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Evaluation of *Punica granatum* L. fruits extracts as anti-fungus infecting Iraqi wheat crop *Triticum aestivum* (L.)

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***Abstract*---**This study was conducted in the laboratories of the Faculty of Science / University of Kufa from October 2021 to March 2022, the Pomegranate granatum (L.) fruits plant was collected from Kufa marked and was cleaned of plankton with tap water and the fruits parts mesocarp (grease) and Peels were cut after separating them into small pieces and dried in shade and room temperature (24±2) and then crushed into a dry powder. A specific weight (10) grams of Mixing of two part (mesocarp and Peels) powder was extracted separately by soxhlet method using ethanol 96%. The qualitative analysis was studied for the detection phytochemicals using chemical reagents and GC-MS analysis was conducted to identify the quality and quantity of phytochemical compounds in crude extraction methods used in this study. Results of chemical tests for the alcoholic extract showed positive results for alkaloids, phenols, terpenes and glucosides, and the ethanolic extract of the mixing of (mesocarp and Peels) using the Soxhlet method showed the highest rate of the chemical compounds diagnosed using GC-MS indicated 72 peaks. Antifungal activity of plant extract determined as growth inhibition evaluation Criteria. Crude extract were tested against three types of plant fungi infection wheat crop in Najaf City. Fungi study were obtained from the Environment /Microbiology Laboratory / College of Science / University of Kufa. Three concentrations of extracts 10, 20 and 30 % were prepared for the antifungal activity at three replicates for each concentration using the wells diffusion method in compare with fungicide at concentration of 30%. Furthermore, it was found that extraction method and types of plant part also gave different data. The results showed that extract concentrations gave fungi growth inhibition of the Aspergillus,

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Fusarium and Alternaria and all fungi species were more affected by increasing these concentration study. The ethanol extracts at 30% crude extract exhibited a 98% growth inhibition percentage of the Aspergillus, Fusarium and Alternaria fungus. While, results show that significantly difference (P<5%) with (control treatment) fungicide at 30% concentration recorded about (15, 10, 5 %) respectively.

***Keywords*---**Punica granatum, extracts, anti-fungus, wheat crop.

Introduction

Medicinal plants which possess a rich source of natural product (Akrout *et al.*, 2012). Today, wide variety of phytochemicals have been extracted from medicinal plants and are utilized by companies in the synthesis of antifungal diseases. About 800. 000 species of plants contain bioactive compounds such as Alkaloids, Phenolic, and terpenes has function as fungicides have been reported. Phytochemicals extracted from such plant consider as biological control agents that affected growth and development of plant pathogens with no side effects on ecosystem or human health .Several studies reported that plant extracts which have gained more attention for synthetic fungicides utilize in the control manner against a wide range of including plant fungal diseases (Srivasata *et al.* 2011; Elsharkawy and El-Sawy 2015). *Fusarium spp, Alternariaand Aspragillus is* the most common fungi infected their wheat (*Triticum aestivum* L.) in Iraq caused plant disease. And major yield losses in the grain yield over different plants areas (Kolmer, 2005). Wheat *Triticum aestivum* (L.) is one of the most important nutritive cereal crops in Iraq in terms of the crop production used for food consumption (Acquaah , 2012). *Punica granatum L.* (Pomegranate) is considered one of the most important types of plant fruits used as an antifungal in order to their biochemical content , (Karumi ,2001). its contains 25% to 28% of Tannins, the chemical compound is Punicallin, , Granatine C, and Tanicacid acid, which is an strong antifungal agent (Majeed *et al.*, 1988).Polyphenols as ellagic acid is a large groups of phytochemicals in pomegranate peels (about 67% of the weight of the fruit) is a rich source of antioxidants, (Mara *et al.*, 2000).Thus , several researchers explore an possible to improve some strategy to against its fungus disease and improves growth and grains yield mainly through phytochemicals as fungicides (Jabel and Bader ,2014).

Goal of this study

The goal of this study was to found the natural compounds produced from medicinal plant *P. granatum* (Pomegranate) having the possibility to against plant pathogenic fungi. These study about the Pomegranate peel and Mesucarp was valuable for developing new uses for the agricultural treatments to produce natural fungicides that may replace the chemical fungicides.

