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Evaluation the Biological Activity of Different Solvent for Orange Peels Extract On Microorganisms That Causes Acute Diarrhea Infections

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Abstract:

The main objective of this study to measure the antibacterial activity of orange (Citrus sinensis) peel extract against clinical isolates from diarrhea infection. As microorganisms are becoming resistant to present day antibiotics, our study focuses on orange peel extract activity against microbials which potentially can be used for the treatment of those cases . We compared in the present study the in vitro potency of extracted orange peel (1-13) samples using chromatography packed by amber lite 200 resin;used the wells diffusion in agar. The extracted orange peel were randomly selected for this study. The isolates of Salmonella spp and E.coli used in this study was previously characterized in our laboratory as resistance to some antibiotics, in addition to used MIC method for fungi that included Rhizopus spp, Fusarium spp, Aspergillus spp and Candida spp. These microorganisms' isolation and identification from sample diarrhea (acute infection).

Tremendous potencies were observed when the compounds used in compared with antibiotic. The effects were high in 1, 4, 5 and 13 compar to other compounds on the bacteria and fungi. The inhibition zone showed that 1(15mm), 4(10mm), 5(33mm) and 13(10 mm) in diameter for E coli. however the Salmonella spp we seen the inhibition zone were 1(25mm), 3(20mm), 4(25mm) and 13(13mm) in diameter. The MIC method was 16µg/ml for each 1, 3 and 4 compound, these compounds were effected on all fungi in to present study and excepted that fungi apparent fully resistance against 13 compounds.

Key words: Orange peel; Solvent Extraction; Agar well diffusion; Antimicrobial activity.

1-INTRODUCTION:

In herbal medicine, there are many herbs used for treating a large variety of diseases. Medical plants are the richest natural drugs than traditional systems of medicine, modern medicines, food complements, folk medicines, pharmaceutical intermediates and chemical units for synthetic drugs [1]. However Citrus fruits are rich source of flavonoids but the peels contain a high concentration of phenolic compounds and nutrients (used as drugs or food supplements) [2,3]. Peels are usually wasted while the fruits are mainly used in juice processing industries. Very large amounts formed as pickings during the production of citrus juices [4]. It Can be extracted and transformed these pickings into edible chemicals consumed either in the form of dietary supplements and incorporated food when the controlled dosages to create functional foods for specific outcomes while masking the unpleasant tastes. Citrus fruit have antimicrobial agents that act against microorganisms, in addition to other materials that input of physiological role because its commercial value in food and pharmaceutical industries of the entire world [5]. The antimicrobial agents are destroy or inhibition growth of microorganisms such as bacteria, fungi and protozoan's [6].

Acute diarrheal infection is a leading cause of outpatient visits, hospitalizations, and lost quality of life occurring in both domestic settings and among those traveling abroad. The Centers for Disease Control and Prevention has estimated 47.8 million cases occurring annually in the United States, at an estimated cost upwards of US$150 million to the health-care economy [7,8]. Acute diarrhea can be defined as the passage of a greater number of stools of decreased form of the normal lasting <14 days. Some definitions require an individual to present with an abrupt onset 3 or more loose or liquid stools above baseline in a 24-h period to meet the criteria of acute diarrhea. Persistent diarrhea is typically defined as diarrhea lasting between 14 and 30 days, with chronic diarrhea generally considered as diarrheal symptoms lasting for greater than a month. Acute diarrhea of infectious etiology is generally associated with other clinical features suggesting enteric involvement including nausea, vomiting, abdominal pain and cramps, bloating, flatulence, fever, passage of bloody stools, tenesmus, and fecal urgency. Acute diarrheal infection is also often referred to as gastroenteritis, and some acute gastrointestinal infections may cause a vomiting predominant illness with little or no diarrhea [9].

2-MATERIALS AND METHODS:

2.1. Plant material and sample preparation: 2 kg of peels and from healthy local oranges were collected in March 2016. All fruits were the undamaged from any rots. The fruits were immediately washed using tap water and sterilization by chloramphnicol before peeled. The remaining peels accounted for 40% of the total fruit and stored at 5^{o}C before use. peels were dehydrated by freeze dryer (CHRIST Alpha 1-2 LD, France) for 72hr at 50^{0}C and 0.001 mbar finely by a coffee grinder (Moulinex, France) was degradation to standard size of particles of approximately 0.315 mm. The peel powder was stored by freezer at 4^{0}C experiments after placed in vacuum packaging bags [10].

