



Comparative study of total phenolic contents, antioxidant and antimicrobial activities of different extracts of *Corchorus olitorius* L. growing in North Cyprus

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Abstract

In this study, antimicrobial and antioxidant activities as well as total phenolic content of dried leaf extracts of *Corchorus olitorius* grown in North Cyprus were determined. The total phenolic content, antioxidant and antimicrobial activity of the extracts were determined using the Folin-Ciocalteu, DPPH free radical and broth dilution methods, respectively. The antimicrobial activity of the extracts was tested against eight different microorganisms (*Bacillus subtilis* ATCC 6037, *Listeria monocytogenes* Scott A, *Enterococcus faecalis* ATCC 29212, *Staphylococcus aureus* 6538P, *Bacillus cereus* No 8, *Escherichia coli* O157:H7 ATCC 43895, *Escherichia coli* ATCC 1103, *Salmonella* Typhimurium NRRL-B-4420). The highest phenolic content was obtained from ethanol extract as 24.61 mg GAE/g. Water, ethanol and methanol extracts of *C. olitorius* were inhibited the DPPH radical by 17.50%, 87.10% and 86.00%, respectively. MIC values of ethanol and methanol extracts of *C. olitorius* against test cultures were varied between 50.00% and 6.25%, while no inhibitory effect was observed for water extract. The most susceptible microorganisms to methanol extract of the plant were *L. monocytogenes* Scott A, *E. faecalis* ATCC 29212 and *B. subtilis* ATCC 6037 (MIC value of 6.25%). All these results showed that *C. olitorius* extracts are important sources showing antimicrobial and antioxidant properties.

Keywords: *Corchorus olitorius*, antimicrobial, total phenolic content, antioxidant

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Kuzey Kıbrıs'ta yetişen *Corchorus olitorius* L.'nin farklı ekstraktlarının toplam fenolik madde içeriği, antioksidan ve antimikrobiyal aktivitelerinin karşılaştırılması

Özet

Bu çalışmada, Kuzey Kıbrıs'ta yetiştirilen *Corchorus olitorius*'un kurutulmuş yaprak ekstraktlarının antimikrobiyal ve antioksidan aktiviteleri ile toplam fenolik madde miktarı belirlenmiştir. Ekstraktların toplam fenolik madde miktarı, antioksidan ve antimikrobiyal aktivitesi sırasıyla Folin-Ciocalteu, DPPH serbest radikal ve sıvı seyreltme yöntemleri kullanılarak belirlenmiştir. Ekstraktların antimikrobiyal aktivitesi, sekiz farklı mikroorganizmaya (*Bacillus subtilis* ATCC 6037, *Listeria monocytogenes* Scott A, *Enterococcus faecalis* ATCC 29212, *Staphylococcus aureus* 6538P, *Bacillus cereus* No 8, *Escherichia coli* O157:H7 ATCC 43895, *Escherichia coli* ATCC 1103, *Salmonella* Typhimurium NRRL-B-4420) karşı test edilmiştir. En yüksek toplam fenolik madde miktarı etanol ekstraktından 24.61 mg GAE/g olarak belirlenmiştir. *C. olitorius*'un su, etanol ve metanol ekstraktları DPPH radikalini sırasıyla %17.50, %87.10 ve %86.00 oranında inhibe etmiştir. *C. olitorius*'un etanol ve metanol ekstraktlarının test kültürlerine karşı MIC değerleri %50.00 ile %6.25 arasında değişirken, su ekstraktının herhangi bir engelleyici etkisi gözlenmemiştir. *L. monocytogenes* Scott A, *E. faecalis* ATCC 29212 ve *B. subtilis* ATCC 6037 (%6.25 MIC değeri), bitkinin metanol ekstraktına karşı en duyarlı mikroorganizmalar olmuştur. Tüm bu sonuçlar, *C. olitorius* ekstraktlarının antimikrobiyal ve antioksidan özellikler gösteren önemli kaynaklar olduğunu göstermiştir.