Materials and Methods

Plant collection

The fruits of *P.granatum* was collected from Kufa /Najaf City marked at duration of November to December 2021, Then mesocarp (grease) and Peels of plant was taken and dried in shad at room temperature for 25 days and milled separately by using electric miller.

Preparation of *P. granatam* mixing Peel and Mesocarp Extracts

Mixing of Peels and mesocarp (10) g was extracted with 200 ml ethanol solvent by Soxhlet apparatus (Continuous extraction) for 24 hours. These extracts were filtered through filter paper. Then the filtrated extract was dried at 45˚C by oven, and then the dried matter was milled by miller and collected in sealed class container and stored in dark cold place. (Liu *et al.,* 2013).

Preparation of plant extracts concentrations

Ethanolic extracts of *P. granatum* Mixing of **(** Mesocarp and Peels) in the soxhlet methods. Three concentrations of plant parts were prepared, weighed 10 mg , 20mg and 30mg of the dry powder, each weight was dissolved in 100ml of distilled water directly, firstly each weight was dissolved with 1ml of Dimethyl sulphoxide (DMSO) (was selected as a safe diluting agent for the oil) and then each weight was dissolved in 100 ml of distilled water, and thus the final concentration for each solvent would be 10 % , 20% and 30% respectively were used in inhibition of fungi growth (Packiyalakshmi *et al.*, 2017). Besides, each of three concentrations of the ethanolic extract, synthetic fungicides solution at a concentration of (30%) used to test the effect as antifungal activity as control treatment (Elbashiti *et al.*, 2011).

Phytochemicals detection using GC-MS technology

In this study, phytochemicals in pomegranate extracts of mixed of Mesocarp and Peel have been studied using (GC-Mass) GC-MS analysis was performed on a Perkin Elmer Turbo mass spectrometer (Norwalk, CTO6859, USA) which included a Perkin Elmer XLGC. The column used was a Perkin Elmer Elite-5 capillary column measuring 30 × 0.25 mm with a film thickness of 0.25 mm and consisting of 95% dimethylpolysiloxane. The carrier gas used was helium at a flow rate of 0.5 ml/min.

Statistical analysis

The study experiments were carried out according to a completely randomized design (C.R.D.). Factorial experiments were used according to the design of CRD and RCBD to implement experiments with two factors. The Least Significant Difference (LSD) test was conducted at the level of significance at 0.05 to compare the results of laboratory experiments and pot experiments (Al-Rawi and Khalaf Allah, 2000). The results were analyzed using the program Genstat was used to organize and extract rates.

Results and Dissections

Qualitative Phytochemicals tests

The phytochemical qualitative detection tests may be useful identify the bioactive properties and may be lead to the drug discovery. (Parivuguna, 2008). Results in the table (1) explain that alkaloids reagent (Dragendorff reagent), gave a positive result for the all ethanolic extracts (fig.1). While Phenols, terpens gave a positive result by Ferric chloride 5% and Sulfuric acid reagent (fig.2 and 3) respectively. As for testing the glycosides, it gave a positive result for all solvents used (fig.4).These results are agreement with(Gupta *et al*, 2014**)** study and with results for (Garba *et al*, 2019), where he was mentioned that they gave a positive result. Work by (Jayaprakash and Sangeetha , 2015) were reported the presence of tannins, phenols, glycosides, terpenoids, alkaloids and carbohydrates, in the pomegranate peel. While, Study by(Karthikeyan and Vidya,2013) was show the presence of different types of Terpenoids, Flavonoids, Alkaloids in pomegranate peel and mesocarp extracts with their strong therapeutic and antibacterial activity . (Middha, *et al* 2013) confirmed the presence of some major phenolic compounds like: gallic acid, ellagic acids and punicalagin, which detect their antioxidant capacity, in *P. granatum* fruit peel. Results in current study agree with study of (Sreedevi, *et al.*, 2017), which found that phytochemical in *P. granatum* were different by using different types of solvents. Several studies by (Trabelsi *et al.*, 2020; Yuan and Fang, 2018) Shown our data of kaempferol, luteolin, apigenin and myricetin, in in ethanolic and aqueous extraction of *P. granatum* fruit peel and Mesocarp.

