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Determination of some chemical compounds and the effect of oil extract from orange peel on some pathogens

تقدير بعض المكونات الكيميائية وتأثير الزيت المستخلص من قشرة البرتقال على بعض الممرضات المجهرية

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Abstract

This research involves detection of the chemical constituents of orange peel; it contains alkaloids, saponins, terpenes, resins, flavonoids, phenols, tannins, and sugars but not contains coumarins and steroids. In addition sugar, proteins, moisture and ash were determined and their percentage were (23.8%, 4%, 11.86%, 5.34%) respectively. Oil of orange peels was extracted and its percentage was 13.12% then its anti-microbial activity was tested against micropathogen that include *Aeromonas hydrophila, Klebsiella pneumonia, Psdumonas aeruginosa, Ecsherichia coli, Psudomonas fluorescenes, Proteus spp. Enterococcus feacalis, Staphylococcus aureus, Candidia albicans* and *Listeria spp.* using agar diffusion wells. Different concentrations of oil were prepared (12.5, 25, 50 and 100,) mg/ml and the diameter of inhibition zone were measured. Oil extract was more effective against *Aeromonas hydrophil* and *Klebsiella pneumonia* than other pathogens were tested. In addition some elements were determined such as (*Fe*, Mn, Zn, Ni, Cu, Cr, Pb, Cd and P). The results demonstrated that orange peels contain these elements at concentrations (125, 88, 13, 1.6, 1.3, 1.2, 0.25, and 0.11) µg/ml. and 0.2% of phosphorus.

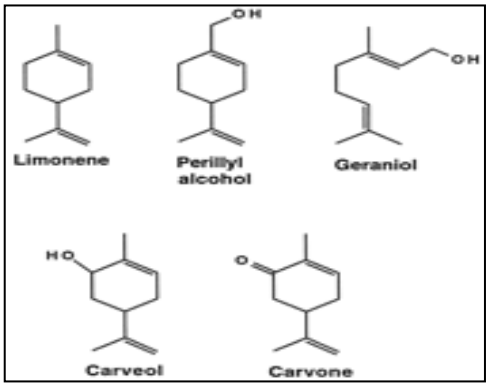
الخـــلاصـــة

تضمن البحث الكشف عن المكونات الكيميائية لقشور البرتقال ووجد بأنها تحوي على الالكلويدات, السابونينات, التربينات, الراتنجات, الفلافونويدات, الفينولات ,التانينات والسكريات لكنها لاتحوي على الكومارينات والستيرويدات. اضافة الى ذلك تم تقدير السكريات, البروتينات, الرطوبة والرماد وكانت نسبهما 23.8% و 4% 11.86% و 5.34% على التوالي. تم استخلاص وتقدير زيت قشور البرتقال وكانت نسبته 13.12% بعدها تم اختبار الفعالية البايلوجية له على بعض الممرضات المجهرية والتي تضمنت *Aeromonas hydrophila, Klebsiella pneumonia Psdumonas aerogenosa, Eschericia coli, Psudomonas florescense, Proteus spp. Enterococcus feacialis, Staphylococcus aureus, candida albicans and Listeria spp* (باستخدام طريقة الانتشار بالحفر. اذ حضرت تراكيز مختلفة من الزيت (100, 50, 25, 12.5) ملغم\ مل وتم قياس قطر منطقة التثبيط اذ وجد ان مستخلص الزيت كان اكثر تاثيرا على بكتريا *Aeromonas hydrophila* و*Klebsiella pneumonia* مقارنة بباقي الممرضات المستخدمة في الدراسة. اضافة الى ذلك تم تقدير بعض العناصر المعدنية مثل (Zn, Ni, Cu, Cr, Pb, Cd, P Fe, Mn,) وقد وجد ان قشور البرتقال تحوي حوي هذه العناصر بتركيز (125 , 88, 13, 1.6, 1.3, 1.2 , 0.25, 0.11) مايكروغرام\ مل على التوالي و 0.2% بالنسبة للفسفور.

