



IRAQI
Academic Scientific Journals



العراقية
المجلات الاكاديمية العلمية

TJAS

Tikrit Journal for
Agricultural
Sciences

ISSN:1813-1646

Tikrit Journal for Agricultural Sciences

Journal Homepage: <http://tujas.tu.edu.iq>

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KEY WORDS:

orange husk extract, *Eruca Sativa* seeds extracts .
Antibacterial efficacy

ARTICLE HISTORY:

Received: 29/05/2018

Accepted: 30/09/2018

Available online: 01/04/2019

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Use of Orange Husk and Watercress (*Eruca sativa*) Seed Extracts as Antimicrobial Natural Preservative for Fruit Juices

ABSTRACT

The natural preservative for foods and juices are become mandatory required instead of artificial or chemical preservatives. That the main goal of our present study. The study included making water and alcoholic extractions of orange husk and *Eruca Sativa* seeds. The inactivation's efficacy of these extracts of 150 , 250 and 400 mg/ml concentrations against some pathogenic bacteria such as *Escherichia coli*, *Staphylococcus aureus*, and *Streptococcus pyogenes* were done by the disc assay method and compared the results of inactivation with Azithromycin antibiotics. Also, the bioactive compounds of these extracts were determined. Orange and pomegranate juice samples which treated with the aquatic extract 400 mg / ml of orange husk and extract of *Eruca Sativa* seeds after 24 hr storage time were sensory evaluated by specialists and teachers to special sensory factors. The results were shown that the aquatic and alcoholic extracts of *Eruca Sativa* seeds have the following active compounds, resin, tannins, comarins, alkaloids, flavons, phenols, glycosides and saponins. However, the orange husk extracts have the same compounds except Comorians, and glycosides were absent. The results of antibacterial efficacy of the aquatic extract of the *Eruca Sativa* seeds of 400 mg/ml shown superior significant bacterial inactivation compared to the orange husk extracts and to the Azithromycin antibiotics with 0.05 probability . The results of the sensory evaluation showed that the orange and pomegranate juice samples which were treated with 400mg/ml water extract of orange husk gave higher scores than the treatment of *Eruca Sativa* seeds extracts .

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INTRODUCTION

The consumption of ready-made fruit juices has increased significantly over the past years, especially in the summer, due to increased demand for low-calorie food products with fresh-like characteristics in addition, there is scientific evidence that the consumption of fresh fruits, vegetables or their juices helps to prevent many diseases such as cardiovascular problems, cancer and diabetes (Jasmine, 2012).

Natural fruit juices are high nutritional value and are important for the health of the human body as they maintain nutritional balance and fight many diseases moreover, the nutrition experts gather these juices contain many vitamins such as vitamin B, C and minerals such as potassium, sodium and iron, enzymes and natural antioxidants, fiber and organic acids, as well as that the colors have health benefits of the heart and skin and the resistance of different types of cancers (Hyson, 2015).

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Pathogenic bacteria usually cause corruption and disease problems in fruit juices and beverages, There have been several cases of food-borne diseases in recent years attributable to the consumption of contaminated commercial juices and beverages (Ashurst, 2016). To address the problems of spoilage and disease transmission, it has been customary use some chemicals to prolong the storage life of fruit juices and saved economic properties such as benzoic, sorbic, and propionic acid recent research has shown that these preservatives may become carcinogenic factors in addition to the ability of some microorganisms to adapt by producing substances that over time can inhibit or impede the work of chemical additives used to conserve food (Mohammed *et al.*, 2015)

Watercress *Eruca Sativa* Herbic plant is belong to the Crusader family. It is cultivated throughout the year except for the cold and very hot months (Al-Janabi, 2004). The seeds are usually yellow but sometimes yellow or spotted with greenish brown spots and be greenish (Koocheki *et al.*, 2012), The seeds of watercress are used for therapeutic purposes. The ancient doctors mentioned in their writings the therapeutic uses of the watercress plant with its leaves and seeds because of its health benefits it was mentioned by Ibn Sina that eating the leaves and mature seeds and drink juice leaves of *Eruca Sativa* gives general activities and it is useful to the human body functions in general also, it is found that watercress leaves and its seeds diuretic and helps digest food and is used for the disintegration of kidney stones and blood purification and speed of rotation (Salem, 1986)