2.2. Chemicals and reagents: High-purity water was produced in the laboratory using an Alpha-Q system(Millipore, MA), Hydrochloric acid was purchased from Fluka (Switzerland), Sodium hydroxide (NaOH), Ethanol, Iron(III) chloride ($FeCl_3$), ammonium solution (NH_3), α-napthol, Sulfuric acid (H_2SO_4), Amberlite 200 Na+ form were purchased from Sigma-Aldrich (Germany) .

2.3. Extraction methods

2.3.1 Primary Extraction by solvent Primary extractions were by two methods. Firstly, the Cold method was taken five grams of citrus peels powder were mixed with 100 ml of 50% ethanol. The mixture was blend by mechanical stirrer in 200 rpm during 24 hours at 25^{0}C under darkness conditions. [11]

2.3.2 Secondary Extraction by column chromatography: In addition to the primary extract separated extract compounds according to PH by colum chromatography system. The characters chromatography was 4 cm diameter of dimension, 33 cm length packed and Amberlite 200 Na^{+} with aqueous solutions of variation pH from 1 to 13 as a table (1). the eluent that used

ethanol in slowly elute different compounds from the plant crude extract **(12)**

Table (1) the extract number

Extract no.	Solution into column	Eluent
1	pH 7	Ethanol
2	pH 9	
3	pH 9	Acetonitrile
4	pH 2	Ethanol
5	Primary extraction in aqueous solution	
6	pH 13	
7	pH 13	and used pH 1 as eluent
8	pH 13	Ethanol
9	pH 13	and used pH 3 as eluent
10	pH 13	and used pH 5 as eluent
11	pH 7Primary extraction	
12	pH 7	and used pH 2 as eluent
13	pH 7	ammonium solution pH12

2.4 Qualitative analysis [13,14]

Test for flavonoids

A few drops of 1% NH3 solution was added to the aqueous extract of each plant sample in a test tube. A yellow coloration confirms the presence of flavonoid compounds**.**

Test for terpenoids

5 mL of aqueous extract of each plant sample was mixed with 2 mL of CHCl3 in a test tube. 3 mL of concentrated H2SO4 was carefully added to the mixture to form a layer. An interface forms with a reddish brown coloration if terpenoids constituent is present.

Test for phenol

2-3 ml of aqueous or alcoholic extract few drops of 5% FeCl3 solution was added. Formation of deep blue-black color indicated the presence of phenols

Test for alkaloids

200 mg plant material in 10 mL methanol, filtered; a 2 ml filtrate + 1% HCl + steam, 1mL filtrate + 6 drops of *Dragendroff* reagent, orange precipitate indicated the presence of respective alkaloids.

2.5 Bacterial isolate: A local isolate bas been isolated from sever diarrhea and fully identified and characterized as drugs resistant bacteria and fungi were identified according to the routine methods to confirm the stability of its diagnostic characteristics.

3.Antimicrobial susceptibility testing: Muller-Hinton agar was used for detection the antibiotics susceptibility by wells diffusion test. The following antibiotics were used with known potency (Himedia): ampecillin, carbencillin, chloramphenicol, and trimethoprim. The results of antibiotics susceptibility were interpreted according to CLSI, 2010, **[15]**.The Minimum Inhibitory Concentration (MIC) and wells diffusion test were used to measurement effect of the compounds (1, 2 …and 13 compound) on the bacteria and fungi which used. 0.1 ml of inoculum was pipetted into each tube containing 2 ml of PDA (potato dextrose agar) along with compounds and incubated at 30 °C and observed on 6th day. All the results were recorded taking endpoint for compound that was defined as the lowest concentration in which the growth score was zero i.e. optically clear. This criteria was used to define the minimum inhibitory concentration (MIC) of the drug [**16**].

4. Results And Discussion

In this study, separate materials from extract and divided to thirteen compounds these compounds were label from one to thirteen based on adsorption and charge. The aqueous solution contains organic compounds in extract and which adsorbed with polystyrene matrix that used as stationary phase . We used an organic solvent as mobile phase. Moreover, The compounds were negative charge because contain hydroxyl and carboxyl group after washing by alkaline solution. These negative compounds did not binding with resin (cation charge).