Table 1
Phytochemical analysis of *P. granatum* extract

Plant part	Phytochemicals	Test name	Indication	Test Result
Mixing Mesocarp and Peels	Alkaloid	Dragendroff' s	Orange coloration	+
	Phenolic	Ferric chloride	Bluish green color	+
	Glycosides	Benedict reagent	Pink or red pellet	+
	Terpenes	Liebermann-Burchard test	Dark pink/green	+

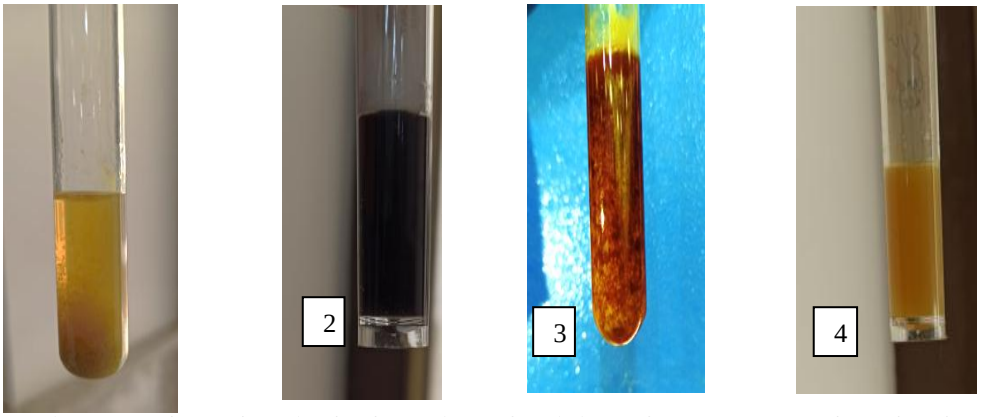
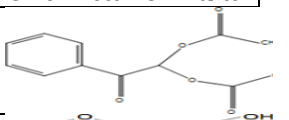
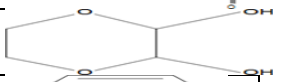
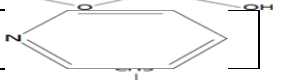
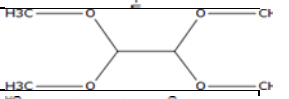
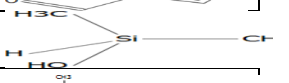
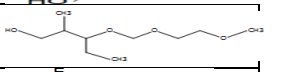
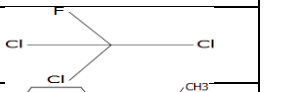
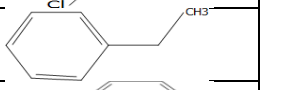
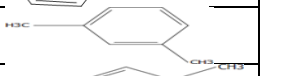
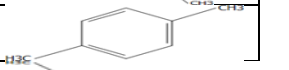
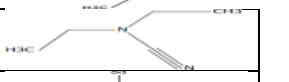
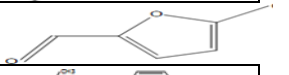
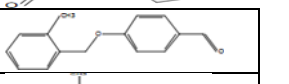
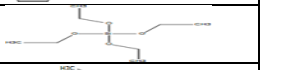
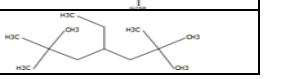


