# CYTOTOXIC AND ANTIOXIDANT ACTIVITY OF FRUIT JUICE OF *ERIOBOTRYA JAPONICA* (THUNB.) LIND PLANT CULIVATED IN IRAQ

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**ABSTRACT**

**This study was conducted to evaluate the anticancer effect of *Eriobotrya japonica* (loquat) fruit juice on cancer cell lines (in vitro) and its antioxidant activity. Study was performed on two cancer cell lines, human cervical cancer (Hela), rhabdomyo sarcoma (RD), and rat embryogenic fibroblast (REF) as a normal cell, at concentrations of 62.5, 125, 250, 500 and 1000 µg/ml of fruit juice were measured at 24, 48, and 72-hr. with three replicates. Free radical 1,1Dyphenyl-2-picrylhydrazyl radical (DPPH) was used for testing the ability as antioxidant on 25, 50, 75, 100, 150 µg/ml concentration of the loquat fruit juice. The results revealed that the juice of *E. japonica* had high antioxidant influence (100%) in 150 µg/ml concentration, followed by 88% in 100 µg/ml concentration. Effect of growth inhibition of cancer cells was depended on juice concentration. The effect of juice on Hela cell line was more than on RD, while the highest effect of juice on Hela was shown on concentration of 500 µg/ml the 48 hr. The cytotoxic effect of exhibited that had a significant effect ($P<0.05$).**

**Key words: *Eriobotrya japonica* (Thunb.), Lind, fruit juice. cancer cell lines, antioxidant.**



## الفعالية السمية والمضادة للاكسدة لعصير ثمار نبات الينكي دنيا المزروع في العراق *Eriobotrya Japonica*

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**المستخلص**

**صممت هذه الدراسة لتقدير تأثيرعصير ثمار نبات الينكي دنيا *Eriobotrya Japonica* كمضاد على الخطوط السرطانية (خارج الجسم الحي) والفعالية المضادة للاكسدة. شملت الدراسة خارج الجسم الحي على نوعين من الخطوط السرطانية (Hela) و(RD) والخط الطبيعي (REF). ان فترة تعرض الخطوط الخلوية لتراكيز العصير 62.5 أو125 او250 او500 او1000 µg/ml كانت 24 أو 48 أو 72 ساعة خلوي ولثلاث مكررات لكل تركيز. استعمل الجذر الحر radical (1,1Dyphenyle-2-picrylhydrazyl (DPPH في اختبار مار كمضاد للاكسدة عند التراكيز 25 أو50 أو75 أو100 أو150 µg/ml. اظهرت النتائج ان لعصير الثمار فعالية مضادة للاكسدة 100% عند التركيز 150 µg/ml ثم تبعها 88% عند التركيز 100 µg/ml. ان تأثير العصير على تثبيط نمو الخلايا السرطانية يعتمد على التركيز وفترة التعرض له ونوع الخلايا السرطانية فقد تفوق تأثير العصير على الخط الخلوي Hela على الخط (RD) فكان اعلى تأثير للعصير على (Hela) عند التركيز 500 µg/ml بعد 48 ساعة تبعها 24 ساعة عند نفس التركيز. اما الخط الخلوي الطبيعي (REF) فكان تأثير ثمار النبات على الخلايا واطيء جداً مقارنة بتأثير على الخطوط السرطانية. ان التأثير السمي العالي للعصير قد أعطى تأثيراً معنوياً ($P<0.05$).**

