ACTIVITY OF *Annona Squamosa* PEELS EXTRACTS AGAINST TWO PATHOGENIC BACTERIA AND TWO BLOOD CANCER CELL LINES

M. F. Altaee[1] R. W. Younis[2] Z. K. Kamona[3]
Assistant Professor Lecturer Lecturer
[1, 2] Biotechnology Department / College of Science/ University of Baghdad/Iraq.
[3] Department of Student Accommodation Affairs / University of Baghdad /Iraq.
ZainAlAbdeenreem@gmail.com

ABSTRACT

This research project was designed to assess the antibacterial and antitcancer activity by using the crude extracts of fresh and dried peels of *Annona squamosa* fruit. The fresh and dried peels were extracted by using three solvents, inculded (water, ethanol, and methanol) at different concentrations (stock, 50, 25, 12.5, 6.25) µg/ml. The antibacterial activity was estimated against Gram-positive bacteria (*Staphylococcus aureus*) and Gram - negative bacteria (*Pseudomonas aeruginosa*) using agar well-diffusion method. Results showed the appearance of different regions of inhibition. Antibacterial activity of alcoholic solvent extracts of the dry and fresh peels showed noticeable inhibitory activity against both tested bacteria when comparing the last one with the aqueous extract of peels. The results of anticancer activity estimation against two hematological blood cancer cell lines showed that the three solvents for fresh and dried peels caused a remarkable inhibitory effects and the ethanolic extracts of dried peels was the best. This encourage for the possibility of using peels extracts of Annona. squamosa as a natural resources for growth inhibiting of different types of bacteria and cancer cells.

Key words: fresh and dry extracts, cytotoxicity, methanol, and ethanol.

فعاليه مستخلصات قشور نبات القشطة ضد اثنان من البكتريا المرضية واثنان من الخطوط الخلوية لسرطان الدم

مها فخري مجيد[1] ريم وليد يونس[2] زيد كاظم كمونه[3]
استاذ مساعد مدرس مدرس
[1, 2] قسم التقنيات الاحيائية \كلية العلوم \ جامعة بغداد /العراق .
[3] قسم شوؤن الاقسام الداخلية / جامعة بغداد/ العراق .

المستخلص

صممت هذه الدراسة لتقديرالفعالية المضادة للبكتريا والفعاليه المضادة للخلايا السرطانيه باسخدام المستخلصات الخام للقشور الرطبه والجافه لنبات القشطة. استخلصت القشور الرطبه والجافه بمذيبات ثلاث شملت (الماء، الايثانول والميثانول) عند تراكيز مختلفه (stock, 50, 25, 12.5, 6.25 µg/ml). قدرت الفعالية المضادة للبكتريا الموجبه (*Staphylococcus aureus*) والسالبه (*Pseudomonas aeruginosa*) باستخدام طريقة نشر الاكار. أظهرت النتائج ظهورمناطق تثبيط مختلفه حيث أظهرت الفعالية المضادة للبكتريا لمستخلصات القشور الجافة والرطبة الكحولية تثبيطاً واضحاً جداً ضد كلا نوعي البكتريا مع مقاومة الاخيرة للمستخلص المائي للقشور. كانت أفضل فعالية مضادة للبكتريا هي ضد (*Pseudomonas aeruginosa*) باستعمال المستخلص الايثانولي للقشور الجافة. كما بينت نتائج تقديرالفعالية المضادة للسرطان ضد خطين خلويين لسرطان الدم ان المستخلصات الثلاثه للقشور الجافة والرطبة لنبات القشطه قد احدثت تثبيطاً واضحاً مع كفاءة المستخلص في التثبيط. وهذا يشجع على امكانيه استخدام مستخلصات قشور كمصدر طبيعي في تثبيط انواع متعددة من البكتريا الخلايا السرطانيه.

كلمات مفتاحيه: المستخلصات الجافه والرطبه, السميه الخلويه , ميثانول و ايثانول.

