

مقارنة المحتوى الكيميائي النباتي والنشاط البكتيري بين المستخلصات
الإيثانولية لقشر الرمان الطازج والمجفف الذي تم جمعه من الزاوية – ليبيا
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الملخص:

تم استخدام الرمان (*Punica granatum L*) لعلاج العديد من المشاكل الصحية مثل السرطان والسكري وأمراض الجهاز الهضمي ومكافحة الملاريا. كما أنه مصدر مهم للعديد من المواد الكيميائية النباتية النشطة بيولوجيًا. وفي هذه الدراسة تم الكشف عن المواد الكيميائية النباتية في مستخلصات الإيثانول لقشر الرمان الطازج والمجفف الذي تم جمعه من الزاوية – ليبيا. تم استخلاص العينات بطريقة الساكسوليت (*soxhle*). تضمنت الفحوصات الكيميائية النباتية الفينولات ، التانينات ، الفلافونويد ، الصابونين ، الستيرويدات ، الجليكوسيدات ، التربينويدات ، القلويدات ، والكربوهيدرات. تم إجراء فحص النشاط المضاد للبكتيريا لمستخلصات قشور الرمان ضد بكتيريا *Staphylococcus aureus* باستخدام طريقة انتشار الآجار. أظهرت نتائج هذه الدراسة أن المستخلص الإيثانولي لقشور الرمان الطازج له تأثير مثبط أكبر على نمو البكتيريا من المستخلص الإيثانولي لقشر الرمان المجفف.

Comparison of Phytochemical Content and Bacterial Activity between the Ethanolic Extracts of Fresh and Dried Pomegranate Peels Collected from Al Zawia – Libya

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ABSTRACT:

Pomegranate (*Punica granatum L.*) has been used for treatment of a various health issues such as cancer, diabetes, digestive system diseases, and to combating malaria. It is an important source of many bioactive phytochemical. In the present study, Phytochemicals were detected in the ethanol extracts of fresh and dried pomegranate peels, collected from Al- Zawia, Libya. The peels extracted using *soxhlet*. Phytochemical analysis investigations included phenols, tannins, flavonoids, saponins, steroids, glycosides, terpenoids, alkaloids, and carbohydrates. Antibacterial activity assay of pomegranate peels extracts, were carried out against *Staphylococcus aureus*, using agar diffusion method. The results of this study showed, that the ethanolic extract of fresh pomegranate peels has a greater inhibitory effect on bacterial growth than the ethanolic extract of dried pomegranate peels.

Keywords: Pomegranate peel, Phytochemical analysis, *Soxhlet* *Staphylococcus aureus*, and Antibacterial activity.

INTRODUCTION

Humans have always relied on natural products, and have continually explored their application, to improving various aspects of our lives. Thus, we have sought to use natural products such as spices, flavoring agents, perfumes, cosmetics, and dyes to control and overcome diseases [1]. Pomegranate (*Punica granatum L.*) derives from the Latin words *pomus* and *granum*, meaning a seeded or a granular apple, is one of the oldest edible fruits known to humankind. The pomegranate is native from Persia Iran, to the Himalayas in northern India and has been cultivated and naturalized over the whole Mediterranean region including Armenia since ancient times [8]. It is one of the landraces and old varieties of fruit trees available in Libya [7]. The edible part of a pomegranate fruit (arils) is mainly consumed directly or used for the preparation of juices and canned beverages, including alcoholic beverages, jellies, and jams, and it is also used for the flavoring and coloring of drinks [6]. Pomegranates fruit peels have a long history in the prevention and treatment of multiple conditions, including gastrointestinal disorders. They contain many bioactive compounds, such as phenols, flavonoids, alkaloids, carbohydrates, tannins, organic acids, vitamins, and trace elements [2]. People have questioned the safety of using synthetic chemicals in food preservation, as they pose toxicological problems and are hazardous to human health [3]. Extracts prepared from pomegranate plant parts have long been used in traditional medicines for the treatment of infectious diseases (4) The gram-negative bacteria, such as *Escherichia coli*, *Enterococcus faecium* and *Staphylococcus aureus* are top listed WHO (World Health Organization) priority pathogens [5]. The

soxhlet extraction is used widely in the extraction of plant metabolites because of its convenience. This method is adequate for both initial and bulk extraction [1].

