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Research Article

The Antioxidant, Antimicrobial, and Total Phenolic Potential of Clove Extracts for Inhibition of Food Pathogens

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Abstract

Food spoilage pathogens cause food waste and consumption of pathogen-contaminated food threatens human health. New approaches that do not harm the environment are needed for decreasing the enlargement of pathogenic microorganisms without using chemical preservatives. The current work intended to appraise the TPC value, anti-oxidant, anti-bacterial, and anti-fungal properties of clove different extracts. The antimicrobial tests were evaluated by disc diffusion, MIC, and MBC tests. Antioxidant potential was conducted using ABTS• and DPPH• radical, and TPC was tested by the Folin–Ciocalteu reagent assay. As a result, methanol extract was found to have the highest antimicrobial activity against *E. faecalis* by 19,30±0,17 mm zone diameter. The lowest activity was obtained from aqueous extract over *S.* Typhimurium by 7,17±0,29 mm zone diameter. MIC and MBC results were examined, and it was determined that clove ethanol extract showed the highest MIC value was 2.5-10 mg/ml. MBC test results also revealed that cloves ethanol extract has the highest activity with 5->10 mg/ml. The antioxidant data of cloves were examined, and the highest DPPH• and ABTS• sweep activities were determined in 60.93±1.67% aqueous and 85.81±1.08 ethanol extract, respectively. The TPC results revealed that the highest content was provided from the aqueous extract with 189.84±2.84 mg/g GA. The results gained from the study bring to light that clove has a high potential for antimicrobial, antioxidant, and total phenolic content.

Keywords: Clove, antimicrobial, antioxidant, total phenolic content

Gıda Patojenlerinin İnhibisyonu için Karanfil Ekstrelerinin Antioksidan, Antimikrobiyal ve Toplam Fenolik Potansiyeli

Öz

Gıda bozan patojenler gıda israfına neden olmakta ve patojenle kontamine gıdaların tüketimi insan sağlığını tehdit etmektedir. Patojen mikroorganizmaların çoğalmasını kimyasal koruyucu kullanmadan azaltmak için çevreye zarar vermeyen yeni yaklaşımlara ihtiyaç vardır. Mevcut çalışma, farklı karanfil ekstraktlarının toplam fenolik içeriğini (TPC), antioksidan, anti bakteriyel ve anti fungal özelliklerini değerlendirmeyi amaçlamıştır. Antimikrobiyal aktiviteler disk difüzyon, MİK ve MBC testleri ile değerlendirilmiştir. Antioksidan potansiyeli, ABTS• ve DPPH• radikali kullanılarak gerçekleştirildi ve TPC, Folin–Ciocalteu reaktif tayini ile test edilmiştir. Sonuç olarak, en yüksek antimikrobiyal aktivite 19,30±0,17 mm zon çapı ile *E. faecalis*'e karşı metanol ekstraktından, en düşük aktivite ise 7,17±0,29 mm zon çapı ile *S.* Typhimurium üzerinden sulu ekstreden elde edilmiştir. MİK ve MBC sonuçları incelendiğinde, karanfil etanol ekstraktının en yüksek MİK değerini 2,5-10 mg/ml gösterdiği belirlendi. MBC test sonuçları da karanfil etanol ekstraktının 5->10 mg/ml ile en yüksek aktiviteye sahip olduğunu ortaya koymuştur. Karanfilin antioksidan verileri incelendiğinde en yüksek DPPH• ve ABTS• süpürme aktivitelerinin sırasıyla %60.93±1.67 sulu ve %85.81±1.08 etanol ekstraktında belirlendiği saptanmıştır. TPC sonuçları açısından en yüksek içeriğin 189.84±2.84 mg/g GA ile sulu ekstresinden sağlandığı ortaya koyulmuştur. Çalışmadan elde edilen sonuçlar, karanfilin antimikrobiyal, antioksidan ve toplam fenolik içeriği açısından yüksek bir potansiyele sahip olduğunu ortaya koymaktadır.