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INTRODUCTION

From the beginning of human development, medicinal plants and herbs were considered as one of the important types of medicinal products. Ventola, (22) reported that a number of medicinal plants used in indigenous medicine have been tested as alternative medicines because of their various applications in cosmetic, food, pharmaceutical and other industries which have obtained considerable attention. In recent years, fruits, which are a rich source of bioactive compounds, have become popular subjects for such investigations besides medicinal plants (17). Annonaceae is one of the biggest families, which containing about 130 genera over 2000 species are Annona (15). *Annona squamosa* is an attractive, slow - growing deciduously shrub or a small tree with a rounded or spreading open crown, belongs to family Annonaceae which carries edible fruits called apples or sweetsops. It reaches a height of 3 - 6 meter. The fruit of the cream also known as Indian cream, cherimoya, custard apple, guanabana or the fruit of the graf tree is one of the most fruit consumed in tropical and warm regions. Its scientific name is Annona cherimola, an evergreen tree belonging to the Annonaceae plant family, whose origins date back to the Caribbean, and Central and South America, and whose presence is concentrated in northern Peru and South Ecuador (6). Each part of the fruit including (peel, pulp and seeds) showed a remarkable medical activities and many researchers work to prove these important activities and thus suggesting it as powerful alternative agent for many diseases (7). Antimicrobial activity means the killing or inhibition process of the microbes that cause disease. For this purpose, several antimicrobial agents are used, such as anti-bacterial, antiviral or anti – fungal compounds. All of them have different modes of action for suppressing of infection (2, 20). *Staphylococcus aureus* is a major human bacterium pathogen causing various clinical manifestations, and found in the environment and also in normal flora on healthiest people's skin and mucous membranes (most often the nasal area) (9). *Pseudomonas aeruginosa* has become a significant reason for gram negative infection, particularly in patients with compromised immunity. It is the most common isolated pathogen in patients hospitalized for more than one week and is a major cause of nosocomial infections. *Pseudomonal infections* can be life threatening and complicated (10). Cancer is one of the lethal diseases; it is characterized by an abnormal proliferation of cells. Lifestyle changes are the most common reason for cancer. It implies high death and incidence as an important public health and economic problem requiring effective prevention (24). Anticancer activity, also called antineoplastic activity, is known to be any drug effective in the treatment of malignant or cancerous disease. Furthermore, there are several drugs not in those classes that show anticancer activity and are therefore used for the treatment of malignant diseases (12). This study was conducted to investigate the antibacterial and anticancer activity of *Annona squamosal.*

MATERIALS AND METHODS

Collection of plant

Fruit of *Annona squamosa* was obtained from the local market of Baghdad city (Baghdad / Iraq) washed gently with water, dried and peeled. The peels were cutted into small pieces and divided into two groups, fresh and dry peels. Dry peels were obtained by allowing the peels to dry at room temperature for few days.

Aqueous extraction

Ten gm of both fresh and dried peels of *Annona squamosa* separately were dissolved in 200 ml of distilled water. The flasks were then putted on hot plate for 2 hrs at 37C°. The extracts were filtrated by filter paper. The supernatant was transferred into test tubes in kept in refrigerator and stored until use.

Alcoholic extraction

Ten gm of dried and fresh peels of *Annona squamosa* separately were dissolved in 200 ml of alcoholic extracts (70% ethanol and 80% methanol). The flasks were plugged with cotton and then leave at room temperature for 2 days. Extracts were filtrated by filter paper and then transferred into petri dish. The dishes were putted into oven at 60C° until dry. The extracts were rub off by spatula and then dissolved in 10 ml of DMSO. Finally the solutions were transferred into test tubes and kept in room temperature until use.

Preparation of Diluted Extract

The solution of alcohol extract was diluted in DMSO and aqueuoes ectract was diluted in distilld water. Different dilutions were made starting from the stock and then several concentrations (50, 25%, 12.5% and 6.25) µg/ml were made (14.).

