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# EFFICIENCY EVALUATION OF ALKALOIDS FROM POMEGRANATE (*Punica granatum*) PEELS TOWARDS SOME PATHOGENIC FUNGI OF HUMAN

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## ABSTRUCT

This study focused on the role and importance of alkaloid compounds in *Punica granatum* peels which is one of many wide distribution medicinal fruits. Two kinds of pathogenic fungi were isolated from patients in Baghdad to be tested, also a type of extracts was prepared, alkaloids were isolated and partially purified and detected by two ways, a classic depended technique also used for determine these alkaloids, results showed an observed differences among extracts or treatments towards the fungi samples. So this study was one of the scientific applications to find natural alternative compounds that inhibit the growth of several pathogenic organisms that cause dangers and harms for human health.

**Keywords**: Spectrophotometer, alternative compounds, antifungal, inhibition rate.

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# تقييم كفاءة القلويدات المستخلصة من قشور الرمان (*Punica granatum*) تجاه بعض الفطريات الممرضة للإنسان

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## الخلاصة

ركزت هذه الدراسة على دور وأهمية المركبات القلويدية في قشور الرمان *Punica granatum* والذي يعد واحد من الفواكه الطبية الواسعة الانتشار، فتم عزل نوعين من الفطريات المرضية من المرضى في بغداد ليتم اختبارهما، وكذلك تم إعداد نمط من المستخلصات، وتم عزل القلويات وتنقيتها جزئيًا واكتشافها بطريقتين، تقنية كلاسيكية معتمدة تستخدم أيضًا لتحديد هذه القلويات، أظهرت النتائج وجود فروق ملحوظة بين المستخلصات او المعالجات نحو عينات الفطريات، وبالتالي فان هذه الدراسة واحدة من التطبيقات العلمية لإيجاد مركبات بديلة طبيعية تحول دون نمو العديد من الكائنات المسببة للأمراض التي تسبب أخطارًا وأضرارًا على صحة الإنسان.

**الكلمات المفتاحية**: المطياف الضوئي، مركبات بديلة، مضاد فطري، معدل التثبيط.

## INTRODUCTION:

Pomegranate is one of the oldest fruits known to humans. Pomegranate fruits have a high nutritional value. They contain large amounts of sugar of about 16%, proteins near to 9%, fatty substances more than 7%, citric acid 1%, fat 3%, fibers 2% and some mineral salts such

as iron and vitamins. Peels, stems and trunks of pomegranate contain four type of alkaloids: pelletierine, Isopelletierine, Ethylpelletierine and pseudo pelletierine, these active compounds of pomegranate use as a treatment in USP9 **(AL-Kunani, 2015)**. In recent years the pomegranate has been the subject of research all over the world. The increasing of storage time of the fruits after they have been harvested give it big attention, due to the main problems of long term storage which is often caused by the presence of fungal inoculums present in the blossom end of the fruit **(Shikha *et al.*, 2017)**.The fruit husk is a rich source of flavonoids, alkaloids and tannins as well as some complex polysaccharides, the main alkaloids present in the pomegranate plant are: (a) Pelletierine (1-(2piperidyl)propan-2-one), (b) Isopeletierine (2-acetonylpiperidine), (c) Punicine. They are all found in the root and bark only, but pelletierine is also present in the husk which is mentioned as pharmaceutical medical compounds **(Michael, 2010)**. Natural products play an important role at the rise of drug development programs in the pharmaceutical industries. Therefore, there has been a great shift from the prescription of antibiotics to the use of medicinal plants **(Newman & Cragg, 2012)**. Pomegranate peels are found to be effective against malaria, various pomegranate supplements are found in the market and can use for helping keep heart diseases such as heart attacks, in addition tannins and elagic acid are among the most potent antioxidants found in peels.Pomegranateextracts are found to increase the hemoglobin count **(Shikha *etal.*, 2016)**. Fungal skin infections are less common than bacterial skin infections. In recent years, however, the incidence of diseases and skin damage due to fungal infections, which can cause problems and malfunction in human organs, has increased significantly. Some types of skin infections cause minor and sometimes serious skin infections. When the body's defenses are low, fungi seize opportunities and invade various body tissues causing disease. Aspergillosis that caused by Aspergillus sp. as well as Candidiasis which caused by yeast, is the most common among people who are suffering from convulsions and immune problems. We find that there is a significant increase in the level of these types of infections, which are attributed to the ability of these fungi and yeast to cause multiple infections of the patient on the one hand and coincided with the immune state of the person and some other environmental factors **(Limper *et al.*, 2011)**. This study aimed to get a natural alternative compounds that inhibit the growth of several pathogenic organisms that cause dangers and harms for human health.

## MATERIAL AND METHODS

### Pathogenic fungi

Two kinds of pathogenic fungi were selected (*Aspergillus sp. and Candida albicans*) for their interaction with a lot of series infections in human deices. *Aspergillus species* isolated from lower respiratory tract specimens (LRTS). While the *Candida sp*. were isolated from digestion tract of sick people and from some skin infections of patients.

### Plant preparing

Pomegranates collected and washing with tab water then with dilute water to remove any impurities after that they dried at room temperature 25$^{o}$C without light, then samples transfer to become powder (**Entessar wtal, 2012**).

### Alkaloid extraction from the plant sample

A method was modified and used to extract alkaloids from the peels of the plant.

1. The plant powder was mixed with water and lime, thus will give it ability for affiliated with acids.
2. Ether (organic solvent) then will add to the alkaloid extraction.
3. The concentrated organic liquid is then shaken with aqueous acid to be separated.

