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The Synergism and Antagonism behavior of Aqueous Extraction for Black Tea, Green Tea and Coffee against the Effectiveness of Certain Antibiotics

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Abstract: Increasing antibiotic resistance is the most common concern in the world. *Escherichia coli* are a well-known causative agent of various infectious diseases. The bacterial resistance on the *E.Coli* was noted against various antibacterial agents. Thus, the objective of this study is to evaluate the assessment of the green, black tea and coffee in improving antibiotic susceptibility towards *E.Coli*. Antimicrobial susceptibility test was performed to determine the sensivity of *E.Coli* to Augmantin, Amikacin, Imipenem, Ciprofloxacin, Nitrofurantoin, Pincillin, Cefixime, Tetracyclin, Chloramphenicol and Ceftriaxone. The results show that the *E.Coli* was resistant to all antibacterial agents, while Pincillin, amikacin and Nitrofurantoin was appeared sensitive to moderate respectively. The aqueous extract of green and black tea have synergistic effect for Chloramphenicol and Nitrofurantoin susceptibility,on the other hand, the antagonistic effect was on Tetracyclin, Ciprofloxacin and Amikacin activities. The aqueous extract of coffee was strongly inhibitory to bacteria at concentrations 5 and 7.5%. These results may suggest using this natural product for increasing some of antibacterial agents' effect.

Keywords: *Escherichia coli*, Antibiotic resistance, Black tea and coffee

Introduction

Black tea, green tea and coffee can be widely consumed in the Middle East; these products contain biological active compounds, which help to reduce microbial infection. The pharmacological validation have been reported the anti-microbial activity of these natural product (Mbuthia et al., 2014); however, there is limited knowledge about the combination effect with some antibiotics against resistant microbes.

Phenomenon of resistance among microorganisms against currently antimicrobial is the most common concern in the world. Although developments in antimicrobial drugs therapy, these therapies associated with resistance and often infectious diseases are hard to treat. For over years, the step of development of novel antimicrobial drugs has slowed down when the prevalence of resistance has grown at a formidable rate, there have been interesting to use combination therapy (Usha et al., 2010; Aiyegoro et al., 2009). Since the Combination therapy can be used to expand the antimicrobial spectrum due to its role in preventing the germination of resistant mutants and minimizing the toxicity, thereby exhibiting antimicrobial activity greater than that would be expected from each antimicrobial drug individually (Si et al., 2008). Thus, the present study was undertaken to evaluate the Synergistic or antagonistic effects of black tea, green tea and coffee extracts with common antibiotics, which might provide natural solution for increasing some of antibacterial agents' effect.

Method

Bacterial Strain

The Escherichia coli was obtained from postgraduate student in biology department- College of science-Mustansiriyah University and diagnostic by morphological characteristic and biochemical tests beside to re-conform by API 20 E system. It was isolated from patient suffering Urinary tract infection; it has been cultured on nutrient agar plate and activated in Brain heart infusion broth then incubated for 24 h at 37°C. Bacterial strain was maintained on nutrient agar slant for 4-5 weeks and in LB broth supplemented with 20-25% glycerol at -20°C for long time preservation. Test bacterial isolate was sub-cultured onto fresh plates of nutrient agar at 37°C for 24 h. Colonies from these plates were suspended in Mueller-Hinton broth to a turbidity matching 0.5 mc McFarland standard (108 cfu/ml) for bacteria (McFarland, 1970).

Antimicrobial susceptibility test

Disc diffusion method was performed to determine the sensivity of *E.Coli* to Augmantin, Amikacin, Imipenem, Ciprofloxacin, Nitrofurantoin, Pincillin, Cefixime, Tetracyclin, Chloramphenicol and Ceftriaxone. The diameters of inhibition zone for individual antibiotics were measured to the nearest millimeters and values were interpreted as resistant and sensitive categories referring to interpretation table of national board for clinical laboratory standard recommended by National Committee for Clinical Laboratory Standards (Ferraro, 2001).

Preparation of aqueous extraction

The black tea, green tea, and coffee used in this study were brought from local markets in Baghdad. Aqueous extraction for above plants were prepared at 2.5, 5 and 7.5 % by mixing the required quantity of dry Leaves and coffee powder with distilled water, then heated with slowly at boiling degree 100 °C for 15 min. The solution was allowed to cool at room temperature for 1- 2 hours and then filtrated by Whatman filter paper no1; the supernatant was collected and used as crud extract and kept in refrigerator at 4 °C until used. 100 ml from all above extraction was mixed together with Mueller-Hinton agar powder and dissolved well and then sterilized by autoclave. The antibiotics discs were fixed on cultured plates and incubated for 24h at 37°C. The diameters of inhibition zone for single antibiotics were measured by millimeters and values were interpreted as resistant and sensitive categories referring to interpretation chart that recommended by National Committee for Clinical Laboratory Standards (Ferraro, 2001).The extract that decreased sensitivity activity it was categorized as antagonism effect, while the extract that gave enhanced of sensitivity greater than the control was considered Synergism.

