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Curcuma longa Ticari Ekstresinin In-Vitro Antibakteriyel Aktivitesinin Araştırılması

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ÖZET

Patojen mikroorganizmalara karşı kullanılan antibakteriyel maddelere direncin artması ve gıda katkı maddelerinin istenmeyen yan etkileri nedeniyle, son yıllarda yeni bitki kaynaklı antibakteriyel madde arayışı artırmıştır.

Bu çalışmada, Hindistan'dan temin edilen *Curcuma longa* bitkisinden hazırlanan ticari ekstrenin, nozokomiyal enfeksiyonların etiyolojisinde de yer alan Genişlemiş Spektrumlu Beta Laktamaz (ESBL) üreten *Escherichia coli, Klebsiella pneumoniae, Pseudomonas aeruginosa, Enterococcus faecalis* ve Metisilin Dirençli *Staphylococcus aureus* (MRSA) üzerine, antibakteriyel aktivitelerinin disk diffüzyon ve minimum inhibitör konsantrasyonlarının broth mikrodilüsyon yöntemleriyle araştırılması amaçlandı.

Disk diffüzyon sonuçlarına göre, 6.24 mg/disk konsantrasyonundaki ekstrenin sadece *S.aureus* 'a karşı üreme inhibisyon aktivite belirlendi.

Broth mikrodilüsyon yöntemiyle ise, *Curcuma longa* ekstresinin bakteriler üzerinde farklı düzeyde minimum inhibitör konsantrasyona (4.87-78 mg/ml) sahip olduğu belirlendi. Her iki yöntemde de *Curcoma longa* ekstresinin uygulanan eşit konsantrasyonlarda sonuçlar uyumlu çıkarken, en düşük MİK değeri *S.aureus* için, en yüksek MİK değeri ise *E.coli, K. pneumoniae* ve *E. faecalis* türlerine karşı belirlendi.

Sonuç olarak, *Curcuma longa* ekstresinin sağlık, farmasötik, kozmetik ve gıda endüstrisi gibi birçok alanda başta MRSA olmak üzere *E. coli, K. pneumoniae, P. aeruginosa, E. faecalis* türlerine karşı etkili olabileceği ve bu konuda yeni in vivo çalışmalara ihtiyaç olduğu düşünülmektedir.

Anahtar kelimeler: Broth mikrodilüsyon, Disk difüzyon, İnhibitör ve Nozokomiyal.

Investigation of In-Vitro Antibacterial Activity of Curcuma longa Commercial Extract

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ABSTRACT

New plant-derived antibacterial agent researches have increased in recent years, because of increased resistance to antimicrobial agents that used against to pathogen microorganisms, and unwanted side effects of food additives.

The aim of this research was to investigate the, in-vitro antibacterial activities and minimal inhibitory concentration of *Curcuma longa* which obtained from India, commercial plant extracts against some nosocomial pathogens (Extended Spectrum Beta Lactamase *Escherichia coli*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Enterococcus faecalis*, Methicillin Resistant *Staphylococcus aureus)*, by disk diffusion and broth microdilution methods.

The diameter of the inhibition zone in the disc-diffusion method shows that *Curcuma longa* extract (6.24 mg/disc) has antibacterial activity only on MRSA (Inhibition zone diameter 8 mm). However, with broth micro dilution method, *Curcuma longa* extract had minimal inhibitor concentration (4.87-78 mg/ml) on all bacteria in different levels. *Curcuma longa* extract showed the minimum MIC value on *S.aureus*, maximum MIC value on E.coli, *K.pneumoniae and E. faecalis.* The results were consistent in both methods at equal concentrations applied from the *Curcuma longa* extract.

In conclusion, particularly *Curcuma longa* leaf extract is thought to be effective against to MRSA, *E. coli*, *K. pneumoniae*, *P. aeruginosa*, *E. faecalis* in many areas like medical and food industries. However, there are need new *in vivo* studies in future.

Keywords: Broth microdilution, Disk diffusion, Inhibition and Nosocomial

INTRODUCTION

Throughout history, plants have been used to treat many diseases. In the present day, many countries continue to be commonly used for treatment and new plants are added to this goal day by day. The majority of medicines used for treatment are of plant origin and very few are used as antimicrobial agents. It has been observed in recent years that studies have increased on antimicrobial activities of plants for the development of alternative medicines against microorganisms (17).

The use of plant extracts and phytochemicals as antimicrobials are the best-known material in therapeutic treatments. Recent studies about antibacterial activity were related to secondary metabolites, which synthesized during the metabolism of plants (1, 5).