Introduction

Orange peel extract may work as a surfactant. A surfactant is a substance that reduces the surface tension of liquids. Orange peel extract may reduce the surface tension of the liquid contents in the stomach, decreasing the potential for the fluid to splash up into the esophagus. Orange peel extract has been studied in several clinical trials. In many of the studies, participants were asked to rate their heartburn for severity and frequency [1].Researchers have determined orange peel extract inhibits the way cancer cells divide and grow. In laboratory studies, orange peel extract

prevented breast, skin, liver, lung, pancreatic, colon and stomach cancers [2-6]. Monoterpenes are nonnutritive dietary components found in the essential oils of citrus fruits, cherry, mint and herbs. They function physiologically as chemoattractants or chemorepellents, and they are largely responsible for the distinctive fragrance of many plants [7]. The essential constituents of orange peel oil include terpenes such as (carveol, carvone, menthol, perillyl alcohol and perillaldehyde) (Fig.1). Citrus, in addition to providing an ample supply of vitamin C, folic acid, potassium, and pectin, contains a host of active phytochemicals. The mutagenicity-reducing activity per weight of peels of citrus fruits was considerably higher than that of their juices. The two main compositional differences between peel and juice components are that the peel contains a higher concentration of ascorbic acid than the juice, and that the peel also contains higher concentrations of active components (d-limonene, hesperidin, naringin, and auraptene) than do the juice and pulp.[8]. D-Limonene, which comprises > 90% of citrus peel oil, has demonstrated chemopreventive activity against a variety of chemically induced rodent cancers[8].



(Fig. 1) components of orange peels

The aims of this study were to know the significant important of orange peels as diet and the antimicrobial effect of oil extract on some pathogenic organisms.

Material and methods

Plant material: The plant material used in this study consists of orange peels which were collected from the orange market. It was dried and grounded into fine powder for further use.

Oil extract: Orange peels were extracted with petroleum ether for 48 hours using soxhlet equipment and then solvent was evaporated using rotary evaporator. Oil extract was stored in deep freezing.

Test microorganisms: Ten microorganisms were used in this study: *E. coli, P. fluorescens, P. aeruginosa, K. pneumonia, A. hydrophila, P. spp., E. feacialis, S. aureus, C. albicans, Listeria spp.* These strains were obtained from biotechnology and genetic engineering institute.

Detection of some Chemical Compound in orange peel

A- Alkaloid test

Dragendorff reagent was used for alkaloids detection and prepared as [9]

B-Coumarin test

This test was tested as [10]

C -Flavonoids test

This test was performed as [11]

D-Resins, Tannins, Terpene and Steroid test

This test was performed as [12]

E- Saponins test

This test was performed as [9,12]

F- Sugar test

This test was performed as [13]

G- Phenolic compound test

This test was performed as [14]

Determination of moisture/ The 2gm of orange peel powder were weighed in porcelain crucibles with its cover and then entered into a thermal oven at 105˚C for 3.5 hours, after that the percentage of moisture was calculated [15].

Determination of ash/ A 2gm of orange peel powder were weighed in porcelain crucibles; the samples was dried in air oven at 110˚C for 15minutes, and then burned in a muffle furnace at 550˚C. The color changed to white or gray. After cooling, the crucible percentage of ash was calculated [16].

Determination of sugars

Sugars of orange peel were extracted according to [15] then the free sugar was determined using colorimetric method [17].

Determination of protein

Proteins were determined as [18].The percentage of the nitrogen was determined using kejeldahal apparatus and the percentage of protein was calculated.

Determination of Phosphorus

Different concentrations of KH_2PO_4 were prepared ranging between (0.2-0.6) µg/ml and standard curve was done then phosphorus in sample was estimated according to [18].

Elements determination

Elements that were determined include Ni, Cu, Fe, Zn, Mn, Cr, Cd, and Pb. The sample was prepared as [19] then the elements were determined using atomic absorption spectroscopy (Perken-Elmer 5000) and air-acetylene flame with hallo cathode for each element.

The Antimicrobial Activity of the orange peel oil

Antimicrobial activity was determined by the well diffusion method according to the [20]. Petri plates containing 20 ml of nutrient agar medium were inoculated by streaking the swab over the entire sterile agar surface. This streaking procedure was repeated and the plate approximately rotated 60 degrees each time so as to insure an even distribution of inoculums. Wells were cut into the agar and 100µl of the orange peel oil were in different concentrations (100, 50, 25. 12.5) mg/ml. The inoculums size was adjusted so as to deliver final inoculums of approximately 1.5×10^7 colony-forming units (CFU)/ml. Incubation was performed at 37°C for 24 hours. The assessment of antibacterial activity was based on measurement of the diameter of the inhibition zone formed around the well.

