Recovery of pure Hesperidin from Iraqi Sweet Oranges Peel and study the effect in some bacteria

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Abstract:

Citrus fruit contain variety of flavonoids such as Hesperidin (the principal flavonoid in oranges and grapefruit). Hesperidin is found in high concentration in fruit peel of oranges and in substantially lower concentration in juice of these fruits.

Hesperidin was extracted from oranges peel by treating the peels with calcium hydroxide. HPLC technique was used to determine hesperidin. Hesperidin was saperated and purified in a purity of about 90.1-95.7% and yield about 1.5 %w/w from oranges peel dry powder.

Both hesperidin and oranges peel extract showed significan antibacterial activity. Sensitivity to hesperidin and oranges peel extracts were not similar for the chosen bacteria Crude orange peel extract gave a various antimicrobial activity agents Gram-positive *Bacillus cereus, Staphylococcus aurous, Str*epto*coccus pyogenus* sp. and Gram-negative (*Escherichia coli, Salmonella typhi)* bacteria strains`. The minimum inhibitory concentration (MIC) values against these bacteria ranged from 45-175µg/disc.for crude orange peel extractand 175-450µg/disc for pure hesperidin In comparison to 30µg/disc reference standards ciproflaxacin and impinme.orange peel extract showed significant antimicrobial activity.

Key words: Hesperidin, orange peel, bacteria

Introduction:

Citrus is the second largest growing fruit in the world, The citrus production is estimated at 80 million tones per year making it an important source for useful to human health components. The main waste of the citrus fruits after processing is the citrus peel. In order to valorize these wastes recently numerous studies were published [1-6] These studys dedicated to a potential use of these peels as a source for natural antioxidants.[7,8]. These studys have several hydroxyls in different position of rings, where there is strong chemical activity. Hesperidin, a poly phenolic bioflavonoid, is the predominant flavonoid in orange peel and other citrus fruits Such component aantioxidant in various biological systems [9,10,11].

Epidemiological studies have suggested the beneficial effects of citrus fruits (rich in Hesperidin) against many degenerative diseases like cardiovascular diseases and some cancers [12].

It is also known to have pharma-cological action as an antiinflam-matory, antihistaminic, antiviral, anti-allergic agent and it can prevent pregnancy [13,14], enhances the action of vitamin C to lower cholesterol levels.[15], inhibit the invitro proliferation of cancer cell [16].

There are numerous reports of HPLC analysis of the composition of commercial juices ,and/ or peels

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concentrates, fresh oranges, and grapefruit [17, 18].

The amount of bioactive compounds in fruit, including citrus flavonoids, are a function of geographical region, climate, soil condition, type of cultivar, growing season, harvest date, storage, low-dose irradiation, and other conditions [12,13].

It is natural that authors who study the bioactive compound content and antioxidant potential of citrus and other fruit from different geographical regions have obtained different results [9, 12, 15, 16].

Materials and Methods:

Hesperidin, Didymin, naringin, calcium hydroxide and hydrochloric acid, were purchased from Fluka, Acetone, methanol, and anhydrous acetic acid pro analysis grade purity were provided by Merck. Gram-posi-tive (*Bacillus cereus, Staphylococcus aureus, Str*epto *coccus pyogenus* sp. and Gram-negative (*Escherichia coli, Salmonella typhi*) bacteria strains**,** were provided from Iraqi Central public hea- lth laboratory

SaperationandPurification

A100 g sample of dry powder of local orange peel was stirred with 200g of distilled water over a period of 90min, while increments of calcium hydroxide were added to maintain the pH at approximately 12.0 The alkaline peel slurry was filtered through cheesecloth and additional liquor recovered by pressing the remaining peel. After combining both liquids the solution was acidified by concentrated hydrochloric acid, the powder optioned was filttared by fine filter paper. Further purification was done by washing the powder with hot distilled water followed by 95% ethanol and finely with ether. The powder was dried in an oven the sample was identified by UV Spectral and analyzed by HPLC technique.

Identification by UV Spectral Analysis

The spectra analysis was accomplished by preparing the samples of orange peel hesperidin and the crude orang pe-el powder in methanol and measured in 1cm path quartz cells by Shimadzu UV 240 (P/N 204-58000) spectropho-meter.