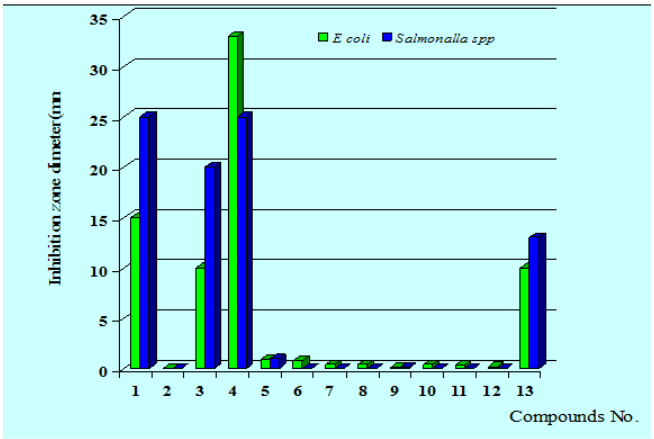
But the compounds : were positive charge which contain amine group after washing by acid solution this results agree with [13] and [12] they reporters the organic compounds that contain hydroxyl, carboxyl and amine groups accord pH medium(12) (13).

Chemically diagnosis was used to limited chemical structure included the flavonoid, terpenoid, alkaloid and phenol table (2). The remarkable results were 1, 3, 4 and 13 as positive effect because it was containing each flavonoid and phenol. These results agree with [12]. And the flavonoid, terpenoid and alkaloid founded in one compound that label 2.

Table (2) the chemical contents of extract compounds.

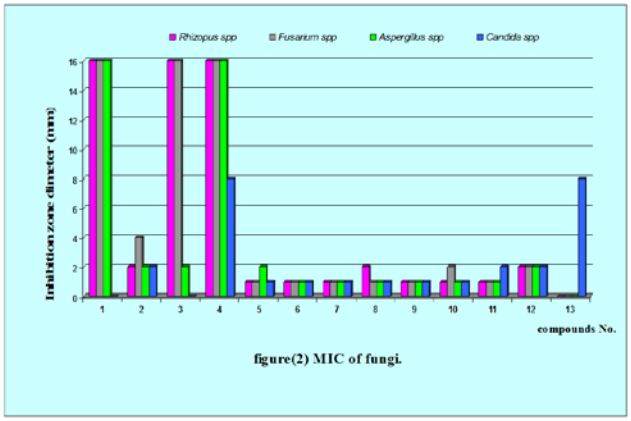
Extract no.	Chemical contents			
	Flavonoid	Terpenoid	Alkaloid	Phenol
1	+	-	-	+
2	+	+	+	-
3	+	-	-	+
4	+	-	-	+
5	+	-	-	-
6	-	+	-	+
7	-	+	+	-
8	-	+	-	-
9	-	-	+	-
10	-	-	-	-
11	-	+	-	-
12	-	-	+	-
13	+	-	+	+

In the well diffusion test was used the antibiotics for the limited sensitive or resistance of the bacteria *(Salmonella spp and E.coli*). *Salmonella spp* appearance fully resistance to antibiotics but the *E.coli* was sensitive to trimethoprim(10mg) and carbencillin(25mg), The inhibitions zones were 6 and 4 (mm) in diameter respectively. Figure (1). The inhibition zone of E-coli (1,3,4 and 13) were (15,10,33 and 10) mm in diameter respectively ,and (25,20,25 and 13)mm of *Salmonella spp* in diameter respectively.



figure(1) Inhibition zone of compounds

Figure (2) MIC results show different effect of compounds on the fungi. The MIC method was 16μg/ml for each 1, 3 and 4 compounds, these compounds were effect on all fungi in present study and the fungi apparent fully resistance against 16 compound excepted *Candida spp* was 8μg/ml of MIC for 13 compound.



figure(2) MIC of fungi.

The results, show four (1, 3, 4 and 13) compounds showed inhibition effect on microorganisms (bacteria and fungi). These results were agreement with Abeer;(2006) who reported that orange peel have highly effect on bacteria and *Candida spp* which contain phenolic and flavonoid contents ranged from 559.3 to 591.8 *mg* [17]. The results revealed that the flavonoid compound has varying degrees of inhibition tested microorganisms ,on the other hand Flavonoids are bactericidal compounds. They cause damage of cell membrane, leading to the inhibition of macromolecular synthesis. these flavonoids are promising leads for further drug development [18].

CONCLUSION:

The best results of this study, *citrus sinensis* peels extract demonstrated *in vitro* antimicrobial activity against microorganisms that causes acute diarrhea Infections. In addition clinical studies to determine the exact dosages and its effectiveness in practical situations. Toxicity studies should be done to determine safety. Need of the hour is to execute more and more screening of natural products or plant parts to set a primary platform for further pharmacological and *in vivo* studies that may open the possibilities of finding new clinically effective antibacterial compounds against Acute Diarrhea Infections and other bacterial resistant pathogens

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