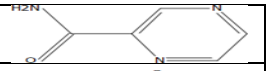
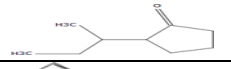
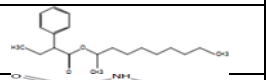
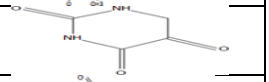
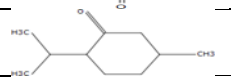
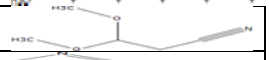
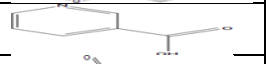
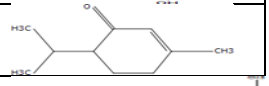
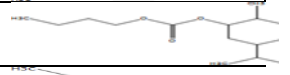
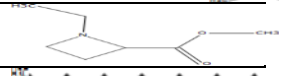
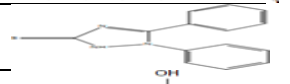
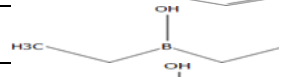
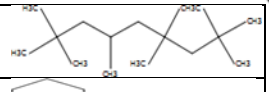
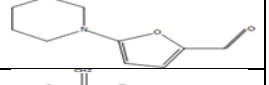
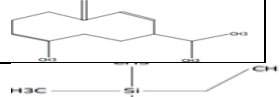
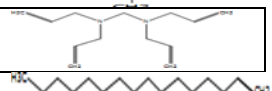
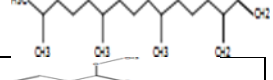
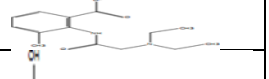
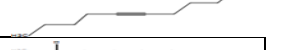
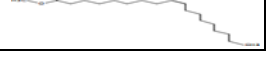
Figure1. Phytochemicals detection of mixing of Mesocarp and Peels of *P. granatum*: 1:Alkaloids : 2: Phenols : 3: Glycosides : 4: Terpene

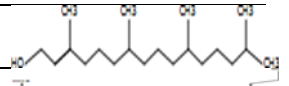
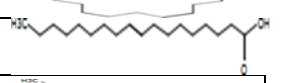
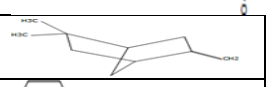
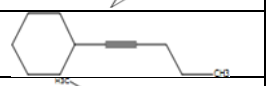
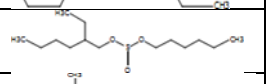
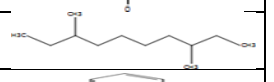
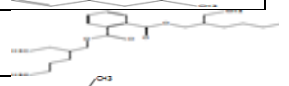
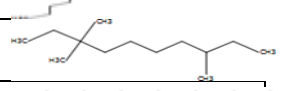
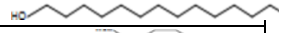
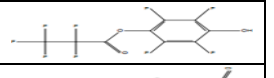
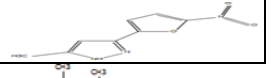
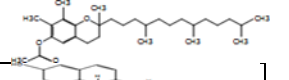
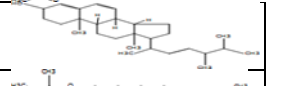
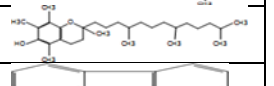
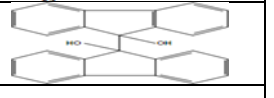
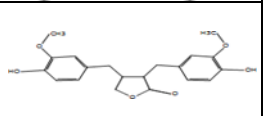
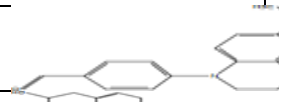
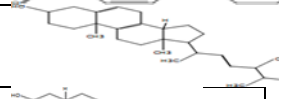
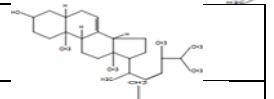
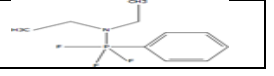
Phytochemicals detection in *P. granatum* by GC-MS chromatography.