Results

Oil of orange peel was extracted with petroleum ether for 48 hours which resulted in 13.21% fixed oil. In addition detection of chemical constituents in orange peels showed that it contains: saponins, alkaloids, tannins, flavonoids, sugars, resins, terpenes, and phenolic compounds but does not contain coumarins and steroids (Table 1). Also the chemical compositions in orange peels were determined as dry matter basis (Table 2). Elements in orange peels were analyzed-using AAS technique (Table3).Nine elements (Fe, Mn, Zn, Ni, Cu, Cr, Pb, and Cd) were detected and their concentrations determined using AAS technique except phosphorus, which was determined by a colorimetric method. The oil of orange peels was diluted in DMSO solvent and was prepared different concentrations then tested against *pneumonia* than other microorganism. microorganism; the results are shown in (Table 4). The oil extract was more effective against *Aeromonas hydrophila* and *Klebsilla*

(Table 1)Chemical compounds of orange peel

Chemical constituents	Reagents	Detection indicator	Results
Alkaloids	Dragendorff	Orange spots	+
Coumarins	Filter paper saturated with dil.NaOH	Yellow–green fluorescence under uv.	–
Flavonoids	50%ethanol+50%NaOH	Yellow color	+
Resins	95%ethanol+4%HCl	Turbidity	+
Saponins	Shaking the extract vigorously	Foaming remaining for long time	+
	$HgCl_2$	White precipitate	
Sugars	Benedect &Fehling	Red precipitate	+
Tannins	1% lead acetate	White mucilage precipitate	+
Phenolic compounds	1% $FeCl_3$	Green color	+
Terpenes	Chloroform+glacial acetic acid	Brown color	+
Steroids		-	-

(Table 2) chemical components of orange peels

Component	Percentage (%)
Ash	5.34
Moisture	11.86
Protein	4
Sugar	23.8

(Table 3) Elements in orange peels

Mineral elements	Concentration (µg/ml)
Iron (Fe)	125
Ma(nganese (Mn)	88
Zinc (Zn)	13
Nickel (Ni)	1.6
Copper (Cu)	1.3
Chromium (Cr)	1.2
Lead (Pb)	0.25
Cadmium (Cd)	0.11
Phosphorus (P)	0.2%

(Table 4) Effect of orange peel oil on the microorganism

Inhibition zones (mm)				
Concentration of oil (mg/ ml)				
	100	50	25	12.5
Ecshericia coli	0	0	0	0
Psudomonas fluorescens	13	12	0	0
Psudomonas aeruginosa	0	0	0	0
Klebsilla pneumonia	16	14	13	12
Aeromonas hydrophila	18	17	15	14
Proteus spp.	15	14	0	12
Enterococcus feacalis	14	13	12	11
Staphylococcus aureus	12	11	0	0
Candida albicans	13	0	13	0
Listeria spp.	14	13	0	0

Discussion

The results indicated that orange peels of citrus spp. are a good source of oil. Also the presence of active ingredients in orange peels makes it useful in folk medicine to treat many diseases. Orange peel extract is an all-natural product and its safety has been evaluated [21]. In fact orange peel extract has been used in cancer studies for many years with no adverse effects [2]. The chemical compositions in orange peels used made it possible to use in herbal medicine for the treatment of many diseases [22]. D-Limonene terpene is derived from the peels of citrus fruits, it is a cyclic monoterpene, Patients use this supplement to prevent and treat cancer [23]. Much research has been focused on the potential use of flavonoids (in citrus and tea) as inhibitors of neoplastic transformation and as free radical scavengers to prevent oxidative skin damage [22]. The oil extract showed antibacterial activity towards gram Gram-positive bacteria (*Aeromonas hydrophila, Enterococcus feacalis, Staphylococcus aureus, Listeria spp.*) and Gram negative

bacteria (*E.coli, P. fluorescens, P. aerugenosa, Klebsilla pneumonia, Proteus spp.*) as well as the fungi (*Candidia albicans*). The resistance may be due to the permeability barrier provided by the cell wall or to the membrane accumulation mechanism [24]. In this study showed that orange peels contain useful elements which human body required such as iron, manganese and nickel and less harmful element like cadmium and lead. Nutritional assessment is important in every patient. Malnutrition is a common problem worldwide, and in developed in countries. Various studies have shown that patients may have evidence, not only of protein-calorie malnutrition, but also of vitamin and mineral deficiencies, especially after major surgery or chronic illness. Iron is an essential element in humans, being the central ion in heam. Iron deficiency causes a failure of heam synthesis, leading to anemia [25]. Copper is an essential trace metal which is a component of a wide range of intracellular metalloenzymes, including cytochrome oxidase, superoxide dismutase, tyrosinase, dopamine hydroxylase and lysyl oxidase. Copper deficiency lead to bone diseases [25]. zinc is an essential element present in over 200 metalloprotein. Zinc deficiency causes characteristic skin rash and hair loss, wound breakdown and delayed healing. [25]. Aluminum, arsenic, antimony ,boron, bromine, cadmium, cesium, germanium, lead, mercury, silver, strontium, Without known nutritional function but toxic in, excess.[26]. Phosphorus contributed in bone formation, energy metabolism, and nucleic acid metabolism. [27].

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