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In vitro Antibacterial Activity of Orange Peel Oil Extract from Citrus Sinensis Fruit in Erbil

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Abstract

Orange is one of the world's most popular fruit crops, contains active constituents that can protect health. In this study orange obtained from Erbil farms and orange peel oil were extracted by using pressing machine-heating systems and hydro-distillation. Two methods have been used to extract orange peel oil including the use of diethyl ether solvent and pressing machine. The obtained results indicate that solvent mode was better (10ml) than pressing machine (1.5 ml) in the extraction of this flavonoid oil from 500 gm of orange peel. The fruit peel extract of orange peel oil showed potent activity against Gram-positive *St. aureus* but lower activity against Gram-negative *E. coli* and *Pse. Aeruginosa*, while showed no activity against *Candida albicans* compared to positive control of amoxicillin. As a result, citrus should be employed as an alternative to synthetic preservatives to minimize their ill effects as they are natural and protect human health.

Kewword: Orange peel oil**;** extracts; antibacterial; plant; herbal medicine.

1. Introduction

The orange peel is thinner than that of the bitter, more yellowish in color and less rough and the taste through pungent and acidic, lacks the Seville peels intense bitterness [1,2]. Orange peel consist of fresh or dried outer peel of citrus sinensis ripe fruits, isolated from the layer of white pith. The citrus not only provides ample supply of vitamin C but also folic acid. The two key variation in composition 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 the juice and the pulp [3,4]. Citrus sinensis peel has historically been rubbed with fresh rind on the face for acne. Membrane and peel substances help destroy bacteria. Dried outer parts of rind have the properties of stomach and tonic. Decoction peel used in dysentery, flatulence, hunger and worms. Citrus sinensis peel is folk toothache treatment. Asian Indians poulyice citrus sinensis skin onto psoriasis [5,

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6] also has antgastric, antihistaminic, orexigenic, secretolytic properties to cure sore throat and cough [7], so it is suggested in anorexia and cramp treatment. Citrus sinensis peel is used as a diuretic and digestive aid citrus sinensis peel extract can also function as a surfactant and citrus sinensis peel extract has a pronounced preventive effect on cancer [8,9]. There is evidence that hesperidin prevents the release of histamine and the degranulation of mast cells which would account for the potential anti-allergic activity of hesperidin [10-12]. The aims of this study were to compare two modes of extract orange oil obtained from Erbil frames and investigate its antibacterial activity against pathogenic microorganisms.

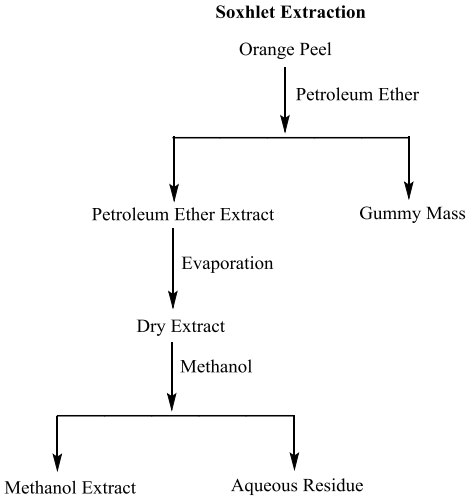
2. Experimental

A. Extraction or separation of Orange peel consists

Orange (Citrus Sinensis) peel extraction consists of the dried outer peel of locally orange ripe fruits, obtained from Erbil farms. Oil separation achieved mechanically by using a pressing machine with heating systems isolated from the white pith sheet [13]. A 100 gm of fresh orange fruit peels were cut to a small piece and placed in a round-bottomed 1-liter flask attached to a reflux condenser. 500 ml petroleum ether (b.p. 40- 60 ^{o}C) has been utilized within 3 hours reflux. The flask contents were filtered while the hot skins were allowed to dry at room temperature to obtain the defatted peels. The dried peels and 500 ml of 70 per cent methanol were returned to the flask. The contents were heated for 4 hours under reflux and then filtered hot and washed 70 per cent methanol with 100ml water. The filtrate or extract was concentrated under reduced dryness pressure and weighed and subjected the dry extract to identification and purification procedures [14].

B. Antimicrobial Activity

The antimicrobial activity was done for fruit peel extracts of *orange* using Brain-Heart infusion broth and agar which prepared accordance to the instructions of the manufacturer. The test microorganisms (*Staphylococcus aureus*, *Escherichia coli*, and *Pseudomonus aeruginosa*) were obtained from local isolates in the clinical microbiology laboratories [15]. The microorganisms were inoculated into Brain-Heart broth and incubated at 35 ± 2 ºC for 4 hours.