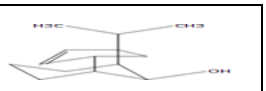
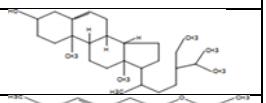
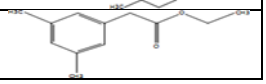
Results in Table (2) and figure (2) show that chemical components identified in GC-MS analysis of ethanolic 96% from pomegranate mixing of Mesocurp and Peels extract there are 72 peaks indicating. By comparison with mass spectra these phytochemicals were characterized and identified .The major phytochemical contents are Silane, ethyltrimethyl-(14.510 %) as antibacterial properties (Gottenbos *et al.*2002) followed by Ethane, 1,1,2,2-tetramethoxy- with ratio of (8.063) third consitituent is Borinic acid, diethyl- with ratio (6.319 %), benzaldehyde,4-(3,4-dihydro-6-methoxy-1(2H)- quinolinyl)- (5.638 %) , 9-Octadecenoic acid with ratio (4.694589 %) ,then, 2(3H)-Furanone, dihydro-3,4-bis[(4-hydroxy-3- methoxyphenyl)methyl]-, (3R-trans)- with ratio (4.404 %) , and 9,9'-bis-(9H-fluorene), 9,9'-hydroxy- with ratio (3.541609 %) followed by n-Hexadecanoic acid with ratio (3.529 %) and Ergosta-4,6,22-trien-3.beta.-ol with ratio (3.217 %), followed by Ethyl 3,5-dimethylphenylacetate (2.340597%) and Phenacylidene diacetate (2.060 %), Heptane, 4-ethyl-2,2,6,6-tetramethyl- (1.747 %) , 1,8,11-Heptadecatriene, (Z,Z)- (1.698 %), Pyrazole, 5-methyl-3-(5-nitro-2-furyl)-(1.678 %), , 2-Isopropyl-tricyclo[4.3.1.1(2,5)]undec-3-en-10-ol (1.491 %), beta.-D-Glucopyranose, 1,6-anhydro- (1.43994 %) , Bicyclo[2.2.1]heptane, 2,2-dimethyl-5-methylene- (1.403 %), and Anthraergostatetraenol p-chlorobenzoate (1.094 %). This study agreement with work by(Vijaya Lakshmi ,2011) found that Twenty six compounds in *Punica granatum* peel ethanolic extracts were Glycerin, Hydroxymethylfurfurole, Guanosine and Pyrogallol. (Barathikannan *et al* .,2016) found that eight-four compounds in *P. granatum* peel ethanolic extracts.(Mossalem *et al.*,2011) reported that the GC-MS analysis of *P. granatum* peel ethanolic extracts presence of 36 compounds were Piperidin-4-ol, 1,3-dimethyl-2,4,6-triphenyl (19.87%), and 6,11-dihydroxy-5,12 naphthacenequinone-1-carboxylic acid (7.80%) were the major components.

Table 2
Qualitative compound report in *P. granatum* mixing of Mesocurp and Peels extractused Soxhlet methods with ethanol solvent 98%

NO.	Name	Formula	RT	%	Chemical formula
1	Phenacylidene diacetate	C12H12O5	4.641	2.060967	
2	p-Dioxane-2,3-diol	C4H8O4	4.662	4.137146	
3	Pyridine	C5H5N	5.013	0.292282	
4	Propane, 2-fluoro-2-methyl-	C4H9F	5.246	0.372524	
5	Ethane, 1,1,2,2-tetramethoxy-	C6H14O4	6.172	8.063373	
6	Tetraethylene glycol	C8H18O5	6.343	0.407	
7	1,3,5,7-Tetroxane	C4H8O4	6.665	0.252207	
8	3-Furaldehyde	C5H4O2	6.736	0.403345	
9	Silanol, dimethyl-	C2H8Osi	6.841	0.918722	
10	3-(2-Methoxyethoxymethoxy)-2-methylpentan-1-ol	C10H22O4	7.017	0.442928	
11	Trichloromonofluoromethane	CCl3F	7.151	0.551631	
12	Ethylbenzene	C8H10	7.274	0.301426	
13	Benzene, 1,3-dimethyl-	C8H10	7.452	0.569686	
14	p-Xylene	C8H10	7.965	0.392081	
15	Butane, 1,1-diethoxy-	C8H18O2	8.268	0.212936	
16	Diethylcyanamide	C5H10N2	8.915	0.178379	
17	Propanoic acid, 2-(benzoylthio)-, ethyl ester	C12H14O3S	9.621	0.346468	
18	2-Furancarboxaldehyde, 5-methyl-	C6H6O2	9.723	0.187444	
19	Benzaldehyde, 4-[(2-methylphenyl)methoxy]-	C15H14O2	10.133	0.17631	
20	Tetraethyl silicate	C8H20O4Si	10.271	0.333748	
21	Heptane, 4-ethyl-2,2,6,6-tetramethyl-	C13H28	10.319	1.747413	