Bacteria Isolates

Two bacterial isolates (*Staphylococcus aureus* and *Pseudomonas aeruginosa*) were kindly provided by labrotary of Department of Biotechnology/ Collage of Science/ University of Baghdad/ Iraq.

Determination of antibacterial activity

Muller-Hinton agar prepared and poured into sterial petridishes and allowed for solidify. Twenty four hours overnight bacterial cultures were swabbed separately on the media using sterile cotton buds, then 2 wells (6-8) mm were made by using sterile cork borer. One hundred microliter of each extract at different concentrations (50, 25%, 12.5% and 6.25) µg/ml from chosen plant were loaded in the wells. The plates then incubated at 37 C for 24hr. After incubation, the diameter of inhibition zone was measure (21).

Cell lines

The cell lines (melanoma and lymphoma) were acquired from the Iraq biotech Cell Bank Unit at Al-Harthia/ Baghdad/ Iraq, and were preserved in RPMI-1640 medium supplemented with 10% Fetal bovine, 100 units/mL penicillin, and 100 µg/mL streptomycin. (3).

Cytotoxicity assays

For determination of the cytotoxicity of the plant extract, the MTT cell viability assay was performed on 96-well plates. The cell lines were cultured at 1 × 10^4cells/well. After 24 hours or achievement of confluent monolayer, cells were treated with three different peel extract of *Annona Squamosa* at a concentrations of (stock, 50, 25, 12.5 and 6.25 µg/ml) and the procedure was completed according to method by (3).

Cytotoxicity = A-B/A *100, Where A is the optical density of the control and B is the optical density of the test.

RESULTS AND DISCUSSION

Antibacterial activity

Each extract showed different patterns of inhibition zones against each bacteria. Antibacterial activity of alcoholic solvent extracts of the peels for both fresh and dry extract showed noticeable inhibitory activity against the two tested pathogens but these pathogens reflect some resistance to aqueous extract of peels. The maximum inhibition activity of dry peel methanol extract against *Pseudomonas aeruginosa* recorded 22mm, followed by ethanolic dry peel extract 19mm and finally low antibacterial activity was seen with aqueous dry peel extract (3mm) as shown in (figure 1). Moreover, ethanolic fresh peel extracts showed highest inhibition rate (16mm) followed by methanolic extract (12mm) with zero inhibition rate for aqueous one as shown in (figure 2). In general stock solution showed the highest inhibition rate comparison with the all test solvent and the inhibition is gradually decreased as dilutions decrease.

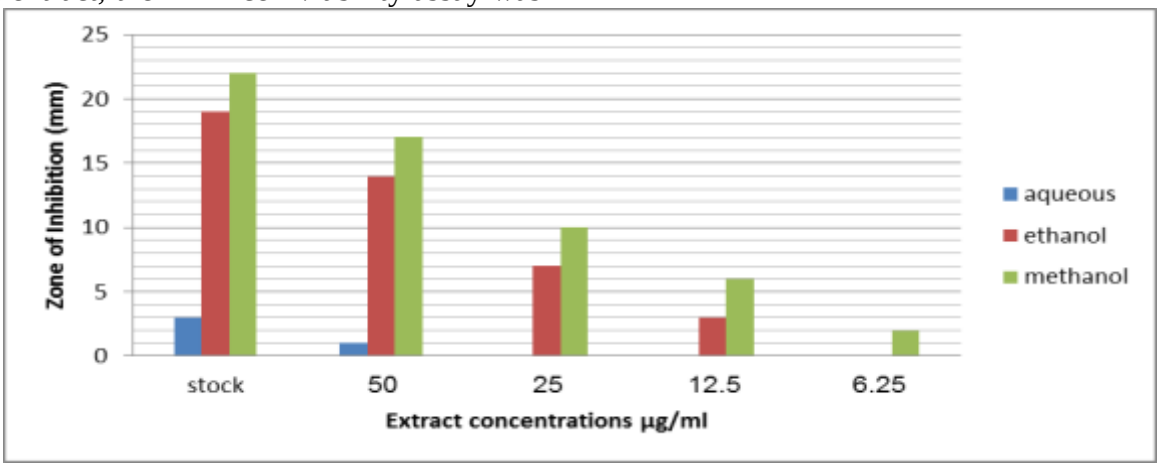

Figure 1. Antibacterial activity of three solvents (aqueous, ethanol, methanol) extracts of dry peel of of *Annona Squamosa* at different concentrations (stock, 50, 25, 12.5, 6.25 µg/ml) against *Pseudomonas aeruginosa*

