



Synergistic effect of green tea and corn hair extract on some pathogenic microorganisms

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Abstract

This study investigates antibiotics or enhances the activity of exciting antimicrobials. Concentrations (200, 100, 50, 25, 12.5, 6.25, 3.121, and 1.56 mg/ml) of green tea (GT) and corn hair (CH) water extracts alone and mixed, were investigated against *Klebsiella pneumonia*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Listeria monocytogene*, and *Candida krusi*. GT shows the best antibacterial activity against *L. monocytogene* 26 and 24 mm by 200 and 100 mg/ml. Mix enhances antibacterial activity to 35 mm against *K. pneumonia* and 33 mm on *P. aeruginosa* using 25 mg/ml from the mixture. Synergistic effect of a mixture (GT and CH) with antibiotics, Ciprofloxacin (Cipro) and Erythromycin (Eryc), was also studied. Mix increased the antibacterial activity of *Cipro* to 38 mm against *K. pneumonia* and *P. aeruginosa*, compared to 18 and 17 mm used alone. Also, the extract mix raised *Eryc* antibacterial activity to 28 and 29 mm, on *K. pneumonia* and *L. monocytogene*, compared to 17 mm used alone. In conclusion, activity of commercial antibiotics can be enhanced by synergistic effect of plant extracts.

Keywords: AMR, Green tea, Corn hair, Antimicrobial effect, Synergistic effect

Introduction

Antimicrobial resistance (AMR) is considered a major health problem issue of our time, and it can spread over international boundaries easily (Naylor et al., 2018). Antibiotics have since it discovered it used to kill or inhibit the growth of bacteria (Gallagher and MacDougall, 2022). For decades antibiotics saved lives and played a major role in changing medicine and surgery forever (Ventola, 2015). Most antibiotics currently used are produced naturally or from natural product derivatives (Ayaz et al., 2019). But antibiotics misuse, overuse, using broad-spectrum antibiotics and using antibiotics in animal foods all these reasons cause the growth of AMR problems (Dabour et al., 2016). Weak or sensitive bacteria are killed or inhibited by antibiotics. Still, resistant

bacteria have the ability to survive and grow, so the new generation may have a great probability of being resistant (Prestinaci et al., 2015). The mechanism of AMR varied among microorganisms and between strains. Some of them used enzyme production, antibiotic-resistant genes, quorum sensing, or multidrug efflux system (Frieri et al., 2017). According to WHO (world health organization) and the UN (United Nations), AMR causes millions of deaths each year, and by 2050, the deaths will be 350 million people (Chanel and Doherty, 2020; Samuel, 2019).

As a new strategy to over AMR, two or more antibiotics combined synergistic effects were studied to obtain a higher impact against acute bacterial infections (Bollenbach, 2015). Medicinal plants have been considered one of the wealthiest

bioresources for drugs since ancient times, and still this time were previously studied in many research (Al-Bakri et al., 2021) and it was found that plant extracts have significant results on several bacterial species, including the resistant bacteria Methicillin-Resistant *Staphylococcus aureus* (MRSA) (Abed et al., 2019). For these reasons, the research on the antibacterial effects of medicinal plants continues. In this study, the antibacterial and antifungal effects of green tea and corn hair water extract will examine twice, once separately or individually and once mixed together. Also, the synergistic effect of these plant extracts will evaluate by mixing them with well-known antibiotics (Ciprofloxacin and Erythromycin). Green tea (*Camellia sinensis*) is one of the tea types according to fermentation degree, it saves more natural compounds in fresh leaves than other types of tea (Xu et al., 2019). The antibacterial activity of green tea was studied in many previous types of research, where it was found to inhibit *Helicobacter pylori*, *Mycobacterium tuberculosis*, *E. coli*, *Staphylococcus aureus*, *Salmonella typhimurium*, and others (Anand et al., 2006; Yee et al., 2000; Hamilton-Miller, 1995).

Furthermore, corn hair (corn silk) is the stigma (female flower) of *Zea mays L.* corn hair is considered a by-product that is usually ignored as agricultural waste or same time, used as food for animals (Aukkanit et al., 2015). Corn hair is locally operated on to treat several diseases, such as inflammation in the urinary bladder and prostate and irritation within the urinary system (Abirami et al., 2021). According to Nessa and colleagues (2012), different extracts of corn hair were found to expose antimicrobial activity against various pathogenic bacteria and fungi. Therefore, this study aims to investigate finding new antibiotics or enhancing the antimicrobial activity of the exciting antimicrobial.

