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Research Paper / Araştırma Makalesi

Total Phenolic Content, Antioxidant and Antimicrobial Activities of Homemade and Industrial Samples of Breakfast Zahter, Herbal Mixture

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ABSTRACT

Breakfast Zahter has been produced in homes and recently in industrial scale especially in the southeastern part of Turkey and some Arabic countries. Total phenolic contents of six different extracts (methanolic or ethanolic) obtained from homemade and industrial productions were determined by a spectrophotometric method while their antioxidant capacity was determined by the inhibition of DPPH radicals. Their antibacterial activity was obtained against *Klebsiella pneumoniae* ATCC700603 and *Stapylococcus aureus* ATTC25923 by the agar diffusion method. The total phenolic contents of methanol and ethanol extracts ranged from 56.4 to 3483.34 and 1274.45 to 5489.03 mg gallic acid equivalents/L, respectively. Antioxidant capacities of extracts were between 78.2% (methanolic extracts) and 91.4% (ethanolic extracts). For antimicrobial activity (100 µL of ethanol or methanol extracts), maximum inhibition zones were observed against tested strains. In general, extracts of homemade Breakfast Zahter samples had higher total phenolic content, antibacterial and antioxidant activity values than those of industrial products.

Keywords: Antibacterial, Antioxidant, Breakfast Zahter, Phenolic content

Ev Yapımı ve Endüstriyel Kahvaltılık Zahter Örneklerinin Toplam Fenolik İçeriği, Antioksidan ve Antimikrobiyal Aktiviteleri

ÖZET

Kahvaltılık Zahter evlerde ve son zamanlarda bazı Arap ülkelerinde ve özellikle Türkiye'nin güneydoğu kesiminde endüstriyel ölçekte üretilmektedir. Ev yapımı ve endüstriyel üretimlerden elde edilen altı farklı ekstrakt (methanol ve etanol) için spektrofotometrik metot kullanılarak toplam fenolik içerik, DPPH radikalleri inhibisyonu ile antioksidan kapasite ve agar difüzyon metodu ile *Klebsiella pneumoniae* ATCC700603 ve *Stapylococcus aureus* ATTC25923 karşı antibakteriyal aktivite belirlenmiştir. Methanol ve etanol ekstraktlarının toplam fenolik içerikleri 56.4 ile 3483.4 ve 1274.45 ile 5489.03 mg/L, gallik asit eşdeğeri olarak belirlenmiştir. Farklı ekstraktların antioksidan kapasitesi %78.2 (methanol) ve %91.4 (etanol) arasında bulunmuştur. Antimikrobiyal aktiviteye için, methanol ve etanol ekstraklarının 100 µL hacminde, test edilen suşlara karşı maksimum inhibisyon zonları gözlemlenmiştir. Ev yapımı Kahvaltılık Zahter örneklerinden elde edilen ekstraktların endüstriyel olanlara göre daha yüksek toplam fenolik içerik, antibakteriyal ve antioksidan aktivite gösterdiği belirlenmiştir.

Anahtar Kelimeler: Antibakteriyal, Antioksidan, Kahvaltılık Zahter, Fenolik içerik

INTRODUCTION

Traditional herbal mixtures are of important food additives in all climates around the world. They almost represent traditional cuisine of the local cultures and countries. Related to interest on traditional foodstuffs and different tastes their importance have increased recently. Besides their traditional production locally, some of them have been produced in industrial scale and supplied to global markets. In this context, Breakfast Zahter is a mixture of spices and nuts consumed mainly at breakfast. Its composition is very rich and changes by cultures and countries. Having a number of spices like zahter (*Thymbra spicata* var. *spicata* L.), aniseed (*Pimpinella anisum* L.), cumin (*Cuminum cyminum* L.), coriander (*Coriandrum sativum* L.), fennel (*Foeniculum vulgare* Mill.), sumac (*Rhus coriaria* L.), black cumin (*Nigella sativa* L.), red pepper, dried unripe grape, sesame and nuts. Its conventional consumption with olive oil at breakfast also support this phenomenon [1]. Ucan et al. [2] investigated some chemical compositions (pH, dry matter, crude protein, fatty oil and inorganic matter) of different Breakfast Zahter products obtained from homemade and industry.

There are many reports indicating high antioxidant capacity and antimicrobial activity of herbs and spices [3, 4]. As a healthy food product with rich spice content, Breakfast Zahter could be high in its antimicrobial activity and antioxidant capacity. Thus, in this study, total phenolic content, antioxidant capacity and antibacterial activities of some Breakfast Zahter products obtained from spice seller and local markets in southeastern part of Turkey were determined.