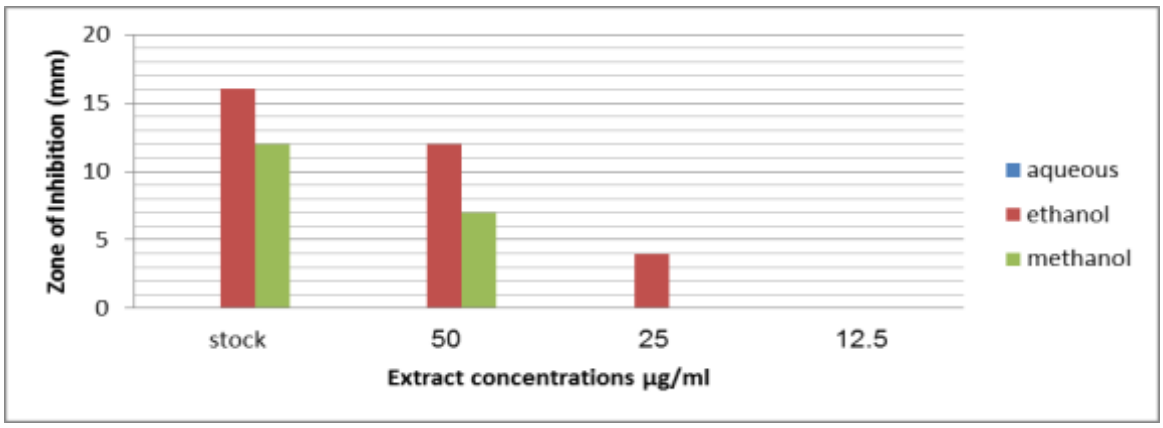

Figure 2. Antibacterial activity of three solvents (aqueous, ethanol, methanol) extracts of fresh peel of *Annona Squamosa* at different concentrations (stock, 50, 25, 12.5, 6.25 µg/ml) against *Pseudomonas aeruginosa*

Also the results of antimicrobial activity of *Annona squamosa* extracts against Staphylococcus aureus showed different inhibition rate with both fresh and dry extracts. For dry peel ethanolic extract showed the highest inhibition zone 15mm followed by aqueous extract 13mm and finally methanolic extract 12mm at stock extract as shown in (figure 3). While with fresh extracts ethanolic extract showed the hightest antibacterial activity 12mm at stock extract with negative results or zero inhibition rate for both aqueous and methanolic extracts for all tested dilutions as shown in (figure 4).