4. Alkaloids will remain in the aqueous liquid, the organic liquid will contain the remain impurities **(Abdul Mushin, 2016)**.

**Alkaloid determination**:

Spectrophotometric determination of total alkaloids with bromocresol (BCG) green is a simple and sensitive method, less time consuming and does not need very special equipment **(Amanlou *et al.*, 2007)**.

**Minimum Inhibitory Concentration (MIC) of the Alkaloid :**

An initial test was performed for the effect of gradient concentrations (1, 2, 3, 4 and 5%) of the alkaloid extracts in the growth of fungi, according for this test, different treatments concentrations from the most smallest extract that inhibit more than 50% of the tow pathogenic fungi, so several concentrations were prepared and considered to be treatments as in the following sentence: Firstly prepare the stock solution of the chosen extract, Secondly several concentrate prepared from this stock were (0.1, 0.3, 0.5,0.7, 1.0, 1. 25, 1.5 and 2.0) to be tested and to determine the MIC **(Kaliyan, 2016)**.

**Antifungal assay**:

The well diffusion assay was used to determine the antifungal effect of extracts against the tested fungal *Candida albicans* by measuring the inhibition zone. The inhibition activity test was used for determine the antifungal effect of extracts against the tested fungal *Aspergillus sp.* by measuring radial growth of fungus. All tested concentration (treatments) were injected with 100 µl of fungi culture incubate for 24-48 hr at 37$^{o}$C after that all set were tested microscopically and a saperoied dextrose agar plate were prepared to be cultivated with only tube that did not contain fungi growth thus to confirm the MIC concentration **(Eksi *et al.*, 2013)**. 10 µg of nystatin (antibiotic drug) was used as a positive control for *Candida albicans* tests. Physiological saline and (DMSO) was used as negative control for *Aspergillus sp.* tests . The plats were incubated for 5 days at 25$^{o}$C. The inhibition activity towards *Aspergells niger* and measured according to abbot equation and the inhibition zone of *Candida sp*. then results were reported **(kannan, 2016)**. All tests were done at these experiment were triplicates.

**Abbot equation**

There is an equation used to determine the rate of growth of the fungi **(Ali & Majeed, 2010)**.

$$\frac{\text{Rate of fungus diameter in a treatment tested plates} - \text{Rate of fungus diameter in control treatment}}{\textit{Rate of fungus diameter in a treatment tested plates}} * 100$$

Pomegranate is the most significant tree which has been domesticated for incalculable human benefits and serves as a producer for drugs with reduced toxicity **(Gow, 2017)**.

**Pathogenic fungi**

Tow kind of pathogenic fungi were tested for their wide distribution and series diseases they caused. Fungi were classified and identified according to their morphological biological characteristics **(Varga, 2007)**, Candida, Saccharomycetaceae, Saccharomycetales, Saccharomycetes, Ascomycota, Fungi. Aspergillus, Trichocomaceae, Eurotiales, Eurotiomycetes, Ascomycota, Fungi.

**Alkaloidsidentification**

20 mL of extract was measured in a test tube to which picric acid solution was added. The formation of orange coloration indicated the presence of alkaloids.

**Calculation of alkaloids**

5 g of sample in a mixture of 200 mL acetic acid methanol (1:15). Add ammonium hydroxide gradually to allow the deposition to be effluent, spray the solution, dissolve the precipitate and spray again **(Varga *etal.*, 2007)**.

## RESULTS AND DISCUSSION

The results of the study were shown in the (Table, 1 and 2). The effects of alcoholic extracts prepared from pomegranate peel in the growth of some fungal species, the results of using several concentrations of pomegranate peel powder which inhibited the growth of fungi on the plant medium. With clear discrete percentages of the comparison treatment. The diameter of fungal was growing *Aspergillus sp*. and *Candida sp.* with (10 and 7 cm) respectively. Candida was clearly consistent with the inhibitory concentrations. The concentration and the first or the initial concentration of the experiment achieved complete inhibition of the two strains respectively and with a 100% inhibition. These two concentrations (MIC) represented both fungi and the concentration used for each fungus. This study was in agree with as tudyby **(Abu-shanab *etal.*, 2008)**.

**Table (1):** The effect of different alkaloid extract concentration on the growth *Aspergillus sp.*

| The extracts conc. mg\ml | The control | 0.1 | 0.3 | 0.5 | 0.7 | 1.0 |
|---|---|---|---|---|---|---|
| Growth diameter (cm) | 10.0 | 4.8 | 4. 0 | 3.6 | 2.5 | 0.0 |
| The rate of inhibition activity (%) | | 4.0 | 55.5 | 65.5 | 75.2 | 100.0 |

**Table (2):** The effect of different alkaloid extract concentration on the growth of *Candida sp.*

| The extracts conc. mg\ml | The control | 0.1 | 0.3 | 0.5 | 0.7 | 1.0 |
|---|---|---|---|---|---|---|
| Growth diameter (cm) | 0.0 | 7.0 | 4.0 | 3.5 | 1.7 | 0.1 |
| The rate of inhibition activity (%) | | 0.0 | 42.7 | 50.0 | 75 | 100 |

With increasing of the alkaloid extract the growth diameter for both fungi, the effect of pomegranate can be explained that a negative impact on the percentage of germination. Alkaloid mechanisms of action as antibacterial. May have respiratory inhibition effects; acts by inhibiting nucleic acid synthesis **(Bukar *et al.*, 2015)** these results were comparatives with a study from **(Kautz *et al.*, 2015)**.

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