MATERIALS and METHOD

Preparation of Zahter Extracts

In this study, 6 different Breakfast Zahter samples homemade produced (4 and 6) and purchased from the different local markets (1, 2, 3 and 5) in Kilis and Hatay, were examined as materials. About 10 g of the powdered materials were extracted in methanol and ethanol (250 mL) by using the Soxhlet apparatus. Following filtration of the final extracts, the obtained extracts were concentrated under vacuum on a rotary evaporator at 35°C. The dry extracts were dissolved in 5 mL of methanol and ethanol. Samples prepared in either methanol or ethanol according to the type of the extract were called as E1, E2, E3, E4, E5, E6 and M1, M2, M3, M4, M5, M6. Samples were stored at 4°C for further use.

Determination of Total Phenolic Contents

The concentration of total phenolic in Breakfast Zahter extracts was measured by using spectrophotometric method [5]. 0.5 mL of the methanolic or ethanolic extracts was mixed with 2.5 mL of 10% Folin-Ciocalteu's reagent and 2.5 mL 7.5% $NaHCO_3$. The reaction mixtures were incubated in a water bath at 45°C for 45 min. Thereafter, the absorbance of samples was spectrophotometrically measured at 765 nm. A standard curve was prepared by using standard gallic acid solution in different concentrations. The content of phenolics in extracts was expressed as gallic acid equivalent (mg/L), according to the measured absorbance.

Radical Scavenging Assay

The antioxidant capacity of the extracts was determined by the stable DPPH (2,2-diphenyl 1-picrylhydrazyl) radical scavenging assay. 100 µL of the extracts and 3.9 mL of the DPPH (0.025 g/L in methanol) solution prepared in either methanol or ethanol according to the type of the extract were mixed. The mixtures were incubated in dark at room temperature for 2h. The remaining DPPH amount was determined by measuring at 515 nm absorbance. In test extracts, the inhibition of DPPH was calculated as percent according to the formula $I\% = [(A_{blank} - A_{sample})/A_{blank}] \times 100$. The blank is the absorbance of the control, which contains all reagents except the extract, and A sample is the absorbance of the test extract or the reference [6, 7].

Antibacterial Activity

Antibacterial activity of different concentrations (10, 30, 50, 70 and 100 µL) of Breakfast Zahter extracts against two bacteria (*Staphylococcus aureus* ATTC25923 and *Klebsiella pneumoniae* ATCC700603) was investigated by the agar well diffusion method. The standard antibacterial agent, Gentamicin (10 µg/disc) and the pure ethanol-methanol were used as control groups.

Statistical Analysis

The software SPSS 20.0 for Windows (SPSS Inc., Chicago, IL, USA) was used for analysis of variance (ANOVA) and Duncan's multiple comparison tests in order to determine significant differences between the samples. Each experiment was repeated at least three times. Statistical analysis was evaluated between samples which obtained from same solvents.

RESULTS and DISCUSSION

Total Phenolic Content

Total phenolic contents in the different Breakfast Zahter extracts were expressed as mg/L of Gallic Acid Equivalents (GAE). In methanol and ethanol extracted samples, the total phenolic contents were revealed to range from 56.4 to 3483.34 and 1274.45 to 5489.03 mg/L, respectively (Figure 1). In methanol extracts of Breakfast Zahter samples by homemade produced, total phenolic contents were determined as 2433.12 (M4) and 2826.13 mg/L (M6). The samples prepared by ethanol, total phenolic contents were detected as 5489.03 (E4) and 3660.77 mg/L (E6). In compared to methanol and ethanol extracts for homemade Breakfast Zahter samples, the highest total phenolic content was observed to E4. For the samples purchased from the different trademarks, while the highest total phenolic contents in the methanol extracts of 2 and 3 numbered samples were calculated as 1254.00 and 56.54 mg/L,

these highest contents for 1 and 5 numbered samples were observed in ethanol extracts (4843.20 and 3654.71 mg/L). When all extracts analyzed, it was exhibited that total phenolic content in homemade Breakfast Zahter samples was quite higher than industrial products. Differences of total phenolic contents in extracts obtained from same Breakfast Zahter samples could depend upon the polarity of solvent used in extraction, especially 3 numbered sample.