Materials and Methods

Sample Collection and Preparation:

Pomegranate fruits collected from garden in Al-zawia Libya, were transported to the Faculty of Education Laboratory at Zawia University , Al-Zawia Libya. The fruit were washed and peeled manually. The half of these Peels was directly extracted, and the other part of the peels left to dry, at ambient temperature and in a room with adequate ventilation and stored for the extraction step.

Soxhlet Extraction:

A 10 g of the fresh pomegranate peels sliced into small pieces and placed in an extraction chamber, which is placed on top of a collecting flask beneath a reflux condenser. A suitable solvent (150 ml ethanol 96%) is added to the flask and the set up is heated under reflux (79 °C) (figure 1), and left for the extraction process. Same steps are repeated for the dried and Grinded pomegranate peel [9].

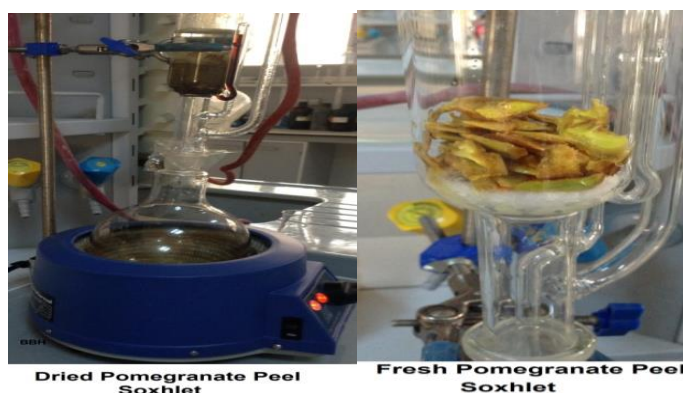


Figure 1: *Soxhlet* extraction for fresh and dried pomegranate peels

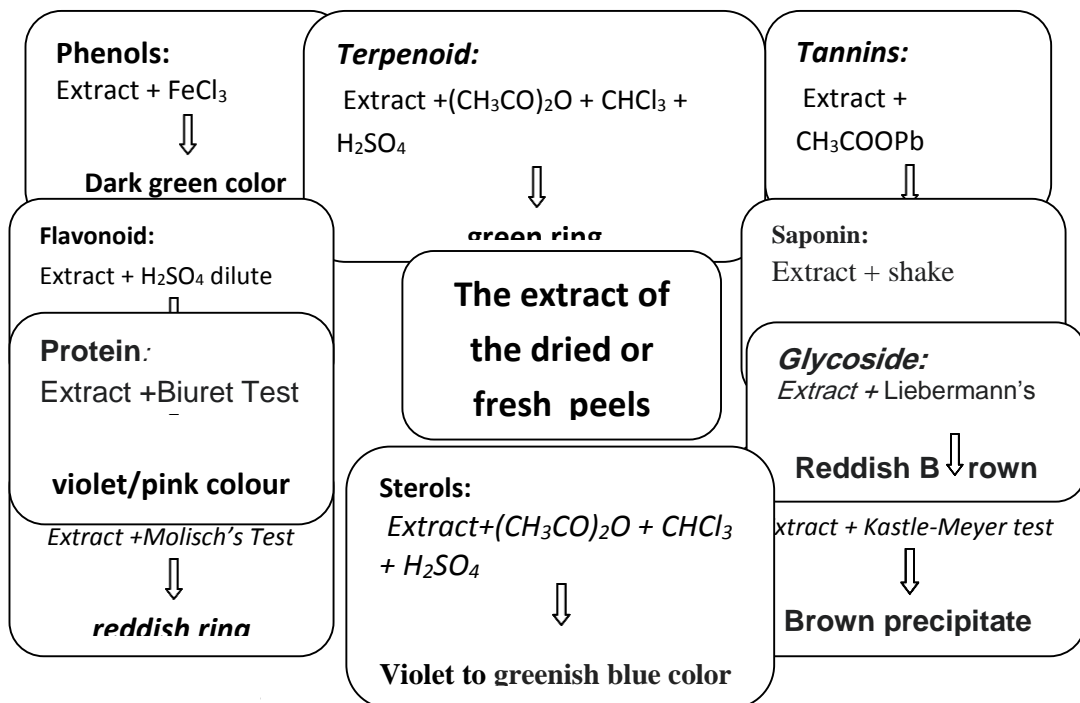
Liquid extract evaporation and condensation:

