

ANTI-MICROBAL ACTIVITY OF ALCOHOL AND WATER EXTRACTS OF POMEGRANATE PEEL

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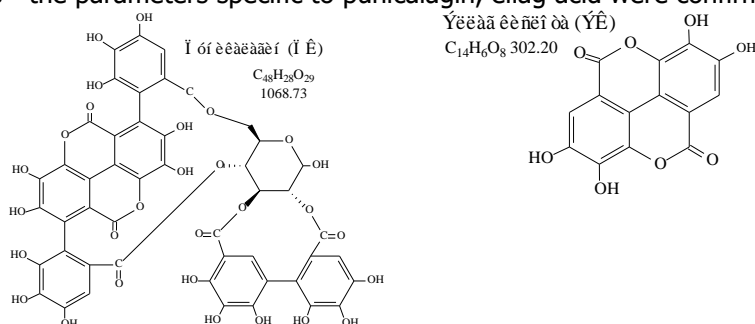
Article history:	Abstract:
<p>Received: 4th February 2022 Accepted: 6th March 2022 Published: 25th April 2022</p>	<p>Pomegranate peel is the remainder of the product in the food industry, ethanol and water extraction was carried out, from which the sum of biologically active compounds (BAC) was isolated. The antimicrobial activity of the extract and fraction was investigated.</p>
<p>Keywords: Pomegranate peel, extraction, fraction, punicalagin, ellagic acid, antimicrobial activity.</p>	

Pomegranate (*Punica granatum* L.) belongs to the family Pomegranate (*anorgulli*) and is a subtropical plant, currently grown in all regions of the country [1].

Pomegranate fruit has long been described in folk medicine as a means of cleansing the blood, and its peel has been described as a remedy for diarrhea, hemostatic, anthelmintic and inflamed skin, mainly for the treatment of chronic dysentery and bleeding. The main active compounds in pomegranate peel are polyphenols (glycosides, ellag and bile acids) in the treatment of wounds in the gastric mucosa and have other properties [2, 3, 4, 5].

Purpose of the study: Given that pomegranate fruit, which is widely used in the territory of Uzbekistan, can be used in the food industry, its peel is considered a residual product, ie the BFB derived from it is not registered as a drug. The main purpose of the study is to isolate the main biologically active compounds in pomegranate peel: polyphenols, coumarins, carbohydrates, organic acids and similar active compounds, to develop methods of qualitative and quantitative analysis and study of biological activity.

Research Materials and Methods: Punicalaginate peel, dried at room temperature and ground into a fine powder, served as the research material. 100 g of crushed raw material of pomegranate peel was extracted three times in 500 ml of ethanol (1: 5 ratio) at room temperature for 12 hours. The ethanol extraction was filtered and a dry extract was obtained by driving ethanol under vacuum in a rotor dryer. 100 ml of water was mixed with the dry extract and it was extracted with hexane, chloroform and ethyl acetate and divided into fractions, the fractions were dried by driving the solvents using a rotor dryer. The aqueous portion was also dried and washed in methanol from a SiO₂ column, and the fractions obtained were controlled on a silica gel plate using thin-layer chromatography. The moving phase was treated with 15% acetic acid and a coloring reagent, ammonia. Using ellagic acid as a sample, the resulting fractions were placed in series on a plate and placed in a chamber with a moving phase. When the plate poured into the chamber reached the front line and was treated with ammonia, it was found that the yellow fractions were present at the same distance from the sample ($R_f = 0.35$) and that the fractions had a second yellow spot on the bottom plate relative to the sample. When the chemical structure of the isolated compounds was studied using physical research methods - the parameters specific to punicalagin, ellag acid were confirmed.



Antimicrobial activity: The antimicrobial activity of the fraction of ethanol extract from pomegranate peel in water and the fraction isolated from the dried extract using methanol as an eluent from the SiO₂ column was determined.