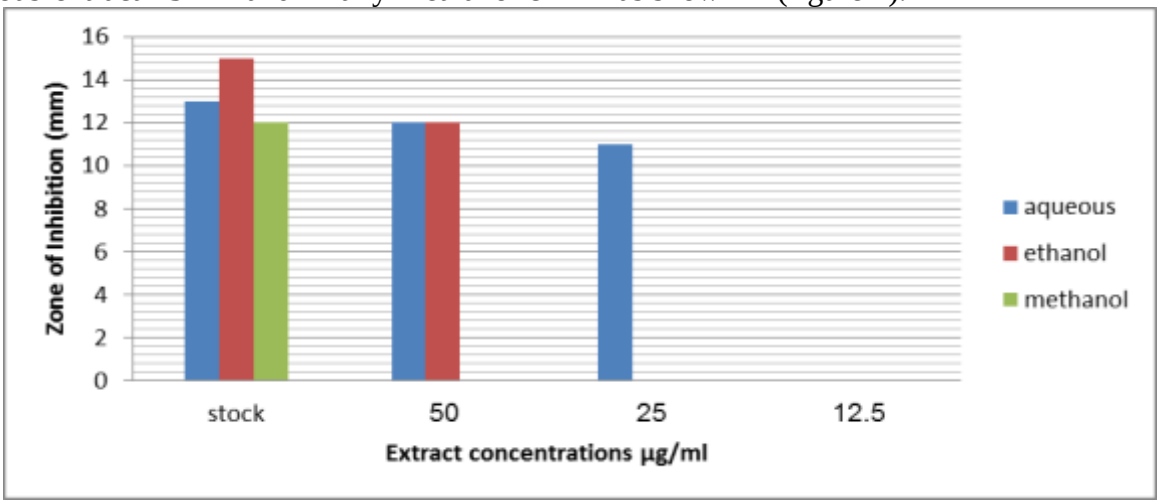

Figure 3. Antibacterial activity of three solvents (aqueous, ethanol, methanol) extracts of dry peel of *Annona Squamosa* at different concentrations (stock, 50, 25, 12.5, 6.25 µg/ml) against *Staphylococcus aureus.*

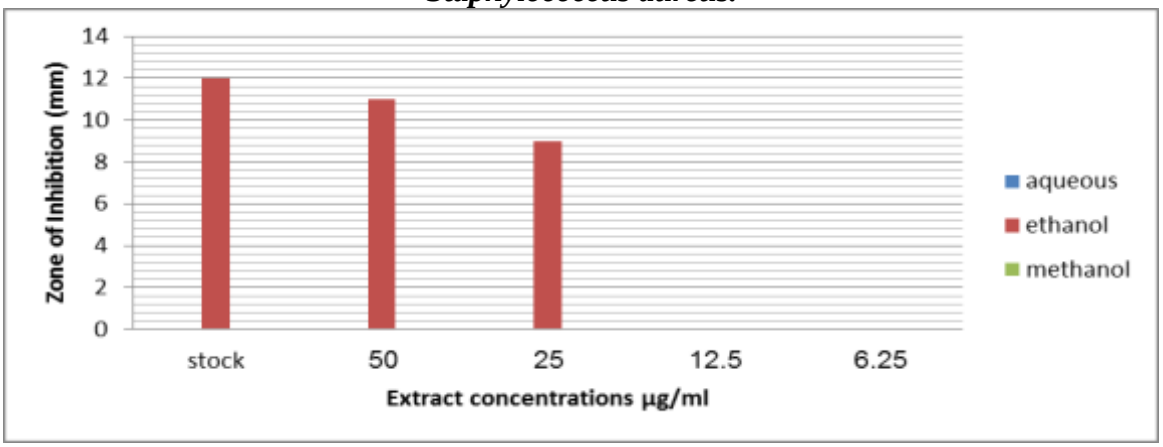

Figure 4. Antibacterial activity of three solvents (aqueous, ethanol, methanol) extracts of fresh peel of *Annona Squamosa* at different concentrations (stock, 50, 25, 12.5, 6.25 µg/ml) against *Staphylococcus aureus.*

Cytotoxicity: The MTT assay results demonstrated that the stock extract of *Annona squamosa* peels is cytotoxic against both cell lines lymphoma and melanoma compared with the all tested concentrations (stock, 50, 25, 12.5, 6.25 µg/ml) of the threesolvents but with different inhibition rates. The cytotoxicity decreased with reduction in extracts concentrations. The cytotoxicity of the dry methanolic extract against both lymphoma and melanoma cell lines showed the highest inhibition rate which reached to (57.23%) and (50. 67%) respectively at the highest tested concentration (stock) of the extract. However stock solution recorded the highest inhibition rate with all studied solvents and the dry extracts were shown to reflect more cytotoxicity than fresh one for the two tested cell line as shown in figures (5) and (6) for dry peels extracts, while (7) and (8) for fresh peels extracts on lymphoma and melanoma respectively.