Materials and methods

Plants extraction

Green tea and corn hair were used in this study. These plants were obtained from the local market in Al Samawah city/ Iraq.

(Khan, 2014). The antibacterial effects of medicinal plants The plants were powdered using an electric grinder. To 50 g from each plant, 350 ml from distal water was added, then boiled for 15 min. The extract was filtered and placed at room temperature until complete dryness (Handa et al., 2008). The crude water extract of green tea (GT) and corn hair (CH) was stored at four °C until use. Then serial dilutions (200, 100, 50, 25, 12.5, 6.25, 3.121, and 156 mg/ml) were prepared from each extract. For preparing the mixture, 2 ml from each extract is mixed well using a vortex.

Microorganisms

The microorganism was isolated from the previous study in the microbiology lab/ Biology department/ college of science/ Al Muthanna university. The bacteria extend spectrum B-Lactamase *Klebsiella pneumonia* and *Escherichia coli*. Also, *Pseudomonas aeruginosa*, *Listeria monocytogene*, and *Candida krusi* were used in this study. The bacterial culture was activated in brain-heart infusion broth overnight.

Antibacterial activity

For each bacterium and fungi suspension, 1x10⁸ cells/ml was calculated by McFarland standard, then 0.1 ml from each suspension was taken and spread on Muller Hinton agar using a class spreader. To study the effect of each plant extract effect on bacteria and fungi individually and in the mixture, filter paper disks saturated with the prepared concentrations of plant extracts by adding 0.5 ml of extract (GT and CH) to 10 sterilized disks in the container. Also, Ciprofloxacin (Cipro), Erythromycin (Eryc), and Amphotericin-B (Amph-B) discs were used in this study to evaluate the synergistic effect of the plant extract mixed with these well-known antibiotics (Kilani et al., 2005), the combination of antibiotic and Mix (GT+CH) prepared by adding 1.5ml from Mix (best result concentration) to antibiotic disk in the container. After that, the culture was incubated at 37°C for 24h, and the inhibition zone (IZ) was taken by diameter ruler for each extract and concertation.

Table 1. The inhibition zone (mm) results from different concentrations of GT water extract on pathogenic microorganisms

Microorganisms	GT water extract concentration mg/ml							
	200	100	50	25	12.5	6.25	3.121	1.56
<i>K. pneumonia</i>	24	20	19	17	14	0	0	0
<i>P. aeruginosa</i>	24	18	17	15	12	10	0	0
<i>E.coli</i>	24	20	19	17	14	10	9	8
<i>L. monocytogene</i>	26	24	18	16	11	10	10	0
<i>C. krusi</i>	18	17	15	12	9	0	0	0

Table 2. The inhibition zone (mm) results from Different Concentrations of CH Water Extract Against Pathogenic Microorganisms

Microorganisms	CH water extract concentration mg/ml							
	200	100	50	25	12.5	6.25	3.121	1.56
<i>K. pneumonia</i>	14	13	13	12	11	12	8	0
<i>P. aeruginosa</i>	10	9	7	6	5	0	0	0
<i>E.coli</i>	9	8	7	7	5	0	0	0
<i>L. monocytogene</i>	11	10	9	8	8	6	0	0
<i>C. krusi</i>	5	4	0	0	0	0	0	0

Results and Discussion

In the present study, green tea and corn hair water extract were examined for their antimicrobial effect and to evaluate the synergistic effect of both extract mixtures combined with existing antibiotics. First, we determined the antimicrobial of GT water extract against the studied pathogenic bacteria and fungi, as shown in Table 1. A more significant antimicrobial effect was found in the high concentration (200 mg), and it is more effective against *L. monocytogene* by IZ, reaching 26 mm. Low concentration didn't expose an antimicrobial effect. Furthermore, Table 2 revealed the results of the CH water extract. Generally, the product was weaker than the GT extract, which gave 14 mm as the highest IZ against *K. pneumonia*.