**الكلمات المفتاحية: نبات الينكي دنيا، عصير الثمار، الخطوط السرطانية، مضادات الاكسدة**

## INTRODUCTION

*Eriobotrya japonica* (Thunb.) Lindl. loquat (Rosaceae) family is medium sized green fruit tree. It attains a height up to 6 m or more, evergreen oval-oblong and the thick leaves. Flowering occur from April to June and the white, covert in the rusty-woolly pile .Fruit pear-shaped, yellow to orange, 4 cm long,with 3 to 4 seeds and sweet test and ripens in late winter or early spring (27).It can grow in a wide range of soil and climatic conditions. The local Arabic name of the plant (Beshmelah); Chinese (luju,biba); English (loquat ); French (bibassier ); German (Loquate) and other names (8). The Ioquat has been cultivated for more than 2000 years, and is indigenous to China and Japan but now commercially cultivated in more than 30 countries worldwide, including Iraq, Japan, Turkey, Italy, Spain, Syria, *etc* (8) (2).Champagne and Golden are varieties which planted in Iraq (2).The harvest season of Loquat fruit in Iraq lasts from May to June and the quality including color, flavor, sweetness and chemical constituents are highly dependent on the ripening degree at harvest. After harvest, loquat fruits are very mortal and prone to mechanical injury and microbial fermentation(19). Loquat plants have been historically used as folk medicines for thousands of years. Water extracts or crude extracts have been used for the treatment of cough, chronic bronchitis, inflammation, diabetes, and cancer in Ayurveda and Chinese folk medicine (5).The fruits are considered sedative and useful in allaying vomiting and thirst, they are used to treat wounds in China (26). The flowers are used as an expectorant and extracted in oil, in cosmetics. Many types of active compounds in fruits and leaves are responsible of the wide range of pharmacological-activity of loquat plant (17). Loquat fruit is edible part of the plant and, it is very rich with bioactive components such as phenols, vitamins A and C, flavonoids and carotenoids…..*etc.*(19)(11).These compounds are different in quantities and qualities depending on the cultivar and conditions of culture soil,and environmental factors.A cytotoxicity assays are widely used by the pharmaceutical research to screen for cytotoxicity in natural or chemical compounds. Researchers are interested in developing a therapeutic that targets rapidly for killing cancer cells, for instance; or they can screen "hits" from initial high-throughput drug screens for unwanted cytotoxic effects before investing in their development as a pharmaceutical (29). An antioxidant compounds are molecules that inhibits the oxidation of other molecules. Oxidation is a chemical reaction that including loss of electrons or an increase in oxidation state. Oxidation reactions can produce free radicals, this radicals are crucial for life, they can also be damaging on organisms cells especially on DNA or onset of many diseases such as cancer, rheumatoid arthritis and atherosclerosis (12)(15). Many types of active compounds in plants have antioxidant and anticancer or cytotoxic activity such as flavonoids, phenols, triterpenoids and vitamins (C, A and E)(7).Loquat plant has rich of active compounds such as polyphenolic compounds, flavonoids, vitamins, carotenoids and other compounds (20).The research aims to achieve qualitative evaluation of active constituents of *Eriobotrya japonica* fruit cultivated in Iraq and test the antioxidant and cytotoxic activity of these compounds.

## MATERIALS AND METHODS

Loquat fruits were collected from fields in Baghdad /Iraq on May 2015 and authenticated by the Iraqi national herbarium. The fruits were washed and stored at 4°C. All chemical materials and solvents used were purchased from Merck (Darmstadt, Germany) or Sigma-Aldrich (St. Louis, USA).

### Preparation of juice

Fifty grams of fruits were freed from seeds, crushed by blender, filtrated and evaporated. The residues obtained were stored in dark bottle in a refrigerator for further use.

### Qualitative analysis (Phytochemical Screening):-

Many types of reagents for detection of the chemical constituents in juice extract, were used:

**Tannins detection**: 1 ml of fruit juice was taken in a test tube and then 1 ml of 0.008 M Potassium ferric cyanide was added. 1 ml of 0.02 M Ferric chloride containing 0.1 N HCl was added and observed for blue-black coloration(25).

**Flavonoids detection**: five ml of dilute ammonia solution was added to a portion of the juice followed by addition of concentrated H2SO4. A yellow coloration observed (25).