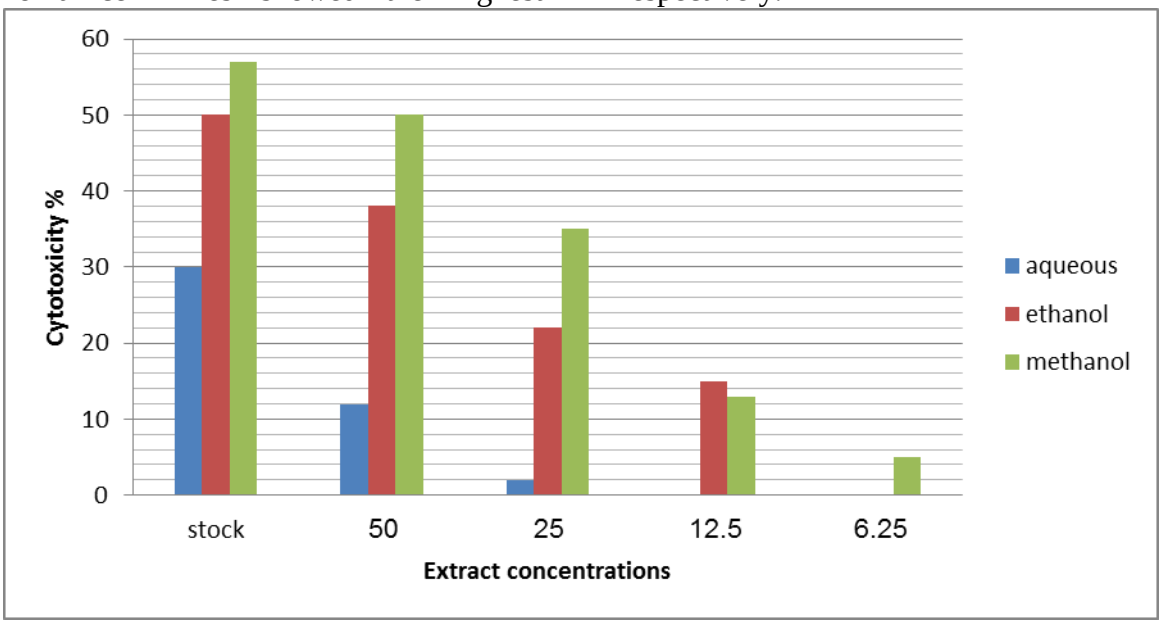

Figure 5. Cytotoxicity of three solvents (aqueous, ethanol, methanol) extracts of dry peels of *Annona Squamosa* at different concentrations (stock, 50, 25, 12.5, 6.25 µg/ml) on lymphoma cell line

