glucose	14.63	2.76
Total		**95.05**

3.6. Cytotoxic Activity

Table 6 showed the results of the cytotoxic activity of 70% methanol extract and the isolated polysaccharides from *Eriobotrya japonica* fruits *in vitro* against different human cancer cell lines. The screening assay revealed significant cytotoxic activities of both extracts against PC-3 (Prostate cancer cell line), HepG2 (liver cancer cell line) and SKOV-3 (Ovarian cancer cell line). Where, both extracts showed decrease in the viability % of the cancer cell in dose dependent manner comparing with a reference anticancer agent (Doxorubicin).

Moreover, the methanol extract is highly potent against the ovarian cancer cell line (SKOV-3) with IC_{50} of (**0.012** µg/mL) compared with doxorubicin (IC_{50} =**0.86** µg/mL) and the isolated polysaccharide (IC_{50} =**4.25**µg/mL). In addition, the70% methanol extract showed a potent cytotoxic activity against the liver cancer cell line (HEPG-2) (IC_{50} =**1.53** µg/mL) as compared with doxorubicin (IC_{50} =**0.78**µg/mL) and the isolated polysaccharide that showed a moderate cytotoxic activity (IC_{50} =**19.35** µg/mL). Moreover, 70% methanol extract showed a moderate activity against the prostate cancer cell line (PC-3) (IC_{50} =**35** µg/mL) by comparing with that of doxorubicin (IC_{50} =**5.48**µg/mL) and the isolated polysaccharide that showed a weak cytotoxic activity (IC_{50} =>**100** µg/mL). So, the results revealed that the 70% methanol extract is more potent than the isolated polysaccharide against the tested human cell lines.

3.7. Antimicrobial Activity

The antimicrobial activities of 70% methanol extract and polysaccharides of *Eriobotrya japonica* fruits were assayed by the well diffusion method against different microorganisms. The results of screening test revealed 70% methanol showed a reasonable antibacterial activity against *Staphylococcus aureus*, *Bacillus cereus*, *Escherichia coli* and *Salmonella typhimurium* with inhibition zone of 32, 25, 23 and 24 mm, respectively, as compared with that of standard amoxicillin (45,38,28, and 28 mm), respectively. On the other hand, the isolated polysaccharides exhibited antibacterial activity against *Staphylococcus aureus* and *Bacillus cereus* only with inhibition zone of 19 and 10 mm, respectively, but did not show any activity against *Escherichia coli* and *Salmonella typhimurium*. Moreover, both tested extracts did not show any activity against the tested fungi *(Candida albicans and Aspergillus brasiliensis*). These results were compiled in Table 7.

Moreover, 70% methanol extract of *Eriobotrya japonica* fruits exhibited a reasonable antibacterial activity against (*Staphylococcus aureus*, *Bacillus cereus*, *Escherichia coli* and *Salmonella typhimurium*) with minimal inhibitory concentration (MIC) values of (100, 200, 200 and 200 mg/ml), respectively. While, the isolated polysaccharides exhibited a reasonable antibacterial activity against *Staphylococcus aureus* and *Bacillus cereus* with equal MIC values (500 mg/ml) Table 8.

Table 6. Cytotoxic activity of 70% methanol extract and polysaccharides of *Eriobotrya japonica* fruits in *vitro* on different human cell lines

Viability %									
Cell lines	**SKOV-3**			**PC-3**			**HEPG-2**		
Conc. (µg/ml)	**MEJF**	**PEJF**	**Dox.**	**MEJF**	**PEJF**	**Dox.**	**M EJF**	**P EJF**	**Dox.**
0	100.00±0.00	100.00±0.00	100.00±0.00	100.00±0.00	100.00±0.00	100.00±0.00	100.00±0.00	100.00±0.00	100.00±0.00
0.01	39.62±0.85	80.22±0.78	97.71±0.77	98.22±0.66	98.29±0.53	97.65±0.29	94.56±0.21	90.96±0.19	89.70±0.93
0.1	32.33±0.79	73.41±0.06	97.30±1.11	96.03±0.87	96.84±0.70	95.81±0.82	71.25±0.38	86.03±0.68	72.12±1.14
1	25.55±0.46	61.45±0.79	50.10±0.53	84.44±1.02	95.70±0.61	78.79±0.54	56.16±0.46	66.72±0.48	52.55±0.82
10	20.78±0.39	47.87±2.25	16.84±0.62	63.31±0.32	93.58±0.63	17.84±0.74	33.20±0.71	55.51±0.83	5.53±0.14
100	20.61±0.66	23.45±0.84	3.12±0.45	32.26±0.12	90.94±0.90	8.57±0.18	15.65±0.91	38.07±0.47	1.57±0.20
IC_{50} µg/ml	**0.012**	**4.25**	**0.86**	**35**	**>100**	**5.48**	**1.53**	**19.35**	**0.78**