22	Pyrazinamide	C5H5N3O	10.813	0.234836	
23	Cyclopentanone, 2-(1-methylpropyl)-	C9H16O	10.952	0.152461	
24	Butyric acid, 2-phenyl-, dec-2-yl ester	C20H32O2	11.453	0.191	
25	2,4,5-Trihydroxypyrimidine	C4H4N2O3	11.539	0.219202	
26	Cyclohexanone, 5-methyl-2-(1-methylethyl)-, trans-	C10H18O	13.136	0.150205	
27	Dodecane	C12H26	13.787	0.429198	
28	Propanenitrile, 3,3-dimethoxy-	C5H9NO2	14.166	0.181486	
29	Niacin	C6H5NO2	14.309	0.258057	
30	2-Cyclohexen-1-one, 3-methyl-6-(1-methylethyl)-	C10H16O	14.689	0.165837	
31	Carbonic acid, (1R)-(-)-menthyl butyl ester	C15H28O3	15.223	0.256144	
32	N-Ethyl-2-carbomethoxyazetidine	C7H13NO2	16.337	0.184265	
33	Tetradecane	C14H30	16.602	0.463491	
34	1,5-Diphenyl-2H-1,2,4-triazoline-3-thione	C14H11N3S	16.847	0.402013	
35	Borinic acid, diethyl-	C4H11BO	17.172	6.319698	
36	.beta.-D-Glucopyranose, 1,6-anhydro-	C6H10O5	17.701	1.43994	
37	Nonane, 2,2,4,4,6,8,8-heptamethyl-	C16H34	19.072	0.464986	
38	Furan-2-carboxaldehyde, 5-(1-piperidyl)-	C10H13NO2	19.771	0.528664	
39	Germacrene D	C15H24	19.871	0.435759	
40	Silane, ethyltrimethyl-	C5H14Si	19.938	14.51019	
41	1,1'-Methylene-bis(di-2-propenylamine)	C13H22N2	20.869	0.170509	
42	Neophytadiene	C20H38	21.714	0.451711	
43	Tolycaine	C15H22N2O3	22.465	0.530928	
44	n-Hexadecanoic acid	C16H32O2	22.913	3.529599	
45	5-Undecyne	C11H20	24.224	0.305189	
46	9-Octadecenoic acid (Z)-, methyl ester	C19H36O2	24.275	0.475787	

47	Phytol	C20H40O	24.391	0.203743	
48	1,8,11-Heptadecatriene, (Z,Z)-	C17H30	24.554	1.698884	
49	9-Octadecenoic acid	C18H34O2	24.603	4.694589	
50	Bicyclo[2.2.1]heptane, 2,2-dimethyl-5-methylene-	C10H16	24.617	1.403152	
51	1-Cyclohexyl-1-pentyne	C11H18	24.881	0.216555	
52	Sulfurous acid, 2-ethylhexyl hexyl ester	C14H30O3S	25.137	0.16759	
53	Decane, 3,8-dimethyl-	C12H26	26.828	0.168333	
54	N,.alpha.,.alpha.'-Trimethyldiphenethylamine	C19H25N	27.994	0.257329	
55	Bis(2-ethylhexyl) phthalate	C24H38O4	28.065	0.974012	
56	Decane, 3,3,8-trimethyl-	C13H28	28.394	0.150963	
57	1,14-Tetradecanediol	C14H30O2	29.124	0.233111	
58	Anthraergostatetraenol p-chlorobenzoate	C35H45ClO2	31.207	1.094936	
59	Tetrafluorohydroquinone, pentafluoropropionate	C9HF9O3	31.457	0.732923	
60	Pyrazole, 5-methyl-3-(5-nitro-2-furyl)-	C8H7N3O3	31.459	1.678386	
61	(+)-.gamma.-Tocopherol, O-acetyl-	C30H50O3	31.716	0.472756	
62	Ergosta-4,6,22-trien-3.beta.-ol	C28H44O	32.18	3.217836	
63	Vitamin E	C29H50O2	32.369	0.158196	
64	9,9'-bis-(9H-fluorene), 9,9'-hydroxy-	C26H18O2	32.502	3.541609	
65	2(3H)-Furanone, dihydro-3,4-bis[(4-hydroxy-3-methoxyphenyl)methyl]-, (3R-trans)-	C20H22O6	32.513	4.404236	
66	benzaldehyde, 4-(3,4-dihydro-6-methoxy-1(2H)-quinolinyl)-	C17H17NO2	32.783	5.63868	
67	Ergosta-5,8,22-trien-3-ol, (3.beta.,22E)-	C28H44O	33.124	1.337621	
68	Ergosta-7,22-dien-3-ol, (3.beta.,22E)-	C28H46O	33.27	1.864576	
69	Diethylamino(phenyl)trifluorophosphorane	C10H15F3NP	33.359	0.6711	