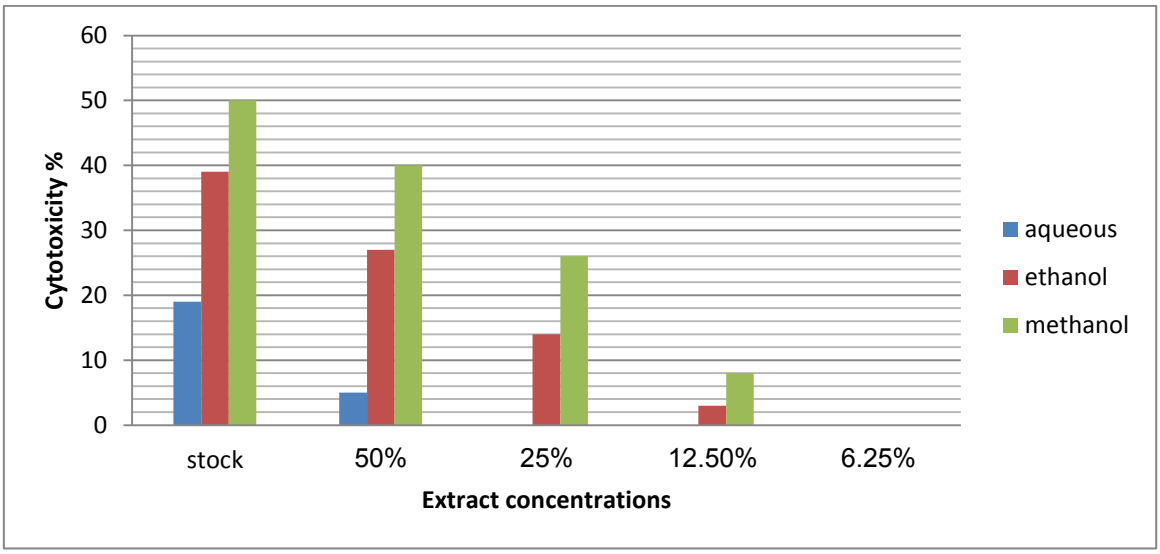

Figure 6. Cytotoxicity of three solvents (aqueous, ethanol, methanol) extracts of dry peels of *Annona Squamosa* at different concentrations (stock, 50, 25, 12.5, 6.25 µg/ml) on melanoma cell line

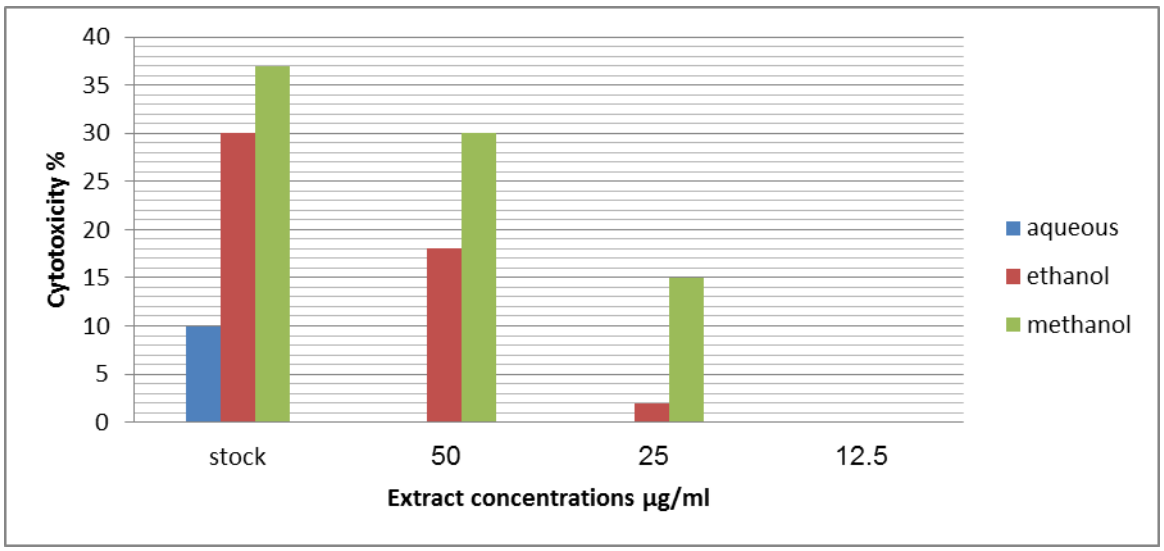

Figure 7. Cytotoxicity of three solvents (aqueous, ethanol, methanol) extracts of fresh peels of *Annona Squamosa* at different concentrations (stock, 50, 25, 12.5, 6.25 µg/ml) on lymphoma cell line