Aqueous and alcoholic solutions with a concentration of 30 mg / ml were prepared from these compounds and their antimicrobial activity was studied using the agar diffusion method [6]. Conditional pathogens and pathogens used to determine the antimicrobial activity of samples (aqueous extract of pomegranate peel, fraction isolated from the column): *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Proteus mirabilis*, *Escherichia coli*, *Serratia marcescens*, *Candida albicans* of the Institute of *Candida albicans*, *Listeria* taken from the collection. In the study of the antimicrobial activity of the samples, a suspension corresponding to 0.5 units according to the McFarland turbidity standard was prepared in a saline solution of one-day conditional pathogens and pathogenic test microorganisms grown in meat peptone agar medium. This bacterial suspension was pushed evenly onto the surface of the meat peptone agar medium using a cotton swab. Holes were drilled in the agar layer of the petri dishes using a sterile metal perforator with a diameter of 6.0 mm, and aqueous and alcoholic solutions of 100 µl of compounds were poured into these holes. A paper disk impregnated with 96% alcohol and 30 mcg cefazolin antibiotic (HiMedia, India) was used as a control to compare the antimicrobial activity of the compounds. Petri dishes planted for good diffusion into the agar layer were kept in the refrigerator at + 4°C for 2 hours. The microorganisms were then grown in a thermostat at 37°C and after 24 h the diameter of the microorganism-free zones around the droplets was measured using a ruler to determine whether the substances had antimicrobial activity.

Analysis of the obtained results: The antimicrobial activity of the collection of compounds isolated from pomegranate peel using different solvents is given in Table 1.1

№	Samples of the obtained unit 30 mg / ml	Үсишни тўхтатиш зонаси диаметри, мм							
		<i>Pseudomonas aeruginosa</i>	<i>Staphylococcus aureus</i>	<i>Proteus mirabilis</i>	<i>Escherichia coli</i>	<i>Serratia marcescens</i>	<i>Candida albicans</i>	<i>Bacillus subtilis</i>	<i>Listeria monocytogenes</i>
1	96% with ethyl alcohol	0	0	0	0	0	0	0	0
2	cefazolin	17,2±0,1	17,6±1,2	0	0	0	0	17,2±0,6	0
3	Ethanol extract of pomegranate peel	26,1±0,5	30,5±1,1	0	25,1±1,3 б.с.	0	0	30,3±0,5	0
4	Aqueous extract of pomegranate peel	26,6±0,8	25,7±0,6	0	27,6±0,4 б.с.	16,5 ±0,5	0	25,1±1,2	0

Note: b.s. - bactericidal effect

CONCLUSION

1. Ethanol and aqueous extraction of pomegranate peel, which is a residual product in the food industry, was carried out, from which a collection of biologically active compounds (BFB) was isolated. Using column chromatography, the biologically active compounds in the collection - punicalagin, ellagic acid - were isolated.

2. The antimicrobial activity of the extract and fraction has a broad spectrum and high antimicrobial effect when studied. These compounds have the potential to be used as effective drugs for the treatment of *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Bacillus subtilis* infections

LITERATURE

1. Ботрус Д.А. Биохимическая характеристика сирийского граната и его промышленное использование: автореф. дис... канд. тех. наук. - Одесса, 1984. - 23 с.
2. Alighourchi H. R. Effect of sonication on anthocyanins, total phenolic content, and antioksidant capacity of pomegranate juices/H. R. Alighourchi, Barzegar M., Sahari M. A. and Abbasi S.//International Food Research Journal, 2013. - vol. 20 (4). - P. 1703-1709.
3. Jia L., Guoliang W., Chen H., Jianke L., Ying L., Baicun L. Punicalagin and ellagic acid from pomegranate peel induce apoptosis and inhibits proliferation in human HepG2 hepatoma cells through targeting mitochondria // Food and agricultural immunology, 2019, Vol 30, No. 1, P. 898-913.
4. Гафизов Г.К., Семочкина Л.Г., Гахраманов М.С. Способ получения танина //Патент СССР № 1531453. - 1987.
5. Гафизов Г.К., Семочкина Л.Г. Способ комплексной переработки корки и перегородок плодов граната // Патент СССР №1733448.1995. - Бюл. № 18.
6. Егоров Н.С. Руководство к практическим занятиям по микробиологии. - Москва: Издательство Московского Университета, 1983. - 220 с.