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Research paper

Ethanol extraction of orange peels from agricultural waste and its activity against four species of the bacteria

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Abstract

The present study was amid the Hot extraction of orange peels. It evaluated its ability to inhibit the bacteria growth against four types, i.e. Bacillus anthracis (B anthracis), Streptococcus pyogenes (S. pyogenes), Yersinia pestis (Y. pestis) and Klebsiella pneumonia (K. pneumonia). Hot-extraction method was used to extract orange peels (EPO) by using ethanol as a solvent. Five concentrations of the extract orange peels were treated against the bacteria. The results showed significant effects against all types of bacteria, where the results were 15mm against B. anthracis, while S. pyogenes showed more effect, reaching 17mm. Moreover, the measurements of the EPO zone against Y. pestis were 12mm, while its zone against K. pneumonia was 15mm. The obtained results can give clear evidence of the ability of EPO to be used as an antibacterial agent. The current study has achieved positive results by utilizing agricultural waste and converting it into useful materials in the biological field, as well as reducing environmental pollution.

Keywords: *Agricultural Waste; Orange Peels; Biological Activity; Bacteria; Extraction.*

1. Introduction

Agriculture has been essential to humankind's ability to produce food and resources for survival from the beginning of humanity. Originally, agriculture relied on using the plants and resources (soil, water, etc.) in the area sustainably in order to provide for the needs of the locals [1]. One important source of bioactive materials with several applications is horticultural waste. Because FVWs may be utilised to extract extremely important biomolecules, horticulture by-products are finally being acknowledged as significant resources. Horticultural by-products are rich in minerals, phenolic compounds, dietary fibre, organic acids, pigments, and sugar by-products. Some of these bioactive substances have characteristics that include cardioprotective, antiviral, anticancer, antibacterial, and antimutagenic effects. A vast amount of waste is produced when fruits and vegetables, including oranges, pineapples, peaches, apples, potatoes, carrots, green peas, onions, artichokes, and asparagus, are used to extract juice or pulp, make jams, and freeze pulp [2].
Citrus fruits are native to tropical and sub-tropical parts of Asia and are classified into six genera: Fortunella, Eremocitrus, Clymendia, Poncirus, Microcitrus, and Citrus. The majority of commercial citrus fruits are of the genus Citrus. Numerous significant fruits, including oranges, mandarins, limes, lemons, and grapefruits, are members of the citrus genus. Large amounts of the essential oil are found in the fruit's peel. Monoterpene hydrocarbons make up the majority of the volatile components in citrus essential oil [3].
The current study picked oranges because it is considered rich in chemicals that can be of great benefit in the medical and biological fields and focused on agriculture waste to help reduce environmental pollution by recycling it and using it to obtain highly effective products that protect bacteria.

2. Methodology

2.1. Preparation of peels of orange

The Oranges were collected from the agricultural waste, washed many times with water and peeled the peels of the Orange. The peels were then dried using the sun-dry method for seven days; after that, the dried peels were used to get powder.

2.2. Extraction of peels of orange

10 g of the peel powder was added to a conical flask with 100 ml of ethanol and boiled to 70 C for 30 minutes, then were cooled to get infused and left for 30 minutes. The solution was then filtered using filter paper to get the crude extract of Peels of Orange; then, it was dried to get the extract as a powder.

2.3. Antibacterial study of peels of orange

The agar diffusion method was used to evaluate the ability of the extract of Peels of Orange against four species of bacteria, including Bacillus anthracis (B anthracis), Streptococcus pyogenes (S. pyogenes), Yersinia pestis (Y. pestis) and Klebsiella pneumonia (K. pneumonia). The test bacteria were first injected individually into nutrient broth tubes and allowed to incubate for eighteen hours at 37 C. Following the adjustment of each culture to the 0.5 McFarland turbidity standard, 0.2 ml of each culture was inoculated onto Mueller Hinton agar (MHA, Oxoid) plates with a diameter of 15 cm. Then, seven wells (6 mm in diameter) with varying extract concentrations were made using a sterile coke borer on each plate, holding cultures of the various test species. Using sterile Pasteur pipettes, the extract was dissolved in pure water at concentrations of 50, 100, 150, 200, and 250 µg/ml before being added to the wells. In a second well, water alone was added as a negative control. As a positive control, wells containing the common antibiotics erythromycin and gentamycin (0.5 mg/ml) were added. After 30 minutes of pre-diffusion on the workbench, the culture plates were incubated for 24 hours at 37°C. Following 24 hours, the antibacterial activity was assessed by measuring the diameter zones of inhibition (mm) around each extract and antibiotic (against the test organisms).