Orange fruits, belongs to the Rutaceae family, a medium-length, evergreen, round-topped tree with thin, fluffy strands, aromatic white flowers, spherical fruit, sweet pulp, It is home to Southeast Asia, Europe, America and most of the world. The husk of the orange fruit act as natural medicine for absorbing gases from stomach and intestines, as well as natural preservative, and anti-cold during cold months. (Chevali, 2010)

Recent research indicates that fruit husk and vegetable leaves and seeds contains natural inhibitors are considered as a container for many anti-microbial compounds on the behavior for the growth of these fruits and vegetables against plant diseases. (Abass *et al.*, 2014). Therefore, increased interest in the use of natural materials to save spoilage of juices and to ensure health benefits and maintain the quality of the juice properties. As well as, the increasing demand for safe and healthy drinks and foods that do not contain chemical preservatives. So that our present research will study antibacterial efficacy of some fruit husk and leaves and seeds of some vegetables.

MATERIALS AND METHODS

Isolation of bacteria from fruit juice :

The contaminated juice samples were collected in clean, sterile laboratory bottles and the necessary blending and transplantation was performed on the following cultivars: Manitol salt Agar, blood agar, MacConkey agar after bacterial growth, microscopic and biochemical tests were performed to diagnose isolated bacteria (Quinn *et al.*, 1994), Where they were isolated from each bacterium *Escherichia coli*, *Staphylococcus aureus*, and *Streptococcus pyogenes*.

the use of antibiotic:

Azithromycin (15 µg) was used in this study to compare inhibitory activity with samples extract.

Preparation of plant extracts:

Watercress seeds and orange husks were collected from local markets of Tikrit and machete-dried and then milled electrical Palmtahn, and packed in plastic containers and stored in the refrigerator until use.

Water extract:

Prepare the water extract according to the method (Harborne, 1973) and melted 20 g of plant powder in 150 ml of distilled water, Mix for one hour by the magnetic stirrer and leave for 24 hours and then spray the extract using the diluted pressure and by the filter paper Type Watman 0.1, Then concentrate the extract with rotary evaporator to obtain the extract Center, followed by the drying process in the oven at 40 °C for dry powder.

Alcoholic extract:

alcoholic extracts attended by method (Nagarajappa *et al.*, 2015) and that 10 g of dissolving vegetable powder in 100 ml of ethyl alcohol, Mix for one hour by the magnetic engine and leave for

24 hours and then nominated the extract using the pressure of the diluted and by the filter paper type Watman then concentrate the extract with rotary evaporator to obtain the center extract, followed by the drying process in the oven 40 °C after placing the concentrated extract in large dishes until dried extract for dry powder.

Detection of bioactive compounds in plant extracts:

Resins, saponnes, tannins and glycosides were detected using the method mentioned in Shihata (1951), and coumarin, flavonate and phenol alkaloids by method (Antherden , 1969).

Sensitivity testing:

Following the method of Disk Diffusion Method (Harley and Prescott, 2002), a colony was taken by a cotton swab and plotted and planted on the center of Mueller Hinton agar, where all sides were plotted so that the quantities were distributed evenly and then left to dry for 30 minutes. The antibiotic tablets were then placed by sterile forceps on the surface of the implanted pods. The same method was used for transplantation of plant extracts, but agar Diffusion Method was used by drilling wells to test the sensitivity of bacteria to plant extracts, as in (tepe *et al.*, 2004), The method consisted of the work of (4) drilling on the center of Mollar Hinton Agar with equal dimensions diameter (5) mm , Using piercing cork so that Cork Borer containing the extract solution by (100) Maekerolter / hole. The dishes were incubated at 37 ° C for 24 hours. The diameter of the inhibition area around the antibody tablets and the millimeter was measured by the normal ruler.