**Phenols detection**: two types of reagents were used for detection the phenolic compounds:

1- half g fast blue salt was dissolved in 100 ml water. The TLC plat was sprayed with 6-8ml of fast blue salt reagent, dried, a red or orange zones observed (28).

2- Folin-ciocalteu reagent: this reagent was prepared depending on (23), with some modified. A blue coloration observed.

**Terpenoids detection**: half ml anisaldehyde was mixed with 10 ml glacial acetic acid, 85ml methanol and 5ml concentrated sulphuric acid and the TLC plate was sprayed with reagent. A red coloration observed (28).

**Antioxidant Activity:** Fruit juice of Loquat plant was assessed by quantifying the scavenging ability to stable free radical 1,1 Dyphenyl-2-picrylhydrazyl radical (DPPH) .Equal volumes (0.5 ml) of DPPH solution (0.4 µM) and 1ml of each concentration (25,50,100,200 and 400 µg $ml^{-1}$) from extract were mixed and allowed to stand for 30 min. at room temperature. The absorbance of samples was recorded at 518 nm, by a spectrophotometer. Ascorbic acid was used as standard. The scavenging activity was calculated according to the formula:

$$\text{scavenging activity \%} = \frac{A_{518}\ \text{control} - A_{518}}{A_{518}\ \text{control}} \times 100$$

Where $A_{518}$ control is the absorbance of DPPH radical + methanol; $A_{518}$ sample is the absorbance of DPPH radical or compound. These experimental repeated triplicate for fruit extract.

**Cytotoxic activity**

The cell lines used in this study were supplied by tissue culture unit/ Iraqi Centre for Cancer and Medical Genetics Research (ICCMGR). Two cancer cell lines were used in this search (HeLa cell line). It was derived from cervical cancer cells taken on February 8, 1951 from Henrietta Lacks, a patient who died of her cancer (22), and (RD cell line) it was derived directly from biopsy specimens of a 7-year-old female with a pelvic rhabdomyo sarcoma previously treated with cyclophosphamide and radiation and found to have refractory disease (18). The (REF) Rat Embryo Fibroblast was used as normal cells. A0.02g of dried Loquat fruits juice was dissolved in 10 ml of solution (media and dimethyl sulfoxide).Cell cultures in microtitration plate (96wells) were exposed to a range of plant extract concentrations during the log phase of growth and the effect was determined after recovery time. Periods of exposure of cell lines were measured at 24, 48, and 72-hr in a microtitration plate under complete sterile conditions. Different concentrations (0, 62.5, 125, 250, 500 and 10000) µg/ml of fruit juice were prepared and tested on each cell line, with three replicates for each concentration.

**STATSTICAL ANALYSIS**

The Statistical Analysis System- SAS (2012) program was used to effect of difference factors in study parameters. Least significant difference –LSD test was used to significant compared between means in this study.

**RESULIS AND DISCUSION**

**Phytochemical Screening**

Results obtained from the general chemical detection showed the presence of phenols, tannins, terpenoids, and flavonoids, in juice extract.

**Antioxidant Activity**

Juice extract of *E. japonica* plant was used as antioxidant and determined by utilizing scavenging action against DPPH as free radical ,and compared with ascorbic acid, as seen in Table (1).

**Table 1. Antioxidant activity of juice of *E. japonica* plant as compared with ascorbic acid**

| Concentrations (µg/ml) | Mean± SD | |
|---|---|---|
| | % Antioxidant activity of Juice extract | % Antioxidant activity of Ascorbic acid |
| 25 | 16.83 ± 0.06 e | 36.91 ± 0.02 e |
| 50 | 33.80 ± 0.10 d | 40.62 ± 0.01 d |
| 75 | 62.60 ± 0.20 c | 42.64 ± 0.16 c |
| 100 | 88.70 ± 0.10 b | 50.24 ± 0.02 b |
| 150 | 100.00 ± a | 59.51 ± 0.01 a |
| LSD 0.05 | 0.357 | 0.234 |