Anahtar Kelimeler: Karanfil, antimikrobiyal, antioksidan, toplam fenolik içerik

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1. Introduction

Worldwide, even in developed countries, microbial spoilage remains a persistent problem affecting many food products [1]. Various factors such as microbial spoilage, are estimated to account for annual global food losses of up to 44% [2]. Bacteria, yeast, and mold are liable for the spoilage of significant numbers of food and food products [3]. In addition to food losses, contaminated food consumption caused foodborne illnesses, and this situation has become an important safety concern for public health [4]. Conventional pasteurization methods are ineffective in eliminating these microorganisms, which can withstand harsh conditions commonly employed for food preservation [5]. Therefore, consumers are concerned about the health risks associated with synthetic additives, which has led to a dropping in the use of these chemicals for food conservation [6]. Thus, there is a demand for novel sustainable approaches to curb the proliferation of pathogenic bacteria while avoiding the use of chemical preservatives, and to extend the safe consumption period of food products, ultimately increasing their storage life. Therefore, the importance of plant-based food preservatives that can serve as alternatives to chemical preservatives and pose lesser risks to public health has become even more significant.

Although not officially recognized by several nations' regulations, the utilization of traditional remedies has become a settled aspect of the customs of various populations. In less developed regions, herbal medicines are frequently resorted to by a significant percentage of individuals [7]. The employment of traditional medicinal productsmarked upswing in developed countries in recent years because of concerns regarding the adverse effects and the efficacy of synthetic drugs [8, 9]. Natural sources with significant antioxidant potential such as medicinal plants, herbs, spices, and oily seeds, are rich in bioactive compounds, including flavonoids, curcuminoids, tannins, terpenoids, and lignans [10].

Clove-like spices have been used for centuries as food preservatives and medicinal plants due to their biological activities [11-13]. Numerous studies have reported that spice plants have antibacterial [14-16], antifungal [17], antiviral [18], antioxidant [19], and anticarcinogenic [20] properties. In particular, clove has drawn attention among other spices due to its strong antioxidant and antimicrobial activities [21].

This study explores the antimicrobial, antioxidant activity, and total phenolic content of the different clove extracts (*Syzygium aromaticum* L.) against food spoilage pathogens. The study aims to provide insights into the potential use of clove extracts as alternative preservatives for foodborne pathogens and their potential benefits in protecting public health against foodborne diseases.

2. Material and Methods

2.1 Plant Sample and Extraction

Clove (*S. aromaticum* L.) as the plant material was commercially obtained from local herbalists and identified by Öğr. Gör. Dr. Olcay Ceylan. The extraction process of the plant sample was performed as described by Okmen et al. [22]. Firstly, the obtained dry material was pulverized into a powder using a blender (Fakir, Türkiye). After pulverization, the samples were preserved in the dark at 4°C until extraction. Then, 50 g of the pulverized samples were weighed (Seles) and placed into a Soxhlet apparatus (Isotex) for extraction using methanol, ethanol, and aqueous solvents (250 ml) for 4 to 8 hours. The extracted samples of ethanol and methanol were dried using a rotary evaporator (Broen) at 45°C. However, the aqueous extract of clove was kept in a fume hood until the solvent evaporated, and the dried extracts were stored at 4°C in sterilized falcon tubes until analysis.

2.2 Bacterial and Fungal Strains

Eight foodborne pathogenic microorganisms were used in this study, including three gram-negative, and four gram-positive bacteria and one yeast strain: Gram-negative, *Escherichia coli* ATCC11229, *Salmonella* Typhimurium RSKK19, *Yersinia enterocolitica* NCTC11174, Gram-positive, *Bacillus subtilis* RSKK245, *Staphylococcus aureus* RSKK2392, *Enterococcus faecalis* ATCC8093, *Listeria monocytogenes* ATCC7644, and *Candida albicans* RSKK02029 used as yeast strain.