The final extract was collected from the *soxhlet* flask and placed into *erlenmeyer* flask, and evaporated at the boiling point temperature of ethanol (79°C) to obtain crude extract for the subsequent steps.

Phytochemical Analysis and Antibacterial assay

Phytochemical Analysis:

It has been described that pomegranate peel is an excellent source of valuable bioactive compounds either secondary or primary Metabolites , including phenolic acids, flavonoids and hydrolyzable tannins , polysaccharides , proteins and amino acid. All of phytochemical with proven beneficial health effects [18, 17, 16]. Some reagents were used to detect the most important compounds in the extract for both fresh and dried peels as shown in the (scheme 1) [10, 11, 12].



Scheme 1: Phytochemical analysis of the pomegranate peels extract.

Antibacterial Assay

Agar Diffusion Test:

A 28 g of agar is dissolved in 1000 ml of water distilled. The materials were dissolved by heating with stirring with a magnetic stirrer, and sterilized with the Autoclave at 121 ° C and 1.5 atmospheres for 30 minutes. Then pour it into sterile Petri dishes, leave to harden. A 1,5 ml aliquots of nutrient broth is inoculated with the test organisms (*Staphylococcus aureus*) and incubated at 37°C for 18 hours [13]. Discs of filter paper of equal diameter (1 Cm) were placed in each type of extract, and left for 10 minutes to absorb, then lifted with the using forceps and distributed to dishes cultivated for bacterial isolation *Staphylococcus aureus*. Diameter of an inhibition zone (ZID) is measured to the nearest mm (the cup size also being noted) [14]. The test was conducted at laboratory of Microbiology - Faculty of Medical Technology Surman-Libya.

RESULTS and DISCUSSION

Soxhlet Extraction and Evaporation:

A 10g of the fresh pomegranate peel extracted by 150 ml ethanol 96% for 4 hours, the extracted solution evaporated and yields a Reddish brown viscous paste (0.5 g). The Same Process of the extraction and evaporation was used for 10 g of dried pomegranate peel to yield Greenish brown viscous paste (0.9 g). The final extracts were collected in an airtight glass vial and kept in the refrigerator for the next step.

Phytochemical Analysis:

The importance of the chemical qualitative detection of the bioactive phytochemical of plant extracts. The phytochemical analysis of fresh and dried pomegranate peels extracts, shows the

presence of important bioactive compounds such as phenols, glycosides, flavonoids, terpenoids, carbohydrates, and tannins. The compounds contained can be used as drugs or prevent various pathogens. Phytochemical constituents afford imperative pharmaceutical properties for human health [19]. The result of the qualitative analysis of the dried and fresh pomegranate peels is presented in Table 1 and figures (2, 3):

Table1: the Result Phytochemical analysis of the Fresh and Dried Pomegranate Peels

Phytochemical	Fresh peels extract	Dried peels extract
Phenols	+	+
Alkaloids	+	+
Terpenoids	+	+
Saponins	+	-
Carbohydrates	+	+
Glycosides	+	+
Steroids	+	+
Flavonoids	+	+
Proteins	-	-
Tannins		+

