



TESTING THE EFFICIENCY OF POMEGRANATE ALKALOID (*PUNICA GRANATUM*) AND SODIUM BICARBONATE AND THEIR INTERACTION IN INHIBITION THE GROWTH OF THE FUNGUS *ASPERGILLUS OCHRACEUS* ISOLATED FROM *ZEAMAYS*

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Abstract

The study included Isolation of alkaloids from pomegranate peel and testing of their activity against of *Aspergillus ochraceuse* isolated from *Zea mays* and testing activity of sodium bicarbonate and interaction effect with. alkaloid against this fungus. the results showed that alcoholic extract of the pomegranate peel have several active substance and this what confirmed by primary test where they contained : alkaloid, glycosides, tannins, phenols, soaps, resins and flavons the value of pH (2.7). The effectiveness of. alkaloid .was also. shown to inhibit radiative. Growth of *Aspergillus ochraeuse* it reached the highest percentage was inhibition at concentration (15mg/ml) (90%), while the highest rate. of inhibition. of sodium. Bicarbonate. Treatment at concentration (15mg/ml) (85.55) while the interaction treatment between alkaloid and sodium Bicarbonate reached (82.22).

Key words : *Zea mays*, pomegranate alkaloid, sodium carbonate, fungus.

Introduction

Pomegranate is one of the Oldest known fruit species mentioned in the holy Quran, where it is used in the treatment of abdominal dieases and diarrhea and contains. Effective substances, including alkaloid and the most important Isopelletierine 'Granatin' Gallotannin. and Punicine as well as the extract of pomegranate. Extract. has a wide effect on microorganisms, whether bacteria, mold, or yeast, it can be used in the production of therapeutic material as found (Gulam, 2014) effect of extract watery of pomegranate peel on broiler. Infected with *Escherichia coli* and can be used in the treatment. of plant. diseases caused by fungi and the pomegranate has anumber of interaction and activities biologic such as anti-tumor has been isolated some of the antioxidants, which found the effect of the biologically oriented (Cerdea *et al.*, 2003) and because of the fact *Aspergillus ochraeuse* is one of the fungus that causes the damage food and feed in addition to its production of toxin ochratoxine A which is responsible for kidney cancer in humans, so we have seen this study amid at :

1. Isolates and diagnosis of fungus *Aspergillus ochraeuse* of *Zea mays*.
2. Diagnosis of *Aspergillus ochraceuse* using PCR technique
2. Isolates Alkaloid Extracted from pomegranate Peel and tested for their effectiveness in inhibition of fungi *Aspergillus. ochraeuse*.
3. Effectively tested Sodium Bicarbonate salt and its inhibition of *Aspergillus ochraeuse*.

Materials and Methods

Isolation of *Aspergillus ochraceuse*

10 samples of *Zea mays* used in the manufacture. from corn different locations from the local markets were placed in a plate containing sodium hypochlorite 2% for 3 minutes. And then washed with distilled water sterilized. Three times and then transferred to the plate on waste paper for drying in the middle of the PDA and by five grains then incubated plate at a temperature of 25 for aperiod of one week and after the end of the incubation

period has been purified isolation by transferring a disc diameter of 5 mm from each colony and transplant again to obtain pure isolates of the fungus and then diagnosed depending on the Morphological features of colony and diagnostic characteristics and confirm diagnosis using the technique PCR.

Collection of sample

Pomegranate. fruits were collected from the local markets and cleaned with water the peel were then separated from the rest of the fruit and dried at room temperature after drying the peel, grind with an electric grinder and then dry pomegranate powder in a sealed glass container, until use.

Preparation of alcoholic extracts of pomegranate peel powder

Attended the alcoholic extracts of pomegranate peel powder for the purpose of conducting chemical statements based on Harborne (1984).

Preliminary chemical detection of some active substances in the experimenter plant

Has been detected Flavonoides according to method of Jaffer *et al.* (1983) and detected Tannins according to method of Jawad (1997) and detected Resins according to Shihata (1951) and detected. Saponins according to method of Harbone (1991) and detected Terpenes, according to method of Edeoga *et al.* (2005) and detected alkaloids according to Dragendroffs Reagent (Harborne, 1984) and detected glycosides according to method of Al-Kazraji (1991).

Extraction of alkaloids

The alkaloids. were extracted. according to method (Ikan, 1969) to separate vegetable. alkaloids.

Diagnosis of *Aspergillus ochraceus* using PCR technique

1. Extracting and Multiplication The DNA

Extract the DNA from the examined fungus by the use of the kit (EZ-10 Spin column .fungal Genomic. DNA mini- Preps Kit), mixture. reaction of PCR has been prepared by the use of (Accupower PCR PreMIX) that is supplied by the Korean company of Bionee.

2- Amplification DNA

PCR Step	Repeat cycle	Temperature	Time
Initial denaturation	1	95C	5min
Denaturation	30	95C	5sec.
Annealing		58C	30sec
Extension		72C	1 min
Final extension	1	72C	5min
Hold	-	4C	Forever

3- Gel electrophorsis

By method of Sambrook and Russell (2001).

