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Comparison of Different Methods in the Extraction of Phenolic Compounds from Bay Leaf (Laurus nobilis L.)

Defne Yaprağından (Laurus Nobilis L.) Fenolik Bileşiklerin Ekstraksiyonunda Farklı Yöntemlerin Karşılaştırılması

Ayca AKYUZ[1*], Idil TEKIN[1], Seda ERSUS[1]

[1]Ege University, Engineering Faculty, Department of Food Engineering, Bornova, İzmir, Turkey.

* akyuzayca@gmail.com, ORCID: 0000-0001-9862-0176

idiltekin.ege@gmail.com, ORCID: 0000-0001-6494-7226

seda.ersus@ege.edu.tr, ORCID: 0000-0003-0475-4099

Abstract

In this study, the microwave-assisted and enzyme-assisted extraction efficiency were compared to solvent extraction. The extraction efficiencies were evaluated in bay leaf extract in terms of phenolic content and antioxidant capacity. The total phenolic content (mg GAE/g) of the extracts from three different extraction methods as a solvent, enzyme-assisted, and microwave-assisted extraction was found 23.29±0.02, 32.45±0.02, and 30.49±0.02, respectively. The highest value for the total phenolic content was found from the enzyme-assisted extraction. DPPH radical scavenging capacity (%) of the extracts from three different extraction methods was found at 36.91%±0.05, 50.72%±0.27, and 41.51%±0.09, respectively. Like the total phenolic content, the highest value for the DPPH radical scavenging capacity was found from the enzyme-assisted extraction. In addition, total dry matter, total ash, total protein, ascorbic acid, and total chlorophyll content of the bay leaf were analyzed.

Keywords: Enzyme assisted extraction, Microwave assisted extraction, Bay leaf, Phenolic compounds

Özet

Bu çalışmada, mikrodalga destekli ve enzim destekli ekstraksiyon verimliliği çözgen ekstraksiyonu ile karşılaştırılmıştır. Defne yaprağı ekstraktında ekstraksiyon verimleri fenolik içerik ve antioksidan kapasitesi açısından değerlendirilmiştir. Geleneksel ekstraksiyon, enzim destekli ve mikrodalga destekli ekstraksiyon olarak üç farklı ekstraksiyon yönteminden elde edilen ekstraktların toplam fenolik içeriği (mg GAE/g) sırasıyla 23.29±0.02, 32.45±0.02 ve

30.49±0.02 olarak bulunmuştur. Toplam fenolik içerik için en yüksek değer enzim destekli ekstraksiyonda belirlenmiştir. Üç farklı ekstraksiyon yönteminden elde edilen ekstraktların DPPH radikal süpürme aktivitesi (%) sırasıyla %36.91±0.05, %50.72±0.27 ve %41.51±0.09 olarak bulunmuştur. Toplam fenolik içerik gibi, DPPH radikal süpürme aktivitesi için en yüksek değer enzim destekli ekstraksiyonda belirlenmiştir. Ayrıca defne yaprağının toplam kuru madde, toplam kül, toplam protein, askorbik asit ve toplam klorofil içeriği belirlenmiştir.

Anahtar Kelimeler: Enzim destekli ekstraksiyon, Mikrodalga destekli ekstraksiyon, Defne yaprağı, Fenolik bileşikler

1. INTRODUCTION

The bay leaf (*Laurus nobilis* L.), an aromatic herb, is one of the oldest and most widely used spice, widely grown in the Mediterranean region (Sayyah et al., 2003). The bay leaf is rich in bioactive compounds which are secondary metabolites that have a positive effect on health (Simić et al., 2003). The plants are known as rich in phenolic compounds which are one of these secondary metabolites. The phenolic compounds are used in food industry because of their nutritional quality, natural colorant, antioxidant function and organoleptic properties such as color, taste, and odor (Li et al., 2008). However, due to the presence of small amounts of these compounds it is necessary to determine the most efficient extraction method.

The extraction is an important stage for the identification and usage of phenolic compounds. The method used for the extraction of phenolic compounds is usually solvent extraction. Since the conventional method has many disadvantages such as the use of excess solvent and high temperature, long application time, alternative methods are under investigation (Pai et al., 2022). In recent years, several alternative methods have been developed such as enzyme-assisted (Anticona et al., 2020; Boulila et al., 2015), microwave-assisted (Muñiz-Márquez et al., 2018), ultrasound-assisted (Chakraborty et al., 2020), and supercritical fluid (Gan et al., 2020; Zulkafli et al., 2014) extraction methods. In this study, microwave assisted and enzyme-assisted methods' extraction efficiency from bay leaves on phenolic content and antioxidant capacity were compared to solvent extraction.