The statistical results shown in Table 1 indicate that juice of *E. japonica* plant have higher antioxidant influence by 100% in 150 µg/ml concentration than 88% in 100 µg/ml concentration as compared with the standard control Ascorbic acid.The results showed that there was significant difference (P≤0.05) between the concentrations in the same juice extract.The results of this study indicate that juice plant have the ability to scavenge free radicals when compared with reference standard, and this is represented by donating their hydrogen atom to quell the free radicals of DPPH, these results matched the study of (14) and (24). The phenolic compounds which were found on fruit juice of *E. japonica* plant especially flavonoids that are able to perform this reaction can be considered as antioxidant (17), and thy approved that radical scavenging activates of the compounds increased with increasing concentrations. The flavonoid heterocycle coud be contribute to antioxidant activity by permitting conjugation between the aromatic rings and the presence of a free 3-OH (6).

**Cytotoxic Activity**

The cytotoxic activity was determined by using five different concentrations of juice of *E. japonica* plant on two cancer cell lines and one normal cell line after 24**,** 48 and 72 hours exposure time. The results are shows in Table 2, 3 and 4. The statistical analysis indicated that the concentrations of the fruit juice have a significant cytotoxic effect on HeLa and RD after 24, 48 and 72 hours at levels (P<0.05) for all concentrations. All concentrations inhibited cell growth at highest concentrations and reduced at the lower concentrations as shown in Table 2. The fruit juice has highest growth inhibition on Hela than RD cell lines at the concentrations from 125 to 1000 µg/ml for the period of 24 hrs. The growth inhibition on RD was higher than Hela on concentration 62.5 .The cytotoxic effect of fruit juice on Ref cell line was lower than Hela and RD cancer cell lines from concentrations 62.5 to 1000 µg/ml ,but the concentration of 62.5 was lowest than other concentration.

**Table 2 Cytotoxic effect of concentrations of *E. japonica* juice on Hela ,RD and Ref cell lines after 24 h**

| Concentration (µg /ml) | Cell line | | | LSD value |
|---|---|---|---|---|
| | Hela | RD | Ref | |
| 0 | 0.00 ± 0.00 | 0.00 ± 0.00 | 0.00 ± 0.00 | 0.00 NS |
| 62.5 | 65.31 ± 3.69 | 67.55 ± 3.86 | 3.83 ± 0.14 | 11.59 |
| 125 | 80.81 ± 4.71 | 82.69 ± 3.98 | 10.29 ± 0.85 | 9.45 |
| 250 | 86.16 ± 4.93 | 81.25 ± 3.47 | 7.29 ± 0.46 | 11.77 |
| 500 | 89.11 ± 3.68 | 83.89 ± 4.55 | 10.29 ± 0.81 | 11.94 |
| 1000 | 87.08 ± 4.61 | 80.05 ± 4.67 | 10.42 ± 0.66 | 10.63 |
| LSD 0.05 | 12.87 | 10.92 | 6.33 | ---- |

Table 3 shows the effect of the fruit juice on cell lines after 48 hrs., highest inhibitory effect on growth of Hela than RD cell lines at the concentrations from 62.5 to 1000 µg/ml. The fruit juice inhibited cell growth at highest concentrations and was reduced at the lower concentrations. The cytotoxic effect on cell lines revealed that showed a significant result (P<0.05) for all concentrations

**Table 3. Effect of concentration of *E. japonica* juice on Hela , RD and Ref cell lines after 48 hours**

| Concentration (µ/ml) | Cell line | | | LSD value |
|---|---|---|---|---|
| | Hela | RD | Ref | |
| 0 | 0.00 ± 0.00 | 0.00 ± 0.00 | 0.00 ± 0.00 | 0.00 NS |
| 62.5 | 79.66 ± 3.67 | 73.46 ± 3.76 | 6.06 ± 0.42 | 9.84 |
| 125 | 85.40 ± 5.75 | 77.49 ± 4.67 | 5.00 ± 0.35 | 10.07 |
| 250 | 87.27 ± 4.58 | 77.25 ± 4.92 | 2.67 ± 0.13 | 10.64 |
| 500 | 89.91 ± 5.67 | 82.22 ± 4.77 | 6.06 ± 0.49 | 11.39 |
| 1000 | 86.65 ± 5.12 | 79.38 ± 4.61 | 4.55 ± 0.24 | 10.96 |
| LSD 0.05 | 11 .69 | 11.76 | 5.25 | ---- |