Scheme 1. Extract Process

A dilution was made in order to obtain standardized inoculums of approximately 4.0 x 10^{8} CFU/ml (16). The agar well diffusion method (17-24) was employed to determine the antimicrobial activity. The medium was inoculated with the test microorganism. Once the agar was solidified, it was punched with a six millimetres diameter wells and filled with 100 µl of the sample. A stock solution was made for the sample by mixing the extracts in DMSO in to get a concentration of 200 µl /ml. A 100 µl volume of each sample was introduced in wells into plates already seeded with the standardized inoculums of the test bacteria cells. All test plates were incubated at 37 ºC for 24-48 hours and then examined [25]. Amoxicillin was used as a positive control. The activity cultures will be developed to transfer 5 ml of brain heart infusion broth from a cultivated lope and to incubate at 37 °C for 24 hours. Culture media were inoculated with activated bacterial growth. The sensitivity of bacteria to antibiotics Amoxicillin (Samarra Pharmaceutical Company-Iraq) were tested by using the method of diffusion by digging with dilutions of 50, 100 and 150 mg/ml of the antibiotic, and it was considered as a positive control. The dishes were kept in the incubator for 24 hours at a temperature of 37°C, and the inhibition areas for antibiotics were measured and compared with the inhibition areas for the used plant concentrations measured in millimeters.

A. Extraction or Orange peel consists

The minimal inhibitory concentration (MIC) was evaluated for the orange peel extracts oil that showed antimicrobial activity. This test was performed at four concentrations of each sample (125, 250, 500, 750 µl/

ml of DMSO) employing the same agar well diffusion assay. A 100 µl volume of each dilution was introduced into plates already seeded with the standardized inoculums of the test bacteria cells. All test plates were incubated at 37 ºC for 24-48 hours. The least concentration of each sample showing a clear zone of inhibition or no growth was taken as the MIC [26].

3. Results and Discussion

A. Extraction Methods of Flavonoid

Two extraction methods were tried to select the best one, from the first experimental work which oil one separation stab done by mechanically separated from the white pith layer using press machine with heating. While in method 2 soxhlet extractor and absolute methanol were used. In addition, the extraction time in method 1 is shorter than that in method 2. Selection of the best method was based on the amount of the extract oil obtained. Results showed that method 2 was the best, because the amount of extract was higher (10 ml) than in method 1 (1.5 ml). This indicates that water is an important solvent used in the extraction of this flavonoid.

B. Antimicrobial study

1. Determination of antimicrobial susceptibility

Three types of bacteria were used, gram positive (*Staphylococcus aureus*) and gram negative (*Escherichia coli* and *Pse. aeruginosa*) and one fungi (*Candida albicans*). Agar well diffusion assay was employed to determine the antimicrobial susceptibility of the fruit peel extracts and pure isolates. The fruit peel extract of *orange* showed activity against Gram positive *St.aureus* but lower activity against Gram *negative E. coli* and *Pse. aeruginosa*. While all samples showed no activity against *Candida albicans* table (1).

Table 1. Antimicrobial activity caused by orange peel extract through agar diffusion method

Samples	Microorganisms			
	Sta. aureus	*E.coli*	*Pse.aeruginosa*	*Candida albicans*
Orange Oil extract	+	±	±	-
Amoxicillin	+	+	+	+

(+) susceptibility (-) absence of susceptibility

2. Determination of minimum inhibitory concentration (MIC)

The MIC was determined for the samples that showed inhibitory activity. Four concentrations of each sample was used (125, 250, 500, 750 µl/ml) employing the same agar well diffusion assay. Results were expressed as the diameter of the inhibition zone around the hole filled with investigated sample (Table 2).

Table 2. The inhibitory activity of the orange peel extract against *St. aureus*.

Sample	Concentration (µl /ml)	Inhibition zone in mm. *St. aureus*
Orange Oil extract	750	14
	500	13
	250	10
	125	7
Amoxicillin mg/ mL	50	14.3
Amoxicillin mg/ mL	100	16.1
Amoxicillin mg/ mL	150	18.5

The MIC of the orange peels extract against *Staphylococcus aureus* were 12.5 µl/ml (7 mm.) and 50 µl/ml (10 mm.) respectively. The possible mechanisms of antibacterial activity are inhibition of DNA chain, inhibition of cytoplasm membrane function, inhibition of energy metabolism and direct disruption of the bacterial membrane and liberation of the cytoplasm contents [27, 28].

4. Conclusions

The essential oil of orange peel was obtaining in good quality and quantity using organic solvent extraction and this method is confirmed to be the best method compared to mechanical pressing method. The Orange peel extract showed potent antibacterial activity against Gram positive bacteria including *St. aureus* and showed moderate antibacterial activity against Gram negative bacteria involving *E. coli* and *Pse.* Aeruginosa compared to positive control of amoxicillin. The findings demonstrated weak

antibacterial activity against *Candida albicans* compared to positive control of amoxicillin.

5. Conflicts of interest

There are no conflicts to declare.

6. Acknowledgments

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