2. MATERIALS and METHODS

2.1. Materials

The dried bay leaves were purchased from a local market in Izmir-Turkey in October 2019. The dried bay leaves were grounded and stored in glass jars at room temperature. The Pectinex Ultra SP-L enzyme used in enzyme assisted extraction was donated by Novozymes (Novozymes, Denmark). All other chemicals and reagents were of analytical grades.

2.2. The Physicochemical Properties of Dried Bay Leaves

The total dry matter, total ash, and total protein content analyses of dried bay leaves were performed according to the official methods of the Association of Official Analytical Chemists (AOAC, 1990). The ascorbic acid (Bajaj & Kaur, 1981) and chlorophyll content (Vernon, 1960) of bay leaves were determined.

2.3. Extraction of Phenolic Compounds from Dried Bay Leaves

The phenolic compounds were extracted by solvent (conventional) extraction, enzyme assisted extraction and microwave assisted extraction methods. The extraction efficiencies were compared.

2.4. Solvent (Conventional) Extraction (SE)

The extraction of phenolic compounds from bay leaf was carried out by the procedure suggested by Muñiz-Márquez et al. (2018) with some modifications. The solvent extraction was carried out using a bay leaf/ethanol (50%), solid/solvent ratio (1:10, w/v), at 25°C extraction temperature for 24 hours extraction.

2.5. Enzyme Assisted Extraction (EAE)

For the enzymatic pretreatment, a 1 g of dried bay leaf powder was dispersed in 10 mL of pure water. The pH value of the sample was adjusted to 5.5 by 0,1 N HCl for the maximum enzyme activity. The selected extraction parameters were the concentration of the enzyme (8%), incubation temperature (45°C) and reaction time (30 min). Extraction was carried out under constant stirring conditions at 150 rpm continuously. After enzymatic pretreatment, solvent extraction with ethanol (50%) was carried out at a bay leaf/ethanol ratio of 1:3 (v/v) for 45 min at 45°C mixing at 150 rpm (Özkan & Bilek, 2015).

2.6. Microwave Assisted Extraction (MAE)

The extraction of phenolic compounds from bay leaf was carried out by the procedure suggested (Zhang et al., 2019) with some modifications. The microwave extraction was carried out using a bay leaf/ethanol (50%), solid/solvent ratio (1:10, w/v), at 500 W power, for 30 s extraction by a microwave oven (Samsung MS23F300EEW/TR).

2.7. Total Phenolic Content (TPC) Analysis

The prepared bay leaf extracts of 0.5 mL were used for total phenols determination. The Folin Ciocalteau reagent was used as an oxidizing agent. The amount of 0.5 mL of extract and 2.5

mL of Folin Ciocalteau reagent (diluted 10 times with water) was mixed for a 4 min, then 2 mL of Na2CO3 (75 g/L) was added to that solution. The samples were incubated at 50ºC for 5 min and then cooled. The absorbance was measured at 760 nm by a spectrophotometer (Cary 50 UV-vis.). The results were expressed in mg GAE/g D.M (Bilek, 2010).

2.8. DPPH Radical Scavenging Capacity

The percentage of antioxidant capacity (AC%) of bay leaf extracts was assessed by DPPH free radical assay (Garcia et al., 2012). The samples were reacted with the DPPH radical in an ethanol solution. The reaction mixture consisted of adding 0.5 mL of sample, 3 mL of absolute ethanol, and 0.3 mL of DPPH radical solution (0.5 mM in ethanol). The changes in color were read at 517 nm by a spectrophotometer (Cary 50 UV-vis.). The mixture of ethanol (3.3 mL) and sample (0.5 mL) was used as blank. The control solution was prepared by mixing ethanol (3.5 mL) and DPPH radical solution (0.3 mL). The scavenging capacity percentage (AC%) was determined according to Equation 1.

$$AC\% = 100 - \left[\frac{(Abs_{sample} - Abs_{blank}) \times 100}{Abs_{control}}\right] \text{ (Equation 1)}$$

2.9. Statistical Analysis

All analyzes were performed in triplicate and the results were given as mean and standard deviation. The difference between the extraction methods based on the total phenolic content and antioxidant capacity was evaluated by the statistical analysis performed with SPSS program (SPSS Inc., Chicago, IL, USA). Significant differences between samples were determined using Duncan's multiple range test at the confidence interval of 95%.