Anahtar kelimeler: *Corchorus olitorius*, antimikrobiyal, toplam fenolik madde, antioksidan

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1. Introduction

Antimicrobials are commonly used in various areas including food production. Hence, some microorganisms became more resistant to antimicrobial agents and infectious diseases are generally caused by these resistant microorganisms, which make treatment difficult [1]. As an alternative to chemically synthesized antimicrobials, there was a need for effective and reliable antimicrobial agents of plant origin [2]. While many drugs originate from plants, various medicinal plants have been used for many years because of their curative effects on diseases [1]. The use of plant-based preservatives instead of chemically synthesized antimicrobials in foods inhibit microbial growth and prolong the shelf life of foods, as well as providing antioxidant effects due to phenolic contents, and shows positive effects on consumer health by increasing immunoglobulin synthesis [3].

Phenolics are secondary metabolite produced by plants to defend themselves against external threats (microbial, physical etc.) [4]. Phenolic contents of plants depend on environmental conditions and endogenous factors such as salinity, light intensity, bacterial infection, drought, temperature, maturity, organ and genotype [5]. Phenolics can be classified as flavonoids, phenolic acids, tannins, stilbenes, coumarins, ligands, quinones and curcuminoids, depending on the amount and position of the carbon element in their chemical structure [6]. Most of the antioxidant activities of fruits and vegetables are associated with their phenolic compounds. Recently, researches on plants containing phenolic compounds have been increased and it has been determined that they have various effects such as antimicrobial, anti-carcinogenic, antioxidant, anti-inflammatory and reducing chronic diseases [7, 8]. For example, flavonoids protect body cells against the harm brought about by oxygen, delivered as a side-effect of vitality digestion [9]. Thanks to the ability of gallic acid to rummaging free radicals and suppress-amyloid oligomerization, it can reduce nerve damage and cerebrum amyloid neuropathology and increase intellectual capacity [10]. In addition to being used as metal ion chelator and protein-precipitating agent, tannins also have antioxidant properties [11]. Hence, in recent years, consumers increase the demand for natural herbal products [12].

Corchorus olitorius is a green leafy plant belonging to Malvaceae family. It is commonly grown in Africa, Europe and Asia and named as molehiya in North Cyprus [13]. Leaves or whole plant are boiled, dried or freshly used in food [14]. As *C. olitorius* is rich in substances such as minerals, vitamins, fiber and ascorbic acid that provide cell renewal and energy production, it is also used in the medical field due to its antimicrobial and antioxidant effects [15, 16]. Studies indicate that this herb is used to cure diseases such as diabetes, asthma, diarrhea, back and stomach pain [17,18]. There are studies investigating the antimicrobial effect of the extracts of *C. olitorius* obtained with different solvents [19, 20]. Compounds such as hexadecanoic Acid, 2,4-di tert-butyl phenol, tetratetracontane, isoheptadecanol, ethyl palmitate, 1-eicosanol and 8-heptadecene have been identified in *C. olitorius* extracts [21].

In addition to the limited number of publications in which the phytochemical properties of *C. olitorius* plant grown in N. Cyprus, there is no study in the literature investigating the antimicrobial effect of this plant in terms of MIC (Minimum inhibition concentration) values. Therefore, in this study, antimicrobial and antioxidant activities as well as total phenolic content of three different extracts obtained from *C. olitorius* by water, ethanol and methanol were investigated.

2. Materials and methods

2.1 Collection and Drying of Sample

C. olitorius collected in July 2018 from Famagusta, N. Cyprus. The leaves removed from the branch were dried using a local conventional method. Fresh leaves allowed to dry for a week on a clean flat surface in the dark room at 30°C and mixed three times a day until drying took place. The dried leaves were powdered in a mortar and stored in sterile jars at room temperature.

