INHIBITION EFFECT OF GREEN APPLE PEEL EXTRACTS ON SOME TYPES OF PATHOGENIC BACTERIA ISOLATED FROM VAGINA

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ABSTRACT

Escherichia coli and staphylococcus aureus pathogens were collected from 65 vaginal sample of patients aged (15-55). Estimating anti-bacterial effect of Ceftriaxone (CT) and ethanol extract green apple peels (EEGAP) versus E.coli and Staph. aureus through variety of concentrations (125, 250, 500, 1000, 1500, 2000, 2500, 3000) µg/ml each one alone. Furthermore, results obtained by the combination test of CT with EEGAP against E. coli and Staph. aureus, showed that when using concentrations of (250, 500, 1000, 2000,3000) µg/ ml treated with disc dilutions of EEGAP and CT each one alone against E. coli and Staph. aureus respectively, only CT showed inhibitory impact versus two pathogens. The minimum inhibition concentration (MIC) of CT at concentration (125) was (1.2 mm for Staph. aureus, R for E.coli), while maximum inhibitory concentration(MXIC) of CT at (3000) was (5.0 mm for Staph. aureus, 4.8 mm for E.coli). In contrast, Staph. aureus and E.coli are R when treated with EEGAP separately. From another hand, the MXIC result seen from the combination of (CT 50% + EEGAP 50% against E.coli) at 3000 µg/ ml was 16.31%, in addition, (CT 50% + EEGAP 50% against Staph. aureus) at 3000 µg/ ml was 16.33%.

Keywords: Green apple peel, Ceftriaxone, E. coli, Staph. Aureus

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INTRODUCTION

Recently aremarkable expansion in food researches was observed, by enhancing food quality with health interest [1].Last 20-30 years the awareness of markets to new natural outputs, havingfortified food qualities that not only supplements the diet but also assists in treating or preventing disease, slow down the risk of illnesses, molecules in products of food, identifying antioxidant characteristics,so provides medical benefits [2]. Polyphenols is an important category of molecules which has considerable health features. Generally in term of nomenclature, they are believed as a “constitutional class of fundamentally natural, organic chemical complexes identified by the existence of huge numbers of phenol structural units”[3].Interesting in natural plant products such as botanicals in food applications researches as nutraceuticalcomponents [4] or natural preservatives [5] and many valuable antioxidant activities [6-8]. Though, until nowthe practical applicationsof manybotanicals have not noticed scientifically by the European Food Safety Authority (EFSA) [9]. In comparison to the wide kinds of fruits, apples considered to have strong...
antioxidant ability and significant source of phenolic compounds. Polyphenols of apple have many bioactivities in vitro that may be in integration with dietary fibre (i.e. reducing the risk of coronary heart disease). Commonly, the pulb of the fruits contain less amounts of polyphenols especially flavonol glycosides compared to large amounts found in the skin.

In perspective of safety, phenols with antibacterial, antifungal and antioxidant activity, terpenes and polyphenolic components are existing in the skins of vegetables and fruits. These phenolic compounds which persist in high percentages around the world in all fruits in general, especially in apples are consuming in various forms for instance in different juices, fresh and cider. Apples contain numerous types of flavonoids and phenolic derivates (flavonols, procyanidins, flavan-3-ols, anthocyanins and chalcones), as we mentioned above apple peels contain flavonoids in addition to all the aforesaid substances i.e. cyanidin glycosides and quercetin glycosides, that they are absent in pulb which contains many components such as chlorogenic acid, caffeic acid, catechin and procyanidin. Eating a diet rich in fruits and vegetables protect us versus a variety of diseases in addition to minimize heart malady and cancer. Low hazard was seen persons who consumed large quantities of fruits enriched with vitamin C and vegetables of green leaves.

Ceftriaxone (CT) is sterile, semi-synthetic, wide-spectrum cephalosporin antibiotic, it’s treatment is clear to protect the body from different pathogens restrictively on bacteria that cause varieties of illnesses or infections. CT mode of action via preventing of cell wall synthesis taking the advantage of possession an altitude degree of steadiness in the existence of beta-lactamases. Cephalosporinases and penicillinases active against both G+ve, G-ve bacteria, previous papers cleared that CT has been vigorous against most strains of Aerobic gram-negative microorganisms (Escherichia coli, Haemophilus influenza, Proteus spp.,), additionally, Aerobic gram-positive microorganisms for example (Staphylococcus spp, Streptococcus spp...) and some other anaerobic microorganisms.