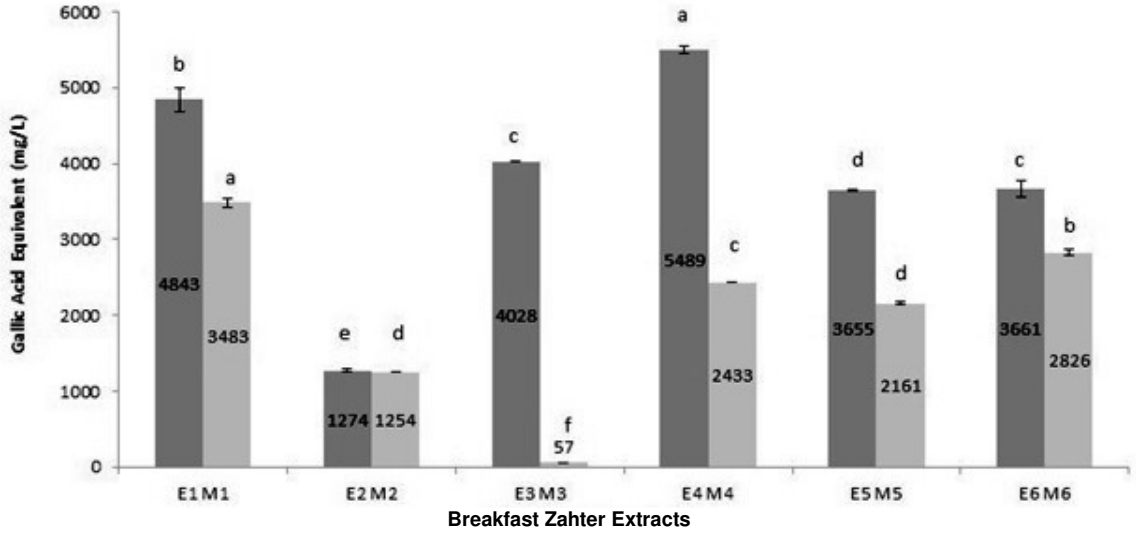

Figure 1. Total phenolic content in extracts, Line over bars with different small letters indicates samples which are significantly different ($p<0.05$). (see the text for sample codes)

High solubility of total phenolic contents in the extracts acquired by using polar solvents indicates high concentration of phenolic substances in Breakfast Zahter samples [5, 8-9]. Homemade and industrial Breakfast Zahter samples had different total phenolic contents and homemade samples had higher levels (Figure 1). Because of homemade Breakfast Zahter products having higher spice content and lower other content than industrial ones, higher total phenolic contents in homemade Breakfast Zahter products are expected. Scientific reports indicate that spices have high total phenolic contents in food plants [3, 4]. In this study, Breakfast Zahter samples had surprisingly lower total phenolic contents in comparison to its spice ingredients. Breakfast Zahter production processes are cleaning, roasting, grinding and packing, and roasting process could be resulted of total phenolic decomposition and losses.

Antioxidant Capacity

Ethanolic and methanolic extracts of different Breakfast Zahter samples were subjected to the DPPH free radical scavenging assay for comparison of their possible antioxidant activities (Figure 2). The results were expressed as inhibition percentage of free radicals, and the highest inhibition percentage exhibited the highest effects of the extracts. Antioxidant capacities of all Breakfast Zahter extracts were between 78.2 and 91.4%. In homemade samples, the highest antioxidant activities were observed for ethanolic extracts (91.4 and 90.3% for E4 and E6). For methanolic extracts, these activities were 78.4 (M4) and 79.3% (M6). In industrial Breakfast Zahter productions, antioxidant capacities for the ethanol extracts of 1, 2, 3 and 5 numbered samples were 90.4, 89.9, 90.2 and 84.7%, respectively. The highest antioxidant activities for methanolic extracts were observed in 2 and 3 numbered samples (88.4 and 81.4%). The solubility of the antioxidant compounds in different chemical structure such as phenolic contents bases on the polarity of solvents [10]. In comparison to total phenolic contents and antioxidant activities of extracts, the highest values for both parameters were obtained for ethanolic extracts. In addition, high antioxidant capacities of the samples, which had high total phenolic contents, showed that these two parameters affect each other (for example, sample E4).

The difference between different Breakfast Zahter samples in total phenolic content and antioxidant capacity analysis for each six samples was statistically significant ($P<0.05$) (Figures 1 and 2).

Antibacterial Activity

The results of antibacterial activities of Breakfast Zahter samples obtained from the ethanol and methanol solvents are shown in Table 1. These activities were evaluated according to clearance zone in around of wells. In 10 µL concentrations of all extracts were not observed an inhibitory effect on standard strains of *Staphylococcus aureus* ATTC25923 and *Klebsiella pneumoniae* ATCC700603. The highest inhibition activities of ethanol extracts on *Klebsiella pneumoniae* ATCC700603 and *Staphylococcus aureus* ATTC25923 were determined in E5 and E4 samples with 14 and 18 mm zone diameter at 100 µL concentrations. As for methanol extracts, the antibacterial activities of M1 sample against *Klebsiella pneumoniae* ATCC700603 and *Staphylococcus aureus* ATTC25923 were

determined with 19 and 20 mm zone diameters, at the same concentration. In addition, these zones are the observed highest inhibition activities. Pure ethanol and methanol (98%) used as negative control groups did not showed inhibitory effect on strains. Antibacterial effect of standard antibiotic gentamicin against tested strains was determined as 13 (*Klebsiella pneumoniae* ATCC700603) and 12 mm (*Staphylococcus aureus* ATTC25923).