2.3 Culture Condition of Microorganisms

The bacterial strains intended for use in the antimicrobial activity assays were cultured at 37°C for 24 hours in Nutrient Broth (Merck) medium. The yeast strain *C. albicans* was cultured in Sabouraud Dextrose Broth (Merck) medium at 30°C for 24-48 hours (Nüve EN400) [26].

2.4 Analysis of Antimicrobial Properties

The antimicrobial efficiency of clove was analyzed using three methods: disk diffusion method, Minimum Inhibitory Concentration (MIC), and Minimum Bactericidal Concentration (MBC). The disk diffusion method was performed according to the Bauer-Kirby [24] method and as detailed by Okmen et al. [25]. The density of microorganisms, which were grown in a liquid medium 24 hours before the study, was adjusted to 0.5 McFarland, and 0.1 ml was inoculated onto the plates under aseptic conditions. Then, empty disks (6mm) (Bioanalyse) were soaked with 25 µl plant extracts and placed on the plate surface. Bacteria were incubated for 24 hours at 37°C on Mueller Hinton Agar (MHA, Merck) plates, and yeast was incubated for 24 hours at 30°C on Sabouraud Dextrose Agar (SDA, Merck) plates to ensure their growth. After 24 hours, the zone diameters were measured in millimeters. Solvents (ethanol, methanol, and aqueous) were used as negative controls, and ampicillin (10 µg) antibiotic for bacteria and nystatin (100 U) antibiotic for yeast were included as positive controls [25]. The MIC and MBC analyses were performed using the method described by Ökmen et al. [26] with slight modifications. For the MIC test, the final concentration of each extract was adjusted to 10, 5, 2.5, 1.25, and 0.625 mg/mL, and the concentration at which no growth was observed was

determined as the MIC. A loop-full sample was taken from the tubes in which MIC and above MIC concentrations were tested and inoculated onto Nutrient agar (NA, Merck) plates. Concentrations at which visible growth was not observed were recorded as the MBC.

2.5 Antioxidant Assay

The non-enzymatic antioxidant activity of the extracts was determined using two methods, namely the 1,1-diphenyl-2-picrylhydrazyl (DPPH) and 2,2'-azino-bis (3-ethyl benzothiazoline-6-sulfonic acid) (ABTS) assays. For the DPPH assay, stable DPPH was used as a free radical scavenger to measure the antioxidant activity of the extracts. A solution of methanol and DPPH (2.9 ml of 0.1 mM) was incubated with the extract (0.1 ml) in the dark for 30 minutes, and the absorbance was measured by spectrophotometry. Methanol was used as the blank, and methanol with DPPH was used as the control (Brand-Williams et al. [27]. Ascorbic acid was used as a positive control and Trolox as a standard. The percentage of inhibition was calculated by equation 1.

ABTS assay was slightly modified from previously reported methods to quantify the non-enzymatic antioxidant activity of the extracts [28]. Briefly, a mixture of 7 mM ABTS solution and 2.4 mM potassium persulfate solution was left in darkness for 12 to 16 hours to maintain the stability of the ABTS radical. Then, 0.1 ml of extract solution was added to 2.9 ml of stable ABTS radical, and the absorbance of the mixture was measured at a wavelength of 734 nm after incubating in the dark for 1 hour. The percentage of ABTS radical scavenging activity was determined using a formula based on the absorbance values. Ascorbic acid was used as a positive control and Trolox as a standard. The percentage of inhibition was calculated by Equation 1.

$$\%I = \left(\frac{\mathrm{Ac}-\mathrm{As}}{Ac}\right) * 100 \quad (1)$$

%I= percentage of inhibition, Ac = absorbance of the control, and As = absorbance of the sample

2.6 Total Phenolic Content (TPC) Analysis

To determine the TPC, the method described by Orhan et al [29] was partially modified and tested. The extract samples (100 µL) were mixed with Folin-Ciocalteu reagent (0.2 ml), followed by dH_2O (2 ml) and 15% Na_2CO_3 (1 ml). After being left in the dark at room temperature for 30 minutes, the blend was measured at 765 nm, and the average of three measurements was calculated. The total phenolic content was reported in mg of gallic acid equivalents per gram, and the determination coefficient was $r^2 = 0.993$.