EJF: *Eriobotrya japonica* fruits, PEJF:Polysaccharide of *Eriobotrya japonica* fruits, MEJF: 70% methanol extract of *Eriobotrya japonica* fruits, Dox.: Doxorubicin

Table 7. The antimicrobial activity of 70% methanol extract and polysaccharides of *Eriobotrya japonica* fruits

Compound	**Diameter of inhibition zone (mm)**					
	***Staphylococcus aureus* ATTCC 6538**	***Bacillus cereus* ATTCC 14579**	***E. coli* ATTCC 8739**	***Salmonella typhimurium* ATTCC 14028**	***Candida albicans* ATTCC 10231**	***Aspergillus brasiliensis* ATCC 16404**
MEJF	32	25	23	24	-	-
PEJF	19	10	-	-	-	-
Amoxicillin (+ve) (10mg/ml)	45	38	28	28	Nd	Nd
Diflucan (+ve) (10mg/ml)	Nd	Nd	Nd	Nd	-	-
DMSO (-ve)	-	-	-	-	-	-

(–): No inhibition, (+ve):Positive control, (-ve): Negative control, ND: Not detected, EJF: *Eriobotrya japonica* fruits, PEJF:Polysaccharide of *Eriobotrya japonica* fruits, MEJF: 70% methanol extract of *Eriobotrya japonica* fruits,

Table 8 The MIC values of 70% methanol extract and polysaccharides of *Eriobotrya japonica* fruits

Test bacteria	**MIC (mg/ml)**	
	M EJF	**P EJF**
***Staphylococcus aureus* ATTCC 6538**	100	500
***Bacillus cereus* ATTCC 14579**	200	500
***Escherichia coli* ATTCC 8739**	200	-
***Salmonella typhimurium* ATTCC 14028**	200	-

EJF: *Eriobotrya japonica* fruits, PEJF:Polysaccharide of *Eriobotrya japonica* fruits, MEJF: 70% methanol extract of *Eriobotrya japonica* fruits; MIC: minimal inhibitory concentration

4. Discussion

The current study revealed the presence of different phytochemical constituents in *Eriobotrya japonica* fruits. This result is similar to what was reported by Zhou which revealed the presence of different phytochemical constituents in different organs of *Eriobotrya japonica* that showed different bioactivities**[15].** Another previous research on *Eriobotrya japonica* stems showed the presence of tannins, terpenes, carbohydrates and flavonoids **[31].** Epidemiological researches revealed that diets rich with vegetables and fruits reduce the risk of cancer as well as microbial infections due to their phytoconstituents **[3].** Many reports revealed that the daily intake of diets rich in flavonoids may have the capacity to reduce the risk of various cancers **[32].** Our results revealed that *Eriobotrya japonica* fruits are rich of phenolic and flavonoidal compounds which play an important role as anticancer and antimicrobial agents. HPLC analysis of polyphenolic compounds revealed the presence of variety of phenolic compounds in the plant extract, where chlorogenic acid was the main phenolic compound, this result is in agreement with what was reported by Ahumada; where chlorogenic acid was the main

phenolic compound in both leaves and flowers of *Eriobotrya japonica* by HPLC analysis **[33].** This result is also similar to what was reported by Rashed and Butnariu, the study showed that Loquat plant has rich of active compounds such as polyphenolic compounds as; quercetin 3-O-α-rhamnoside, kaempferol 3-O-β-glucoside, naringenin and quercetin **[31].**