3. RESULTS and DISCUSSION

3.1. The Physicochemical Composition of Dry Bay Leaf

The total dry matter, total ash, and total protein content of dry bay leaf were given in Table 1. In the study of Tainter and Grenis (1993), the total dry matter, total ash, and total protein content of bay leaves were determined as 94.56%, 3.62% and 7.61%. In this context, the physicochemical properties of the bay leaf are compatible with the literature. The bay leaf is a plant rich in ascorbic acid. According to United States Department of Agriculture data, the ascorbic acid content of bay leaves is 46.5 mg/100 g, and it constitutes 77.5% of the daily intake (USDA, 1997). The ascorbic acid content of the bay leaf used in the study was found to be relatively low. This may be caused by the type of bay leaf, the time of harvest and the geographical region where it grows (Molina-Alcaide & Yáñez-Ruiz, 2008).

Table 1. The physicochemical composition of dried bay leaf.

Properties	Dried bay leaf
Total dry matter (%)	95.39±0.39
Total ash (%) (dry sample)	3.67±0.33
Protein content (%) (dry sample)	8.74±0.12
Ascorbic acid (mg/100 g dry sample)	30.43±0.007
Chlorophyll (mg/100 g dry sample)	79.57±0.28

3.2. Comparison of SE, EAE and MAE Methods

The total phenolic content and antioxidant capacity of the extracts were given in Table 2. The enzyme assisted extraction showed the highest efficiency results in terms of total phenolic content and DPPH radical scavenging capacity. It was observed that the Total phenolic content (TPC) extraction yield increased 39.29% comparing with conventional extraction method. By using enzymatic extraction, glycolytic bonds in pectin chain are broken due to the pectolytic activity of Pectinex Ultra SP-L that contains polygalacturonase, pectinesterase and pectin trans-eliminase, hemicellulase, and cellulase, thus the cell wall of bay leaf could be disrupted. The extraction of phenolic compounds can be facilitated with the breakdown of the cell wall (Boulila et al., 2015).

Table 2. Total phenolic content and DPPH radical scavenging capacity of bay leaf by different extraction techniques

Analysis	SE	EAE	MAE
Total phenolic content (mg GAE/g dry sample)	23.29±0.02[a]	32.45±0.02[b]	30.49±0.02[c]
DPPH radical scavenging capacity (%)	36.91%±0.05[a]	50.72%±0.27[b]	41.51%±0.09[c]

a-c- The difference between the groups marked with different letters in the same column is statistically significant ($p < 0.05$).

The microwave assisted extraction (MAE) is also efficient for extraction of phenolic compounds and the extraction yield increased 30.87% according to conventional extraction method. This can be due to in microwave heating, heating was occurred simultaneously, homogeneously, and quickly in contrast to conventional heating. The water inside the cell begins to evaporate with microwave heating. The evaporating water exerts pressure on the cell wall and eventually causes the cell rupture. Thus, the bioactive components pass into solvent and extraction efficiency increases (Mandal et al., 2007). Also, another advantage of MAE shortens extraction time. While the total phenolic content extraction took 24 hours with SE,

MAE was performed in a short time such as 30 s and with higher efficiency. However, the temperature-time relation was important in microwave assisted extraction. The improper relation of temperature-time may cause the degradation of bioactive compounds therefore the efficiency can decrease (Yağcioğlu, 2015). The differences of the extraction methods changed significantly ($p<0.05$). That difference was determined using Duncan's multiple range test at the confidence interval of 95%.

4. CONCLUSION

Overall, the alternative extraction methods comparing to conventional one increased the extraction efficiency. Especially, this study demonstrated that the enzyme assisted extraction was the most efficient method in the TPC extraction and high DPPH radical scavenging capacity. The enzyme assisted extraction revealed 6.43% higher yield than microwave assisted extraction in terms of TPC. However, the fact that enzymes are expensive biological catalysts and the cost of setting up for microwave equipment require feasibility studies of these technologies. Also, there must be a further research about optimization of extraction parameters to improve the extraction efficiency for each method.

DECLARATIONS

The authors declare that they have no conflicts of interest.

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