2.7 Statistical analysis

All activities were performed using Statistica (StatSoft Inc., Tulsa) software, and values were given as mean ± SD. All tests were performed in triplicate.

3. Results and Discussion

3.1. Antimicrobial activity

The antimicrobial potential of clove methanol, ethanol, and aqueous extracts against 8 microorganisms was examined and the results are given in Table 1. The study determined that only the aqueous extract did not show activity against *E. faecalis*, *Y. enterocolitica*, and *C. albicans* microorganisms among the tested extracts. The highest activity was revealed against *E. faecalis* from the methanol extract (19.30±0.17 mm), while the lowest activity was determined against *S.* Typhimurium (7.17±0.29 mm). The extracts tested for their activity against the *C. albicans* exhibited low inhibitory potential. While the water extract showed no activity, it was determined that the ethanol extract showed activity with a zone diameter of 9.00±1,00 mm and the methanol extract with a zone diameter of 7.30±0,14 mm. (Table 1). Although the aqueous extract has a higher total phenolic content compared to the other extracts, it is seen that it has lower activity in terms of antimicrobial activity (Table 5). Therefore, although the total phenolic content obtained from the aqueous extract is high, it is thought that it may be related to the low phenolic content showing antimicrobial activity. Several researchers have investigated the effectiveness of clove against food pathogens, and have demonstrated that clove has a high antimicrobial activity against these pathogens. Mostaqim et al. [30] noted that clove aqueous and ethanol extracts zone diameters were 30.5 mm and 38 mm over *S. aureus* and *P. aeruginosa*, respectively. Another study found that plant ethanol extract showed inhibitory activity of 16, 20, and 18 mm against the food pathogens *S. aureus, P. aeruginosa*, and *E. coli*, respectively [31]. El-Maati et al. [10] found high antibacterial efficacy against *S. aureus*, *L. monocytogenes*, and *E. coli* for clove. Similarly, another study discovered that the cloves ethanol extract exhibited bactericidal activity over *S. aureus* and *P. aeruginosa* [32]. Additionally, there is more research on clove antimicrobial activity [33-35]. The data obtained from the literature are consistent with our study.

Table 1. Inhibition zone diameters (mm) of Clove different extracts against food pathogens

	Extracts (300 mg/ml)			Antibiotics		Solvents		
Food Pathogens	ET	MT	AQ	AMP	NY	E	M	A
E. coli	9,00±2,00	13,35±0,58	8,00±1,00	17,00±1,50	NT	ND	ND	ND
S. aureus	7,90±0,14	8,67±0,29	10,33±1,15	20,35±0,92	NT	ND	ND	ND
S. Typhimurium	10,20±1,93	12,50±0,87	7,17±0,29	17,60±0,85	NT	ND	ND	ND
E. faecalis	9,85±1,03	19,30±0,17	ND	18,00±1,00	NT	ND	ND	ND
B. subtilis	11,37±1,96	18,70±2,03	9,00±0,50	19,50±0,70	NT	ND	ND	ND
L. monocytogenes	8,65±0,92	9,25±0,35	7,33±0,58	21,70±1,20	NT	ND	ND	ND
Y. enterocolitica	9,55±1,63	9,70±1,54	ND	19,30±1,98	NT	ND	ND	ND
C. albicans	9,00±1,00	7,30±0,14	ND	NT	17,55±2,11	ND	ND	ND