3. Results and discussions

Current studies were conducted to extract Peels of Orange (EPO) collected from agricultural waste and evaluated its biological activities against four types of bacteria. The obtained crude was assessed against two positive controls, including erythromycin and gentamycin, and one negative control was distilled water.

3.1. Negative and positive controls against gram-positive of bacteria

The controls were treated with two gram-positive bacteria, including B. anthracis and S. pyogenes. As shown in Figure 1, distilled water (DW) didn't show any effects on either of the bacteria, and that is normal because water doesn't have any effect on the bacteria [4], while the positive controls were showed significant effects at all concentrations against both of the bacteria. Erythromycin (ER) showed inhibition for the growth of the bacteria, reaching 26mm and 24mm against B anthracis and S. pyogenes, respectively. In comparison, gentamycin (GE) showed 28mm and 26mm against B. anthracis and S. pyogenes, respectively.

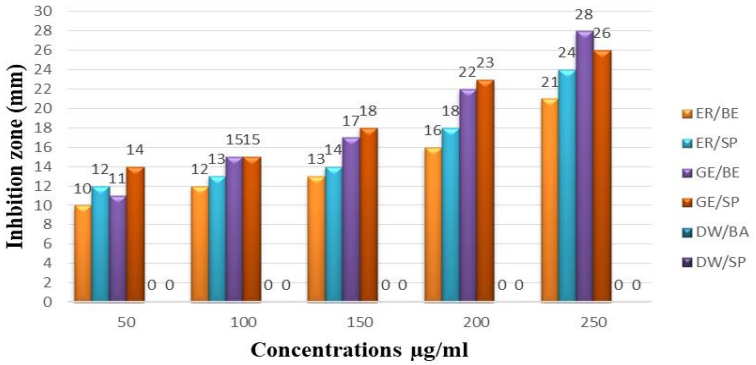

Fig. 1: Antibacterial Activities of Positives Controls Against B Anthracis and S. Pyogenes.

(Key; Erythromycin / B. anthracis (ER/BE), Erythromycin/ S. pyogenes (ER/SP), gentamycin/ B.anthracis (GE/BE) gentamycin/ S. pyogenes (GE/SP), Distilled water/ B. anthracis (DW/BA) and Distilled water/ S. pyogenes (DW/SP))

3.2 Negative and positive controls against gram-negative of bacteria

EE, GR and DW were treated with Two gram-positive bacteria, including Yersinia pestis (Y. pestis) and Klebsiella pneumonia (K. pneumonia). As shown in Figure 2, DW didn't show any effect against bacteria, while both of the antibiotics showed a significant effect on inhibition of the bacteria growth. GE showed higher ability against both bacteria compared with ER; it showed inhibition of bacteria 24mm and 22 mm against Y. pestis and K. pneumonia, respectively.

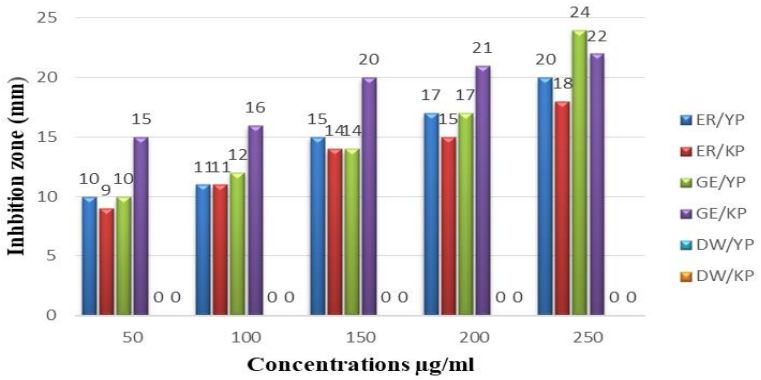

Fig. 2: Antibacterial Activities of Negatives Controls Against Y. Pestis and K. Pneumonia.

(Key; Erythromycin / Y. pestis (ER/YP), Erythromycin/ K. pneumonia (ER/KP), gentamycin/ Y. pestis (GE/YP) gentamycin/ K. pneumonia (GE/KP), Distilled water/ Y. pestis (DW/YP) and Distilled water/ / K. pneumonia (DW/KP))

Figures 1 and 2 show the ability of the picked antibiotics to inhibit bacteria growth of the four species. According to the obtained results, these antibiotics showed more effect on the gram-positive bacteria compared with the gram-negative and the reason for that was mentioned in the previous studies due to the composition of the bacteria negative [5-7].