Test the efficacy of pomegranate alkaloids in inhibiting the radative growth of *A. ochraceus*

Attended three concentrates of alkaloids(5, 10, 15) mg/ml in the PDA with addition of the antibiotic 250mg/ l and then pouring PDA in the plate.the controlling treatment included PDA only after hardening of the medium, the plate were spatially with 5 mm from the edge of the fungus colony *A. ochraceuse* at 7 days placed on the center of the plate and incubated at 25°C for 7 days the percentage of inhibition was calculated by equation (Aboot, 1925).

Test the efficacy of sodium bicarbonate in inhibiting the radative growth of *A.ochraceus*

To determine the efficacy of tested sodium bicarbonate followed method of Dixit *et al.* (1976) the method of toxic food technique.

Effect of interaction between alkaloid and sodium bicarbonate in inhibition the radative growth of *A.ochraceus*

To determine the efectiveness the interaction between alkaloids and sodium bicarbonate in inhibition radative growth of *A.ochraceuse* ,the concentration of 15 mg/ml of both alkaloid and salt was selected. interaction was performed by mixing 7.5mg/ml alkaloids and 7.5 mg /ml salt.

Statistical analysis

Results were subjected to Statistical analysis to determine the significant differences at the 5% including the analysis of binary variation (Anova) two way analysis of variance and the reselts were analyzed statistically by the program spss in the computer.

Results and Discussion

Preliminary chemical detection of some active substances in the experimenter plant

The reselt of table 1 showed the chemical. detection of active in the pomegranate extract containing alkaloids, tannins. Flavonoides, Saponins, Glycosides and resins most medical herbs are effective in the active substances, which benefited most pharmacists. Pomegranate peels are an ideal stock for most alkaloids and tannins for containing theses substances and other amino and organic. acid.

Dignosis of fungus *A. ochraceuse* in the form of polymer chain reaction (PCR)

The resulted is showed that the primer 18SrRNA

Table 1 : Represents the primer that is used in this study with the nucleotide sequence and the output of the test PCR.

Amplicon	Sequence		Primer
423bp	TCCCACCCGTGTATACCGTA	F	<i>A. ochraceus</i>
	CCTACAAGAGCGGGTGACAA	R	

Table 1 : The presence of active ingredients in pomegranate extract.

Alkaloids	+	Resins	+
Glycosides	-	Saponins	+
Flavonoid	+	Tannins	+

Table 2 : Efficacy of pomegranate alkaloids in inhibiting the radative growth of *A. ochraceus*.

Concentration	Average colony diameter	Inhibition ratio
0	90	0
5	22	75.55
10	15	83.33
15	9	90
LSD 0.05	3.64	

Table 3 : Efficacy of sodium bicarbonate in inhibiting the radative growth of *A. ochraceus*.

Concentration	Average colony diameter	Inhibition ratio
0	90	0
5	21	76.66
10	17	81.11
15	13	85.55
L.S.D 0.05	4.68	

Table 4 : Effect of interaction between alkaloid and sodium bicarbonate in inhibition the radative growth of *A. ochraceus*.

Treatment	concentration	Average. colony. diameter	Inhibition ratio
Control	-	90	-
Alkaloid+sodium bicarbonate	Alkaloid 7.5mg/ml + sodium bicarbonate 7.5mg/ml	16	82.22
Alkaloid	15mg/ml	9	90
Sodium bicarbonat	15mg/ml/7	13	85.55
L.S.D 0.05	5.28		

used in the materials and the working methods showed success, in the process of amplification of the DNA of the fungus *A. ochraceus*, as the package showed abaise pair and this indicates that the isolates under study belong to the fungus *A. ochraceus* confirmed Aref *et al.* (2012) the possibility of using molecular. diagnostics using PCR technology to support diagnosis based on Morphological.

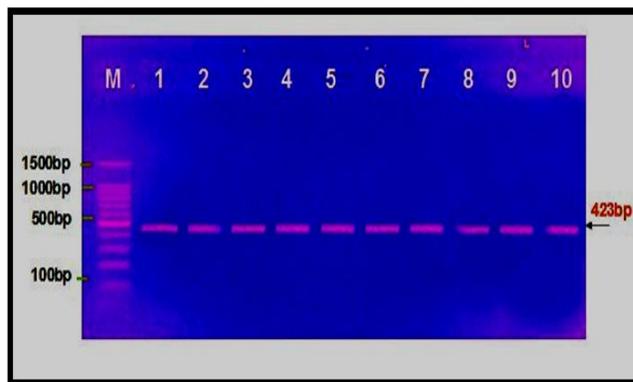


Fig. 1 : Electrophoresis the. use of agarose gel that shows the results of the test of PCR that is concerning the gene 18SrRNA of fungus. *A. ochraceus*, where (1500-100bp) M:Marker ,the. wholes. from(1-10)are the positive isolates for the test with production of 423bp.

features of colony.