2.2 Extraction of Sample

In the study, water, ethanol and methanol extracts were obtained. For the preparation of water extract, 500 mg of *C. olitorius* leaf was dissolved in 10 mL of distilled water and mixed at 25°C for 24 h. The prepared suspension was centrifuged at 4000 rpm for 10 min and filtered through a 0.22 µm sterile filter (Minisart Syringe Filter, Cellulose Asetate, Sartorius Stedim Biotech) [22]. For methanol extract, 5 g of *C. olitorius* were shaken with 100 mL of 85% methanol solution in the shaker (Mettler GF 4931, Germany) at room temperature for 24 h. After filtration, methanol was removed by treating the filtered liquid at 40°C. methanol and then the extract was diluted with water to 50 mL [23]. Ethanol extracts were prepared from 10 g of *C. olitorius* leaves. Extraction was carried out with ethanol and distilled water (40:60, v/v) in a ratio of 1:50 (m/v-solute/solvent), respectively. The medley liquid was shaken with shaker (Mettler GF 4931, Germany) at 25 °C for 24 h, then centrifuged at 5000 rpm for 25 min. The clear extracts were taken and dried in a vacuum evaporator at 40°C to eliminate ethanol [24]. Then, the final extract was used in the analysis as ethanol extract.

2.3 Total Phenolic Content

Total phenolic content of *C. olitorus* extracts was determined using the Folin-Ciocalteu method. 5 mL of Folin-Ciocalteu solution (10%, v/v) and 1 mL filtered sample were transferred into tube and stored in the dark for 3 min. Then 10 mL of saturated NaCO₃ was added and the mixture was filled to 100 mL with distilled water. The resulting mixture became ready to be analyzed after being kept at 25°C for 90 min in a non-light environment. Finally, absorbance of the sample was measured by using a spectrophotometer (Agilent Technologies, Carry60 UV-Visible) at 760 nm. The results were taken as mg gallic acid equivalents (GAE)/L [25].

Using the equation below, the results were converted from mg GAE/L to mg GAE/g [26].

$$T \text{ (mg GAE/g)} = (C \times V)/M$$

In this formulation, C is the concentration of the acid in which the results are expressed (gallic acid) (mg/mL), V is the volume of the extract solution (mL), and M is the weight of the plant extracted (g).

In this formula, C is the concentration of gallic acid established from the calibration curve in mg/mL, V is the volume of the extract solution in mL, M is the weight of the extract in g.

2.4 Antioxidant Capacity

DPPH radical scavenging activity method was used to determine the antioxidant activity of *C. olitorus* extracts. 4 mL of DPPH solution dissolved in methanol was mixed with 0.1 mL of the filtered sample in a tube. The mixture obtained was kept in non-light environment for 15 minutes before being analyzed in the spectrophotometer (Agilent Technologies, Carry60 UV-Visible) at 517 nm [27]. The control sample was methanol (1 mL). The obtained DPPH radical scavenging activity results converted to percentage using by this formula:

$$\text{DPPH scavenging activity (\%)} = (A_c - A_s)/A_c \times 100$$

In this formula, A_s and A_c are defined as absorbance of sample and control, respectively.

2.5 Antimicrobial Activity

The antimicrobial activity of three different extracts of *C. olitorus* was tested on eight microorganisms including 5 Gram positive (*Bacillus subtilis* ATCC 6037, *Listeria monocytogenes* Scott A, *Enterococcus faecalis* ATCC 29212, *Staphylococcus aureus* 6538P, *Bacillus cereus* No 8) and 3 Gram negative (*Escherichia coli* O157:H7 ATCC 43895, *Escherichia coli* ATCC 1103, *Salmonella* Typhimurium NRRL-B-4420) bacteria using broth dilution method. Test cultures obtained from University of Ege, Department of Food Engineering, Laboratory of Food Microbiology Research. Tryptic Soy Broth (TSB, pH 7.3±0.2, Oxoid) was used for the activation of glycerol stock cultures and the cultures were activated by incubating at 37°C for 24 h. Then, activated cultures were used in the analysis after being adjusted to 0.5 Mc Farland turbidity grade using DEN-1 Mc Farland Densitometer.