3.3. Extract of peels of orange against gram-positive of the bacteria

Five concentrations of the crude extract of Peels of Orange (EPO), i.e. 50, 100, 150, 200, and 250 µg/ml, were treated against two species of the bacteria, including B. anthracis and S. pyogenes. All concentrations of EPO showed promising effects against both of the bacteria species. The results showed inhibition of the growth of the bacteria, 6mm, 8mm, 9mm, 11mm, and 15mm against B. anthracis, while against S. pyogenes showed more effect reaching 7mm, 9mm, 12mm, 13mm, and 17mm.

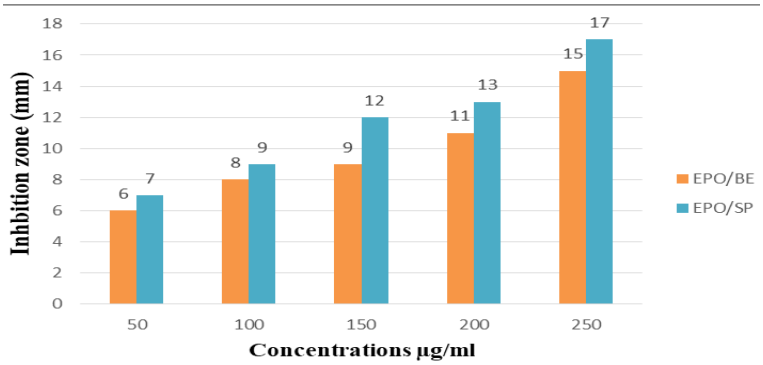

Fig. 3: Antibacterial Activities of Peels of Orange Against B. Anthracis and S. Pyogenes.

(Key; Peels of Orange / B. anthracis (ER/BE), Peels of Orange / S. pyogenes (ER/SP))

Figure 3 shows the ability of EPO to inhibit the growth of the bacteria against S. pyogenes is more than its ability against B. anthracis; the reason for that is the type of the bacteria B. anthracis is resistant bacteria, and it can resist the drugs [8-10].

3.4. Extract of peels of orange against gram-negative of the bacteria

Two species of gram-negative bacteria were used to evaluate the ability of five concentrations of EPO against them to inhibit their growth. The results showed a significant effect for all concentrations against Y. pestis and K. pneumonia. The measurements of the EPO zone against Y. pestis were 4mm, 6mm, 7mm, 10mm, and 12mm, while its zone against K. pneumonia was 5mm, 7mm, 10mm, 11mm, and 15mm.

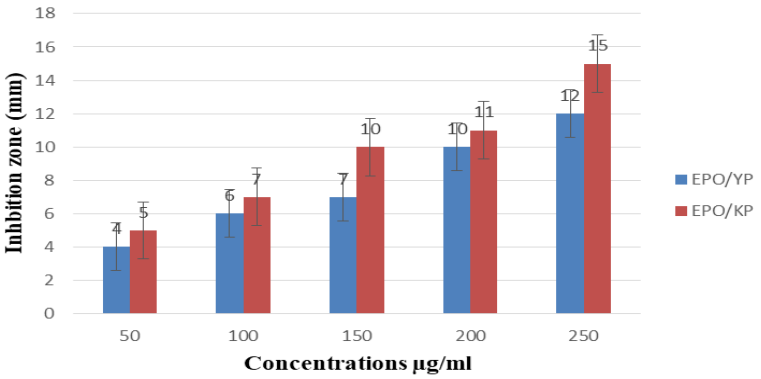

Fig. 4: Antibacterial Activities Peels of Orange Against Against Y. Pestis and K. Pneumonia.

(Key; Peels of Orange / Y. pestis (ER/YP), Peels of Orange / K. pneumonia (ER/KP))

Figure 4 shows that the ability of EPO to inhibition of the growth of the bacteria was more effective against K. pneumonia than Y. pestis, and the reason for that was because of the resistance of Y. pestis against drugs which is have a good ability to resistance [11], [12].
According to Figures 3 and 4, the effect of the EPO showed a high ability to inhibit the growth of gram-negative and gram-positive bacteria. Moreover, the effect of the EPO was observed to have a higher effect against gram-positive than gram-negative bacteria; the reason for that was because of the compost ion of gram-negative bacteria, which gave them a good ability to resist the effect of the drugs [13-14].

4. Conclusion

The present study aimed to extract the Peels from the agricultural waste. Ethanol as a solvent was used to extract the Peels of Orange using a hot extraction method and evaluated its antibacterial properties against four bacteria species. The present study showed a promising effect for the extract of the Peels of Orange against both strands of the bacteria. The current study demonstrated the recycling of agricultural waste and utilizing it in the medical fields through the effectiveness of these natural materials against bacteria, which contributes greatly to preserving the environment on the one hand and obtaining useful natural materials on the other hand.

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