Test the efficacy of pomegranate alkaloids in inhibiting the radative growth of *A.ochraceus*

The reselts in table 2 showed the effect of pomegranate alkaloids in inhibiting the radative growth of the fungus. *A. ochraceus* and at different rates according to the concentration of alkaloids the highest percentage of inhibition of the fungus was observed at 15 mg/ml (90%) and the lowest inhibition ratio at 5mg / ml concentration. These findings are consistent with Hamid *et al.* (2009) and the reason. is that to the effect. of active pomegranate peel in the fungal cell and its interaction with cellular structures and metabolic processes (Mishra and Vinit, 2012), where it leads to changes in the exact structure of the fungal cell and these changes are related to the loss of cell wall strength

responsible for the strength integrity and shape of the cell (Gandomi *et al.*, 2009), also the cell wall is one of the target sites for the work of active substances and changes arise from the direct interference between active substances and cellular wall components or with the construction of the wall (Otang *et al.*, 2011), also these results are consistent with the results obtained by Hassan

(2011) that alkaloids obtained from the leaves of the datura and rhizome seeds had a disincentive effect in the growth of a number of plant pathogenic fungi and inhibition rates ranged between (0-96%).

Test the efficacy of sodium bicarbonate in inhibiting the radative growth of *A. ochraceus*

The results in table 3 effectiveness of sodium bicarbonate salts the growth radius of the tested fungus was 21mm at 5mg/ml concentration and inhibition 76.66% and 17mm at 10 mg/ml concentration and inhibition ratio 81.11% and 13mm at the concentration of 15 mg/ml and inhibition ratio 85.55% , these results were consistent with Alsaïdi (2013) finding that sodium bicarbonate inhibited the fungus *A. niger*, the inhibitory effect of sodium bicarbonate is that it is base salts it effect its pH values, which negatively affects the fungal growth (Al-dulaimi, 1978).

Effect of interaction between alkaloid and sodium bicarbonate in inhibition the radative growth of *A. ochraceus*

The results in table 4 effectiveness of sodium bicarbonate effected of the fungus *A. ochraceuse* and inhibition ratio 88,88 where it was lower than individual treatments and this may be to the effect of these two active substances on each other or the formation of a compound with low inhibitory activity for growth of microorganism.

Conclusion

From this research results, that extract of *Punica granatum* have very important antimicrobial activities and will be possible to use of pomegranate peel extracts to, formulate new products, to be used, in food industry as natural antioxidant.

References

Afrah, A. W. Gulam (2014). The effect watery extracts of pomegranate peel (*Punica granatum*) with different antifungal Substance of flower (*Rose indica*). *Economic Botany*, **30** : 71- 73.

- Arif, M., S. Chawla, N. W. Zaidi, J. K. Raya, M. Variar and U. S. Singh (2012). Development of specific primers for genus *Fusarium* and *solani* using Rdna Sub-unit and transcription elongation factor (TEF-la) gene. *African Journal of Biotechnology*, **11(2)** : 444-447.
- Barnett, H. L. and B. B. Hunter (1972). Illustrated genera of imperfect fungi. 3rd ed . Burgess Publishing Company, U.S.A, pp241 .1972.
- Gandomi, H., A. Misaghi, A. A. Basti, H. Hamed and Z. R. Shirrani (2009). Effect of *Zataria multiflora* Boiss. Essential oil on growth and aflatoxin formation by *Aspergillus flavus* in culture media and cheese. *Foodchem. Toxicol.*, **47** : 2397-2400.
- Harbone, J. B. (1982). *Introduction to ecological biochemistry*. Academic press. london 4th Ed . Pp:278 .
- Harborne, J . B. (1984). *Phytochemical methods*. A guide to modern techniques of plants analysis London, New York, chapman & Hall. 2nd ed.
- Harborne, J . B. (1984). *Phytochemical methods*. A . guide to modern techniques of plants analysis London . New York, Chapman & Hall, 2nd ed.
- Ikan, R. (1969). *Natural products alabrotory guide*. Academic press London & Newyork, 3135.
- Jaffer, H. J., M. J. Mahmood, A. M. Jawad and A. Al-Naib (1983). Phytochemical and Biological screen of some plants. *Fitoterapi*, **LIX** : 229.
- Mishra, A. K. and K. M. Vinit (2012). Field survy for some fungal diseases on eggplant. *International Multidisciplinary Research Journal*, **2(9)** : 23.
- Otang, W. M., D. S. Grierson and R. N. Ndip (2011). The effect of the acetone extract of *Arctotis arctotoides* (Asteraceae) on the growth and ultra structure of some opportunistic fungi associated with HIV/AIDS. *Int. J. Mol. Sci.*, **12** : 92269235.
- Sambrook , J . and D. W. Russell (2001). *Molecular cloning*. A laboratory manual 3th ed cold spring Harbor (NY): cold spring Harbor Laboratory Press , N.Y.