Additionally, Table 3 shows the mixture (GT+CH) antimicrobial effect. The results of the Mix were increased

more than the separate effect of GT and CH. The highest antimicrobial was exposed in 25 mg concentration, reaching 35 mm against *K. pneumonia*. The final part of the current study evaluated the synergistic effect of the Mix combined with the antibiotics. Results indicated in Table 4 were just the best concentration of Mix taken 25 mg and mixed with the antibiotics. Also, the effect of antibiotics was determined. The IZ of the antibiotic was clearly increased by combined with Mix, where Cipro gave 18 and 17 mm when used alone with *K. pneumonia* and *P. aeruginosa*, respectively. In contrast, when combined with Mix, the same antibiotic gave 38 mm, against both mentioned bacteria. Similarly, Eryc antibiotic effect increased from 17 mm to 28 and 29 mm against *K. pneumonia* and *L. monocytogene*, respectively. But the synergistic effect of a Mix with Amph-B against *C. krusi* was ineffective.

Table 3. The inhibition zone (mm) results from Different Concentrations of Mixture GT +CH Against Pathogenic Microorganisms

Microorganisms	Mix (GT+ CH) water extract concentration mg/ml							
	200	100	50	25	12.5	6.25	3.121	1.56
<i>K. pneumonia</i>	24	22	22	35	20	18	16	12
<i>P. aeruginosa</i>	25	22	18	31	15	14	12	11
<i>E.coli</i>	28	27	24	33	18	18	17	14
<i>L. monocytogene</i>	25	24	22	28	16	14	12	10
<i>C. krusi</i>	28	25	20	20	17	16	15	13

Table 4. Synergistic Effect of Mix With Antibiotic Against Microorganisms

Microorganisms	Mix	Cipro	Eryc	Amph-B	Mix+ Cipro	Mix+ Eryc	Mix+ Amph-B
<i>K. pneumonia</i>	35	18	17	-	38	28	-
<i>P. aeruginosa</i>	31	17	14	-	38	22	-
<i>E.coli</i>	33	19	16	-	28	25	-
<i>L. monocytogene</i>	28	19	17	-	31	29	-
<i>C. krusi</i>	28	-	-	15	-	-	8

Green tea is considered one of the highest plants in phytochemicals content, including epigallocatechin-3-gallate (EGCG), epicatechin, epicatechin-3-gallate, epigallocatechin (Butt and Sultan, 2009). Caffeine and L-theanine are also found at a high level in green tea components (Dietz and Dekker, 2017). The antibacterial activity of green tea was reported in numerous previous studies. The recent one found a synergistic effect of green tea extract with antibacterial ciprofloxacin against (MRSA) (Dicle et al., 2022). Furthermore, the synergistic antibacterial activity of phytochemicals from green tea, such as catechin and Alkyl gallates, were reported with ciprofloxacin and B-lactam antibiotic (Lee et al., 2005; Shibata et al., 2005). Previously, the mechanism of antibacterial properties of catechin was found to be related to reducing membrane fluidity (Tsuchiya, 1999). Conversely, corn hair is reported to contain a high level of polyphenols, flavonoids, and ascorbate. Therefore, it's considered an important natural source for many pharmaceutical purposes (Singh et al., 2022).

The medical properties of medicinal plants result from the joint activity of a group of phytochemicals (Chen et al., 2007). Many works reported that secondary metabolites could inhibit drug efflux pumps that are used by bacteria to resist antibiotics. These compounds include alkaloid lysergol, flavonoids rotenone, chrysin, and carotenoids capsanthin (Cushnie et al., 2014; Molnar et al., 2010). Additionally, primary metabolites that destroy antibiotic-resistant, such as mannitol, fructose, and glucose were found to lower the antibiotic resistance of bacteria and make them more sensitive to being killed by antibiotics, especially aminoglycoside (Allison et al., 2011).

Conclusion

The results of the current study proved the antibacterial of green tea and corn hair against the studied microorganisms. Also, the synergistic effect of the mixture of the two-extract work to raise the level of effectiveness of both extracts. Furthermore, the mix of green tea and corn hair water extract made a clear difference in the action of antibiotics combined. These results strongly indicate the development of highly effective antibacterial agents by combining them with natural compounds that are safe to use to counteract the AMR problem. Further studies will be established to screen more synergistic effects of other plant extracts against antibiotics.

Conflict of Interest

The author hereby declares no conflict of interest.

Consent for publication

The author declares that the work has consent for publication.

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