70	2-Isopropyl-tricyclo[4.3.1.1(2,5)]undec-3-en-10-ol	C14H22O	34.26	1.491872	
71	.gamma.-Sitosterol	C29H50O	34.273	1.027854	
72	Ethyl 3,5-dimethylphenylacetate	C12H16O2	35.166	2.340597	

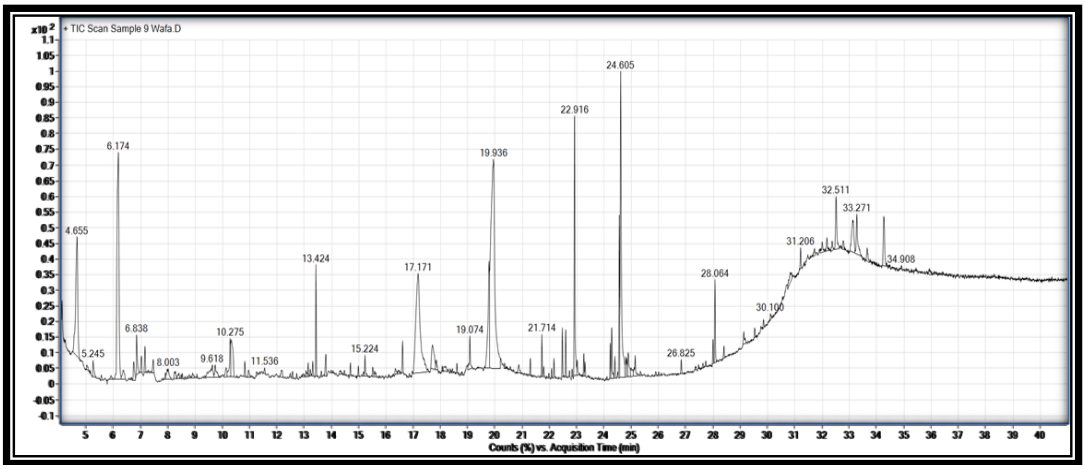


Figure 2. Chemical Compounds in *P. granatum* mixing of Mesocarp and Peels in GC-MS chromatography

Effect of pomegranate mixing of peels and Mesocarp extracts and fungicide on fungal growth inhibition

Fusarium, Aspergallus and altalnaria, is the most common fungi infected their wheat *T. aestivum* in Iraq, causing major yield losses in the grain yield over different plants areas . Because the toxicity and limited manufacture fungicides of the commonly used antifungal there is a need for the isolation of new naturally antifungal agents especially from plant extracts. (Helmerhorst *et al*, 1999). In this study we evaluated the antifungal activity of pomegranate mixing of peels Mesocarp extracts used ethanol solvent by Soxhlet method and synthetic fungicide on *in vitro* fungal growth inhibition percentage.

Results in this work Table (3) and figure (3) reported that most of the growth inhibition percentage increased significant (P<5%) with increasing crude extract concentration application. The ethanol extracts at 30% crude extract exhibited a 98% growth inhibition percentage of the *Aspergillus* , *Fusarium* and *Alternaria* fungus (Figure 3 D) and significantly difference (P<5%) with (control treatment) fungicide at 30% concentration recorded about (15,10, 5 %) respectively and also significantly difference (P<5%) with control treatment(Figure 3A). Also, Plant Crude extracts at 20% concentration recorded about (90%), (90%) and (80%) of all fungi study respectively (Figure 3C) and significantly difference (P<5%) with fungicide at 30% concentration of all fungi study . Also, Plant Crude extracts at 10% concentration treatments recorded lowest growth inhibition percentage about