Currently, with development in medical technologies, the strength of diseases has also raised. Recent studies appreciate that utilizing medical drugs of natural products are more effective than chemical synthetic rival part, nevertheless the advances in cemical-medical drugs industry, but it is still correlated to many side effects ranging from low to heavy intensity.

MATERIALS AND METHODS

Bacterial strains

Escherichia coli and staphylococcus aureus were isolated from 65 human vagina aged (15-55) years. Specification media were used are methylene blue agar (MBA) and mannitol salt agar (MSA) as differentiation growth media respectively, using nutrient agar medium (NA) as general growth enrichment medium. Incubation at 35-37°C for (24-48) hrs.

Extraction process

Extracts were prepared of ethanol green apple peels (EEGAP), dissolving 30 g in 300 ml of Diethyl ether (DEE) concentration (99%) at a rate (10-1) Weight to volume. Grinding the specimen by the use of an
electric mixer, after that leaving the mixture in the vessel, then refrigerated 1 day in order to be soaked, continually crossing the sample through gauze consisting of several layers and filtration were used twice again by Buchner and special (Whatman) no. 2 filter paper utilizing (Rotary vacuum evaporator) under evacuation at 40°C. Finishing the drying by Lyophilizer after that samples conserved in freeze until we use [23].

Preparing range of dilutions for both natural product, chemical drug and combination of both (natural extract, drug), calculating anti bacterial effects of (EEGAP) and Ceftriaxone (CT) against \textit{E. coli} and \textit{staph. aureus} at concentrations (125, 250, 500, 1000, 1500, 2000, 2500, 3000) µg/ml each one alone, then testing the combination effect of (CT) compound with (EEGAP) against \textit{E. coli} and \textit{staph. aureus} at concentrations of (250, 500, 1000, 2000, 3000) µg/ml.

Determination of bacterial resistance to antimicrobials is an important part of the management of infections in patients by using standard disk diffusion protocol Following incubation, by measuring diameters of inhibition zones in millimeter around all the colonies either resistant or sensitive using a ruler or caliper; include the diameter of the disk in the measurement [24].

RESULTS AND DISCUSSION

At the end of incubation period and examining the antibacterial disc in different concentrations of (CT) and (EEGAP) each one alone against \textit{E. coli} and \textit{Staph. aureus} separately, we can see that only CT showing inhibition zones versus two types of bacteria (table 1). On the one hand, regarding to \textit{E. coli} : (MIC) of CT is (2 mm) at concentration (250 µg/ml) and (MXIC) is (4.8 mm) at concentration (3000 µg/ml), whereas \textit{E. coli} showed noticeable resistance to (EEGAP) for all concentrations. Relating to \textit{Staph aureus} : (MIC) of CT is (1.2 mm) at concentration (125µg/ml) and (MXIC) is (5 mm) at concentration (3000 µg/ml) and reassembly to \textit{E. coli}, \textit{Staph aureus} showed clear resistance to (EEGAP) for all concentrations. On the second hand, for \textit{E. coli} and \textit{Staph. aureus}: incorporation test demonstrated that combination effect of [(CT 50%+EEGAP 50%)] µg/ml] MXIC at concentration (3000 µg/ml) is (16.31%, 16.33%) for \textit{E. coli} and \textit{Staph aureus} respectively (table 2, 3) and (Fig. 1).