From the results shown in table 4 it appears that the effect of fruit juice on RD cell line have highest effect than Hela for all concentrations. The statistical analysis showed that concentrations of the fruit juice have a significant cytotoxic effect on HeLa and RD after 72 hours for all concentrations, but these concentrations were not significant on Ref cell line, or in another meaning the fruit juice has not effect on normal cell

**Table 4. Effect of *E. japonica* juice on Hela ,RD and Ref cell lines after 72 hours**

| Concentration (µ/ml) | Cell line | | | LSD value |
|---|---|---|---|---|
| | Hela | RD | Ref | |
| 0 | 0.00 ± 0.00 | 0.00 ± 0.00 | 0.00 ± 0.00 | 0.00 NS |
| 62.5 | 67.21 ± 2.97 | 72.64 ± 4.57 | 6.67 ± 0.35 | 10.75 |
| 125 | 71.29 ± 3.76 | 71.17 ± 3.89 | 2.40 ± 0.09 | 9.86 |
| 250 | 70.64 ± 3.60 | 77.36 ± 4.08 | 2.43 ± 0.12 | 10.39 |
| 500 | 77.65 ± 3.92 | 78.66 ± 3.68 | 5.33 ± 0.32 | 10.85 |
| 1000 | 69.82 ± 3.09 | 74.92 ± 4.22 | 9.66 ± 0.75 | 9.22 |
| LSD 0.05 | 9.45 | 11.64 | 6.34 | ---- |

After treatment with different concentrations of *E. japonica* juice during 24, 48 and 72 hours the optical densities (OD) for the stained cell lines plate, revealed differences of (OD) between concentrations, the high concentration gave low value of OD, indicating maximum response because the affected (dead) cells are removed by washing during staining procedure leaving a light colour represented the attached viable cells. The low concentration gave high value of OD, which indicates minimum response in proportion to high percentage of viable cells (4). These results indicate that *E. japonica* juice have more constituents like phenols, tannins, terpenoids, and flavonoids, capable to effect on cells. Studying the effect of *E. japonica* juice on cancer cell lines explained the highest sensitivity of cell lines by the activation of some glutathione-S- transferase enzymes (GSTs) via several compounds in plant extract, especially the polyphenolic compounds(25,26). Those authors also mentioned during their studies, the effect of Polyphenols and terpenoids from some plant medicine on cancer cell lines. The (GSTs) acted as an antioxidant causing cellular detoxification by enhancing their combination with reduced glutathione leading to the cancer cell toward programmed cell (death, apoptosis)(25). The differences in Hela and RD cell lines response toward different treatments might indicate a presence or absence of specific cellular receptors in each type of cell lines; making the cells interact at same concentration in different manner. Furthermore, the path ways of metabolism in response to each treatment differed from one line to another. Many researchers which were referred to in this study about this fact, have investigated different plant extracts in treating many types of cell lines (1, 10, 29). The results of this study agree with many researchers who emphasized that active compounds on herbs or plant medicine have many physiological effects on the cells, tissue and organs. The active compounds phenols, tannins, alkaloids, flavonoids, vilamins and terpenes have cytotoxic character against many diseases, including cancer cell lines and antioxidant (29,30). These results mention to uses the fruit or fruit juice of *E. japonica* plant to protective the human body against free radicals and cancer better than use of the methods extraction, which may lead to lose some effective compounds during the extraction process and get rid of the poisonous of solvent.

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