Chromatographic Analysis

Fifty mg of hesperidin and the crud powder was accurately weighed in 25ml beaker, 10ml of dilute sodium hydroxide was added, the solution was heated to 50 °C with stirring until the whole powder was dissolved and the cooled solution was extracted with dimethyl sulfoxide (DMSO) three times, the final volume of DMSO was 25ml. The DMSO fraction were subjected to HPLC analysis for determination of hisperdin

In HPLC analysis, the mobile phase was methanol: deionised water :glacial acetic acid (10:1:14) at a flow rate of 1 ml/min, the column temperature was 25 °C and sample volume injected was 10 µL of 2µg/mL purified Hesperidin, crude hesperidin and Hesperidin standard, the optimum detecting wavelength for Hesperidin was 283 nm. Under above conditions Hesperidin gave a peak at 32.7 min.[19].

Determination of Minimum Inhibi-tory Concentration (MIC):

The MIC of the tested fractions was determined against some selected microorganisms gram-positive (*Bacill subtilis, Staphylococcus aureus, Streptococcus pyogenus* sp.) and gram-negative (*Escherichia coli, Salmonella typhi*). by agar diffusion technique. Serial dilutions of the crud peels extract and the purified hesperidin with different concentrations (50 to 800 µg) were placed in Sterile filter-paper discs (Whatman no. 1, 6 mm diameter).

Incubation was done for 24 h at 37°C. Growth was determined by visual examination. MIC was calculated by plotting log concentration against the diameter of inhibition zone the corresponding is the MIC. MIC was expressed as the lowest concentration of plant extract that produced a complete inhebitiors of colony growth[19].

Results

Hesperidin exists largely in citrus peels and is considered as the main active component. It is known that hesperidin is the most abundant glycosides flavanone in peel of orange fruits. Hesperidin purfied from orange peel was indentified by spectroscopy data. The UV. Spectrum of methanol showed maximum absorption at 283 nm. The profile of spectrum was the same us standard. No additional peaks were seen in HPLC spectroscopy for the purified sample and this result mains the purified sample of hesperidine is almost pure.fig 1

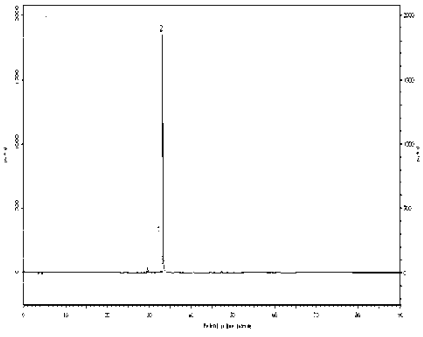


Fig.1: HPLC chromatography of hesperidin purfied from Iraqi oranges peel.

Hesperidin was recovered in a purity of about 90.1-95.7% and yield about 1.5 % w/w from dry orange peel powder.

HPLC chromatograms of orange peel crude extract obtained from the DMSO fraction show that many peaks are detected at 283 nm The retention times (RT) of naringin (32.76 min) hesperidin (33.2 min) and Didymin (35.6min) (Fig. 2).

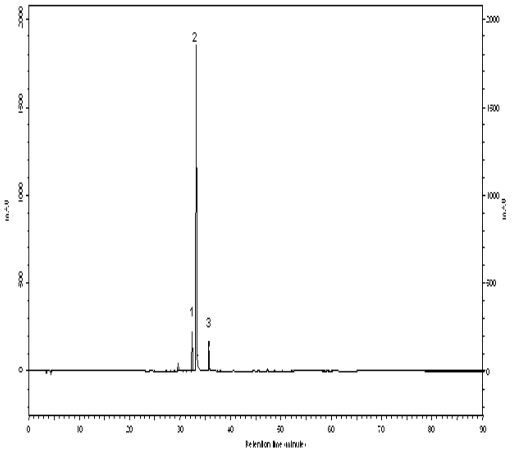
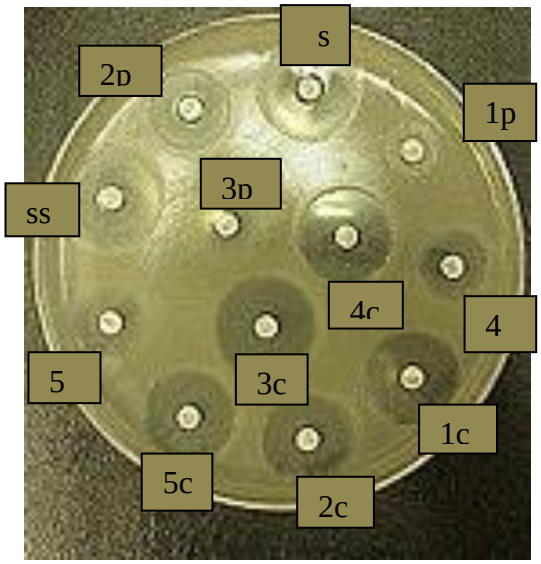


Fig.2: The HPLC chromatography flavanone glycosides:1-narirutin,2-hesperidin, 3- Didymin in Iraqi orange peel crude extract.