(+)=Presence, (-)=Absence

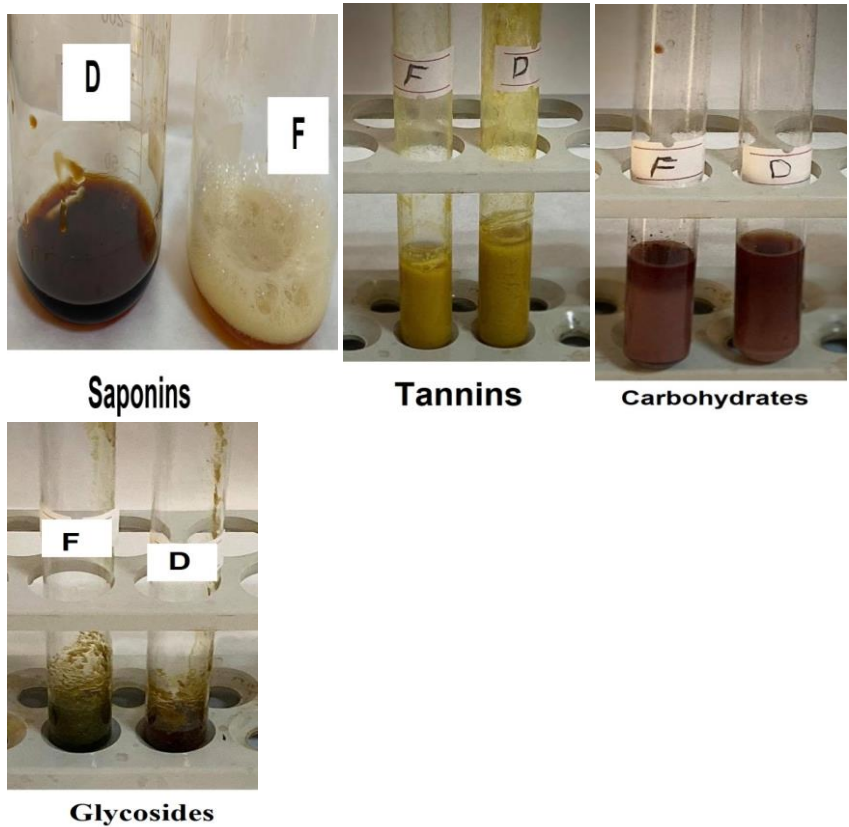


Figure 2: Phytochemical analysis of fresh and dried pomegranate peel extract.