The cytotoxic assay revealed that the 70% methanol extract is more potent than the isolated polysaccharide against the tested human cell lines. This result may be due to the synergistic effect of the phytoconstituents in *Eriobotrya japonica* fruits which possess a crucial role as cytotoxic agents. According to a previous research, polyphenolic compounds include gallacatechins, resveratrol, curcumin, tannins and flavonoids all considered to be anticancer compounds **[34].** Purified flavonoids showed anticancer activities against breast cancer (MCF-7), cervical carcinoma (Hela) and hepatoma (Hep-G2) **[35].** The antiproliferative activity of 14 oriental medicinal herbs was evaluated, *Eriobotrya japonica* leaves exhibited the strongest cytotoxic activity against estrogen receptor-negative breast cancer (MDA-MB-231), cervix epitheloid (HeLa) and lung (A549) carcinoma **[36].** Different procyanidin oligomers from *Eriobotrya japonica* leaves exhibited selective cytotoxicity against human salivary gland tumor cell and human squamous cell (HSC-2) carcinoma **[13].** In another research, betulinic acid, 3-O-(E)-p-coumaroyl tormentic acid, ursolic acid and δ-oleanolic acid isolated from the methanol extract of *Eriobotrya japonica* leaves showed a potent cytotoxic activity against human HL60 cells **[37].** Various researchers suggest that plant polysaccharides are effective in managing different type of cancers [30]. Estimation of polysaccharides as antitumor agents has become an important field in drug discovery **[38].** Various studies have investigated the capability of plant polysaccharides in inhibiting the growth of tumor and induction of the apoptosis in cancer cells, the results revealed enhancement in the treatment of cancer **[7, 12, 39, 40].**

Despite our results showed that methanol extract is highly potent against the ovarian cancer cell line (SKOV-3) , that indicated its cytotoxicity against the tested cancer cell line, previous studies reported that *Eriobotrya japonica* fruit exhibited no mortality or toxicity in *in vivo* studies in the tested animals, as well as it was safe against normal cell line so it might be used clinically as a safe traditional medicine **[8, 41].**

In addition, the results revealed that the 70% methanol extract of the plant showed a reasonable antibacterial activity against all tested bacteria (*Escherichia coli*, *Staphylococcus aureus*, *Bacillus cereus* and *Salmonella typhimurium*). While the isolated polysaccharides exhibited antibacterial activity against *Staphylococcus aureus* and *Bacillus cereus* only. On the other hand, the plant extract as well as the isolated polysaccharides did not show any antifungal activity against *Candida albicans* and *Aspergillus brasiliensis.* These results are in contrary with what was reported by Rashed and Butnariu where, the antimicrobial activity of methanol 80% extract of *Eriobotrya japonica* stems was evaluated against bacterial and fungal strains. The extract exhibited a potent antifungal activity against *Candida albicans*, while it did not show any activity against the other bacterial and fungal strains **[31].**

The current results confirm that 70% methanol extract of *Eriobotrya japonica* fruits is more potent than the isolated polysaccharides as antimicrobial agent. This effect may be due to the presence of bioactive components in 70% methanol extract of the plant such as; flavonoids, alkaloids, tannins, and terpenoids in addition to carbohydrates. And due to synergistic effect, 70% methanol extract is more potent than the isolated polysaccharides. The current result is in agreement with what was reported by Rita and et al., the study stated that medicinal plants contain several phytochemicals such as flavonoids, alkaloids, tannins, carbohydrates and terpenoids, which possess antimicrobial activity **[4].** Another study showed that proanthocynidin was isolated from *Eriobotrya japonica* leaves; it has anti *Helicobactor pyroli* activity **[42].** From *Eriobotrya japonica* leaves, ursolic acid was isolated that showed antimutagenic activity as it decreased the numbers of *Salmonella typhimurium* TA 100 revertants per plate **[43].**

5. Conclusion

Based on our results and discussion, it can be concluded that the 70% methanol extract of *Eriobotrya japonica* fruits possess a highly potent cytotoxic activity against ovarian cancer cell line (SKOV-3) more than that of doxorubicin, and it showed a reasonable cytotoxic activity against all tested human cell line more than that of the isolated polysaccharides. Moreover, 70% methanol extract exhibited a promising antimicrobial activity against different number of microorganisms more than that the isolated polysaccharides. This activity is due to the presence of bioactive compounds as polyphenolic compounds that were found in different concentrations and were confirmed by HPLC analysis. Further researches are needed by which the extract can possibly be exploited for pharmaceutical use.

Conflicts of Interest
There are no conflicts to declare

Funding
This research received no external funding

Acknowledgments
The authors would like to appreciate National Research Centre for providing technical knowledge, assistance and facilities for this study.

Authors' contributions
AAS suggests the point, GFA performed all the phytochemical part, writing and editing the manuscript, with the interpretation of the results, SAI performed the Antimicrobial assay. All authors read and approved the final manuscript.

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