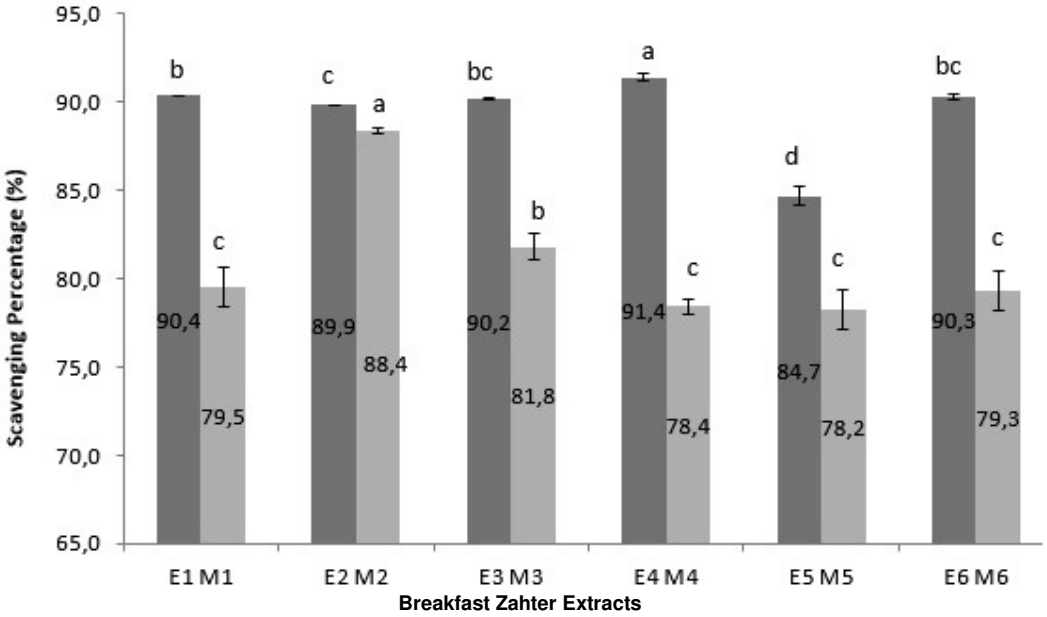

Figure 2. Antioxidant capacity of extracts, Line over bars with different small letters indicates samples which are significantly different ($p<0.05$)

Moreover, antibacterial activities of homemade Breakfast Zahter samples on standard strains were generally detected to be more effective compared to industrial productions. However, the results of total phenolic content, antioxidant capacity and antibacterial activity analyses were indicated that Breakfast Zahter extracts produced by traditional methods were to be efficient than industrial productions. Higher spice ingredients of homemade Breakfast Zahter than industrial ones could result in higher antibacterial activity.

Table 1. Antibacterial activities of Breakfast Zahter extracts on *K. pneumoniae* ATCC700603 and *S. aureus* ATTC25923 in terms of zone diameter (mm)

	Pathogen strains									
	K. pneumonia ATCC700603					*S. aureus* ATTC25923				
*SA** / *S***	10µL	30µL	50µL	70µL	100µL	10µL	30µL	50µL	70µL	100µL
E1	-	-	-	-	-	-	10	12	13	17
E2	-	-	-	-	11	-	-	-	-	-
E3	-	-	-	-	-	-	-	12	15	17
E4	-	-	-	-	13	-	10	12	14	18
E5	-	-	-	11	14	-	-	-	10	12
E6	-	-	-	12	13	-	11	12	15	15
M1	-	10	12	14	19	-	10	14	17	20
M2	-	-	-	-	-	-	-	10	11	12
M3	-	-	-	-	-	-	10	14	17	19
M4	-	11	12	13	17	-	10	10	11	13
M5	-	-	-	-	-	-	10	11	13	17
M6	-	10	12	13	14	-	12	19	19	20
Pure methanol	-	-	-	-	-	-	-	-	-	-
Pure ethanol	-	-	-	-	-	-	-	-	-	-

*: Sample amount, **: Sample code

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