Research on the biological activities of plant extracts has accelerated due to the fact that plant-based therapeutic agents have natural and less side effects, inexpensive, exhibit synergistic effects, easily biodegradable and increased resistance to antibiotics.

Curcuma longa is a yellow-flowered, large-leafed perennial plant in *Zingiberaceae* family that grows in Asia's tropical regions, especially in Pakistan, India, China and Bangladesh. It is commonly known as 'turmeric'. A polyphenolic flavonoid Curcumin, is the most important active component in *Curcuma longa*. Curcumin has been reported to be used as spice and food coloring (food additive number E100). Also curcumin uses in

the treatment of acute and chronic diseases like inflammatory bowel diseases, diabetes and asthma. And curcumin has been reported to have antibacterial activity against various bacteria such as *Helicobacter pylori, Vibrio vulnificus,* and *Pseudomonas aeruginosa.* However, there are not many studies about the antibacterial activities of *C. longa* extract against multiple antibiotic resistant bacteria (7, 8, 12, 13, 15 and 18).

Because of its several efficiencies for health, we aimed to investigate the *in-vitro* antibacterial activity of commercial turmeric extracts against Extended Spectrum Beta Lactamase (ESBL) producing *Escherichia coli*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa Enterococcus faecalis* and *Methicillin Resistant Staphylococcus aureus* (MRSA) with disc diffusion and minimal inhibitory concentration (MIC) tests.

MATERIALS and METHODS

In this study, commercial ethanol-extracts of *Curcuma longa* (CI) were used. Commercial plant extracts prepared with the active ingredient content of 312 mg/ml (w/v) were sterilized using 0.45 µm filters. Until antibacterial activity tests were carried out in the sterile samples were labeled to put 1.5 ml eppendorf tubes and stored in the refrigerator at + 4 ° C.

Bacterial strains

The ethanolic extracts of *Curcuma longa* were individually tested against a panel of nosocomial infections agents including gram-negative Extended Spectrum Beta Lactamase (ESBL) producing *Escherichia coli* (ATCC 35218), *Klebsiella pneumo*niae (ATCC 700603), *Pseudomonas aeruginosa* (ATCC 27853) and gram positive *Enterococcus faecalis* (ATCC 29212) and Methicillin Resistant *Staphylococcus aureus* (MRSA) (isolate of clinic). Bacterial strains were cultured 24 h at 37 ° C in nutrient agar (Merck-Cat No. 105450).

Disc Diffusion Test

Disc diffusion test was performed according to Clinical and Laboratory Standards Institute (4). The bacterial suspensions whose turbidity was adjusted to McFarland 0.5 (~10^8 cfu/ml) in Mueller-Hinton Broth (Merck-Cat No. 110293), were spread over a Mueller-Hinton agar (Merck-Cat No. 105437) surface. In the following phase, the discs containing maximum 20 µl (6,24 mg/disk) of plant extracts were dried at 30 °C and placed on an agar surface by sterile forceps. Gentamycine (10 µg/disc) (Bioanalyse, Cat No. ASD04301) discs were used as reference antibiotics and 5% DMSO were used negative control. The medium was incubated at 37°C for 24 hours and in the end the zones of inhibition greater than 7 mm was recorded. The experiments were repeated three times, and the mean and standard deviation were calculated.

Determination of minimum inhibitory concentration (MIC)

MICs of the extracts against the test organisms were determined by broth microdilution method. All the tests were performed in duplicate and repeated twice. Broth microdilution test was performed according to Clinical and Laboratory Standards Institute (3). All wells of 96-well microplates were loaded with 100µl cation-adjusted Mueller-Hinton broth (BBL-Cat No. 296164). Then 100 µl of the previously prepared sterile plant extract (312 mg / ml) was added to the first line of wells and two-fold serial dilutions were made to obtain *Curcuma longa* extract concentrations between 156 mg / ml and 2.43 mg / ml.

Bacterial suspensions were added to all wells to a final density of $5x10^5$ cfu/ml and these were confirmed by viable counts. A well without a plant extract was reserved for growth control of each bacteria and another well without containing bacteria or a plant extract was reserved for sterility control. To ensure bacterial concentration, 0.1 ml of nutrient agar was inoculated with a 1: 1000 dilution from the reproductive control well. The MIC of Gentamycine (40µg/ml) (Sigma Cat no. G1272) was individually determined in parallel experiments in order to control the sensitivity of the test organisms. The micropleyts were incubated at 37 ° C for 24 hours. In addition, 50 µl of 1 % 2,3,5-Triphenyl-tetrazolium chloride solution (Sigma- Cat no. 17779) was added to each of the well, the plates were incubated for 30 min at 37 ° C. The MIC values were verified depending on the occurrence of color change (6, 14).