Table 1: (CT) with \textit{E. coli} and \textit{staph. aureus}, (EEGAP) with \textit{E. coli} and \textit{staph. Aureus}, each one alone.
Table 2: Combination (CT 50%+ EEGAP 50%) for *E. coli*

<table>
<thead>
<tr>
<th>Conc* CT µg/ml</th>
<th>Inhibition Zone (mm)</th>
<th>Conc. (EEGAP) µg/ml</th>
<th>Inhibition Zone (mm)</th>
<th>Comb.** (CT 50%+EEGAP 50%) µg/ml</th>
<th>Inhibition Zone (mm)</th>
<th>Synergistic percentage %</th>
</tr>
</thead>
<tbody>
<tr>
<td>125</td>
<td>R</td>
<td>125</td>
<td>R</td>
<td>250</td>
<td>R</td>
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<tr>
<td>250</td>
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<td>R</td>
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<td>2.1</td>
<td>4.8</td>
</tr>
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<td>2.7</td>
<td>500</td>
<td>R</td>
<td>1000</td>
<td>3.1</td>
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<td>1000</td>
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<td>1000</td>
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<td>2000</td>
<td>3.8</td>
<td>14.48</td>
</tr>
<tr>
<td>1500</td>
<td>3.85</td>
<td>1500</td>
<td>R</td>
<td>3000</td>
<td>4.6</td>
<td>16.31</td>
</tr>
</tbody>
</table>

- Conc*: Concentration
- Comb.**: Combination

Table 3: Combination (CT 50%+ EEGAP 50%) for *staph. Aureus*

<table>
<thead>
<tr>
<th>Conc* CT µg/ml</th>
<th>Inhibition Zone (mm)</th>
<th>Conc. (EEGAP) µg/ml</th>
<th>Inhibition Zone (mm)</th>
<th>Comb.** (CT 50%+EEGAP50%) µg/ml</th>
<th>Inhibition Zone (mm)</th>
<th>Synergistic percentage %</th>
</tr>
</thead>
<tbody>
<tr>
<td>125</td>
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<td>125</td>
<td>R</td>
<td>250</td>
<td>1.22</td>
<td>1.64</td>
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<tr>
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<td>2.2</td>
<td>250</td>
<td>R</td>
<td>500</td>
<td>2.25</td>
<td>2.23</td>
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<tr>
<td>500</td>
<td>2.8</td>
<td>500</td>
<td>R</td>
<td>1000</td>
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<td>9.68</td>
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<tr>
<td>1000</td>
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<td>1000</td>
<td>R</td>
<td>2000</td>
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<td>14.51</td>
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<tr>
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<td>4.1</td>
<td>1500</td>
<td>R</td>
<td>3000</td>
<td>4.9</td>
<td>16.33</td>
</tr>
</tbody>
</table>

- Conc*: Concentration
- Comb.**: Combination
Results showed that *E. coli* is resistant to CT at (125 µg/ml) due to that MIC of CT against *E. coli* is (250 µg/ml) \(^{[25]}\). Nevertheless the same concentrations were used for antibiotic and natural extract, both pathogens showed sensitivity to CT alone when treated, in contrast they were resistant to EEGAP alone at numerous discs dilutions, attributed to few quantities of apple skin extract in addition to scarcity of active compounds within extracts that we treated with tiny quantities.

It is cleared from (table 2,3 and fig.1) that synergistic effect has been raised by increasing the combined concentrations attributable to the presence of several polyphenolic compounds of 3 categories (phenolic acids, flavanols, dihydrochalcones)\(^{[26]}\). To conclude that We preferred to utilize apple peel instead of pulp due to incredible contents of antioxidants and phenolic compounds which are averaged around (297 mg/100g) in peels to (25.6 mg/100g) in pulps, counting on previous HPLC studies\(^{[27-29]}\).

Overall, synergism to pathogens acting by natural extracts of plants enriched with antibacterial compounds\(^{[30]}\), essentially it's content of phenolic acids disrupt cytoplasmic membrane causing damage to microbial cell nucleotides and energy transmission deficiency leading to death\(^{[31]}\). Finally, the best synergistic ratio was (50:50)% that flavonoids, acidic components and phenols of green apple peel extracts to obtain the highest pathogenic restriction when using lower dose of CT combined with temperate level of extract that preventing toxicity of higher CT dose to human alone, this results supported by \(^{[32]}\).

I recommend future researchers to increase the ratio of (CT-EEGAP)% to (40-60)% and (30-70)% towards peel extracts. We motivate women for consuming green apples daily without peeling, with a view to ensure their reproductive system safety.
ETHICAL CLEARANCE

The Research Ethical Committee at scientific research by ethical approval of both environmental and health and higher education and scientific research ministries in Iraq

CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

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REFERENCES