Test the inhibitory efficacy of plant extracts on yeast and bacteria in juice:

Making Orange Juice according to Harrigan and McCance (1976) After obtaining fresh and pure juice, the juice was distributed on 100 ml glass bottles with 50 ml / flask and blocked with cotton caps, a pasteurization process was carried out at 63 ° C for 30 minutes, and then the bottles were refrigerated and the juice was tested for infection with fungus, yeast and bacteria as a result of the growth of the potato-dextrose (PDA) and Nutrient agar, the extracts were added (0.15, 0.25, 0.4) % to the flasks and the bacterial larvae were fertilized by 0.1 µl The bottles were then stored at 38 ° C for 24 hours, and the total numbers of the bacteria were calculated in a haphazard manner.

RESULTS AND DISCUSSION

The seeds of watercress gave a clear inhibitory effect to the bacteria used in this study. The diameters ranged between (4 - 25) mm for the water extract and (7 - 19.5)mm for the alcoholic extract as shown in Table (1). This result is better than the researcher reached (Rizwana *et al.*, 2016) as it found that the highest rate of diameters inhibitory extract alcohol raw seeds 14.33 mm, and the water extract showed a higher inhibitory effect than the alcohol extract of *S. aureus* and *Str. Pyogenes* where the diameters were 25 and 20.5 mm, respectively, this is accepted with Rizwana *et al.* (2016) who indicated that all alcohol extracts used showed a weak inhibition of *Str. Pyogenes*, While the alcohol extract had a high impact on the *E. coli* and reached 17 mm.

Table (1) The inhibitory effect of watercress seeds extract on some bacterial isolates

Bacteria	Type of extract	The average of the inhibition diameters (mm) of the concentration series used (mg / ml)		
		150	250	400
<i>streptocooccus pyogenes</i>	Water extract	0	10	25
	Alcohol extract	7	14	16
<i>Staphylococcus aureus</i>	Water extract	12	12	20.5
	Alcohol extract	11	15	19.5
<i>Escherichia coli</i>	Water extract	4	7	10
	Alcohol extract	7	10	17

The results of this study of inhibitory activity towards the microorganisms used indicate that the Gram positive bacteria were more affected by the Gram negative bacteria, This is accepted with Rizwana *et al.* (2016) who found that the Gram-positive bacteria were more sensitive than the Gram-negative bacteria, this response by the Gram-positive bacteria is due to peptidoglycan, which has high permeability, While lipopolysaccharide membrane in the Gram-negative bacteria acts as an effective permeability barrier that prevents the introduction of the plant extract into the microbial cell.

Table (2) shows that the diameters of the orange husk extract, the alcohol extract has a higher inhibitory effect than the water extract for each bacteria, the inhibition of bacteria *Str. Pyogenes*, *S. aureus* and *E. coli* (15, 19 and 16.5), respectively, the concentration of 150 mg / ml of the water extract had no inhibitory effect on both *Str. Pyogenes* and *E. coli*. The results of this study showed that the higher concentration of the extract. Is greater the inhibitory effect against the isolated bacteria, where the highest inhibitory rate at 400 concentration of water extract at 10 mm for *Str. pyogenes* and 19 mm for the alcohol extract of bacteria *S. aureus*.

These results agree with (Hameed *et al* ,2010) they reported that the higher the concentration of the extract, the greater the diameter of the inhibition area against the bacteria used by *S. aureus* , *B. subtilis* and *Sal. typhimurium* and *E. coli*. This effect was evident against the Gram positive bacteria more than negative bacteria, it was also agreed with Shetty *et al.* (2016) who noted that the water extracts of orange husk were effective in very high concentrations against the tooth decay bacteria (*Str. mutans* and *Lactobacillus acidophilus*).