RESULTS

Disc diffusion test results

According to the disc diffusion test, the inhibition zone diameter of five bacteria species exposed to *C. longa* plant extracts are indicated in Table 1. According to the size of the inhibition zone diameter, *Curcuma longa* extract was found to have antibacterial activity only on *S. aureus*. ESBL *E. coli*, *K. pneumoniae*, *P.aeuroginosa* and *E. faecalis* were found to be resistant to the applied disc dose (6.24 mg / disc).

Table 1. Growth inhibition zone diameters (mm) created by plant extracts according to disc diffusion test

Plant Extract (6,24 mg/disc)	**Antibacterial activity zone diameters (mm)**				
	E.coli*	***P.aeuroginosa***	***K. pneumoniae****	***S.aureus*****	***E.faecalis***
Curcuma longa	R	R	R	8±0,47	R
Gentamycine (10 µg/disk)	18±1,69	19±0,47	17±0,81	20±1,24	17±1,63

*ESBL, ** Methicillin resistance, R: Resistant**,** Values; mean ± standard deviation.

Microdilution test results

For the broth microdilution method, the MIC values of plant extract (312 mg/ml) against five different bacteria are indicated in Table 2. It was determined that the MIC value of *C. longa* plant extract was 4,87 - 78 mg/ml against five different bacteria. *C. longa* plant extract was determined the lowest MIC value of on *MRSA* and was found to be the highest MIC value on *E.coli, K.pneumoniae* and *E.faecalis*.

Table 2. Minimum Inhibitor Concentration (MIC) values according to broth microdilution method

Plant Extract	**Minimum Inhibitor Concentration (MIC) values (mg/ml)**				
	E.coli*	***P.aeruginosa***	***K.pneumoniae****	***S.aureus*****	***E.faecalis***
Curcuma longa	78	19,5	78	4,87	78
Gentamisin (40µg/ml)	0,0025	0,005	0,0025	0,005	0,005

*ESBL, ** Methicillin resistance,

DISCUSSION and CONCLUSION

According to the results of disc diffusion studies, it was determined that *Curcuma longa* (*CI*) extract showed antibacterial activity against only MRSA at 6.24 mg/disc concentrations. According to broth microdilution study results; *CI.* was found to have antibacterial activity on all bacteria at different concentrations in the range of 4.87-78 mg / ml. According to MIC values *S. aureus* was the most sensitive bacteria and *E.coli, K.pneumoniae* and *E.faecalis* bacteria were observed to be the highest MIC values.

In both methods, it was determined antibacterial activity on *S. aureus* at a concentration of 6.24 mg/ml, but undetermined against other bacterial strains. When the doses were equal in the two test, antibacterial activity was determined to be the same. Thus, it was seen that the two methods support each other.

As a result of the broth microdilution test of the *Cl.* extract, the MIC values were determined 4.87 mg/ml for *MRSA*, 19.5 mg/ml for *P. aeruginosa*, 78 mg/ml for *E. coli, K.pneumoniae* and *E. faecalis*. In the researches related to *Cl.* extract; Singh *et al.* (19) reported that *S. aureus, E. coli* and *P. aeruginosa* are susceptible to different concentration of *Cl.* extracts. Saleem et al. (16) reported that *Cl.* extract showed antibacterial activity against *MRSA, E.coli*, vancomycin-resistant *E. faecalis (VRE)* and *P. aeruginosa.*

Chakraborty et al. (2) reported that *Cl.* ethanol extract (30 mg/disk) had antibacterial activity to *K. pneumoniae* (16.5 mm), *S.aureus* (15.6 mm), *P.aeuroginosa* (10.4 mm) and *E. coli* (10.3 mm) in disc diffusion method and MIC values of 17,63 µg/mL, 20,32 µg/mL, 18,90 µg/mL and 34 µg/mL in microbroth dilution method, respectively. Marasini et al. (11) reported that MIC values of *Cl.* ethanol extract were determined against to 6.25 mg/ml for *E. coli* and *MRSA*, and 0.098 mg / ml for *E. faecalis*.

Previous studies on the antibacterial activity of these plant extract has often found similar antibacterial activity, but have been to determine different results, especially in MIC values. These differences thought to be related to the changes in the chemical substances in the contents depending on the extraction method, the antimicrobial activity test used, the dose applied, the geographical characteristics of the plant, the developmental stage and the extraction process (9, 10, 11).

As a result of this research; it was thought that the *Cl.* plant extract could be use as an antibacterial agent in many fields such as health, pharmaceutical, cosmetic and food industries. But primarily it has to be supported by in vivo and toxicity studies.

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