ET: Ethanol Extract, MT: Methanol Extract, AQ: Aqueous Extract, AMP: Ampicilin, NY: Nystatine, E: Ethanol, M: Methanol, A: Aqueous, NT: Not Tested, ND: Not Determined

3.2. Minimum inhibition concentration (MIC) analysis

The study examined the MIC of clove extracts on foodborne pathogens (Table 2). The ethanol extract exhibited the highest activity against the tested microorganism with MIC diverse spectrum from 2.5-10 mg/mL, while the water extract showed the lowest activity with MIC values ranging from 5->10 mg/mL (Table 2). Hoque et al. [36] reported ethanol and aqueous

extracts of cloves MIC values ranging from 0.5-5.5 mg/mL and 0.8-5.5 mg/mL, respectively, against foodborne pathogens. Ishaq et al. [37] revealed that clove had the highest MIC value against L. monocytogenes. Shukla et al. [38] stated that cloves exhibited high MIC efficacy against foodborne pathogens, while Mostafa et al. [32] obtained that the ethanol extract of cloves showed high MIC activity against food-spoiling pathogens. Saeed et al. [39] showed that the methanol extract of cloves had a high MIC value against foodborne pathogens. These data from the literature are consistent with our findings and indicate the high MIC potential of ethanol and methanol extracts of clove.

Table 2. Minimum inhibition concentration (MIC) of Clove different extracts against food pathogens

	MIC (mg/ml)		
Food Pathogens	ET	MT	AQ
E. coli	5	5	10
S. aureus	5	10	5
S. Typhimurium	2,5	5	>10
E. faecalis	5	10	NT
B. subtilis	5	5	5
L. monocytogenes	2,5	10	>10
Y. enterocolitica	2,5	5	NT
C. albicans	10	2,5	NT

3.3. Minimum bactericidal concentration (MBC) assay

In this study, the MBC test was also evaluated, and the ethanol extract of clove presented the highest MBC activity against *L. monocytogenes* and *Y. enterocolitica* bacteria at 5 mg/ml. For the methanol extract, the highest activity was found against the yeast pathogen *C. albicans* at 5 mg/ml. Accordingly, it was observed that the ethanol extract had higher potential than other extracts, and the water extract had low MBC activity (Table 3). Yassin et al. [40] discovered MBC values of 2 mg/disc and 1 mg/disc for the dichloromethane extract of clove against *S. aureus* and *E. coli*, respectively. Mostafa et al. [32] revealed an MBC value for the ethanol extract of clove was 10 mg/ml over *S. aureus*, and similarly, According to Witkowska et al. [41], the ethanol extract of cloves exhibited an MBC value of 10 mg/ml against both *E. coli* and *S. aureus*. The data provided from the literature are consistent with our findings.

Table 3. Minimum bactericidal concentration (MBC) of Clove different extracts against food pathogens

	MBC (mg/ml)		
Food Pathogens	ET	MT	AQ
E. coli	>10	10	>10
S. aureus	10	>10	>10
S. Typhimurium	10	10	NT
E. faecalis	10	>10	NT
B. subtilis	>10	10	>10
L. monocytogenes	5	>10	NT
Y. enterocolitica	5	10	NT
C. albicans	>10	5	NT

ET: Ethanol Extract, MT: Methanol Extract, AQ: Aqueous Extract, AA: Ascorbic acid