To determine MIC (Minimum Inhibitory Concentration) value of each *C. olitorus* extracts, the broth-dilution method was performed by using 96 well ‘‘U’’ type sterile microplates [28]. Firstly, a hundred µL of Mueller Hinton Broth (MHB, pH 7.3 ± 0.2, Oxoid) turned into brought to all wells, then a hundred µL of extract turned into brought to all wells in horizontal alignment. The amount of the extract was diluted to contain half of the previous well. The extraction rates from the first well were 50%, 25%, 12.5%, 6.25%, 3.125%, 1.563%, 0.781%, 0.391%, 0.195%, 0.098% (v/v), respectively. 10 µL of the culture to be tested was added to the homogenized mixed wells and incubated at 37°C for 18 h. After incubation period, each well filled with 20 µL of 0.5% 2,3,5-triphenyltetrazolium chloride and incubated at 37°C for 30 min. The lowest concentration that does not cause color change on test cultures was determined as MIC value. The sample taken from the well containing the lowest concentration of extract without growth was used to determine the MBC (Minimum Bactericidal Concentration) value by spreading to Mueller-Hilton Agar (MHA, pH 7.3 ± 0.2, Oxoid).

2.6 Statistical analysis

The statistical analysis was applied using the one-way ANOVA method, with SPSS 20 package program, at the significance level of $P \leq 0.05$. Results are given as mean and standard deviation [29].

3. Results

Solvents such as methanol, acetone, hexane, chloroform, ethyl acetate, ethanol and water are used to obtain extracts from various plants. During extraction, the dissolution of phytochemicals varies according to the type of solvent used. In the other studies, it has been determined that although solvents are recommended for the extraction of phenolic compounds and saponins, water can dissolve non-phenolic antimicrobial substances and also the best option for the extraction of trypsin inhibitors [30]. Besides, water extraction could be preferred due to the safety and environmental concerns. In the study, the total phenolic content of water, ethanol and methanol extracts of *C. olitorus*

was the highest (24.61 mg GAE/g) in ethanol extract, while it was the least (9.86 mg GAE/g) in water extract ($P < 0.05$) (Table 1). Besides, no statistical difference was observed between phenolic contents of ethanol and methanol extracts ($P > 0.05$). Hence, ethanol appears to be the most suitable solvent for the dissolution of phenolic compounds of *C. olitorius*. Ademiluyi et al. [31] reported that the total phenolic amount of the water extract of dried leaves of *C. olitorius* grown in Nigeria was determined as 5 mg GAE/g.

Table 1. Total phenolic content of *C. olitorius* extracts

Extracts	Total phenolic content (mg GAE/g)
Water extract	9.86 ± 2.07 ^B
Ethanol extract	24.61 ± 1.97 ^A
Methanol extract	21.14 ± 0.69 ^A

*The capital letters (A, B) in the table show the statistical difference between samples ($P < 0.05$).

It was determined that water, ethanol and methanol extracts of *C. olitorius* inhibited the DPPH radical in the range of 17.50% - 87.10% (Table 2). The highest value was observed in ethanol extract, while there were no statistical differences between ethanol and methanol extracts ($P > 0.05$). These results were in parallel with the total phenolic content results. In the study of Yakoub et al. [32] antioxidant activity of water and ethanol extracts of dried leaves of *C. olitorius* grown in Tunisia was 77.64% and 91.65%, respectively. Obeng et al. [33] investigated the antioxidant activity of *C. olitorius* grown in Ghana after drying the leaves in an oven at 55°C for 6 h. The results showed that methanol extract of *C. olitorius* inhibited the DPPH radical by %22.20, which is very low than our results.

Table 2. Antioxidant activity of *C. olitorius* extracts

Extracts	DPPH (%)
Water extract	17.50 ± 2.59 ^B
Ethanol extract	87.10 ± 1.41 ^A
Methanol extract	86.00 ± 0.54 ^A

*The capital letters (A, B) in the table show the statistical difference between samples ($P < 0.05$).