Table (2) The inhibitory effect of orange husk extract on some bacterial isolates

Bacteria	Type of extract	The average of the inhibition diameters (mm) of the concentration series used (mg / ml)		
		150	250	400
<i>streptococcus pyogenes</i>	Water extract	0	4	10
	Alcohol extract	2	11	15
<i>Staphylococcus aureus</i>	Water extract	5	7	9
	Alcohol extract	9.5	14.5	19
<i>Escherichia coli</i>	Water extract	0	5.5	7.5
	Alcohol extract	6	11.5	16.5

Table 3 show the rates of Azithromycin inhibition of bacterial growth where *S. aureus* and *Str. pyogenes* higher sensitivity at 18 and 16 mm inhibition, respectively, while *E. coli* has not been affected by antibiotic. These results are accepted with the global standard tables (NCCLS, 2002).

Table (3) Effect of Azithromycin inhibition on bacteria

Bacteria	Average diameters are measured (mm) for the antibiotic AZM
<i>Staphylococcus aureus</i>	18
<i>Escherichia coli</i>	0
<i>Streptococcus pyogenes</i>	16

These results of the effective groups of plants used in this study showed that the watercress seeds contained all the active substances, this result was agreed with (Mohammed and Abdullah, 2013) they found the seeds of watercress contain all the active substances, while the orange husk is free of the Saponins, the glycosides and the comarines. This result is accepted with (Al-Saadi *et al.*, 2009 : Al-Tikriti, 2017 : Shetty *et al.*, 2016).

Also the inhibitory effect of the extracts used in this study bacteria was designed to demonstrate the effect of the extracts with a concentration of 150, 250 and 400 mg / ml to reduce contamination of

bacterial load in sterilized juice contaminated with a number of *S. aureus*, *E. coli* and *Str. Pyogenes*, and this experiment is based on the exposure time to extract a statement on the development of the preparation of bacteria with increased exposure time for 24-hour effect. Table (5 , 6) show a decrease the number of bacteria remaining in the juice after 24 hours of storage significantly, and the increase in concentration leads to a reduction in the number of bacteria remaining in the juice, and it reached the preparation of the bacteria at the treatment of seeds Watercress water and alcohol and orange husks alcohol concentration of 400 mg / ml was $0 * 310$ unit colony formation / ml of juice for both *S. aureus* and *Str. Pyogenes*, the main reason for the ability of extracts to inhibit bacteria because they contain a wide range of secondary metabolic compounds, Flavonoids for example, due to the effectiveness of bacteria viability in the formation of a complex compound with soluble cell proteins and overlap with the cell wall of the cell bacteria, and alkaloids working on interfering with the DNA cell, while tannins and phenolic compounds act to inhibit the action of enzymes and transport proteins (AL-bayati and Almola, 2008).

Table (4) Detection of active aggregates in watercress seeds and orange husk

Active compounds	User Detector	Result detection	Seeds of watercress	Orange husk
Tannins	Ferric chloride	Greenish blue colo	+	+
Resins	Hydrochloric Acid 4%	Be reversible	+	+
Saponins	Mercury chloride	White precipitate	+	-
Flavonoids	Ammonia solution	yellow color	+	+
Phenols	Ferric chloride	blue color	+	+
Alkaloids	Drakendov	Orange color	+	+
Glycosides	Benedict	Red residue	+	-
Coumarin	sodium hydroxide	Yellow – green	+	-

+ Positive result of detection - negative result of detection

Table (5) Effect of different concentrations of water and alcohol extracts of watercress seeds on the preparation of bacteria (CFU) in juice

Bacteria	Concentrations the control	150	250	400	
		CFU * 10 ³	CFU * 10 ³	CFU * 10 ³	
<i>streptococcus pyogenes</i>	Water extract	400	25	6	0
	Alcohol extract		42	9	0
<i>Staphylococcus aureus</i>	Water extract	256	76	43	0
	Alcohol extract		64	38	0
<i>Escherichia coli</i>	Water extract	205	141	80	38
	Alcohol extract		106	44	11

Table (6) Effect of different concentrations of water and alcohol extracts of orange husk on the preparation of bacteria (CFU) in juice