3.4. Antioxidant tests

Antioxidant tests of clove are indicated by testing its DPPH and ABTS scavenging potential. DPPH radical sweep results detect the activity as aqueous>methanol>ethanol extract, and the highest activity is obtained from 60.93±1.67% aqueous extract (Table 4.). In addition, in terms of ABTS radical sweep activity, it is seen that this order is ethanol>aqueous>methanol extract and the highest activity was obtained from 85.81±1.08% ethanol extract (Table 4). Phenolic molecules are essential antioxidant components responsible for deactivating free radicals based on their ability to donate hydrogen atoms to free radicals. From this point of view, when the data obtained within the scope of the study are examined, it is seen that the highest content is obtained from aqueous and methanol extracts, respectively (Table 5). This explains why aqueous and methanol extracts have higher DPPH scavenging capacity. For ABTS, the opposite is the case. This shows that it may be related to the change in the phenolic content of the plant. Mashkor [42] found that the clove's DPPH radical scavenging activity was 87.5%, Baghshahi et al. [43] reported that the DPPH radical scavenging activity of clove ethanol extract at increasing concentrations (0.1-12.5 µg/ml) ranged from 5.9 to 97.9%. In a similar study, it was noted that cloves showed high antioxidant and radical scavenging potential [44]. In addition, Hidayati et al. [45] stated that both the DPPH and ABTS radical scavenging potential of clove methanol extract is high (IC50 44.35 mg/ml and 17.69 mg/ml, respectively), and Kutlu et al. [46] reported the clove ethanol extract shows the highest antioxidant activity. Unlike the literature, it was assessed that the highest efficiency was detected in aqueous, and ethanol extract, respectively, for DPPH and ABTS activity. This may be due to the harvest, storage, extraction and chemical content of the plant.

Table 4. Antioxidant Inhibition (%) of Clove extracts by the DPPH, ABTS

Extracts (300 mg/ml)	Inhibition%			
	DPPH	TE (mM/g DW)	ABTS	TE (mM/g DW)
ET	25,80±1,54	1,47	85,81±1,08	2,16
MT	36,20±1,87	1,63	34,58±1,48	0,19
AQ	60,93±1,67	2,02	47,98±2,63	0,71
AA	82,46±2,77	2,36	88,35±2,26	2,35

ET: Ethanol Extract, MT: Methanol Extract, AQ: Aqueous Extract, AA: Ascorbic acid, TE: Trolox equivalent dried weight, NT: Not tested

3.4. Total phenolic content (TPC) evaluation

In this study, we also performed TPC analysis on clove's aqueous, ethanol, and methanol extracts. The highest TPC value was revealed from the aqueous extract by 189.84±2.84 mg/g GA, while the methanol extract had the lowest TPC content at 125.45±1.38 mg/g GA (Table 5). Previous studies have discovered different TPC ratios for clove extracts. Witkowska et al. [41] found a TPC ratio of 185.30±6.80 mg/g GA for clove ethanol extract, whereas Turgay and Esen [47] documented a TPC ratio of 560 mg/g GA for clove. Radha krishnan et al. [48] noted TPC ratio of clove aqueous extract was 24.65±0.83 mg/g GA. Our results are consistent with the previous works regarding the TPC ratios of clove extracts.

Table 5. Total phenolic content (TPC) of Clove different extracts

Extracts (100 µl)	TPC (mg/g GA)
ET	129,72±1,28
MT	125,45±1,38
AQ	189,84±2,84
AA	NT

ET: Ethanol Extract, MT: Methanol Extract, AQ: Aqueous Extract, AA: Ascorbic acid, NT: Not tested

4. Conclusion

Global warming and climate crisis create various problems in the production and storage of food. Climate change causes a decrease in food yield, leading to an increase in food supply with the increasing world population. Crop production also depends on good quality soil and water, as well as predictable weather and an adequate growing season. With the increase in demand for food, the decrease in food supply for various reasons (such as microbial factors) has increased the interest in food preservation. Recently, natural methods for food preservation and reduction of food loss have been of great importance. Therefore, the trend towards natural products is increasingly emerging. The clove sample used in this study has been shown to exhibit high efficacy against microorganisms that cause food loss. Furthermore, due to its high antioxidant, antimicrobial and phenolic content, clove can be considered suitable for use in food preservation. In addition, there is a clear need for further comprehensive studies on this topic. Moreover, additional studies on this subject will provide significant medical and economic benefits.

Ethics in Publishing

There are no ethical issues regarding the publication of this study.

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