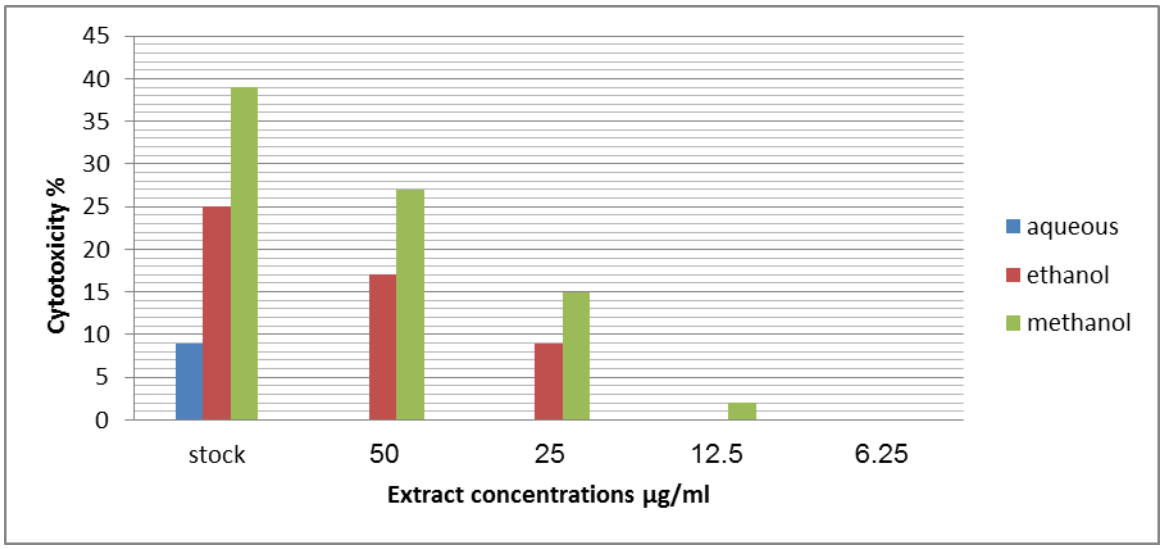

Figure 8. Cytotoxicity of three solvents (aqueous, ethanol, methanol) extracts of fresh peels of *Annona Squamosa* at different concentrations (stock, 50, 25, 12.5, 6.25% µg/ml) on melanoma cell line

In the light of analysis of the present study, the following points have been concluded that *Annona squamosa* was shown to be a powerful antibacterial and anticancer agent. These results came in agreement with other studies who tried to prove the biological activity of this fruit with different extraction methods and different solvents. Fatin and Vijayalakshmi (11, 23) showed that also alcoholic extract was better than watery extracts in reflecting the highest antibacterial activity against some gram positive and negative bacteria. Anticancer activity against some cancer cell lines and normal cell lines were also recorded (6). However no previous studies were recorded for examine the cytotoxicity of *Annona squamosa* on hematological blood malignancies and thus this research can be considered as the first one who proved the cytotoxicity of this fruit on such cell lines. However this greatest biological activity of *Annona squamosa* could be due to it is chemical composition. Many studies showed that each part of the fruit (peel, pulp, and seeds) having different chemical composition such as (vitamins, minerals and antioxidants) and each part reflect different biological activity. Peels were shown to be rich with a long list of nutrients, vitamins, minerals and antioxidants such as vitamins C, A and E, soy sterols and polyphenols. It is also a good source of potassium, fiber, folic acid, manganese, phosphorus, zinc, copper, iron, and calcium. Many previous study tested for

antioxidant efficacy, the researchers found that the highest concentration of antioxidants was due to the high concentration of vitamins A and C (16, 18). highest antibacterial and anticancer activities of this fruit were correlated with methanolic and to lower extent with ethanolic extracts this may suggest that many of it is chemical compounds could be released when treating with alcohol while negative or zero results were reported by aqueous which may indicate the deactivation of these chemical compounds during boiling (1). The anticancer activity of *Annona squamosa* could be explained by different mechanisms mediated by three mechanisms: (1) stimulation of fatal differentiation of altered cells, (2) antiproliferative effect, and (3) alteration of antigens on the surface of tumor cells that leads to the induction of the immune system (5). While antibacterial activity could be suggested by it is ability to destroy cell wall or cell membrane or even genetic material degradation (13 19) .

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