Results and Discussion

The results are illustrated in Table 1. Showed that *E.coli* isolate was sensitive to Imipenem, Ciprofloxacin, ceftriaxone, Tetracyclin, Cefixime, Chloramphenicol and Augmantin respectively, while to amikacin and Nitrofurantoin was moderate, but against to Pincillin it was appeared resistance. This pattern was changed when the *E.coli* was cultured on different aqueous extraction plate agar for green tea, black tea and coffee which regulated with gradient concentration. The results were exhibit all concentration of green tea and Back tea appeared synergistic for Chloramphenicol and Nitrofurantoin susceptibility; but antagonistic effect was shown on Tetracyclin, Ciprofloxacin and amikacin activity. The inhibition effect of coffee was appeared strongly when the bacteria was cultured on 5 and 7.5% of coffee and no growth was appeared.

The increased level of inhibition zone diameter on combination of the aqueous extract with Chloramphenicol and Nitrofurantoin might be related to the presence of two binding sites on the bacterial surface for antibiotic and extract. Furthermore, the enhanced level might be explained by capacity of extracts to induced damage of the bacterial cell wall and the possible interference, resulting in the inhibition its biosynthesis (Esimone et al., 2006; Hosseini et al., 2006). The result in this study is in agreement with outcomes reported by Souto de Oliveira et al who reported the effect of combination of extraction plant with antimicrobial drugs on pathogenic bacterial growth, the synergistic activity of tetracycline, norfloxacin, and erythromycin combined with ethanol extract of Mangifera indica L. peel was investigated against different S. aureus strains. Single extract did not show considerable antibacterial activity at (MIC $\geq$ 2048 μg/ml), but it adjust the activity of antibiotics at (MIC = 512 μg/ml) when combined with antibiotics, a four-fold drooping in the MIC values for erythromycin and

tetracycline was detected. The study demonstrates that mango peel may act as a source of potential in enhancement of antibiotics, which adds significant value to mango by-product. Toroglu who proved that the effective of various spices and herbs include Coriandrum sativum, Rosmarinus officinalis, Cumium cyminum, Micromeria fruticosa L.,and Mentha piperita were combined with different antibiotics such as cephalothin, gentamicin, ceftriaxone , nystatin ; these combination were investigated against thirty bacterial species. The results were pointed that the combination of antibiotics with plant extract further decreased drug resistance. The synergistic effects acquired may progression to new choices for the treatment of diseases that caused by pathogens (Toroglu et al., 2000;.Falcão-Silva et al., 2000).

Table 1. Combination effect of salts with antibiotics

Antibiotic	control mm on Mueller Hinton Agar	Green tea%			Black tea %			Coffee %		
		2.5	5	7.5	2.5	5	7.5	2.5	5	7.5
Imipenem(10ug/ml)	30	33	36	34	27	24	26	28	No growth	No growth
Chloramphenicol(30ug/ml)	22	26	25	27	25	24	33	26	No growth	No growth
ceftriaxone(5ug/ml)	30	27	40	30	30	27	35	39	No growth	No growth
Cefixime(30ug/ml)	24	25	35	35	24	25	32	35	No growth	No growth
Augmantin(30ug/ml)	21	22	25	30	20	25	30	30	No growth	No growth
Nitrofurantoin(300ug/ml)	17	27	35	25	25	26	33	30	No growth	No growth
Penicillin(10ug/ml)	9	12	11	19	12	17	20	8	No growth	No growth
Tetracyclin(30ug/ml)	29	13	9	10	19	19	14	20	No growth	No growth
Ciprofloxacin(5ug/ml)	30	8	7	9	11	8	0	14	No growth	No growth
amikacin(30ug/ml)	16	10	10	16	10	8	0	8	No growth	No growth

Synergism Antagonism

Conclusion

In conclusion, the combination of aqueous extract of green tea, black tea and coffee with different antibiotics may increase their synergistic effectiveness resistance against pathogenic E.coli but other antibiotic was reduced their effectiveness This study has suggested use these extraction during infection with antibiotics.

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