Antimicrobial activity of the crude peels extract and the purified hesperidin was expressed by the diameter of inhibition zone in micro organism.plate picture and table 1



Antibacterial activity (Plates) of hesperidin and orange pee extractl showes

the inhibition zone by agar disc diffuit-ion method.
P.Pure hesperdin C.orange peel extract.
1. *Bacillus subtilis* 2. *Escherichia coli* 3. *Salmonella typhi.* 4. *Staphylococcus aureus* 5. *Str*epto *coccus pyogenus* sp. S. pincilin stander. Ss, stander

Table 1. the diameter of inhibition zone In hesperidin and orange peel extract .

Microorganisms	Inhibition diameter zone (mm)at24h		
	Crude sample	Pure hesper-idin	Standerd sample
Bacillus subtilis	13.1	7	13.3
Escherichia coli	13.5	8.8	13.1
Salmonella typhi	8.7	nil	13.1
Staphylococcus aureus	13.8	7.6	13.1
*Str*epto *coccus pyogenus* sp	12.8	7	13.1

the inhibition zone diameter of hesperidin and orange peel extract microorganisms by purified hesperdin and crud peels extract showed strong antimicrobial activity on *Staphylococcus aureus, Escherichia coli,* followed by *Str*epto *coccus pyogenus* and *Bacillus aerus*, Data showed weak activity against *Salmonella typh.* The purified hesperidin showed a weak antimicrobial activity agents all micro organisms. Crude peels extract exhibited the most powe-rful antimicrobial activity and the lowest minimum inhibitory concentration (MIC) values against tested bacteria ranged from 75-275 µg/ml. tabel 2.

Table2 minimum inhibitory concen -tration values of purified hesperidin and oranges peel extract against tested bacteria S – Standard ciproflaxacin drug, Ss- Standard impinme drug.

Microorganisms	Minimum inhibitory concentration (MIC) µg/disc		
	Crude sample	Pure hesperidin	Standerd sample
Bacillus subtilis	45	175	30 Ss
Escherichia coli	75	160	30 S
Salmonella typhi	175	---	30 S
Staphylococcus aureus	130	420	30 S
*Str*epto *coccus pyogenus* sp	175	450	30 S

The crud peels extract sampl are considered a bioactive fractions.
Didymin, naringin and hespiridin, were present in crud peels extract These data also showed that hesperidin can easily be extracted and purified from Iraqi orange peel. So, it is economic to use oranges peel as source of hesperidin for pharmaceutical use.

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عزل مادة الهسبردين النفية من قشور البرتقال العراقي ودراسة التأثير القاتل على انواع من البكتريا المرضية

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الخلاصـــة:

تعتبر الحمضيات من الفواكه الغنية بفتامين ج المعروفة فوائده للصحة العامة كما تعتبر مصدرا غنيا بعدد من الفلايفونات اهمها الهسبردين الموجود بتركيز عالي في قشور الحمضيات وبتركيز اقل في عصيرها. يعمل الهسبردين كمضاد للتاكسد فهو يثبط انتاج الخلايا السرطانية ،ويعمل كمضاد للحساسية ومضاد للالتهابات ويعالج تصلب الشرايين.

تم في هذا البحث عزل الهسبردين بشكل نقي من قشور البرتقال العراقي بطريقة تحويل الفلايفون الى ملحه وتنقيته وتقيسه بطريقة كروماتوكرافيا السائل عالي الاداءHPLc حيث اعطت طريقة الفصل نقاوة للهسبردين بنسبة(95.7-90.1)% واعطت نسبة وزن للهسبردين النقي 15% من وزن قشور البرتقال الجافة.

تم دراسة التأثير القاتل والمثبط لانواع من البكتريا السالبة والموجبة لصيغة كرام لكل من الهسبردين النفي ومستخلص قشور البرتقال وقد بينت النتائج اختلافا في حساسية المادتين للبكتريا المستخدمة حيث اعطى مستخلص قشور البرتقال تثبيطا عاليا وبتركيز 45-175 مايكروغرام /دسك من المستخلص واعطى الهسبردين النقي تثبيطا اقل وبتراكيز 175-450 مايكروغرام/دسك للهسبردين بالمقارنة مع impinime وciproflaxcin كمضادات قياسية