(80%) , (80%) and(67%) of all fungi study respectively (Figure 3 B) and significantly difference (P<5%) with fungicide at 30% concentration of all fungi study. Meanwhile, Result in the same tableand figure reported that all treatments in the synthetic fungicides (30%) recorded lowest growth inhibition percentage compare with other treatment (Figure 3 A) . Therefore, a comparative study has been carried out to identify the effect of natural fungicide of pomegranate extracts on the growth inhibition of *fusarium , altalnaria and Aspergallus* fungi were show reduced urediniospore germination of all fungi study at a different concentration of plant extract . Data in Table (3) indicates higher inhibitory percentage at (30%) concentration of plant extract with ethanolic solvents by soxhlet methods prepared from pomegranate mixing peels and Mesocarp extracts .10% crude extract exhibited lower values than other plant extracts, respectively . In addition, data in the current study reported that plant extracts possessed strong inhibitory fungi growth percentage was found much stronger than the synthetic fungicide at 30% concentration . Moreover ,Results revealed that the application of pomegranate mixing peels and Mesocarp extracts of all the wheat fungi showed high efficacy for reducing disease severity comparing with the control treatment . Several phytochemicals (glycosides) have been isolated from pomegranate peels and mesocarp were identified to be cytotoxicity for fungi growth treatment.

Table 3
The percentage of fungal growth inhibition of the extract pomegranate mixing peels and Mesucarp by Soxhlet methods

Extracts	concentrations %	*Aspergillus*	*Fusarium*	*Alternaria*
		Inhibition %		
Fungicides	30	15	10	5
Ethanol	10	80	80	67
	20	90	90	80
	30	98	98	90
L.S.D.(0.05)	0.963	1.1 8		

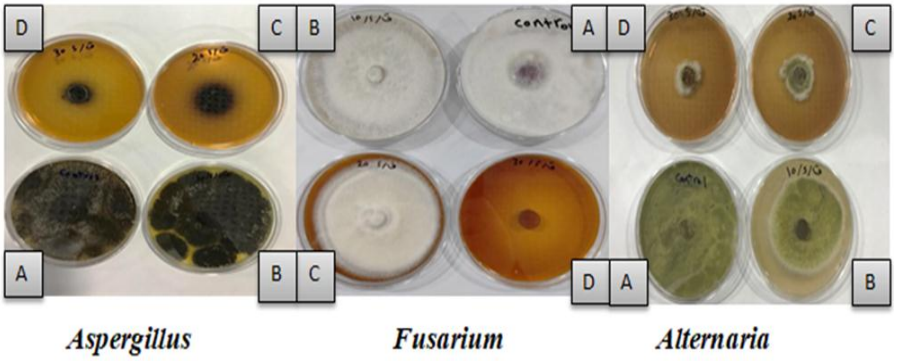


Figure 3. The percentage of fungal growth inhibition of the extract pomegranate peels by Maceration **(A)** control (**B**) con.(10%) **(C)** con.(20%) (D) con. (30%) .

For example, glycosides which has been reported being a multiple bioactive was displayed as an anti- fungal activities .One of the suggested mechanism that possibly against fungal growth by flavonoids and its glycosides was by inhibiting cell cycle . Based on the above-mentioned results, extracts of pomegranate mixing peels and Mesocurp were may be selected for field experiment to study their ability to against wheat fungal growth. (Zaker and Mosallanejad ,2010) reported that some Phytochemicals extracted from Eucalyptus and lavender extracts Controlled plant pathogen *Alternaria alternate.* Study by (Avasthi *et al.* ,2010) investigated that natural product from black pepper, garlic and onion extracts Controlled plant pathogen *Aspergillus niger* Work by(Cowan, 1999) notes the Mechanisms of action of phytochemicals Alkaloids compounds Intercalate into cell wall ,Tannins Bind to proteins, enzyme inhibition, substrate deprivation , Flavonoids Bind to adhesins, complex with cell wall, Inactivate enzymes, Coumarins Interaction with eucaryotic DNA Lectins Form disulfide bridges , Simple Phenols Membrane disruption, substrate deprivation ,Phenolic acids Bind to adhesins, complex with cell wall, inactivate enzymes Terpenoids Membrane disruption Essential oils Membrane disruption Alkaloids Intercalate into cell wall.

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