Bacteria	Concentrations	the control	150	250	400
			CFU * 10 ³	CFU * 10 ³	CFU * 10 ³
<i>streptococcus pyogenes</i>	Water extract	400	356	165	77
	Alcohol extract		18	0	0
<i>Staphylococcus aureus</i>	Water extract	256	229	83	35
	Alcohol extract		102	31	0
<i>Escherichia coli</i>	Water extract	205	178	121	90
	Alcohol extract		108	67	17

Sensory evaluation of juices:

Table (7) shows the results of the sensory evaluation of the orange and pomegranate juice treatments, with the addition of orange husk and waercresst seeds with concentration of 400 mg / ml. The results show the extent to which consumers accept the color, taste, aroma and general acceptance parameters of the treatments and in conjunction with the decrease in microbial load to approximately 100% under the concentration of 400 mg / ml in the juices through the inhibition experiment.

Table (7): Sensory evaluation of the types of orange juice and pomegranate supplemented with water extracts (400 mg/ml)

Sensory qualities	pomegranate juice				Orange juice			
	color	Taste	Smell	Public acceptance	color	Taste	Smell	Public acceptance
Treatment	10 degrees				10 degrees			
watercress seeds	9	7	6.5	7.5	8	6	6.5	6.8
orange husk	10	8	9	9	10	9	9.5	9.5

We observed the superiority of the sensory evaluation of orange juice and pomegranate samples treated with orange husk compared to the juice samples treated with watercress seeds. The treatment with orange husk extract obtained the highest rating for the general acceptance rate between 9 and 9.5 for pomegranate and orange juice, respectively. The result of the sensory evaluation of this orange husk extract was not parallel to the microbial inhibition rates of this extract.

The sensory evaluation of the watercress extract was low from 6.8 to 7.5 (Table 7) and is not parallel to the microbial inhibition rates (Table 5).

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استعمال مستخلصات قشور ثمرة البرتقال وبذور نبات الجرجير (*Eruca Sativa*) كمضادات ميكروبية طبيعية لحفظ عصائر الفاكهة

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المستخلص

أصبح الطلب على المواد الحافظة الطبيعية ضروريا بدلا من المواد الحافظة الكيماوية الصناعية وكان ذلك الهدف الأساسي لهذا البحث. شملت الدراسة تحضير مستخلصات مائية وكحولية لقشور البرتقال و بذور الجرجير بتركيز 150, 250 و 400 ملغم/مل ضد بعض أنواع البكتريا المرضية مثل بكتريا *Streptococcus pyogenes*, *Staphylococcus aureus*, *Escherichia coli* وقورنت نتائج التثبيط مع قابلية التثبيط بالمضاد الحيوي Azithromycin . كذلك قدرت المركبات الفعالة في هذه المستخلصات. كما اجري التقييم الحسي لعصير البرتقال والرمان المضاف لهما مستخلصات قشور البرتقال وبذور الجرجير بتركيز 400 mg/ml بعد فترة خزن 24 ساعة.

أظهرت النتائج بأن المستخلص المائي والكحولي لبذور الجرجير قد احتوى المركبات الفعالة التالية: الراتنجات، التانينات، كومارين، قلويدات، فلافونات، فينولات، كلايكوسيدات والصابونيات. بينما مستخلص قشور البرتقال احتوت على نفس المركبات ماعدا مركبي الكومارين و الكلايكوسيدات و . نتائج كفاءة التثبيط الميكروبي باستعمال المستخلص المائي لبذور الجرجير 400 ملغم /مل أظهرت تفوق معنوي مقارنة مع التثبيط باستعمال مستخلص قشور البرتقال والمضاد الحيوي Azithromycin (p < 0.05). نتائج التقييم الحسي أظهرت بأن عصير البرتقال والرمان المخلوط مع المستخلص المائي لقشور البرتقال بتركيز 400 ملغم/مل حصلت على درجات تحكيم متفوقة على نماذج العصير المخلوط مع مستخلص بذور الجرجير .

الكلمات المفتاحية: مستخلصات قشور البرتقال وبذور الجرجير، التثبيط البكتيري