Antimicrobial activities of the extracts obtained from *C. olitorius* had been decided against 8 different microorganisms by using broth dilution method. The water extract of *C. olitorius* showed no antimicrobial effect against any of the microorganisms tested. All test cultures had MIC value of 25.00% against ethanol extract. The results showed that antimicrobial activity of methanol extract is higher than ethanol extract ($P < 0.05$). In addition, the extracts did not show bactericidal effect against any of the microorganisms tested.

Table 3. Antimicrobial activity of *C. olitorius* extracts

Test Microorganisms	MIC value (% v/v)		
	Water extract	Ethanol extract	Methanol extract
<i>L. monocytogenes</i> Scott A	>50	25.00	6.25
<i>E. faecalis</i> ATCC 29212	>50	25.00	6.25
<i>B. subtilis</i> ATCC 6037	>50	25.00	6.25
<i>S. aureus</i> 6538P	>50	25.00	12.50
<i>E. coli</i> O157:H7 ATCC 43895	>50	25.00	12.50
<i>S. Typhimurium</i> NRRL-B-4420	>50	25.00	12.50
<i>E. coli</i> ATCC 1103	>50	25.00	12.50

B. cereus No 8

>50

25.00

12.50

There is no study in the literature in which the antimicrobial effect of *C. olitorius* extracts was determined by MIC analysis. In the study performed by Mohammed [34], *C. olitorius* was extracted in 96% ethanol and its antimicrobial effect was examined for four different concentrations (1.00, 0.75, 0.50, 0.25 mg/mL) by agar diffusion using 10 mm discs against *B. subtilis*, *E. coli* and *E. faecalis*. Zones formed against *B. subtilis*, *E. coli* and *E. faecalis* are reported as in the range of 18-20 mm, 14-20 mm and 13-15 mm, respectively. In another study, the antimicrobial effects of methanol petroleum ether and ethyl acetate + water extracts of *C. olitorius* harvested in N. Cyprus were determined against twelve different bacteria (*B. cereus* NRRL B-3711, *S. aureus* ATCC 25923, *Pseudomonas gingeri* 3146, *B. subtilis* NRRL B-209, *Enterococcus faecium* NRRL B-3502, *Micrococcus luteus* NRRL-B 1018, *E. faecalis* ATCC 29212, *E. coli* ATCC 25922, *Enterobacter aerogenes* NRRL-B 3567, *Proteus vulgaris* NRRL B-123, *Yersinia enterocolitica*, *S. Typhimurium* ATCC 14028) using agar diffusion method with 10 mm discs and it was observed that the extracts were formed inhibition zones ranging from 10 to 20 mm [35]. All these results showed that *C. olitorius* have antimicrobial activity in various ranges depending on the test cultures used.

According to the results, the antioxidant and antimicrobial effects of the *C. olitorius* extracts were different, as well as the total phenolic substance content. This difference is thought to be due to the solvent's ability to dissolve different compounds of the plant.

4. Conclusions and discussion

Various compounds found in plants can be extracted by different methods. The performance of extracting process varies according to the solvent and the type of plant used. In the current study, it was observed that as the amount of phenolic content increased, the antimicrobial effect also increased. As similar with our results, the inhibitory effect of *C. olitorius* ethanol extract against test microorganisms was observed in another study [34], which also shows that ethanol is a suitable solvent for extracting the antimicrobial effective compounds from the plant. However, the total phenolic content of *C. olitorius* water extract obtained in another study [31] was almost half of our result. This result may be due to the geographical difference in which the plant grows.

In this study, it was determined that *C. olitorius* extracts have antimicrobial and antioxidant activities as well as rich in total phenolic content. The total phenolic content and antioxidant activity results showed that ethanol extract was the most efficient sample when compared to water and methanol extracts, while antimicrobial activity was the highest in methanol extract against all test cultures. Hence, *C. olitorius* has the potential to be used as an alternative to chemical antimicrobial and antioxidant agents. The results showed that the antimicrobial and bioactive properties of *C. olitorius* may change depending on the geographical region where the plant is grown, the drying processes applied to the plant, as well as solvent type used during plant extraction. In the future studies, *C. olitorius* will be used in various foods for detecting its effect on food quality and safety.

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