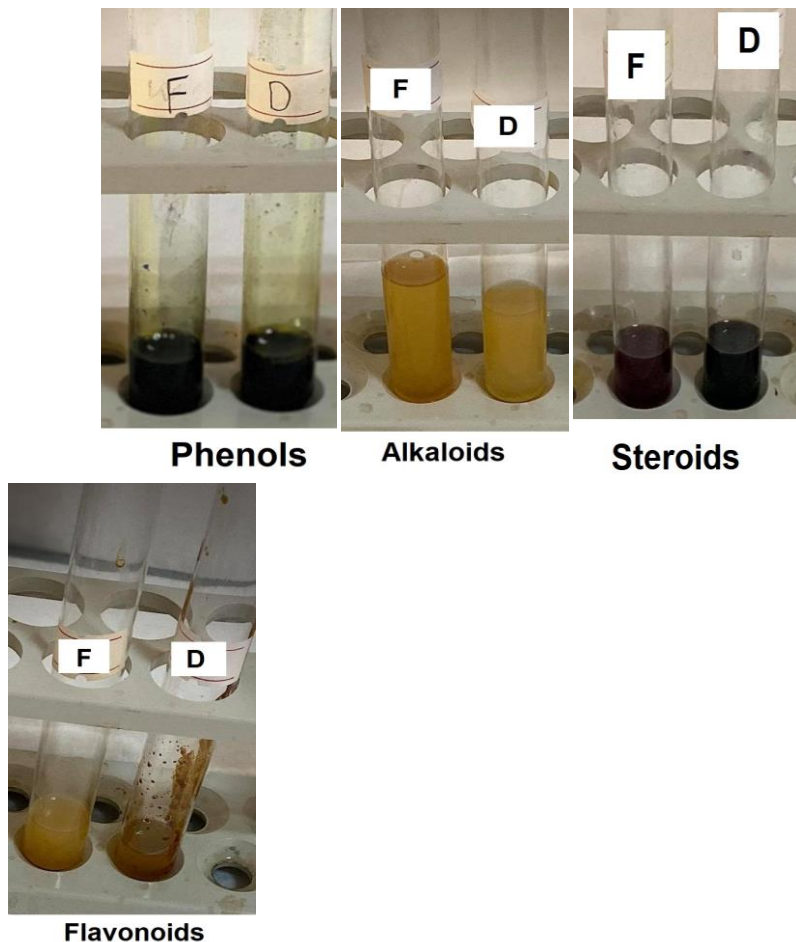


Figure 3: Phytochemical analysis of fresh and dried pomegranate peel extract

Antibacterial Activity:

The potency was initially determined by the agar diffusion method using *Staphylococcus aureus* which is gram-positive bacteria. The diameter of inhibition zones (DIZ) for both extracts was 16 mm, and 20 mm as presented in Table 2 and Figure 4. The fresh pomegranate peels extract showed susceptible inhibition (20mm), while the dried pomegranate peels showed intermediate inhibition (16 mm), according to zone diameter interpretation

chart which is as follows: 13mm or less resistant, 14-16 intermediate and 17 or more susceptible inhibition [15]. The efficiency of pomegranate peel in inhibiting bacterial growth is that, it contains various compounds, as shown by phytochemical analysis. The most important reason for the anti-bacterial activity of fresh pomegranate peels is that, they contain saponins (Figure 2), which are effective against microbes [20].

Table 2: Diameter of Inhibition Zones (DIZ) (mm) of *Staphylococcus aureus* for dried and fresh Pomegranate peels.

Diameter of Inhibition Zones(DIZ) (mm) <i>Staphylococcus aureus</i>	Pomegranate peels extracts
16	Dried peels
20	Fresh peels

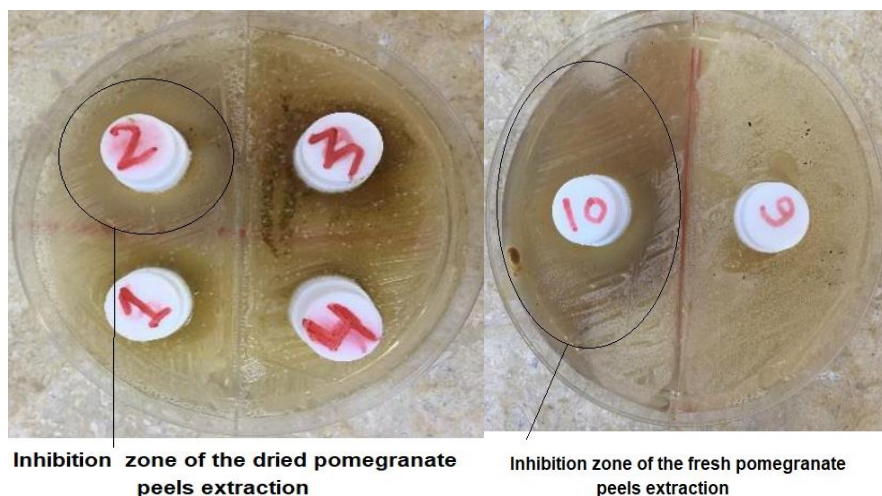


Figure 4: Inhibition zone of *Staphylococcus aureus* of pomegranate peels extracts

CONCLUSION

According to this study we can conclude that, fresh ethanolic extract of the pomegranate fruit peels displayed an antibacterial activity against *Staphylococcus aureus* gram-positive bacteria greater than the dried peels extract. Phytochemical analysis showed an important bioactive compounds with are useful for treating different ailments and it also have a potential of providing useful drugs for human use. Traditional medicines extracted from natural products were used in the past and are still in use today because of the hazardous caused by chemically prepared medicines.

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